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Neonicotinoids transference from the field to the hive by honey bees: Towards a pesticide residues biomonitor

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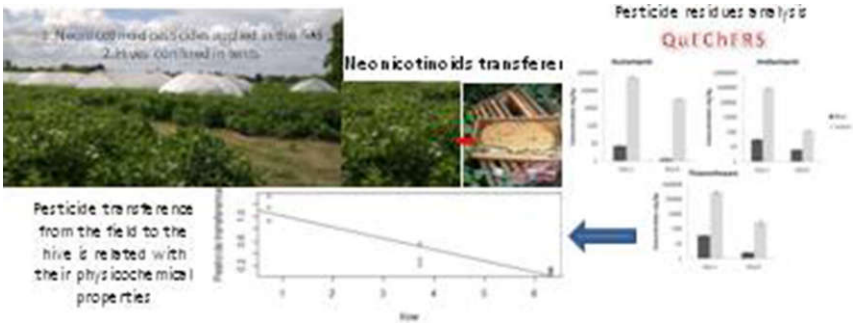
HIGHLIGHTS

- New insights to support beehives as environmental monitors for pesticide residues
- It is shown that bees carry pesticides applied in the field to the hive.
- Pesticide transference was calculated for acetamiprid, imidacloprid, thiamethoxam.
- Transferences had a linear inverse trend with their Kow.
- Relationship with Vp was also observed.

GRAPHICAL ABSTRACT



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ABSTRACT

The beehive as a quantitative monitor of pesticide residues applied over a soybean crop was studied through a semi field experiment of controlled exposure of honey bees to pesticides in macro tunnels. The distribution within exposed beehives of pesticides commonly used in soybean plantation, was assessed. Residue levels of insecticides in soybean leaves, honey bees, wax, honey and pollen were analyzed. The transference from pesticides present in the environment into the beehive was evidenced. The obtained results allow relating pesticide concentrations present in the environment with traces found in foraging bees. Therefore, pesticide transference ratios could be calculated for each detected compound (acetamiprid, imidacloprid and thiamethoxam) which showed a linear inverse trend with their 1-octanol/water partition coefficient (Kow). The least transferred pesticide to the hive (acetamiprid) has the highest vapor pressure (Vp). This study gives new insights on the usefulness of monitoring the environment through beehives aiming to evaluate if agroecosystems remain sustainable. It also contributes to generate valuable information for model building aiming to predict environmental quality through beehive's analysis.

1. Introduction

South America is the region with the fastest growing area planted with soybean. During the last four decades, it has increased its

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production area almost 30-fold (from 1.44 to 42.75 million hectares from 1970 to 2009). As it holds the largest area planted with soybean, it also currently produces the largest volume of this crop (FAOSTAT, 2011). In 2009, out of the 222.94 million tons of soybean harvested globally, 43% (equivalent to 94.91 million tons) was produced in the region. The vast majority of its production takes place in the Southern Cone countries: Argentina, Bolivia, Brazil, Paraguay and Uruguay.

Honey bees and bee products have been studied as environmental quality indicators in several reports. In Italy, monitoring stations consisted of sets of two hives with dead honey bee traps placed in different agroecosystems. When a settled threshold was exceeded they performed chemical analysis in dead honey bees to identify the active ingredient (Porrini et al., 2002; Porrini et al., 2003). This interesting approach of biomonitoring using honey bees gives information about toxic compounds, mainly insecticides with low honey bee $LD_{50,}$ but excludes information about pesticides present at sublethal concentrations. In a recent study colony strength was added to the research (Porrini et al., 2014). A study conducted in agricultural areas of Greece, indicates that useful information about the occurrence and the distribution of pesticide residues due to crop protection treatments can be derived from the analysis of randomly collected honey samples, used as bioindicators (Balayiannis and Balayiannis, 2008). On the other hand, an assessment of honey bee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France concluded that given the results (highest frequency of presence) and practical aspects (easy to collect; matrix with no turnover, unlike with honey bees that are naturally renewed), pollen loads were the best matrix for assessing the presence of pesticide residues in the environment in their given conditions (Chauzat et al., 2011). The main drawback of this matrix (pollen loads) is that it is collected using pollen traps placed at the entrance of the hive during a week, four times per year (Chauzat et al., 2006). This is the maximum period these traps are recommended to remain installed (a week) because they prevent the colony from their pollen supply as only honey bees can pass through, while pollen is collected in the trap outside the hive. Therefore, the contaminants gathered together with pollen loads will come exclusively from the species honey bees were able to visit during that week. A separate study conducted in France during 2008–2009 concluded that honey bees, honeys and pollens are appropriate sentinels for monitoring pesticide and veterinary drug environmental pollution. This study revealed the widespread occurrence of multiple residues in beehive matrices and suggests a potential issue with the effects of these residues alone or in combination on honey bee health (Lambert et al., 2013). In a recently conducted study in Egypt, residues of organochlorine and synthetic pyrethroid pesticides in honey were assessed as an indicator of environmental contamination (Malhat et al., 2015).

