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Comparison of APIStrip passive sampling with conventional sample techniques for the control of acaricide residues in honey bee hives

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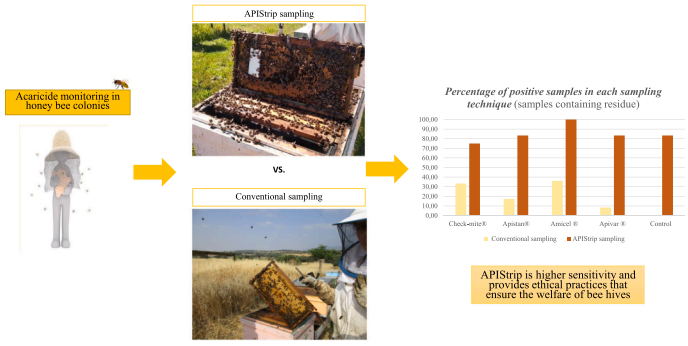
HIGHLIGHTS

- APIStrip has higher sensitivity than conventional methods of residue sampling in bee hives.
- Conventional sampling methods underestimate contamination levels.
- APIStrip provides concentration that captures residues circulating within the hives.
- APIStrip provides ethical practices that ensure the welfare of bee hives.

GRAPHICAL ABSTRACT



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ABSTRACT

Western honey bees are very sensitive bioindicators for studying environmental conditions, hence frequently included in many investigations. However, it is very common in both research studies and health surveillance programs to sample different components of the colony, including adult bees, brood and their food reserves. These practices are undoubtedly aggressive for the colony as a whole, and may affect its normal functioning and even compromise its viability. APIStrip-based passive sampling allows long-term monitoring of residues without affecting the colony in any way. In this study, we compared the effectiveness in the control of acaricide residues by using passive and conventional sampling, where the residue levels of the acaricides coumaphos, tau-fluvalinate and amitraz were evaluated. Conventional and APIStrip-based sampling differ in methods for evaluating bee exposure to residues. APIStrip is less invasive than conventional sampling, offers more efficient

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measurement of environmental contaminants, and can be stored at room temperature, saving costs and minimizing operator error.

1. Introduction

The Western honey bee (*Apis mellifera iberiensis*) faces major threats, in particular the parasite *Varroa destructor* (Smith et al., 2013). Given the ineluctable dependence on acaricide treatments to control *Varroa destructor* infestation, it is necessary to control their residues within the honey bee colonies (Nazzi and Le Conte, 2016). In recent years, some acaricide residues have been found to be persistent, which can lead to their accumulation in the bees wax and even migration to the colony's food reserves (Chauzat and Faucon, 2007; Mullin et al., 2010; Boi et al., 2016; Dai et al., 2018). Residues of acaricides such as coumaphos have been shown to migrate from the bees wax to the pollen on which the bee brood feed (Premrov Bajuk et al., 2017; Dai et al., 2018). It has been estimated that there is a high risk for the bee brood (larvae) by ingestion of bee bread when the residue concentration is >251.31 ng/g (Luna et al., 2023).

It is not only critical to control colony exposure levels to acaricide residues for their health and welfare, but also in beekeeping products for human consumption. However, so far in Europe, only maximum residue limits (MRLs) for acaricide residues in honey have been established, without considering pollen, which is also considered a functional food, highly valued by consumers of beekeeping products (Regulation (EC) 396/2005). It is therefore important to monitor acaricide residues properly, for the welfare of the colony and for the safety of beekeeping products.

To date, in both research studies and honey bee colony health monitoring programmes in many countries, exposure assessment to acaricide residues has been carried out using conventional sampling (Martinello et al., 2021; Murcia-Morales et al., 2021b). These techniques are based on the sampling of adult bees, honey and pollen-containing comb pieces, and brood-containing comb pieces (Chauzat et al., 2011; Lambert et al., 2013; Reeves et al., 2018; Kammoun et al., 2019; Lozano et al., 2019; Qadir et al., 2021). These practices applied in conventional sampling are undoubtedly aggressive. Areas of comb with honey or pollen that have been removed during sampling are often not rebuilt by the adult bees, and lead to a depletion of food reserves. In turn, both the sampling of adult bees and, above all, the sampling of brood bees, may affect the welfare of the colony, its normal social functioning and even compromise the viability of the hive depending on the health status of the colony (Chauzat et al., 2011; Lambert et al., 2012; Lozano et al., 2019; Murcia-Morales et al., 2021a; Murcia-Morales et al., 2021b). There is no doubt that there is a need to evolve towards ethical beekeeping practices without causing any harm to the bees and allow them to maintain their natural behaviour as much as possible. Conventional sampling has other limitations that must also be considered, such as the uncertainty related to small and moderate sized datasets (EFSA, 2013). Additionally, it can be quite challenging to normalise results from samples connected to bees because of possible heterogeneous toxic compounds dispersion, varied sizes, contamination history, etc. (Murcia Morales et al., 2020; Murcia-Morales et al., 2023). There are numerous matrices with various physicochemical characteristics, which contributes to an irregular distribution of accumulated contaminants (Lozano et al., 2019). In addition, it requires a wide range of analyses adapted to the different matrices collected from the hives.

The first-generation passive samplers were placed in tubes outside the hive entrance. When leaving or entering the hive, the bees passed through the tube and came into contact with the sampler. Its exposure to weather conditions, which could affect the binding capacity and contact moments, is a potential danger or drawback of using the outside-hive passive sampler (Van der Steen et al., 2017; Clarke and Robert, 2018). Another passive sampler consists of capturing images to count bees entering and leaving the hive, and to see the impact of acaricides on their behaviour (Ngo et al., 2019) but it does not capture the actual contamination migration into the hive. APIStrip ® (Adsorb-Pesticide-Inhive Strips) is a new passive sampling technique developed to evaluate the exposure of honey bee colonies to residues of pesticides, acaricides and other contaminants (Murcia-Morales et al., 2020).

The objective of this field study is to compare the use of conventional sampling with passive sampling (APIStrip) for the evaluation of honey bee colony exposure to acaricide residues. Bee hives were treated with acaricides including tau-fluvalinate (Apistan ®), coumaphos (Check-mite ®) and amitraz (Amicel ® and Apivar ®) according to the recommended doses to control Varroa infestation. This 6-month field study used the same hives for both APIStrip installation and conventional sampling for comparison.

2. Experimental

2.1. Management and sampling

The field study was carried out from July to December 2019, at the experimental apiary located at the University of Córdoba (37° 55′33.5″N, 4° 43′26.1″W), Córdoba, Spain. Twenty native honey bee (*A. m. iberiensis*, Engel) colonies were used in the experiment. The honey bee colonies were kept in wooden Langstroth hives, and spaced about 50 cm each other. The colonies were separated into five groups. Each group of four colonies received a different treatment for Varroa control: Check-mite ® (coumaphos), Apistan ® (tau-fluvalinate, 0.8 g), Apivar ® (amitraz 0.5, g/strip), Amicel ® (amitraz, 25 mg/mL) and controls treated with oxalic acid. In each case, the treatments were carried out in accordance with the label. The treatment was applied during the summer period, when the bee hives have few brood (Hernando et al., 2018; Flores et al., 2019). All treatments were applied to the bee hives on July 26 and remained continually in the hives until September 4 with the exception of the Amicel ®, which was applied two times on August 12th. Samples of bee brood, honey and bee bread were taken from each bee hive four times: i) before applying the treatment (July), ii) just after the treatment (September), iii) in November, and iv) in December. Comb samples were kept at −20 °C for further residue analysis. 495 samples were examined.

