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Studies on the botanical origin and the residues of pesticides in corbicular pollen loads and bee bread of bee colonies in the proximity of apple orchards in South Tyrol

Untersuchungen zur botanischen Herkunft und den Rückständen von Pflanzenschutzmitteln in Pollenhöschen und Bienenbrot von Bienenvölkern im Einzugsgebiet des Obstbaus von Südtirol

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Abstract

Pollen is the primary source of protein for honeybee colonies. Foragers collect pollen from different plants depending on the availability of the surrounding environment, which varies seasonally in wild and urban areas or agricultural landscapes. In agricultural systems, honeybees can play a particular role as pollinators, especially where entomophagous pollination is important, such as in pome fruits. In intensively managed agricultural systems, honeybees and other pollinators are often exposed to pesticides. The exposure to pesticides via corbicular pollen load or bee bread has been analysed in several studies; previously, some studies also included the analysis of the botanical origin of the chemically analysed corbicular pollen load samples. In this study, we tried to focus on the accumulation of pesticide quantities in bee bread portions in the hives from March to October and simultaneously the dynamics behind the incoming pesticide residues on the daily collected corbicular pollen load samples from March to June. In addition, palynological analyses were performed on some corbicular pollen loads.

From 2016 to 2020, we collected corbicular pollen loads from two honeybee colonies from March to June in three different apiaries in South Tyrol. Every three weeks, starting with calendar week 11 in March, we took bee bread samples from other colonies in the same apiary, next to those which were used for corbicular pollen load collection. We performed chemical residue analyses on 154 corbicular pollen load and 217 bee bread samples. In 84.3% of the corbicular pollen load samples and in 76.4% of bee bread samples, we found contamination with pesticide active substances (PAS) used in plant protection products harmful to bees (according to the Italian etiquette). On 152 samples of the corbicular pollen loads, we also performed palynological analyses to determine the botanical origin of the pollen samples. In the case of some samples, even if most of them belonged to species not grown in agricultural fields, the quantity of active substances harmful to bees detected on them was higher than 1 mg/kg, as in the case of chlorpyrifos-methyl. On the example of chlorpyrifos-methyl and chlorpyrifos-ethyl (the latter was used only until 2016 and was then mostly replaced by chlorpyrifos-methyl for the period 2017-2020), it was possible to show in which amounts these active substances were present in corbicular pollen loads and bee bread samples of colonies placed near apple orchards in South Tyrol over a period of five years.

Keywords

honeybees, corbicular pollen loads, bee bread, pesticides harmful to bees

Zusammenfassung

Blütenpollen stellt die Hauptproteinquelle von Honigbienenvölkern dar. Sie sammeln ihn von verschiedenen Pflanzen, abhängig von der Umgebung (z. B. wild-wachsende oder in Hausgärten vorzufindende Pflanzen sowie landwirtschaftliche Kulturen) und der Jahreszeit. In landwirtschaftlichen Kulturen können Honigbienen eine besondere Rolle einnehmen, vor allem z. B. bei Kernobst, wo die entomophage Bestäubung eine wichtige Rolle spielt. In intensiv bewirtschafteten Kulturen sind Bienen und andere Bestäuber oft Pflanzenschutzmitteln ausgesetzt. Verschiedene Studien haben die Exposition über Pollenhöschen und Bienenbrot untersucht und einige hatten dabei zuletzt auch die botanische Zusammensetzung der Pollenhöschen bestimmt. Wir versuchten im Rahmen dieser Studie die sich im Bienenbrot ansammelnden Rückstände von bienengefährlichen Pflanzenschutzmitteln von März bis Oktober zu bestimmen und gleichzeitig die Dynamik der eingetragenen Rückstände über Pollenhöschen von März bis Juni zu erfassen. Einige der Proben von Pollenhöschen wurden auch palynologisch bestimmt.

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Im Zeitraum von März bis Juni 2016–2020 wurden an drei verschiedenen Standorten in Südtirol Pollenhöschen mithilfe von 2 Bienenvölkern je Standort gesammelt. Zusätzlich wurden ab Kalenderwoche 11 im März alle drei Wochen Bienenbrot-Proben von Völkern, welche neben den Pollenhöschen sammelnden Völkern standen, gezogen. Insgesamt konnten an 185 Proben von Pollenhöschen und 250 Proben aus Bienenbrot Rückstandsuntersuchungen durchgeführt werden. Dabei wurden in 84,3 % der Pollenhöschen und 76,4 % der Bienenbrot-Proben Rückstände von bienengefährlichen Pflanzenschutzmitteln festgestellt.

Stichwörter

Honigbienen, Pollenhöschen, Bienenbrot, bienengefährliche Pflanzenschutzmittel

Introduction

The total area of agricultural land in South Tyrol is about 209,232 ha. The most important fruit productions are apple orchards (18,438.9 ha), vineyards (5,553 ha), stone fruits (cherries 108 ha and apricots 81 ha), and berries (strawberries 110 ha and raspberries 25 ha) (Autonome Provinz Bozen – Abteilung Landwirtschaft, 2020). Orcharding has a long history in this area and it is still important also today. South Tyrol has developed to become the largest contiguous apple growing area in Europe and within this process also the management of these orchards has continuously evolved (Dalla Via & Mantinger, 2012).