The analysis and occurrence of pesticide residues in honey bees or bee products has been widely reported without aiming to study them as biomonitors (Bargańska and Namieśnik, 2010; Fernández et al., 2002; Jiménez et al., 2004; Karazafiris et al., 2008; Kasiotis et al., 2014; Panseri et al., 2014; Pérez-Parada et al., 2011; Rissato et al., 2007; Rodríguez López et al., 2014; Rossi et al., 2001; Stoner and Eitzer, 2013; Walorczyk and Gnusowski, 2009; Wiest et al., 2011; Yáñez et al., 2013).

The ectoparasitic honey bee mite *Varroa destructor* was originally confined to the Eastern honey bee *Apis cerana*. After a shift to the new host *Apis mellifera* during the first half of the last century, the parasite dispersed worldwide and is currently considered the major threat for honey bee health (Rosenkranz et al., 2010). Among the many organisms that plague honey bees, *V. destructor* is by far the most damaging (Locke, 2016). Beekeepers are required to use regular *Varroa* mite control management to avoid colony death. Control methods, however, can often have adverse effects on bees (Haarmann et al., 2002), leave residues in honey and can be expensive for beekeeper. Uruguay suffers from the devastating effects of *Varroa*, and its presence has been associated with colony losses (Antunez et al., 2015).

Foliar applications of combinations of neonicotinoids and pyrethroids are commonly used to control stink bugs (Hemiptera: Pentatomidae) in soybean (*Glycine* max) (Baur et al., 2010), considered a major pest of this crop in various parts of the world (Kogan and Turnipseed, 1987). The high doses required due to the low susceptibility of stink bugs (Temple, 2011) and the broad action spectrum of the products commonly used, cause impact on non-target species such as honey bee and wild pollinators, which could have other way enhanced soybean productivity (de O. Milfont et al., 2013).

The occurrence of pyrethroids of agricultural use in honey bees or bee products has not been as reported as other chemical classes. Permethrin has been found as a frequent residue in a honey quality study (Malhat et al., 2015), and as responsible of honey bee kill incidents while other pyrethroids such as fenpropathrin, esfenvalerate and bifenthrin were findings with lower frequency of detection (Mullin et al., 2010).

Over the last few years great attention has been paid to the risk that pesticides pose to honey bees, particularly neonicotinoids (Goulson, 2013). These chemicals mimic the acetylcholine neurotransmitter and are highly neurotoxic to insects. Their systemic mode of action inside plants means xylem and phloem transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators at sublethal concentrations for most of the year (van der Sluijs et al., 2013).