A previous study provides a detailed description of how the APIStrip sampling devices are prepared and installed inside the bee hive (Murcia-Morales et al., 2020). In brief, a thin polystyrene plastic layer (5x10x0.2 cm) was coated on both sides with 6 mL of a concentrated (125 mg/mL) Tenax solution in dichloromethane. The upper portion of the strip is perforated to create a small hole after the solvent has evaporated under a moderate nitrogen current. The produced APIStrip has 0.75 g of Tenax in a homogeneous film layer covering the sampler surface (0.375 g each side). The APIStrips are then placed into the central zone of each bee hive, where they stay for 14 days (sampling period), using a thread or wire to make the process easier. Afterwards, the strips were removed for analysis. The samples of APIStrip were kept at room temperature in aluminium foil and in zip-lock plastic bags.

2.2. Reagents and standards

The pesticide standards of tau-fluvalinate, and coumaphos, with a purity ≥95 % were purchased from Sigma-Aldrich (Steinheim, Germany), and amitraz were obtained from Reidel-de Haën (Seelze, Germany) and, the metabolites 2,4-dimethylformanilide (DMF) and N′-(2,4-dimethylphenyl)-*N*-methylformamidine (DMPF) were obtained from Sigma-Aldrich and Dr. Ehrenstorfer with a purity ≥97 %. Individual

pesticide stock solutions (1000–2000 mg/L) were made in methanol and stored in amber screw-capped glass vials in the dark at −20 °C. The stock standards were utilised to prepare both the individual standard solutions that were used for the optimization and the standard-mix solutions that were used for the calibration.

Merck (Darmstadt, Germany) provided the optima HPLC grade water and acetonitrile. Fluka Analytical produced LC-MS grade methanol and ethyl acetate for pesticide residue analysis (Steinheim, Germany). Fluka Analytical also provided the formic acid (98 % purity) and ammonium formate (Steinheim, Germany). It purchased sodium chloride, anhydrous magnesium sulphate, sodium hydrogenocitrate sesquihydrate, and sodium citrate tribasic dihydrate from Sigma-Aldrich (Steinheim, Germany). Sodium acetate from Fisher Scientific (Waltham, USA). Scharlab (Barcelona, Spain) provided the PSA and C18 (Bulk Adsorbents Extra Bond) sorbents.

2.3. APIStrip extraction procedure

The APIStrips were sliced into small pieces and placed into 50-mL PTFE centrifuge tubes as per the method outlined in a prior work (Murcia-Morales et al., 2020) for the desorption of the pesticides from the Tenax coating on the APIStrip surface. Following the addition of 10 mL of acetonitrile, the samples were automatically shaken at 1250 rpm for 3.5 min (Geno/Grinder, 2010; SPEX) and centrifuged at 4000 rpm for 5 min. The 10-fold dilution required for this extraction procedure – 1 APIStrip to 10 mL acetonitrile – was undone during the creation of the injection vials. To monitor de effectiveness of the extraction process, procedural internal standards such as dichlorvos-D6, malathion-D10, carbendazim-D3, and triphenyl phosphate (TPP) were utilised. For liquid chromatography vials, 1 mL of the extract was first evaporated using a moderate nitrogen stream before being reconstituted with 100 μL of acetonitrile and 400 μL of ultrapure water. Five hundred microliters of extract were evaporated and then reconstituted with 50 μL of ethyl acetate for use in gas chromatography. The changes in injection volume were checked using internal standard for injection (lindane-D6 for GC and dimethoate-D6 for LC).

2.4. Bee bread, honey and bee brood extraction procedure

The extraction has been carried out according to the previously described methodology with minor modifications (Lozano et al., 2019; Murcia Morales et al., 2020). A 30 mL PTFE centrifuge tube was filled with 3 mL of ultrapure water after 1 g of each sample – bee brood and honey – was weighed. The samples were shaken and let to stand for 5 min. The samples were then shaken horizontally for 15 min in a multi-tube shaker (Benchmark Scientific in Sayreville, New Jersey), after 3 mL of acetonitrile had been added. The samples were then sonicated for 60 s. The mixture was then centrifuged for 4 min at 3500 rpm with the addition of 1.2 g $MgSO_4$, 0.3 g NaCl, 0.3 g trisodium citrate dehydrate, and 0.15 g sodium hydrogencitrate sesquihydrate. Finally, a 3 mL aliquot of the honey extract was added to a PTFE centrifuge tube that already contained 60 mg of PSA and 60 mg of C18. Using 0.45 g $MgSO_4$, 75 mg PSA and 75 mg C18, the bee brood extract was purified. A freezing-out process followed by d-SPE with PSA and C18 was employed as the clean-up method for bee bread. After shaking the samples for 30 s, they were centrifuged for 5 min at 5000 rpm, transferred to amber vials, and formic acid 5 % (10 μL per mL of extract) were added. To evaluate extraction effectiveness, dichlorvos-D6 was utilised as an internal standard.

2.5. GC-QqQ-MS/MS and LC-QqQ-MS/MS analysis for APIStrip samples

An Agilent Intuvo 9000 GC system (Agilent Technologies, Palo Alto, CA, USA) outfitted with an Agilent 7693 autosampler and an Agilent 7010 GC–MS/MS triple quadrupole was used to conduct the gas chromatography analyses. Agilent Technologies 6490 Triple Quad LC/MS and Agilent UPLC 1290 Series were utilised for the LC-MS/MS analyses. The chromatography, acquisition, and processing guidelines for the GC–MS/MS and LC-MS/MS pesticide residue analyses are described elsewhere (Murcia-Morales et al., 2020). Table 1 provides specifics on retention time (Rt), transitions, and collision energy (CE) for the examined compounds. The LOQ (Limit of Quantification) was set as 0.5 ng/g for all compounds. The detailed information about the validated methods are in supplementary material S1.

2.6. GC–MS and LC-MS/MS analysis for honey, bee bread and brood samples

A 6410 Triple Quad LC/MS was connected to an Agilent HPLC 1200 Series (Agilent Technologies, Palo Alto, CA, USA). Agilent MassHunter QQQ Quantitative Analysis and Acquisition software version B.01.04, which involves use of dynamic MRM software, was used for processing and data acquisition. A Kinetex 2.6 EVO C18 column (Phenomenex, Torrance, USA), 100 × 3.0 mm, was used for the chromatographic separation, which was carried out at a constant temperature of 30 °C on both sides. The injection volume was 5 μL. The mobile phase consisted of (A) 0.1 % formic acid in acetonitrile and (C) 10 mM ammonium formate in MilliQ water, with a gradient of 50 % to 30 % of C over 10 min and a drop to 5 % C over 2 min. The system used nitrogen as the nebulizer gas and has an electrospray ionization (ESI) ion source. The following ion source characteristics were used: capillary voltage 4000 V, fragmentor voltage 100 V, drying gas flow rate 9 L/min, nebulizer pressure 35 psi, drying gas temperature 300 °C. The collision gas used was nitrogen, which is 99.99 % pure. Positive and SRM (selected reaction monitoring) modes with unit resolution were used to operate the system.