In order to ensure continuously high crop yields, a minimum of pest management, such as chemical plant protection, is a general approach in agriculture adopted by farmers to also ensure adequate quality of the yield (Cooper & Dobson, 2007; IDM Südtirol – Alto Adige, 2017). At the same time, different strategies (e.g. integrated pest management) have been developed to avoid negative impacts on the environment (IDM Südtirol – Alto Adige, 2017; Godfray, 2014). A major challenge in the use of plant protection products is the protection of pollinators to ensure the environmental service of pollination. Pollination is one of the most important services provided by wild pollinators and honeybees in agro-ecosystems (Gallai et al., 2009; Klein et al., 2007; Lautenbach et al., 2012). In 2019, 3,473 beekeepers with 37,957 honeybee colonies were registered in South Tyrol (Autonome Provinz Bozen – Abteilung Landwirtschaft, 2020).

For honeybees, the main routes of pesticide contamination are direct contamination during spray application (Koch & Weißer, 1997), dust abraded from treated seeds during sowing in arable farm land (Pistorius et al., 2009), and contaminated water puddles (Samson-Robert et al., 2014; Rolke et al., 2016). In addition, the exposure through collected pollen, nectar, and guttation droplets should also be considered (Rolke et al., 2016; Girolami et al., 2009; Reetz et al., 2011), also on wildflowers (Böhme et al., 2018a; Botías et al., 2016; Böhme et al., 2018b). As a consequence, pesticides can accumulate in bee matrices in the hive (Böhme et al., 2018a; Johnson et al., 2010; Mullin et al., 2010; Traynor et al., 2016). Several studies have shown contamination with pesticides through a chemical residue analysis of bee bread samples (Mullin et al., 2010; Orantes-Bermejo et al., 2015; Porrini et al., 2016; Kruse-Plaß et al., 2020). Bee bread is relatively easily accessible in the hive, but it is a mixture of many pollen pellets where the contamination is not expected to be homogeneous (Böhme et al., 2018b). More detailed information can be obtained by analysing pollen pellets (Rolke et al., 2016; Smodis Skerl et al., 2009; Chauzat et al., 2009; Stoner & Eitzer, 2013; Böhme et al., 2018b; Favaro et al., 2019). Pollen pellets can be collected daily during the active agronomic season, and it is also possible to identify (at least partially) the botanical origin of some pollen portions. Some studies have used this approach to show that some portions of the pollen pellets collected daily by foragers had different levels of contamination with pesticide active substances (PAS) (Friedle et al., 2021; Böhme et al., 2018b; Favaro et al., 2019).

With some exceptions, most of the measured PAS residues were found at sub-lethal concentrations considering LD_{50} values and maximum pollen consumption rates (Rortais et al., 2005). Nevertheless, some studies have shown that even sub-lethal concentrations of pesticides can cause effects such as impaired behaviour and performance, changes in social interactions, effects on growth, development, or gene expression of honeybees (Andrione et al., 2016; Tosi et al., 2017; Alkassab & Kirchner, 2017; Wu et al., 2017).

Considering the important role of honeybees in South Tyrolean agriculture, we investigated PAS residues in corbicular pollen loads and bee bread samples at selected sites to obtain information on the entry of such PAS into beehives. In some samples of corbicular pollen loads collected in spring, the botanical composition was also analysed in order to better understand from which plants collection occurred when the contamination was measured. During the active season for five years, at three different sites, and under common agricultural practice, corbicular pollen load samples were analysed from March to June to identify their pesticide contamination and their botanical composition. Simultaneously, we extracted and chemically analysed bee bread samples from March to October in order to additionally monitor the accumulation and degradation of pesticides within the hive.

Materials and methods

Seven honeybee colonies were placed at three different apiaries in the villages of Lana, Tirol, and Rabland in the region Trentino-South Tyrol (northern parts of Italy; exact positions and exemplary apiary can be found in Table S1 and Figure S1). Two colonies were used to collect the corbicular pollen load samples with front porch pollen traps, and the other five colonies were used to extract three samples of bee bread every three weeks. All apiaries were located near apple orchards, which are the predominant agricultural form on the valley floor. In order to monitor a possible influence of plant protection measures in vineyards, the apiary in Rabland was moved in mid-July to a location in Kaltern, closer to the vineyards, in the years 2019 and 2020.

Corbicular pollen load samples were collected from March to June. The collected daily samples were placed in a plastic beaker to be stored at -20 °C before possible further analysis. Starting from calendar week 11 from March to October 2018–2020, every 3 weeks until calendar week 40, samples of bee bread were collected from the five colonies located next to the two colonies used for pollen collection with the pollen traps. Principally, on each sampling day, a comb containing bee bread was collected from at least one, but no more than three, of the five colonies. Samples of bee bread were then stored in a beaker at -20 °C for further analysis. Residue analysis was performed by two different laboratories (Greit and Lufa). The analytical method was based on the multi-residue sample preparation technique QuEChERS (Anastassiades et al. 2003) followed by GC-MS (/MS) and LC-MS/MS analysis. To estimate the risk to honey bees from the measured PAS contamination in bee matrices, the Pollen Hazard Quotient (PHQ) max was calculated according to Stoner & Eitzer (2013). The analysis focused on the highest measured concentrations of the most harmful PAS (LD50 < 10 µg/bee) found in the samples, as these were expected to pose the highest risk.