At field realistic doses, neonicotinoids cause a wide range of adverse sublethal effects in honey bee and bumblebee colonies, affecting colony performance through impairment of foraging success, brood and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene among others (Brandt et al., 2016; Farooqui, 2013; Piiroinen and Goulson, 2016; Pisa et al., 2015; Simon-Delso et al., 2015). Neonicotinoids exhibit a toxicity that can be amplified by various other agrochemicals and they synergistically reinforce infectious agents such as *Nosema ceranae* which together can produce colony collapse (van der Sluijs et al., 2013). Furthermore, it is important to consider that neonicotinoids may present synergistic effects when combined with triazole and imidazole fungicides which modify metabolism in cytochrome P450 enhancing the neonicotinoid toxicity (Johnson et al., 2013; Schmuck et al., 2003; Thompson et al., 2014). Neonicotinoid insecticides have been reported to enhance the impact of pathogens, in particular, the molecular mechanism through which clothianidin adversely affects the insect immune response and promotes replication of a viral pathogen in honey bees bearing covert infections has been reported (Di Prisco et al., 2013). The limited available data suggest that neonicotinoids are likely to exhibit similar toxicity to virtually all other wild insect pollinators (van der Sluijs et al., 2013). Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of honey bees was investigated finding that thiamethoxam had no effect under the conditions used and that acetamiprid impaired a particular vulnerability. Specifically, after oral consumption acetamiprid increased sensitivity to antennal stimulation by sucrose solutions at doses of 1 μg/bee and impaired long-term retention of olfactory learning at the dose of 0.1 μg/bee (El Hassani et al., 2008). The evaluation of acute, semi-field and field toxicity of pesticides to *Apis cerana* and *A. mellifera* was reported in 2015: acetamiprid and endosulfan were found to be safer than the other assayed insecticides and did not cause any repellent effect on honey bees in field trials, while thiamethoxam (among others) was found highly toxic (Stanley et al., 2015). In a study conducted during 2012–2013 to study sub-lethal exposure to neonicotinoids of honey bees it was observed that when honey bees were exposed to either imidacloprid or clothianidin at a dose of 0.73 ng/bee/day for 13 consecutive weeks from July to September 2012, six of twelve previously healthy neonicotinoid-treated colonies died and all progressed to exhibit CCD symptoms during the winter months (Lu et al., 2014).

Recently, in Spain the influence of pesticide use in fruit orchards during blooming on honey bee mortality in 4 experimental apiaries was

Table 1
Population, brood, honey storage and *V. destructor* infection percentages of beehives at their initial biological conditions.

Beehive tag	Population	Brood	Honey storage	*V. destructor* infection (%)
1	7	48	2.5	0.9
2	7	10	3.5	0
3	8	52	3	0.9
4	7	39	3.5	0.4
5	8	46	3	0.4
6	7	34	4	0
7	8	53	3	0.8
8	6	27	4.5	0
9	6	40	4	0.8
10	6	43	3.5	0
11	7	52	2	4
12	7	38	4	0

studied. Among other pesticides (coumaphos, chlorpyrifos, dimethoate, omethoate, fluvalinate and carbendazim), the neonicotinoids imidacloprid and acetamiprid were detected. Imidacloprid was the fourth insecticide most frequently detected in the extracts of honey bees. The concentrations found (mean 53 ng/g and maximum 223 ng/g) are above of those considered sublethal and could be responsible of honey bee losses or even acute intoxication of forager honey bees (Calatayud-Vernich et al., 2016b).

Despite the huge amount of information on the occurrence of pesticides in the hive, little is known on the processes and mechanisms that rule the transport of agrochemicals from the field to the hive. Physicochemical properties of pesticides have been used to model the distribution within the hive or to understand the mechanisms of their toxicity to honey bees (Devillers, 2014; Dulin et al., 2012; Tremolada et al., 2011). Nevertheless, no data linking the concentrations found in the field with those detected in the hive has been reported. The aim of the present work is to study the transference of neonicotinoids from the field to the beehive done by foraging honey bees and their distribution within the beehive matrices: pollen, honey and beeswax. Data generated during this study about realistic field exposure of honey bees will be useful for refined risk assessment and modeling.

2. Materials and methods

2.1. Chemicals and standards

Acetonitrile (MeCN) of HPLC quality was from Pharmco Products Inc. (Brookfield, CT, USA). Water was deionized in the laboratory using a Thermo Scientific (Marietta, OH, USA) EASYpure RoDi Ultrapure water purification system. Magnesium sulfate anhydrous, reagent grade was from J.T. Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA) and formic acid p.a. 88% was purchased from Macron chemicals (Netherlands). A solution of 5% formic acid (v/v) was prepared in acetonitrile. The bulk amino sorbent (PSA, 40–60 μm), RP-C18 and graphitized carbon black (GCB) were from Scharlab (Barcelona, Spain). Analytical standards, of purity ≥ 95%, were from Dr. Ehrenstorfer (Augsburg, Germany).