The Agilent GC System 7890 A and MSD 5977 A (both from Agilent Technologies, Palo Alto, California, USA) were used. Data was collected using the Agilent MassHunter GC/MS acquisition B.07.01.1805 and processing QQQ Acquisition and Quantitative software version B.07.00. A multimode injector inlet in splitless mode, with an ultra-inert liner made of glass wool frit, was used to inject the samples (Agilent 5190–2293). 2 μL of sample was injected, and the injector's temperature was 250 °C. The column was an Agilent Technologies HP-5MS UI 30 m × 250 μm × 0.25 μm film thickness column. The oven was set to 70 °C for 1 min, 230 °C for 9 min at 20 °C/min, 280 °C for 8 min at 40 °C/min, and finally 300 °C for 10 min at 40 °C/min. The run time was 27 min in total. The instrument's flow rate was constant at 1.4 mL per minute. As a carrier and quenching gas, helium (99.999 % purity) was used. The gas flow was 20 mL/min (gas saver). The ion source and the transfer line were kept at 230 °C. The temperature of the quadrupole analyser was set at 150 °C. A 2.5-min solvent delay occurred. The system was operated in single ion monitoring (SIM) mode.

Operational parameters for the analysis of acaricide residues by LC-MS and GC–MS: retention time (Rt), transitions, and collision energy (CE) are provided in Table 2. The LOQ was set as 15 ng/g for all compounds. The detailed information about the validated methods are in supplementary material S2.

Method validation for the identification and quantification of

Table 1
Acquisition parameters for the compounds under study.

Compound	Rt (min)	Transitions	CE (eV)	Technique
Amitraz	14.19	294.1–162.9	5	LC-MS/MS
		291.1–253.0	15	
Coumaphos	9.73	362.0–334.0	5	GC–MS/MS
		362.0–109.0	15	
DMF	7.87	150.0–107.0	20	LC-MS/MS
		150.0–132.0	10	
DMPF	4.75	163.0–122.0	15	LC-MS/MS
		163.0–106.9	20	
Tau-fluvalinate	10.92	250.0–200.0	20	GC–MS/MS
		250.0–55.0	15	

Table 2
Acquisition parameters for the compounds under study.

Compound	Rt (min)	Transitions	CE (V)/EI (eV)	Technique
Amitraz	12.05	294.0–253.0	15	LC-MS/MS
		294.0–163.0	19	
		294.0–122.0	41	
Coumaphos	7.45	363.0–307.0	16	LC-MS/MS
		363.0–227.0	28	
		363.0–211.0	20	
DMF	1.54	150.0–132.0	15	LC-MS/MS
		150.0–122.0	10	
		150.0–107.0	18	
DMPF	1.08	163.0–132.0	15	LC-MS/MS
		163.0–122.0	23	
		163.0–107.0	32	
Tau-fluvalinate (sum of isomers)	14.78	252.0	70	GC–MS
		250.0	70	
		181.0	70	

residues in bee bread, honey and brood was carried out according to quality control standards (European Commission, 2021).

2.7. Statistical analysis

Data were statistically processed using SPSS (Statistical Package for the Social Sciences) Statistics software for Windows, IBM Corp, 2016. Version 24.0. IBM Corp, Armonk, NY, USA. Parametric statistics were applied when possible. When data resulted none normally distributed, or there was no variance homogeneity (heteroscedasticity), non-parametric statistics were used. The tests are specified in the results.

3. Results and discussion

3.1. Residue levels of the acaricides coumaphos, amitraz and tau-fluvalinate detected by using conventional and APIStrip sampling through a six-month period

The colonies were separated into five groups. Each group of four colonies received a different treatment for Varroa control. Of the four hives in each group, four treated hives and one control hive were lost. Several factors have been suggested as contributing to honey bee losses, including pests and pathogens, inadequate apiary management, pesticides or habitat loss, although in reality these factors tend to overlap and interact with each other, making them difficult to assess (Goulson et al., 2015). In this study, this could be due to the high levels of Varroa found initially.

3.1.1. Bee hives used as control in the field study

The mean concentration of residues detected in the three control hives is shown in Table 3. No samples collected by conventional sampling in the control hives showed any residues of amitraz (amitraz concentration defined as the sum of DMF and DMPF), coumaphos, or tau-fluvalinate in none of the samples collected including bee bread, honey and bee brood. Instead, residues of all acaricides were detected in the control hives by APIStrip. Passive sampling eliminated the risk of non-detection of residues as happened in the conventional sampling. The residue levels were: amitraz (sum of DMF + DMPF) from 28.47 to 693.57 ng/g, tau-fluvalinate from 9.04 to 61.55 ng/g, and coumaphos from 3.25 to 187.87 ng/g (Figs. 1 and 2, and Table 3). APIStrips provided a higher sensitivity for the detection of residues, since passive sampling allows to obtain a time-weighted average concentration over a deployment period, which can vary between several days and weeks, although the recommended sampling time is two weeks (Murcia-Morales et al., 2020). After the detection of residues over six months indicates a persistent residual contamination in the commercial bees wax that was initially placed in the hives. As has already been seen in several previous studies, the commercial bees wax used regularly by beekeepers is not free of residues, since the conventional treatments that are still carried out for its reuse are not effective for the removal of residues (Chauzat and Faucon, 2007; Smodiš Škerl et al., 2011; Lozano et al., 2019). Obtaining detailed information on residues in hives is important because the presence of residues has been shown to have implications for the generation of resistance by Varroa against acaricide treatments, reducing their effectiveness and aggravating the situation suffered by beekeepers, since they only have a very small number of authorized treatments to be able to keep hives healthy, has what can further exacerbate the problems facing bee colonies (Medici et al., 2015; Higes et al., 2020).

3.1.2. Bee hives treated with the acaricides

Amitraz was applied in two different commercial forms (Amicel ® and Apivar ®), coumaphos using the strips Check-mite ® and tau-fluvalinate, with the strips Apistan ®. As shown in Fig. 1, the percentage of samples where acaricide residues have been detected is, by far, higher in the sampling carried out with APIStrip than with the conventional sampling. In conventional sampling, the percentage of samples where acaricide residues were detected was between 8 and 33 %, while with APIStrip, the percentage was between 75 and 100 %.

With conventional sampling, as in the bee hives used as control, in the bee hives selected to be treated with acaricides, and during the period prior to their treatment, residues were not detected in any of the collected samples of bee bread, honey and bee brood. In contrast, residues of the three acaricides applied were detected with APIStrip in all bee hives (Figs. 2 and 3). Since their detection is observed throughout

Table 3
Residue levels (ng/g) of amitraz (sum of DMF + DMPF), coumaphos and tau-fluvalinate by using conventional and APIStrip sampling in the control bee hives.