The palynological analysis was performed as in the master thesis of Jacob Geier (2021).

Results

Of the 371 chemical analyses, 217 were performed on bee bread and 154 on corbicular pollen loads. Residues of PAS harmful to bees were found in 84.3% of the corbicular pollen load samples and 76.4% of the bee bread samples. The corbicular pollen load samples contained 17 different PAS harmful to bees, while the bee bread samples contained 14 (Table 1). Flupyradifuron, spirodiclofen and thiamethoxam were found only once in the corbicular pollen load, while phosmet was detected most frequently (80), followed by imidacloprid (67) and chlorpyrifos-methyl (56). The highest absolute concentrations were found for phosmet (4.2 mg/kg), chlorpyrifos-ethyl (2 mg/kg), and imidacloprid (1.27 mg/kg), while the highest mean concentrations were found for chlorpyrifos-ethyl (0.49 mg/kg), phosmet (0.47 mg/kg), and chlorpyrifos-methyl (0.19 mg/kg). In the bee bread samples, a lower number of PAS harmful to bees and also lower concentrations were found compared to the corbicular pollen load samples. Fenoxycarb, flupyradifurone, pyrethrins, spirotetramat were not detected in bee bread, while pyriproxyfen, sulfoxaflor and dimethoate were found only once. In contrast, residues of spinosad (12 detections) were found only in bee bread samples. The highest absolute concentrations were measured for phosmet (2.2 mg/kg), chlorpyrifos-methyl (0.64 mg/kg) and chlorpy-

Table 1. Mean and maximum concentration of products harmful to bees and number of detections in the analysed corbicular pollen load (p.l.) and bee bread (b.b.) samples.

active substance	concentration [mg/kg]						LD50 oral* [µg/bee]	PHQ max (p.l.)	PHQ max (b.b.)	no. of detections (p.l.)	no. of detections (b.b.)
	max (p.l.)	max (b.b.)	mean (p.l.)	mean (b.b.)	± st. dev. (p.l.)	± st. dev. (b.b.)					
abamectin	0.02	0.05	0.01	0.02	0.00	0.01				4	12
chlorantraniliprole	0.05	0.08	0.03	0.02	0.01	0.01	> 104.1			7	39
chlorpyrifos-ethyl	2.00	0.13	0.49	0.09	0.45	0.06	0.25	8,000	520	48	2
chlorpyrifos-methyl	1.20	0.64	0.19	0.12	0.24	0.12	0.18	6,667	3,556	56	84
dimethoate	0.01	0.01	0.01	0.01	0.00		0.10	100	100	4	1
etofenprox	0.26	0.05	0.06	0.02	0.05	0.01	0.366	710	137	29	24
flupyradifurone	0.01		0.01				1.20	8		1	
imidacloprid	1.27	0.07	0.08	0.02	0.17	0.01	0.0037	343,243	18,919	67	20
indoxacarb	0.25	0.02	0.05	0.02	0.06	0.00	0.232	1,078	86	12	2
phosmet	4.20	2.2	0.47	0.14	0.87	0.28	0.37	11,351	5,946	80	127
pyrethrins	0.05		0.03		0.03					2	
pyriproxifen	0.10	0.02	0.04	0.02	0.03		> 100			8	1
spinosad		0.03		0.02		0.01	0.057		526		12
spirodiclofen	0.02	0.03	0.02	0.02		0.01	> 196			1	7
spirotetramat	0.02		0.02		0.00		> 107.3			10	
sulfoxaflor	0.08	0.02	0.05	0.02	0.02		0.146	548	137	7	1
thiacloprid	0.54	0.35	0.05	0.09	0.10	0.10	17.32	31.2	20.2	31	25
thiamethoxam	0.35		0.35				0.005	70,000		1	

*LD_{50} values were obtained from the University of Hertfordshire pesticide properties database, the University of Hertfordshire bio pesticide properties database and the US EPA ecotoxicology database. For substances where the LD_{50} was indicated > as x, no calculation of PHQ max was performed. No LD_{50} oral values were found for abamectin and phyretrins.

rifos-ethyl (0.13 mg/kg) – as well as for the highest mean concentrations (0.14 resp. 0.12, resp. 0.09 mg/kg). The most frequently detected substances were phosmet (127), chlorpyrifos-methyl (84), and chlorantrarniliprole (39).

To get a clearer idea of that what these contaminations mean, the concept of the PHQ is useful. The great advantage of the PHQ is that the hazard quotient of each PAS can be calculated and compared to each other. In general, a high PHQ (high risk to bees) can be the result of a very low LD_{50} or a high detected concentration of an active substance.

The highest PHQ was 343,243 in corbicular pollen loads and 18,919 in bee bread. In both cases, this was the result of contamination with the active substance imidacloprid (LD_{50}: 0.0037 µg/bee), which was the most toxic substance in our samples, according to the oral LD_{50}, after thiametoxam (LD_{50}: 0.005 µg/bee) and spinosad (LD_{50}: 0.057 µg/bee).