2.2. Apparatus

Automatic pipettes suitable for handling volumes of 1–10 μL, 100–1000 μL and 1–10 mL were from Socorex (Lausanne, Switzerland). Analytical balances capable of weighing to 0.1 mg or to 10 mg were from Shimadzu (Kyoto, Japan). The centrifuge, suitable for use with the centrifuge tubes employed in the procedure and capable of achieving at least 3000 × *g*, was a SL16 by Thermo Electron (Langenselbold, Germany). The hand blender used was a Philips HR1616 Mixer.

LC–MS/MS was performed with an Agilent 1200 LC system coupled to a 4000 QTRAP® LC/MS/MS System from AB SCIEX™ run in the Scheduled® MS/MS-mode. LC-Separation was performed on a ZORBAX Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) column from Agilent. The operation of the LC gradient involved the following elution program: A, water/HCOOH 0.1% (v/v) and B, MeCN. It was run at 600 μL min^{-1} starting with 10% component B at injection time during 1 min and gradually changing to 100% B over 15 min. This mobile phase was kept for 10 min and then shifted back to the starting conditions (10% component B) and kept constant for 9 min giving a total run time of 35 min after injection. The injection volume was 5 μL and MeCN was the solvent used. MS/MS detection was performed in the multiple reaction monitoring (MRM) mode using an electrospray ionization interface in the positive ion mode. The ionization voltage was 4500 V, the nebulizer gas was synthetic air at 70 psi, and the curtain gas was nitrogen at 30 psi. The solvent evaporation in the source was assisted by a drying gas (heated synthetic air at 425 °C.50 psi^{-1}).

GC–MS was performed with a HP 6890 GC system coupled to a HP 5973 MS detector, GC-separation was performed on a HP-5 (5% methylsiloxane; 30 m; 0.25 mm id; 0.25 μm) column. Oven temperature was programmed: 120 °C from 0 to 5 min; 120 to 190 °C at 10 °C min^{-1}; 190 °C 1 min; 190 to 250 °C at 5 °C min^{-1}; 250 °C 5 min; 250 to 300 °C at 5 °C min^{-1}; 300 °C 5 min ($t = 45$ min). Helium was the carrier gas. Injector temperature was 280 °C, constant flow at 1 mL min^{-1}, transference temperature to MS was 280 °C and injection volume was 1 μL. Detection was performed in Single Ion Monitoring mode.

2.3. Experiment

Langstroth beehives were selected and prepared previously so that all of them had similar biological conditions for the experiment. Biological conditions were studied by estimating colony strength, honey storage and beehive health, specifically *Varroa* mite incidence. Adult honey bee population and the brood area in each colony were estimated. Hives were carefully opened with very little smoke, spaces between frames were inspected in detail, and frames completely covered by adult honey bees were recorded. Once the adult population was registered (number of frames completely covered), combs were removed from the hive and the brood area was estimated as quarters of frame faces covered by brood (with ¼ frame face = 210 cm^2). The area occupied by honey, pollen or empty was not included in the estimation. All determinations were visually performed by the same operator (Carrasco-Letelier et al., 2012; Mendoza et al., 2014). The percentage of *Varroa* infection was determined based on a reported methodology (De Jong et al., 1982). Briefly, a sample of 250–300 honey bees from the brood camber is taken and kept in freezer at −18 °C in plastic bags. Then they are placed in hot water in order to be able to count *Varroa* mites detached from honey bees and calculate their percentage.

Initial conditions for each beehive used in the experiment are summarized in Table 1.

Twelve macro tunnels as the ones used in horticulture production but specially constructed to individually cover 24 m^2 (6 × 4 m), were installed over an experimental plot, consisting of a flowering soybean crop. Each crop area was either maintained as blank or treated only once with formulated insecticides using a previously calibrated experimental CO_2 backpack pesticide sprayer using water as solvent with a 3 m arm which had 6 spraying flat fan nozzles in a volume of 120 L

Table 2
Dead honey bees collected in mortality traps and neonicotinoids concentration ranges in them 24 h after application.

Pesticide	Number of dead honey bees/beehive	Concentrations (μg/bee)	LD_{50} (μg/bee) contact (Lewis et al., 2016)
Control	(3,25,31)	Nd	–
Imidacloprid	(31,80,174)	(0.010–0.072)	0.081
Acetamiprid	(8,15,46)	(0.012–0.041)	8.09
Thiamethoxam	(61,343,632)	(0.001–0.003)	0.024

Table 3
LOQs and instrumental parameters for each studied compound.