Acaricide residues	Control hives (code)	Conventional sampling				APIStrip sampling			
		Pre-treatment-JULY	Post-treatment-SEP	Post-treatment-NOV	Post-treatment-DEC	Pre-treatment-JULY	Post-treatment-SEP	Post-treatment-NOV	Post-treatment-DEC
		Avg conc.[a] (ng/g)	Avg conc. (ng/g)	Avg conc. (ng/g)	Avg conc. (ng/g)	Avg conc. (ng/g)	Avg conc. (ng/g)	Avg conc. (ng/g)	Avg conc. (ng/g)
Amitraz (sum of DMF + DMPF)	A62	<LOQ	<LOQ	<LOQ	<LOQ	80.15	42.65	28.47	43.64
	A906	<LOQ	<LOQ	<LOQ	<LOQ	693.57	161.47	112.37	56.65
	A958	<LOQ	<LOQ	<LOQ	<LOQ	123.87	147.85	<LOQ	<LOQ
Coumaphos	A62	<LOQ	<LOQ	<LOQ	<LOQ	187.87	114.83	39.55	78.63
	A906	<LOQ	<LOQ	<LOQ	<LOQ	56.24	71.13	13.64	8.76
	A958	<LOQ	<LOQ	<LOQ	<LOQ	7.71	3.25	<LOQ	<LOQ
Tau-fluvalinate	A62	<LOQ	<LOQ	<LOQ	<LOQ	29.41	52.72	14.71	32.29
	A906	<LOQ	<LOQ	<LOQ	<LOQ	37.53	36.41	13.41	9.04
	A958	<LOQ	<LOQ	<LOQ	<LOQ	61.55	30.27	<LOQ	<LOQ

[a] Avg conc.: average concentration.

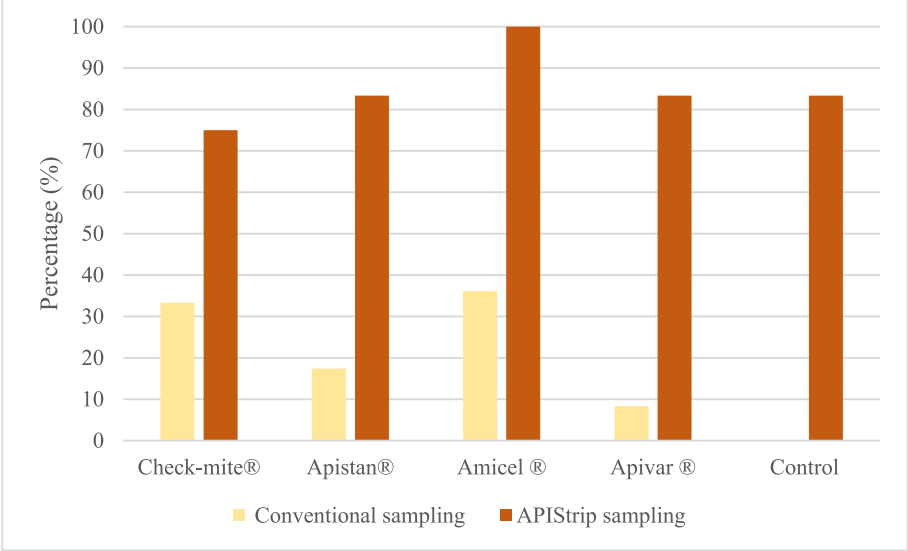

Fig. 1. Percentage of samples where acaricide residues were detected using APIStrip and conventional sampling.

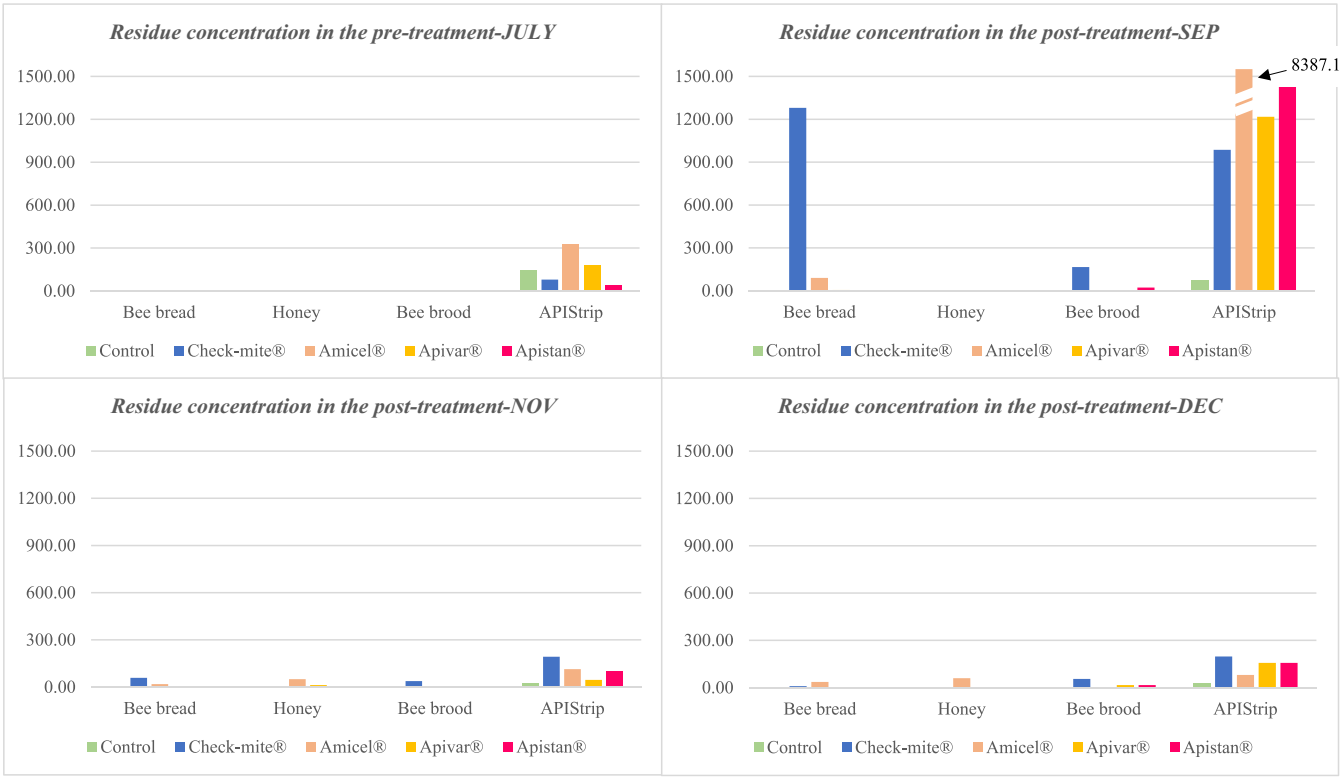

Fig. 2. Comparison of residue concentration (ng/g) in the two trials and in the different treatments. The concentration of bee bread, honey and bee brood from conventional sampling is shown compared to the concentration in APIStrip, from the pre-treatment, and post-treatment.

the entire study period, this reveals their persistence inside the bee hive.