To better understand when contamination with PAS occurred in the studied matrices from 2016 to 2020, only results for chlorpyrifos-methyl (and for 2016 chlorpyrifos-ethyl) are shown in the following plots (Figure 1 and Figure 2). Chlorpyrifos-ethyl was mainly used in horticulture in South Tyrol only in 2016 and from 2017 to 2020, chlorpyrifos-ethyl was replaced by chlorpyrifos-methyl. The choice of these two PAS was advantageous because they were expected to be used in practice (especially in apple orchards) in a more or less constant way during the five years of observation.

In the following, chlorpyrifos is used as a synonym for chlorpyrifos-ethyl and chlorpyrifos-methyl.

Most of the corbicular pollen load samples analysed were from the late April/early May period (see in the row "no. of analysed samples per cw" in Figure 1). The highest chlorpyrifos contaminations in corbicular pollen load samples were observed in the

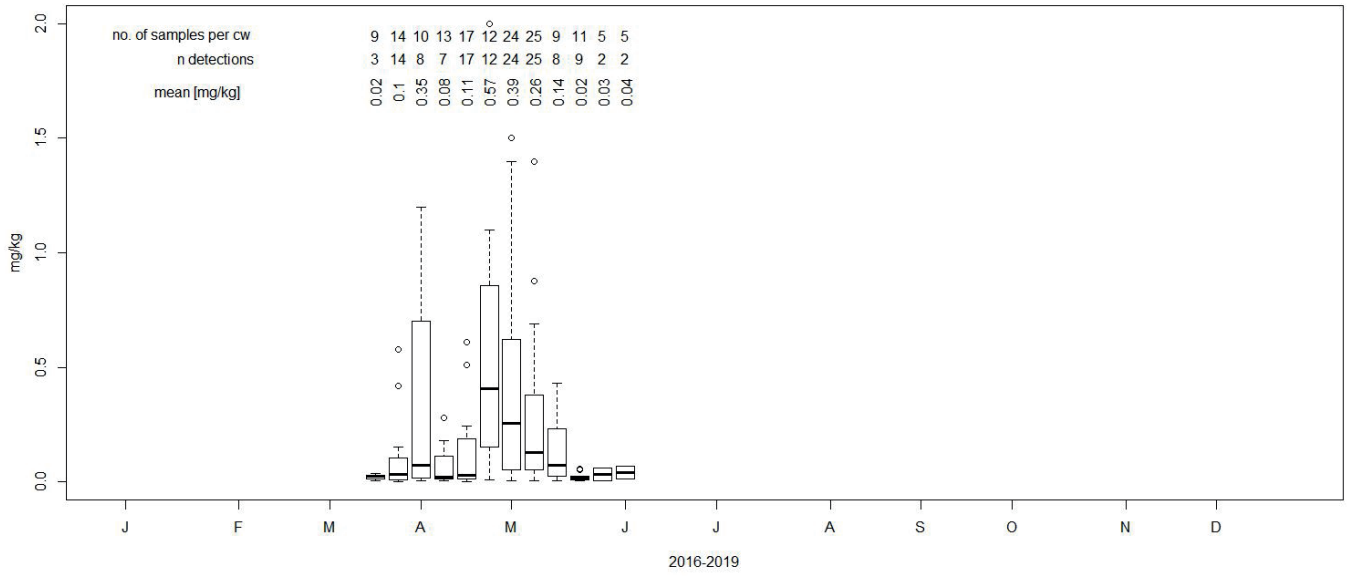

Figure 1. Residues of chlorpyrifos in corbicular pollen loads per week from March to June 2016–2020.