LC-MS/MS			
	Acetamiprid	Imidacloprid	Thiamethoxam
LOQ (mg kg^{-1}) bees	0.0001	0.0001	0.0001
LOQ (mg kg^{-1}) wax	0.001	0.010	0.010
LOQ (mg kg^{-1}) pollen	0.001	0.001	0.010
LOQ (mg kg^{-1}) honey	0.001	0.001	0.001
LOQ (mg kg^{-1}) leaves	0.010	0.010	0.010
LOD (mg kg^{-1}) bees	0.0001	0.0001	0.0001
LOD (mg kg^{-1}) wax	0.001	0.001	0.001
LOD (mg kg^{-1}) pollen	0.001	0.001	0.001
LOD (mg kg^{-1}) honey	0.001	0.001	0.001
LOD (mg kg^{-1}) leaves	0.002	0.002	0.002
First transition $m/z > m/z$	223 > 126	256,1 > 175	292 > 211
First transition DP,CE,CXP	55,25,10	85,23,10	88,15,10
Second transition $m/z > m/z$	223 > 90	256,1 > 209	292 > 181,2
Second transition DP,CE,CXP	55,48,10	85,22,10	88,24,10

GC–MS			
	Cyhalothrin-lambda	Cypermethrin	Cyfluthrin
LOQ (mg kg^{-1}) bees	0.05	0.10	0.10
LOQ (mg kg^{-1}) wax	0.10	0.10	0.10
LOQ (mg kg^{-1}) pollen	0.05	0.10	0.10
LOQ (mg kg^{-1}) honey	0.10	0.10	0.10
LOD (mg kg^{-1}) bees	0.01	0.03	0.03
LOD (mg kg^{-1}) wax	0.03	0.03	0.03
LOD (mg kg^{-1}) pollen	0.01	0.1	0.1
LOD (mg kg^{-1}) honey	0.03	0.03	0.03
Selected ions (m/z)	181, 197, 208	163, 181, 209	163, 206, 226
Confirmation ion (m/z)	208	181	226
Quantification ion (m/z)	181	163	163

per ha so that the whole area was treated in a single walk through the macro tunnel. The experiment was arranged in a randomized complete block experimental design with three replicates: [imidacloprid (100 g L^{-1}) + beta-cyfluthrin (15 g L^{-1})] at 800 mL ha^{-1}, [thiamethoxan (141 g L^{-1}) + lambda-cyhalothrin (106 g L^{-1})] at 220 mL ha^{-1} and acetamiprid (200 g L^{-1}) at 270 mL ha^{-1} with cypermethrin (250 g L^{-1}) at 100 mL ha^{-1}. Each hive was placed in the extreme opposite to the door of the macro tunnel which ended in triangular shape with enough space for this purpose so that the 24 m^2 of the covered crop started outside the hive entrance and finished at the macro tunnel door. Each hive was provided with 1 L of water in a Doolittle feeder of 1.5 L total volume (not present during pesticides´ application), and a Gary dead honey bee trap (Gary, 1960) to evaluate mortality 24 h after pesticide applications. Samplings were performed before the application and following it at day 1, 6, 12 and 39. Samples (honey bees, honeycombs and soybean leaves from the top third of the plant) were stored in freezer at −18 °C until analysis, honey, pollen and wax were manually separated from each honeycomb sample. Honey bees' pesticide exposure was concluded at day 6 when the macro tunnels were opened.

2.4. Methodologies

Pesticide multiresidue analysis of hive matrices were performed using previously developed and validated methodologies (Niell et al., 2014; Niell et al., 2015). Briefly, 2 g of beeswax is extracted with 10 mL MeCN at ~80 °C. Then, the extract is freezed-out, liquid-liquid partitioned with hexane and an acetonitrile aliquot cleaned-up with 25 mg of PSA primary –secondary amine (PSA) and 25 mg of C18 sorbent per milliliter of extract.

Briefly, 5 g of honey are extracted with 10 mL water and 10 mL MeCN. Then the mixture of citrate buffer salts is added. The extract is cleaned-up with PSA 25 mg per milliliter and $MgSO_4$ 150 mg per milliliter.