After treatment, residues continued to be detected in Nov and Dec with the conventional sampling. The levels detected, as shown in Figs. 2 and 3, increased during the *post-treatment* period although this occurred unevenly between treatments, matrices and evaluations, observing a reduction in some cases, so a trend pattern cannot be defined after the treatments. Residues of the three acaricides were found in the bee bread and/or brood, but were not detected in the honey samples. In the *November* evaluation, residues of coumaphos were detected in bee bread, honey and brood. Metabolites of amitraz were found in bee bread and honey but not in brood from bee hives treated with Amicel®. In those bee hives treated with Apivar®, residues were only detected in honey. There were not fluvalinate residues in any sample collected in Nov. In *December*, coumaphos residues were detected in bee bread and brood. Residues of amitraz were detected in bee bread and honey when hives were treated with Amicel®, and only in honey, in the bee hives treated with Apivar®. Residues of tau-fluvalinate were only found in brood (Fig. 3). Due to the lipophilicity of tau-fluvalinate, residues were no detected in honey as it happened in previous studies (Lozano et al., 2019). Tables S1, S2 and S3 show levels of residues detected in the bee

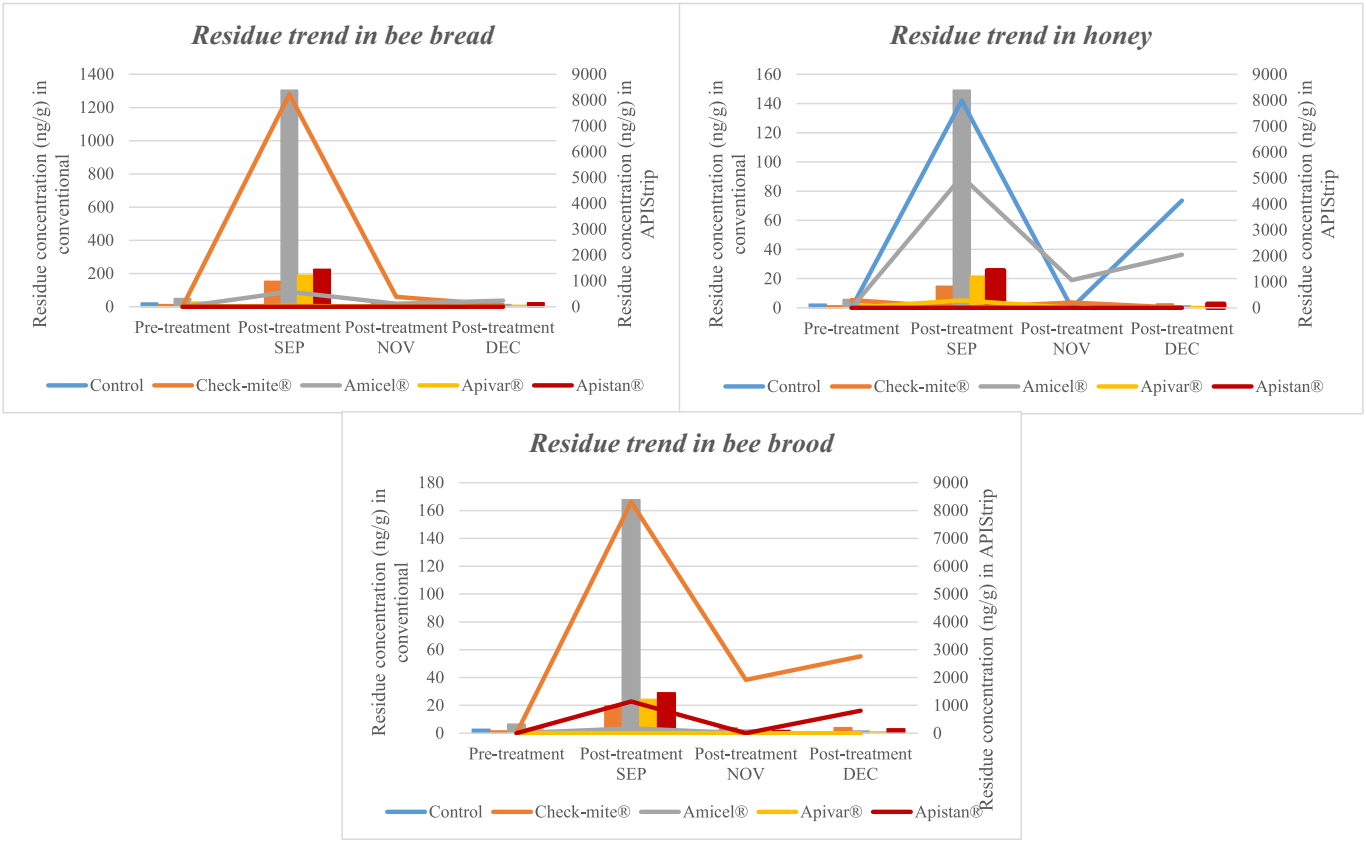

Fig. 3. Comparison of the residue trend in conventional (primary axis, lines) and APIStrip (secondary axis, columns) samplings, in each matrix (bee bread, honey and bee brood) and treatment or not (control, Check-mite ®, Amicel ®, Apivar ® and Apistan ®).

hives treated (supplementary material). As previously mentioned, with the results obtained by conventional sampling, a trend pattern cannot be defined. Depending on where the sample is taken from, the results can be very different. For example, samples of honey collected from different parts of a bee hive could have been stored at different times, some coinciding with a more recent treatment, and some not, so different samples from the same hive could have different residue concentrations. And it would really be very difficult and laborious to know when a honey was stored by the bees several months ago, considering the natural evolution that occurs in the hive over time. The same could be said of bee bread reserves, which change over time according to the needs of the bees and the pollen input. Or in the case of the bee brood, for which the bees use different combs, each with a different level of bees wax contamination, and the possible migration of residues and bee brood exposure as pointed out in previous studies (Dai et al., 2018; Murcia Morales et al., 2020; Luna et al., 2023).

With APIStrips, residue levels of acaricides below 320 ng/g were detected during the pre-*treatment* period (Fig. 2). Residues of coumaphos increased first during the post-treatment period, then decreased to finally stabilize. A similar trend was observed in the case of amitraz residues. There was a significant increase in the residues of amitraz (Amicel ®) after post-treatment to rapidly decline to levels lower than those detected in the pre-treatment period. In the bee hives treated with Apivar ® there was also an increase once the treatment was applied, although at a lower level than the observed with Amicel ®. The level of amitraz residues were generally higher in the form of Amicel ® than in the form of Apivar ®. An increase occurred after treatment and after, residual contamination decreases or disappears. Therefore, a drastic increase in contamination was seen, yet afterwards, it decreased in the bee hives. This trend was also observed in tau-fluvalinate (Fig. 3).

Overall, APIStrips show results in line with expectations, with an increase after application of the treatments, followed by a reduction of the residues found in the subsequent evaluations. When comparing the residues quantified from the analysis of the different matrices versus those quantified with APIStrip, significantly more residues were always found in the APIStrips for all treatments and in each of the evaluations, except for the Check-mite® (coumaphos a.s.) treatment in the post-treatment evaluations, during November and December (Mann-Whitney U test, $p \leq 0.05$) (Table 4). It is evident that APIStrip sampling is significantly more effective than conventional sampling and provides more information about the number of pesticides that can be found in the various matrices of a bee hive. In addition, because the APIStrip

Table 4
Residues quantified in each of the evaluations, for each treatment in the analysis of all matrices (sum of residues in bee bread, bee brood and honey) and the APIStrip. Results are shown as the mean ± s.d. of all hives and all matrices of each treatment comparing the mean of the residues detected with the APIStrip in all hives of each treatment.