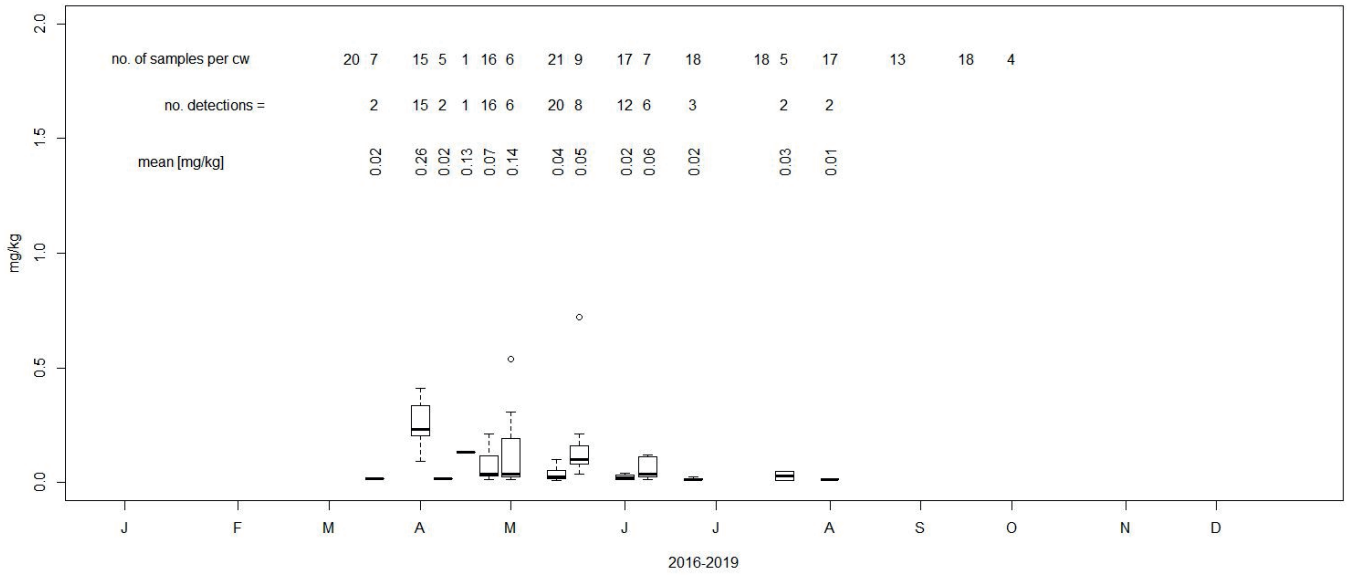

Figure 2. Residues of chlorpyrifos in analysed bee bread samples from 2016 to 2019.

late April/early May period. In particular, in mid-April (roughly corresponding to the annual apple bloom in the valley floor) and from mid-May to early June, concentrations were lower than in the periods from late March to the first half of April and from late April to mid-May. The highest concentration for chlorpyrifos was 2.00 mg/kg at the end of April. No residues of chlorpyrifos were found at the beginning of the observations in the three samples taken around 10th of March. In early June, chlorpyrifos residues were found in 2 out of 5 samples. During the rest of the spring, the active ingredient was present in more than 50% of the samples when analyses were performed.

Figure 2 concerns the residues of chlorpyrifos in the bee bread samples. No residues of chlorpyrifos were found in most of the bee bread samples from calender week 11 and 12 in March chlorpyrifos (0.02 mg/kg, n=2/27). At the end of March and the first days of April, chlorpyrifos residues were detected in 15 out of 15 samples analysed, reaching a maximum median concentration of 0.26 mg/kg. From late April to mid-June, chlorpyrifos was usually present in more than 50% of the daily samples analysed.

In the 75 samples from July to October, chlorpyrifos was detected in only four samples (the last one in the first days of August). The highest concentration measured in July on day 208 (~ July 27) in one of those four samples was 0.048 mg/kg. No residues were found from the end of August until the last samples were analysed in early October.

At the same time as the PAS residues in the corbicular pollen and bee bread samples were examined, the former were also analysed palynologically in order to identify the botanical origin of the pollen collected daily. Figure 3 shows the mean quantities of pollen (if several samples per day (day of the year) were analysed, otherwise it is the result of a single analysis), that could be assigned to individual plant species. The overall composition and abundance of certain species in the samples, varied throughout the ongoing season. In March