Briefly, 2 g of previously homogenized honey bees are extracted with 5 mL of water and 10 mL MeCN. Then the mixture of citrate buffer salts is added. The extract is freezed-out overnight and cleaned-up with PSA and C18 25 mg per milliliter and $MgSO_4$: GCB (59:1) 150 mg per milliliter.

Finally all extracts were acidified with 5% formic acid solution in MeCN (v/v) (10 μL per mL extract) and injected in LC-MS/MS. Four milliliters were evaporated until dryness under a gentle stream of N_2 and redissolved in 1 mL ethyl acetate and injected in GC/MS.

Briefly, 5 g of previously homogenized pollen are extracted with 5 mL of water and 10 mL MeCN and 1 g sodium acetate, 4 g anhydrous magnesium sulfate, and 100 μL acetic acid are added. The extract is cleaned-up with PSA and C18 50 mg per milliliter and $MgSO_4$ 150 mg per milliliter and injected in LC-MS/MS. Two milliliters of toluene were added to 4 mL of extract and evaporated until dryness under a gentle stream of N_2 and redissolved in 1 mL ethyl acetate and injected in GC/MS.

Soybean leaves were extracted using a QuEChERS based method (Anastassiades et al., 2003): each entire freezed sample was thoroughly comminuted and homogenized with a hand blender. The sample amount was 2.5 g; $MgSO_4$ and NaCl were added during the salting out step. Water addition and dispersive Solid Phase Extraction clean up with alumina, C18 and $MgSO_4$ was performed. This methodology was previously validated by our group obtaining acceptable accuracy according to the guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed from the European Commission (European Commission, 2015).

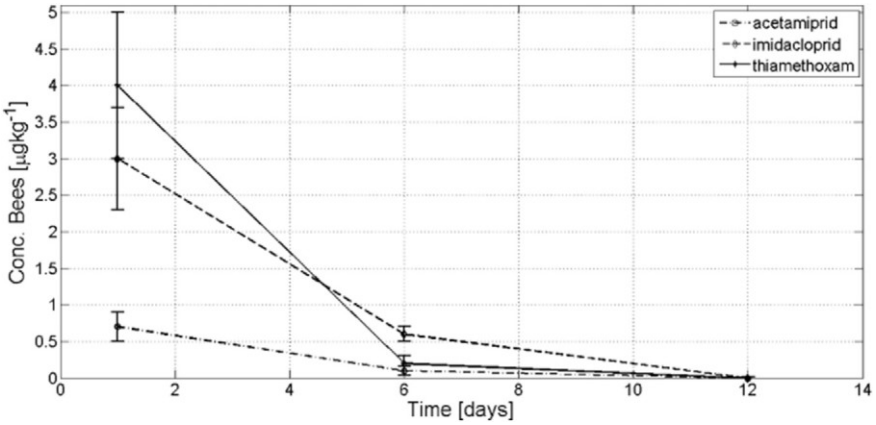

Fig. 1. Pesticide residues concentrations (μg kg^{-1}) evolution with time (days).