Trials	Sampling	Check-mite ®	Amicel ®	Apivar ®	Apistan ®
Pre-treatment JULY	Matrices	<LOQ	<LOQ	<LOQ	<LOQ
	APIStrip	79.43 ± 78.56	322.25 ± 66.90	177.70 ± 57.76	39.25 ± 23.76
Post-treatment SEP	Matrices	481.87 ± 1026.23	31.14 ± 50.55	<LOQ	<LOQ
	APIStrip	934.00 ± 254.70	8387.10 ± 3979.60	1217.21 ± 114.09	1448.47 ± 324.14
Post-treatment NOV	Matrices	33.65 ± 51.10	23.18 ± 42.93	<LOQ	<LOQ
	APIStrip	177.05 ± 197.12	113.77 ± 35.80	46.13 ± 37.78	97.95 ± 74.89
Post-treatment DEC	Matrices	21.64 ± 44.25	32.10 ± 40.24	<LOQ	<LOQ
	APIStrip	181.20 ± 201.75	80.59 ± 7.23	50.04 ± 37.86	157.04 ± 125.19

captures the residue circulating in the hives, and this can come from all the matrices and all the combs in the hive, the previous problems would be avoided. Also, tau-fluvalinate has a lipophilic nature due to its log Kow value of 7.02, which gives it a high affinity for bees wax and a high persistence inside the bee hive (Murcia Morales et al., 2020). The use of APIStrip, a more sensitive method of sampling, resulted in higher concentrations of tau-fluvalinate than conventional sampling. This indicates that using conventional methods may underestimate the actual levels of residues present in bee products (Murcia-Morales et al., 2021a). Similarly, coumaphos is also a lipophilic compound with a log Kow value of 3.86, which explains its affinity for bees wax and its high persistence (Lozano et al., 2019). One interesting finding from table S1 is the high concentrations of coumaphos in bee bread compared to honey and bee brood. This is consistent with previous studies that suggest that bee bread is a reservoir for coumaphos residues due to its role in food storage and consumption by larvae and adult bees (Lozano et al., 2019). The migration of coumaphos from bees wax to food reserves and bee brood could also explain this trend (Premrov Bajuk et al., 2017; Kast and Kilchenmann, 2022).

Additionally, the correlations between the residues quantified in the different matrices (bee bread, honey and bee brood) and the residues quantified in the APIStrip were studied. The study found no significant correlations between trials or in the various matrices in the pre-treatment (Table 5). However, correlations between the conventional sampling and APIStrip were seen in bee bread and brood after the treatment had been given (Spearman's Rho correlation test, $P \leq 0.05$). This is probably favoured because at this time, high concentrations of residues occurred in the bee hives, and conventional sampling and analysis was able to detect it. The study further noted that coumaphos is the only treatment with a very high correlation coefficient comparing APIStrip and conventional sampling, according to Spearman's Rho correlations (Table 6) after the treatment has been applied, and that its residual capacity is very high, making it easy to detect in both sampling types and remains over time. As mentioned above, the lipophilic nature of coumaphos (log K_{ow} 3.86) also makes it one of the most frequent residues detected in apiary surveillance programs carried out in several countries due to accumulation in bees wax (Mullin et al., 2010; Fulton et al., 2019; Lozano et al., 2019).

This statistical analyses raise several important points for discussion. Firstly, the lack of significant correlations between trials or in the various matrices in the pre-treatment suggest that the initial conditions were well controlled and that the study was conducted rigorously. The observed correlations between the conventional sampling and APIStrip after treatment indicates that these two trials can be used interchangeably to detect pesticide residues in bee bread and brood.

3.2. Advantages and disadvantages of APIStrip vs. conventional sampling

Conventional and passive sampling with APIStrip are two different methods for collecting information about the exposure of honey bee colonies to residues of pesticides, acaricides or other contaminants. With a conventional sampling, there is a direct collection of samples of bee brood, honey and bee bread from the bee hives that need to be store immediately at −20 °C for further residue analysis. APIStrip is placed for 14 days to give time weighted average (TWA) concentration of residues inside the bee hives (Murcia-Morales et al., 2020). This period has been established as the optimum period to maintain and obtain more information about the contamination circulating inside the hive. After this 14-day period has elapsed, APIStrip can be kept at room temperature in aluminium foil and in zip-lock plastic bags until its processing for residue analysis. Conventional sampling can provide detailed information on the health and status of bee colonies, such as the presence of diseases, the level of mite infestation (Lee et al., 2010; Glenny et al., 2017), or the level of punctual contamination of a specific matrix as in this case of bee bread, honey and brood. Instead, the passive with APIStrip is an integrated passive sampler, mainly used to measure the exposure of bees to pesticides and other chemical compounds in the environment (Murcia-Morales et al., 2020), so it is not able to identify the specific matrix in which the contamination is found. To effectively use bees as indicators in ecological studies on bee colonies, it is crucial to take samples throughout the entire season and over multiple years. This approach helps ensure that the data collected accurately reflects the overall bee population and its behaviour, providing a more comprehensive understanding of the community's ecological status (Oertli et al., 2005). Conventional sampling is generally more expensive and requires more time and technical skills to be carried out correctly. Passive sampling with APIStrip is easy to use, reduces the cost of sampling and what it is more important, reduces the risk of operator errors during the sampling (Murcia-Morales et al., 2021a). Conventional sampling is invasive since bees feel stressed while samples are taken from the colony what surely affects their social behaviour and productivity of beekeeping products. Passive sampling with APIStrip does not require direct handling of the bees and therefore has no impact on their behaviour, functioning and productivity of the bee hive. Both methods also differ in the posterior processing during the extraction and analysis of residues. With APIStrip, only one extraction method is needed, while in the conventional method, several extraction methods need to be adapted according to the type of sample. This undoubtedly is time-consuming and implies more cost. With APIStrip, as it is a passive sampling that is deployed for 2 weeks, the number of samples to be taken and analysed is significantly reduced. In addition to what was discussed above, in beekeeping it is necessary to continue advancing towards better practices that are more

Table 5
Spearman's Rho correlation coefficients in the different matrices (bee bread, honey, bee brood), comparing APIStrip and conventional assays in the different evaluations (pre-treatment, post-treatment in July, November and December).