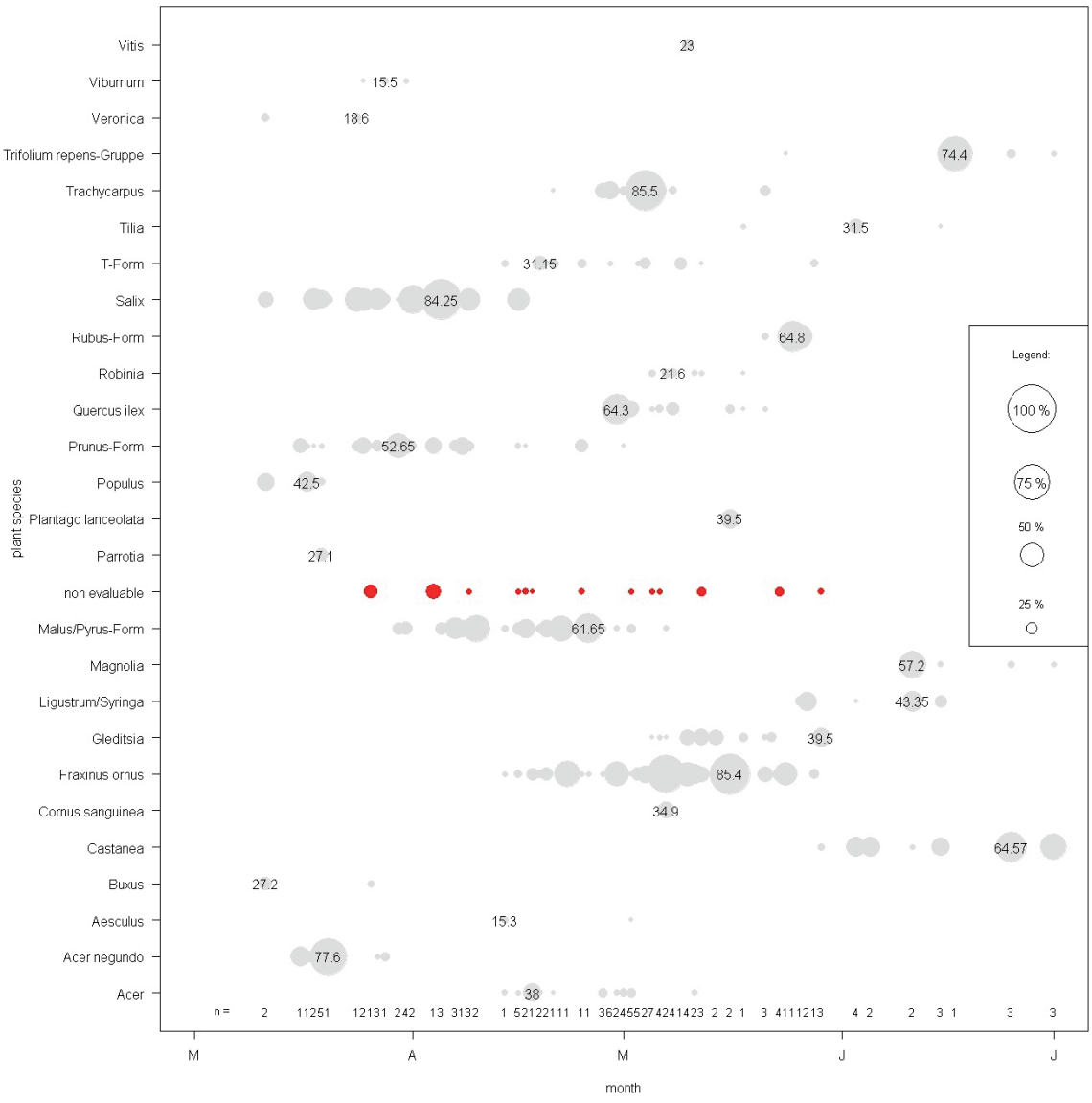

Fig. 3. Mean quantity of pollen per day (only if > 10%), which could be assigned to individual plant species from the total amount of a honey bee colony. The size of the circle corresponds to the number of the plant species (maximum values achieved by the single plants are plotted on the points). In the legend, circles are plotted that indicate 100, 50, 75, and 25%, respectively. The quantities that were not possible to identify are represented in red.

for example, we observed higher amounts of poplar (*Populus sp.*), willow (*Salix* sp.), acer (*Acer negundo*) and Prunus (*Prunus sp.*). Prunus, willow and acer (*Acer sp.*) continued to play an important role in April. Some other new plant species such as Malus/Pyrus-form (*Malus sp. or Pyrus sp.*) or T-form (from *Asteraceae*) reached maximum amounts of 61.65% resp. 31.15%. In May, ash (*Fraxinus ornus*), palm tree (*Trachycarpus sp.)*, oak tree (*Quercus ilex)*, locust *(Robinia sp.)*, honey locust *(Gleditsia sp.)* and rubus (*Rubus-Form)* were regularly present in higher amounts, while *Cornus sanguinea* and *Plantago lanceolata* appeard punctually in amounts of more than one third of the daily collected amount. In June, chestnut (*Castanea sp.*), clover (*Trifolium repens-group*), Magnolia, linden tree (*Tilia sp.*), and privet (*Ligustrum/Syringa*) served as major pollen sources. For some other species such as *Buxus (Buxaceae)*, *Parrotia (Hamamelidaceae), Aesculus (Sapindaceae)*, Veronica *(Scrophulariaceae)*, *Vitis sp. (Vitaceae)* and *Cornus sanguinea* (*Comaceae*) only punctual detections of more than 10% were observed.

Discussion

The aim of this study was to investigate in more detail the dynamics of the entrance of PAS entry into honeybee colonies, after some results were obtained in a preliminary study in 2015. During the first two years (2016 and 2017), the focus was more on the contamination of corbicular pollen loads with PAS in spring (70 chem. analyses from 2016 to 2017 resp. 84 chem. analyses from 2018 to 2020), whereas from 2018 to 2020, the focus was on the contaminations of bee bread (24 chem. analyses from 2016 to 2017 resp. 193 chem. analyses from 2018 to 2020) and the palynological composition of the corbicular pollen loads (49 palyn. analyses from 2016 to 2017 resp. 103 palyn. analyses from 2018 to 2020) to better understand if it is possible to assign contaminations to a certain group of plant species.

This study describes for the first time the contamination of corbicular pollen loads and bee bread samples with PAS in South Tyrol over a period of five years. It was possible to describe the changing PAS concentrations (as exemplified by chlorpyrifos-ethyl and -methyl in this paper) in both matrices. The continued analysis of bee bread samples in summer and autumn allowed an estimation of how long and in what concentrations PAS remain in the stores (i.e. bee bread) within the hives.

At the same time, the botanical origin of some of the corbicular pollen loads collected daily in spring was analysed; the results showed that on most days, two or three woody plants could account for more than 50% of the daily collected pollen. Species that are sometimes present in apple orchards (except Malus/Pyrus-Form at the time of its bloom), such as *T-Form* from *Asteraceae*, *Veronica* or *A/H-Form* from *Apiaceae*, contributed only in small amounts to the pollen collected in one day.