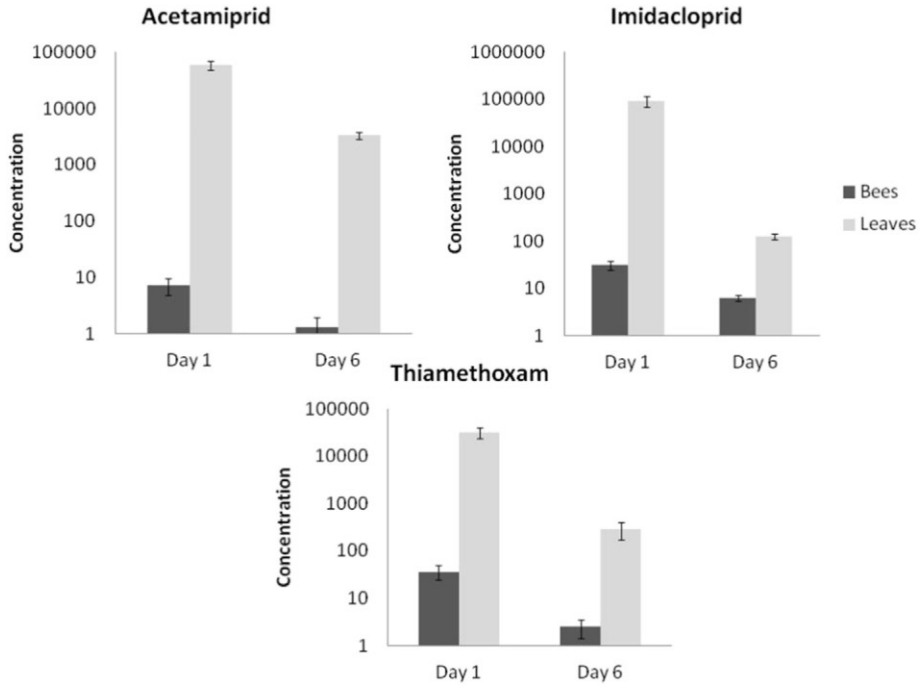

Fig. 2. Pesticide residue concentrations in honey bees and leaves sampled simultaneously. Note that pesticide concentrations in mg kg^{-1} of both honey bees and leaves were multiplied by 10^4 and presented in log scale in order to be able to show comparatively pesticide contents in honey bees and leaves at day 1 and 6.

Neonicotinoids were analyzed by LC-MS/MS and pyrethroids by GC–MS.

Statistical analyses were performed using R, which is a free software environment for statistical computing and graphics (R Core Team, 2014).

3. Theory

Honey bees collect pesticide residues present in the different environmental compartments during their flights and transfer them inside the hive. When pesticides have been applied over a crop and honey bees forage in it the transference can be quantified. It is related to the physicochemical properties of the compounds (Kow and Vp), which is very useful for predictive modeling of pesticide fate within the hive. Therefore, bees quantitatively monitor pesticide residues.

4. Results and discussion

Beehives presented similar initial biological conditions (Table 1) regarding population, brood and honey storage (estimations were performed as explained in Section 2.3). All beehives were healthy, as it can be observed they presented a small percentage of *Varroa destructor* infection: <4% in every case and <1% for 92% of the beehives.

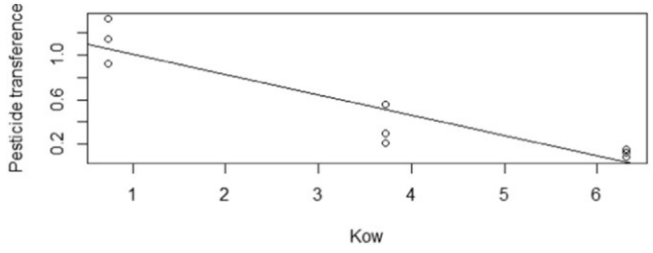

Fig. 3. Pesticide transference from soybean crop to honey bees plotted against each Kow (thiamethoxam = 0.741; imidacloprid = 3.72; acetamiprid = 6.31). Note that pesticide transference values were scaled (multiplied by 10^3).

None of the pesticides in any of the studied matrices were detected before the exposure.

Pesticide concentrations in dead honey bees collected in the mortality traps 24 hs after application were between 0.001 and 0.072 μg/bee (Table 2). They were below each neonicotinoid's contact LD_{50}. Dead honey bees collected in the mortality traps 24 h after application accounted for 4% of the whole population (based on the initial estimation) in the case of highest mortality (632 honey bees).

Neonicotinoid residues were detected while pyrethroids were not. Probably, as pyrethroids have a knock down effect, the honey bees that were exposed to higher concentrations (which could have been detected) remained dead in the field and could not return to the hive. The ones sampled alive presented concentrations below the instrument (GC/MS) detection limit, which are higher than the limits for the neonicotinoids by LC-MS/MS (Table 3) Each bee foraging in the field within the macro tunnel, 24 h after pesticide application, collected an average of 0.05 ng of acetamiprid, 0.3 ng of imidacloprid and 0.3 ng of thiamethoxam, from the 130, 192, and 74 mg applied over each crop portion respectively. These values are below each pesticide bee oral acute 48 h LD_{50} (they represent 1/10 of thiamethoxam's and imidacloprid's) (Lewis et al., 2016).