			APIStrip			
			Pre-treatment JULY	Post-treatment SEP	Post-treatment NOV	Post-treatment DEC
Conventional	Pre-treatment JULY	Bee bread	–	–	–	–
		Honey	0.100	0.020	0.383	0.383
		Bee brood	–	–	–	–
	Post-treatment SEP	Bee bread	0.591**	**0.694****	0.540*	0.558*
		Honey	–	–	–	–
		Bee brood	−0.012	**0.546***	0.593**	0.569**
	Post-treatment NOV	Bee bread	0.414	0.455*	**0.532***	0.461*
		Honey	0.507*	0.407	0.412	0.350
		Bee brood	0.100	0.099	0.383	0.383
	Post-treatment DEC	Bee bread	0.438	0.484*	0.515*	0.436
		Honey	0.503*	0.440	0.255	0.189
		Bee brood	0.035	0.117	0.528*	**0.528***

In bold the coefficients that compare the conventional and APIStrip assays. $N = 20$.
* The correlation is significant at the 0.05 level (bilateral).
** The correlation is significant at the 0.01 level (bilateral).

Table 6
Spearman's Rho correlation coefficients in Check-mite ® (coumaphos) comparing APIStrip and conventional assays at the different evaluations (pre-treatment, post-treatment, monitoring November and December).

		APIStrip			
		Pre-treatment JULY	Post-treatment SEP	Post-treatment NOV	Post-treatment DEC
Conventional	Pre-treatment JULY	0.447	0.141	0.141	0.141
	Post-treatment SEP	0.693	**0.876***	0.876*	0.876*
	Post-treatment NOV	0.933**	0.984**	**0.984****	0.984**
	Post-treatment DEC	0.693	0.876*	0.876*	**0.876***

In bold the coefficients that compare the conventional and APIStrip assays. $N = 6$.
* The correlation is significant at the 0.05 level (bilateral).
** The correlation is significant at the 0.01 level (bilateral).

ethical for the proper care and management of hives. It is important to be aware to evolve towards ethical beekeeping practices to allow the bees to maintain their natural behaviour as much as possible.

4. Conclusions

Conventional sampling and APIStrip-based sampling are different methods for collecting information regarding the exposure of bees to residues. Each type of sampling has its own advantages and disadvantages in terms of cost, complexity, type of information collected, sample extraction and analysis, and impact on bees. The findings of this study highlight the importance of using more effective and comprehensive sampling techniques. APIStrip provides a time-weighted average concentration over a deployment period. On the contrary, conventional sampling techniques may underestimate the actual levels of contamination inside the bee hives. APIStrip is a good approach for measuring the contamination due to residues or other contaminants inside the hives, since this method captures the residues that are circulating in the hives, whereas the analysis of specific matrices (conventional), as those included in this study, only provides information about the residues accumulated in these matrices, which can be a disadvantage from an overall point of view. The APIStrip reduces the number of samples needed for the evaluation of residues inside hives. APIStrip samples can be stored at room temperature, saves costs and minimize the operator errors during sampling. In addition, APIStrip is not invasive, nor does it produce any type of harm or alteration for the honey bee colony. All this makes the APIStrip an advance in the development of ethical beekeeping practices for ensuring the welfare of the honey bee colonies.

CRediT authorship contribution statement

Alba Luna: Methodology, Validation, Investigation, Writing – original draft. **María Murcia-Morales:** Methodology, Validation, Investigation, Writing – review & editing. **María Dolores Hernando:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jozef J.M. Van der Steen:** Project administration, Funding acquisition, Visualization. **Amadeo R. Fernández-Alba:** Project administration, Funding acquisition, Supervision. **José Manuel Flores:** Investigation, Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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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.2023.167205.

References

Boi, M., Serra, G., Colombo, R., Lodesani, M., Massi, S., Costa, C., 2016. A 10 year survey of acaricide residues in beeswax analysed in Italy. Pest Manag. Sci. 72 (7), 1366–1372. https://doi.org/10.1002/ps.4161.

Chauzat, M.P., Faucon, J.P., 2007. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. Pest Manag. Sci. 63 (April), 1100–1106. https://doi.org/10.1002/ps.1451.

Chauzat, M.P., Martel, A.C., Cougoule, N., Porta, P., Lachaize, J., Zeggane, S., Aubert, M., Carpentier, P., Faucon, J.P., 2011. An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. Environ. Toxicol. Chem. 30 (1), 103–111. https://doi.org/10.1002/etc.361.

Clarke, D., Robert, D., 2018. Predictive modelling of honey bee foraging activity using local weather conditions. Apidologie 49 (3), 386–396. https://doi.org/10.1007/s13592-018-0565-3.

Dai, P., Jack, C.J., Mortensen, A.N., Bustamante, T.A., Ellis, J.D., 2018. Chronic toxicity of amitraz, coumaphos and fluvalinate to *Apis mellifera* L. larvae reared in vitro. Sci. Rep. 1–9. https://doi.org/10.1038/s41598-018-24045-3.

European Commission, 2021. SANTE/11312/2021.

European Food Safety Authority (EFSA), 2013. Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, Bombus spp. And solitary bees). EFSA J. 11 (7) https://doi.org/10.2903/j.efsa.2013.3295.

Flores, J.M., Gil-Lebrero, S., Gámiz, V., Rodríguez, M.I., Ortiz, M.A., Quiles, F.J., 2019. Effect of the climate change on honey bee colonies in a temperate Mediterranean zone assessed through remote hive weight monitoring system in conjunction with exhaustive colonies assessment. Sci. Total Environ. 653, 1111–1119. https://doi.org/10.1016/j.scitotenv.2018.11.004.

Fulton, C.A., Huff Hartz, K.E., Fell, R.D., Brewster, C.C., Reeve, J.D., Lydy, M.J., 2019. An assessment of pesticide exposures and land use of honey bees in Virginia. Chemosphere 222, 489–493. https://doi.org/10.1016/j.chemosphere.2019.01.156.

Glenny, W., Cavigli, I., Daughenbaugh, K.F., Radford, R., Kegley, S.E., Flenniken, M.L., 2017. Honey bee (Apis mellifera) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLoS One 12 (8), e0182814. https://doi.org/10.1371/journal.pone.0182814.

Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347 (6229), 1255957. https://doi.org/10.1126/science.1255957.

Hernando, M.D., Gámiz, V., Gil-Lebrero, S., Rodríguez, I., García-Valcárcel, A.I., Cutillas, V., Fernández-Alba, A.R., Flores, J.M., 2018. Viability of honeybee colonies exposed to sunflowers grown from seeds treated with the neonicotinoids thiamethoxam and clothianidin. Chemosphere 202, 609–617. https://doi.org/10.1016/J.CHEMOSPHERE.2018.03.115.

Higes, M., Martín-Hernández, R., Hernández-Rodríguez, C.S., González-Cabrera, J., 2020. Assessing the resistance to acaricides in Varroa destructor from several Spanish locations. Parasitol. Res. 119 (11), 3595–3601. https://doi.org/10.1007/s00436-020-06879-x.