Variety and concentrations of PAS detected in the corbicular pollen loads were generally higher than those in the bee bread samples. For example, the maximum concentrations of chlorpyrifos-methyl in corbicular pollen pellets (2 mg/kg) was three times higher than the maximum concentration in bee bread samples (0.64 mg/kg). However, this was expected considering the results of other studies (Mullin et al., 2010; Traynor et al., 2016; Orantes-Bermejo et al., 2015; Porrini et al., 2016; Böhme et al., 2018b; Friedle et al., 2021). This is probably because bee bread samples are always a combination of pollen portions collected over several days, if not weeks. Colonies do not collect pollen in approximately the same amount over days; often the composition of plant species is different the next day, or at least the proportions assigned to individual plant species change (Roncoroni et al., 2019). Therefore, in general, a mixture of a more contaminated portion with a less or no contaminated portion is more likely to occur in bee bread, resulting in dilution. Moreover, the sampling method used took only a small portion (at least 5 g) from a much larger reservoir of bee bread within a hive for chemical analysis. These are probably the main reasons why the corbicular pollen loads were almost 10% more frequently contaminated with PAS harmful to bees than the bee bread samples (84.3% vs. 76.4%) and why the diversity of PAS harmful to bees is generally lower in bee bread (17 vs. 15).

Less than half of the PAS found in corbicular pollen loads (seven substances: chlorpyrifos-ethyl, chlorpyrifos-methyl, etofenprox, imidacloprid, indoxacarb, phosmet, and thaiclоprid) were found in more than 10 samples. They accounted for most of the contaminations. Substances such as flupyradifurone or sulfoxaflor were rarely found because these products were first registered in 2019 and 2020, while phosmet, imidacloprid, chlorpyrifos-ethyl and –methyl, thiacloprid or etofenporx were most common, indicating a wider use in agricultural practice.

A similar situation can be observed for the bee bread samples, where also only 7 PAS were present in more than 10 samples. Most of the substances were the same as those found in the corbicular pollen loads (chlorpyrifos-ethyl, etofenprox, phosmet and imidacloprid), with the exception of the three products abamectin, chlorantraniliprole and spinosad. They were more present in samples of bee bread and in the period from summer to autumn, the time when no analysis of corbicular pollen loads is available. However, their maximum concentration reached 0.08 mg/kg (chlorantranliliprole) and exceeded a PHQ of 500 only in the case of spinosad, with a maximum PHQ of 526 for one time. They were therefore present only in very low concentration in comparison to other pesticides in other moments of the year. The timing of the spinosad contaminations (9 times in September, 1 time in October and for 4 times in the first samples analysed in March at the beginning of the season) and the presence of this PAS at sites with more vineyards, lead to the conclusion that this is the result of its use in vineyards to protect the grapes against of *Drosophila suzukii* attack in autumn.

In the case of bee bread samples, it was possible to observe how long contaminations of an exemplary PAS, such as chlorpyrifos, were present until the end of the season. Figure 2 shows that most of the contaminations are present from the end of March to mid-June, followed by only two detections in July and two in August at quite low concentrations (max. 0.048 mg/kg). No residues of chlorpyrifos were found in the samples from September, October and the first half

of March (first half of March, in total 20 samples analysed). This means that, at the end and the beginning of the season (during the overwintering period), the bee bread samples were free from contaminations of these PAS harmful to bees, which were found regularly during the season (highest contaminations in May). However, this study shows that the contamination of the corbicular pollen load and bee bread samples with PAS in spring went hand in hand: when corbicular pollen load samples showed higher contaminations, more residues were subsequently found in the bee bread samples. If this assumption holds true for other months as well (which seems to be obvious), the lower contaminations of bee bread samples from July to October (shown for example for chlorpyrifos in Figure 1 and Figure 2) means that the risk of PAS contaminations of the pollen diet would be higher in spring than in summer or autumn.

It is remarkable that the analysed mid-April corbicular pollen load samples showed less frequent residues and lower concentrations of chlorpyrifos than the samples before (late March to early April) and after this period (late April to mid-May) (Figure 1). The median concentration was 0.017 mg/kg on April 14 and 0.018 mg/kg on April 15. In contrast, no chlorpyrifos residues were found in the five samples analysed on April 13 and in two samples analysed on April 16, and this period (mid-April) corresponds more or less to the main flowering period of apples in the years observed. The application of products harmful to bees is not allowed during the flowering period and therefore no residues or very low concentrations were found in corbicular pollen loads. The reasons for these very low levels of contamination could be the whirling up of dust particles on the ground by wind, as foehn is a very common phenomena in South Tyrol during spring, also in the Burggrafenamt district (Linhart et al., 2019; Kruse-Plaß et al., 2020), or drift (Böhme et al., 2018b; Sartori et al., 2020; Favaro et al., 2019; Lötscher & Ehrler, 2020; Prechsl et al., 2022). This would also explain the contamination of corbicular pollen load samples, even if most of the collected pollen comes from plants that are not present in apple orchards (see Figure 3). However, there is certainly also an overlap of these phenomena, coupled with the fact that small amounts of plants which are present in agricultural fields are highly contaminated (Böhme et al., 2018b; Favaro et al., 2019). For example, we observed contamination of corbicular pollen loads collected directly from the legs of foraging bees on *Taraxacum officinale* in the apple orchards at concentrations of 0.21 mg/kg chlorpyrifos-methyl. A similar report was made by Böhme et al. (2018b), who stated that some weed species in cereals were contaminated with high levels of pesticide residues due to direct application in the field (Böhme et al., 2018b). Consequently, the daily collected corbicular pollen loads show relevant contaminations, although most of the pollen collection originates from woody plants growing somewhere outside the agriculturally managed area.