In beeswax recently produced (cut from the external layers of the comb), sampled at day 6 of the experiment, the three neonicotinoid residues were detected at their limit of detection (1 μg kg^{-1}). These results indicate that honey bees exposed to neonicotinoids effectively transferred the three of them they had collected. Pesticide residues were detected neither in honey nor in pollen separated from the sampled combs; concentrations in these matrices could have been below 0.001 mg kg^{-1} which is the method's LOQ shown in Table 3. Similar LOQs were obtained for honey and honey bee pesticide residues analysis using QuEChERS and other compared methods in a recently published work (Calatayud-Vernich et al., 2016a).

In a recent reviews on the effect of neonicotinoids on bee and colony health, Sanchez Bayo et al. gathered information on the negative effects of chronic exposure of these insecticides on honey bees and as a consequence on their performance. The impairment of some basic functions for bee work as well as the lowering in their immune system, can

have consequences on the whole colony, threatening its survival (Sánchez-Bayo and Desneux, 2015; Sánchez-Bayo et al., 2016).

Fig. 1 shows the evolution with time of average pesticide residues concentration in honey bees sampled from inside the beehive for each treatment. It is observed a decrease in pesticide concentration, being not detectable from day 12 for any of the investigated compounds.

With the obtained results from the pesticide multiresidue analysis performed, a look at the relation between the variables pesticide residue concentration in the crop with pesticide residue concentration in the honey bees sampled simultaneously is shown in Fig. 2.

It was observed that the degradation for each compound in the plant matrix (DT_{50}) for the experiment is <6 days which is within the range reported in the Pesticides Properties DataBase (Lewis et al., 2016).

This data allows to calculate a pesticide transference ratio from the crop to honey bees = pesticide concentration in honey bees/pesticide concentration in leaves at day 1. This relationship is particularly important because it allows relating quantitatively the amount of pesticide which was applied in the field (pesticide concentration in leaves) to the amount honey bees sample (pesticide concentration in honey bees) and will therefore enter into the hive.

Acetamiprid was found in honey bees collected either after 24 h or six days, at levels ten times lower than the other two neonicotinoids, therefore, its transference to the hive is lower. A possible explanation to this observation could rely on the differences of vapor pressure (Vp) between the three pesticides: thiamethoxam = 6.60×10^{-06}; imidacloprid = 4.0×10^{-07}; acetamiprid = 1.73×10^{-04} mPa (Lewis et al., 2016). The Vp of acetamiprid is two to three orders higher than thiamethoxam and imidacloprid respectively. Due to the higher volatility of the insecticide, probably honey bees detected it and avoid visiting soybean flowers contaminated with acetamiprid. Although the transference of the pesticides did not show a general trend with their water solubilities, the most soluble pesticide thiamethoxam has the highest transference.

Interestingly, the calculated pesticide transference shows an inverse linear trend with Kow for each molecule (Fig. 3).

When trying to fit a linear model ($n = 9$) the following were obtained: Adjusted R-squared = 0.84; Intercept = 1.192; Slope = −0.183; *P* value: 0.00031; which indicates that it can be rejected the hypothesis that the slope is equal to 0 and that 84% of the variability in the transference can be explained by the Kow. The highest pesticide transfer is observed for the pesticide presenting the lowest Kow: thiamethoxam = 0.741; imidacloprid = 3.72; acetamiprid = 6.31 (Lewis et al., 2016). The importance of the relationship between the physicochemical property Kow and the pesticide transference is that it allows to predict the behavior of other compounds. For example, for pyrethroids which have a much higher Kow than neonicotinoids, a transference much lower is expected, therefore, their concentrations in honey bees will be small, which is concordant with the non-detection of pyrethroids in bee samples from the experiment.

5. Conclusions

New insights on honey bee and bee products as environmental monitors of agroecosystems sustainability are given. The distribution of pesticides commonly used in soybean plantations within the beehive was studied. Residue levels of insecticides in soybean leaves, honey bees, wax, honey and pollen in hives with controlled exposure in semi field conditions were analyzed. The obtained results allow relating pesticide concentrations present in the hive surroundings with traces found in foraging honey bees and in beeswax. Therefore, a pesticide transference ratio could be calculated for each detected compound (acetamiprid, imidacloprid and thiamethoxam) which shows a linear inverse trend with their physicochemical property Kow. Although three pesticides are not enough to make general statements, they do show a tendency, which gives evidence that it is worthy to further expand this research to other relevant molecules.

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