Kammoun, S., Mulhauser, B., Aebi, A., Mitchell, E.A.D., Glauser, G., 2019. Ultra-trace level determination of neonicotinoids in honey as a tool for assessing environmental

contamination. Environ. Pollut. 247, 964–972. https://doi.org/10.1016/j.envpol.2019.02.004.
Kast, C., Kilchenmann, V., 2022. An in vitro model for assessing the toxicity of pesticides in beeswax on honey bee larvae. Chemosphere 287 (P2), 132214. https://doi.org/10.1016/j.chemosphere.2021.132214.
Lambert, O., Piroux, M., Puyo, S., Thorin, C., Larhantec, M., Delbac, F., Pouliquen, H., 2012. Bees, honey and pollen as sentinels for lead environmental contamination. Environ. Pollut. 170, 254–259. https://doi.org/10.1016/j.envpol.2012.07.012.
Lambert, O., Piroux, M., Puyo, S., Thorin, C., L'Hostis, M., Wiest, L., Buleté, A., Delbac, F., Pouliquen, H., 2013. Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of Western France. PLoS One 8 (6), 1–12. https://doi.org/10.1371/journal.pone.0067007.
Lee, K.V., Moon, R.D., Burkness, E.C., Hutchison, W.D., Spivak, M., 2010. Practical sampling plans for <I>Varroa destructor</I> (Acari: Varroidae) in <I>Apis mellifera</I> (Hymenoptera: Apidae) colonies and apiaries. J. Econ. Entomol. 103 (4), 1039–1050. https://doi.org/10.1603/EC10037.
Lozano, A., Hernando, M.D., Uclés, S., Hakme, E., Fernández-Alba, A.R., 2019. Identification and measurement of veterinary drug residues in beehive products. Food Chem. 274 (July 2018), 61–70. https://doi.org/10.1016/j.foodchem.2018.08.055.
Luna, A., Flores, J.M., Miguel, E., Fernández-Alba, A.R., Hernando, M.D., 2023. Coumaphos residue transfer to honey bee brood (Apis mellifera) in realistic scenarios. Res. Vet. Sci. https://doi.org/10.1016/j.rvsc.2023.04.016.
Martinello, M., Manzinello, C., Dainese, N., Giuliato, I., Gallina, A., Mutinelli, F., 2021. The honey bee: an active biosampler of environmental pollution and a possible warning biomarker for human health. Appl. Sci. 11 (14), 6481. https://doi.org/10.3390/app11146481.
Medici, S.K., Maggi, M.D., Sarlo, E.G., Ruffinengo, S., Marioli, J.M., Eguaras, M.J., 2015. The presence of synthetic acaricides in beeswax and its influence on the development of resistance in V *arroa destructor*. J. Apic. Res. 54 (3), 267–274. https://doi.org/10.1080/00218839.2016.1145407.
Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One 5 (3). https://doi.org/10.1371/journal.pone.0009754.
Murcia Morales, M., Gómez Ramos, M.J., Parrilla Vázquez, P., Díaz Galiano, F.J., García Valverde, M., Gámiz López, V., Manuel Flores, J., Fernández-Alba, A.R., 2020. Distribution of chemical residues in the beehive compartments and their transfer to the honeybee brood. Sci. Total Environ. 710, 1–10. https://doi.org/10.1016/j.scitotenv.2019.136288.
Murcia-Morales, M., Van der Steen, J.J.M., Vejsnæs, F., Díaz-Galiano, F.J., Flores, J.M., Fernández-Alba, A.R., 2020. APIStrip, a new tool for environmental contaminant sampling through honeybee colonies. Sci. Total Environ. 729, 138948. https://doi.org/10.1016/j.scitotenv.2020.138948.
Murcia-Morales, M., Díaz-Galiano, F.J., Guitérrez-Tirado, I., Flores, J.M., Van der Steen, J.J.M., Fernández-Alba, A.R., 2021a. Dissipation and cross-contamination of miticides in apiculture. Evaluation by APIStrip-based sampling. Chemosphere 280 (February). https://doi.org/10.1016/j.chemosphere.2021.130783.
Murcia-Morales, M., Díaz-Galiano, F.J., Vejsnæs, F., Kilpinen, O., Van der Steen, J.J.M., Fernández-Alba, A.R., 2021b. Environmental monitoring study of pesticide contamination in Denmark through honey bee colonies using APIStrip-based sampling. Environ. Pollut. 290 (July) https://doi.org/10.1016/j.envpol.2021.117888.
Murcia-Morales, M., Vejsnæs, F., Brodschneider, R., Hatjina, F., Van der Steen, J.J.M., Oller-Serrano, J.L., Fernández-Alba, A.R., 2023. Enhancing the environmental monitoring of pesticide residues through Apis mellifera colonies: honey bees versus passive sampling. Sci. Total Environ. 884, 163847. https://doi.org/10.1016/j.scitotenv.2023.163847.
Nazzi, F., Le Conte, Y., 2016. Ecology of Varroa destructor, the Major Ectoparasite of the Western Honey Bee, Apis mellifera. Annu. Rev. Entomol. 61, 417–432. https://doi.org/10.1146/annurev-ento-010715-023731.
Ngo, T.N., Wu, K.-C., Yang, E.-C., Lin, T.-T., 2019. A real-time imaging system for multiple honey bee tracking and activity monitoring. Comput. Electron. Agric. 163, 104841. https://doi.org/10.1016/j.compag.2019.05.050.
Oertli, S., Müller, A., Dorn, S., 2005. Ecological and seasonal patterns in the diversity of a species-rich bee assemblage (Hymenoptera: Apoidea: Apiformes). Eur. J. Entomol. *102* (1), 53–63. https://doi.org/10.14411/eje.2005.008.
Premrov Bajuk, B., Babnik, K., Snoj, T., Milčinski, L., Pislak Ocepek, M., Škof, M., Jenčič, V., Filazi, A., Štajnbaher, D., Kobal, S., 2017. Coumaphos residues in honey, bee brood, and beeswax after Varroa treatment. Apidologie 48 (5), 588–598. https://doi.org/10.1007/s13592-017-0501-y.
Qadir, Z.A., Idrees, A., Mahmood, R., Sarwar, G., Bakar, M.A., Ahmad, S., Raza, M.M., Li, J., 2021. Effectiveness of different soft acaricides against honey bee ectoparasitic mite varroa destructor (Acari: Varroidae). Insects 12 (11), 1–11. https://doi.org/10.3390/insects12111032.
Reeves, A.M., O'Neal, S.T., Fell, R.D., Brewster, C.C., Anderson, T.D., 2018. In-hive acaricides alter biochemical and morphological indicators of honey bee nutrition, immunity, and development. J. Insect Sci. 18 (5) https://doi.org/10.1093/jisesa/iey086.
Smith, K.M., Loh, E.H., Rostal, M.K., Zambrana-Torrelio, C.M., Mendiola, L., Daszak, P., 2013. Pathogens, pests, and economics: drivers of honey bee colony declines and losses. EcoHealth 10 (4), 434–445. https://doi.org/10.1007/s10393-013-0870-2.
Smodiš Škerl, M.I., Nakrst, M., Žvokelj, L., Gregorc, A., 2011. The acaricidal effect of flumethrin, oxalic acid and amitraz against Varroa destructor in honey bee (Apis mellifera carnica) colonies. Acta Vet. Brno 80 (1), 51–56. https://doi.org/10.2754/avb201180010051.
Van der Steen, J.J.M., Bergsma-Vlami, M., Wenneker, M., 2017. The perfect match: simultaneous strawberry pollination and bio-sampling of the plant pathogenic bacterium Erwinia pyrifoliae by honey bees Apis mellifera. Sustain. Agric. Res. 7 (1), 25. https://doi.org/10.5539/sar.v7n1p25.