Two or three woody plants dominated most of the palynologically analysed samples of a single day and corresponded to the currently available flowering plants, e.g. in early spring *Populus sp.*, *Salix sp.*, *Prunus sp.* and *Acer sp.*. This means that, for many days, the majority of the corbicular pollen loads collected were from plants outside of intensively managed agricultural fields, such as apple orchards or vineyards, where pesticides are regularly used. Some species, such as the pollen type T-Form of *Asteraceae*, grow both in and outside the agricultural fields, and therefore the place of effective collection remains unclear (Geier et al., 2023). In addition, unfortunately, not all pollen grains can be assigned to a single plant species (e.g. the pollen types Malus/Pyrus-Form, A/H-Form, T-Form, J-Form, etc.) or were not evaluable – this makes it even more difficult to understand where certain portions were collected.

However, for plants, such as *Ligustrum sp.* or *Trachycarpus sp.*, it can be concluded that part of the pollen collection takes place in urban areas or gardens. Contamination with pesticides due to non-professional use – and thus a risk to honeybees – cannot be excluded.

The highest risk in our observations based on the evaluation of PHQ came from imidacloprid (maximum PHQ in corbicular pollen loads: 343,243 and in bee bread samples: 18,919). For the active ingredient thiacloprid, for example, the maximum PHQ calculated in corbicular pollen loads and bee bread never exceeded the value of 50, which Stoner & Elitzer (2013) considered to be a relevant threshold. Compared to the work of Favaro et al. (2019) on corbicular pollen loads in the same region (Trentino-South Tyrol), we calculated a 4-fold higher maximum PHQ for imidacloprid (343,243 versus 82,051), and this value is also more than 2-fold higher than their maximum PHQ of 160,000 due to a contamination with chlorpyrifos-ethyl. Focusing on the highest PHQ calculated for bee bread, our maximum values for imidacloprid achieved an approximately 4-fold higher maximum PHQ than that measured by McArt et al. (2017) in apple orchards (18,919 versus >4,000). In another study by Traynor et al. (2016) on bee bread near citrus plantations, the maximum PHQ exceeded 2,000. However, there are some critical aspects regarding PHQ that need to be mentioned. This method does not take into account the fact that toxicity is not the same at different developmental stages of a bee (different susceptibility between larvae and adult bees) or that nursing bees consume more pollen than foragers (Stoner & Eitzer 2013; Rortais et al., 2005; Aupinel et al., 2007; Tasei, 2001). Another point to consider is that the published LD_{50} values on which our calculations are based were obtained from feeding trails with a sugar solution, whereas the concentrations in the study were measured in corbicular pollen loads or bee bread samples (Stoner & Eitzer, 2013). Furthermore, pesticide interactions (e.g. synergism or antagonism) are not considered, too (Traynor et al., 2016). The combination of different pesticide substances in one sample has also been shown in some other studies (Mullin et al., 2010; Traynor et al., 2016; Pettis et al., 2013; Tosi et al., 2018; Böhme et al., 2018b; Favaro et al., 2019; Sartori et al., 2020), as well as the possible adverse effects of sublethal concentrations, such as impairment of behaviour, learning, memory, homing performance (Andrione et al., 2016; Tosi et al., 2017; Teeters et al., 2012; Decourtye et al., 2005; Fischer et al., 2014; Urlacher et al., 2016; Smagghe et al., 2013), growth development (Wu et al., 2011), queen fecundity (Wu-Smart & Spivak, 2016), and social interactions (Forfert & Moritz, 2017; Medrzycki et al., 2003).

On the one hand, it is good news that the PHQ (and consequently the concentrations of active substances) in bee bread are lower than in corbicular pollen loads; on the other hand, this is a permanent or at least longer lasting contamination exposure route parallel to that of corbicular pollen loads, as shown in this study by the example of chlorpyrifos (-ethyl and -methyl).

In any case, the superorganism honeybee colony is somehow able to tolerate and buffer the negative effects of pesticide exposure better than single individuals or wild bees do (Straub et al., 2015). Considering that wild bees or bumble bees are an important complement to entomological pollination, it should therefore be noted that they are even more at risk because they use few pollen pellets to supply single larvae that consume pollen directly without a nurse bee in between (Böhme et al., 2018b).

This study supports the findings of other researchers who have studied pesticide contamination of corbicular pollen loads and bee bread. In addition, this study illustrates the botanical origin of some corbicular pollen loads collected during the spring and was able to demonstrate that most of the collected pollen comes from plants outside agricultural fields. Unfortunately, the botanical origin of the bee bread samples was not analysed. The analysis of the contamination of corbicular pollen loads with PAS and their palynological composition, as done in this work during spring, should be extended in the future to the summer and autumn. A further publication will show the amount of corbicular pollen loads that colonies at our sites are able to collect per day with the traps used in this study. Further research is needed to clarify in more detail how much of the contaminations of corbicular pollen loads or bee bread is due to drift, resuspension and transport by wind, or direct application on weeds.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

Supplementary information

The supplementary information for this article can be found online at https://doi.org/10.5073/JfK.2023.09-10.01.

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