Bull Environ Contam Toxicol (2010) 85:125–128
DOI 10.1007/s00128-010-0069-y

Exposure to Pesticides at Sublethal Level and Their Distribution Within a Honey Bee (*Apis mellifera*) Colony

Maja Ivana Smodiš Škerl · Veronika Kmecl · Aleš Gregorc

Received: 17 March 2010 / Accepted: 8 July 2010 / Published online: 18 July 2010
© Springer Science+Business Media, LLC 2010

Abstract Honey bee colonies were exposed to pesticides used in agriculture or within bee hives by beekeepers: coumaphos; diazinon; amitraz or fluvalinate. Samples of bee workers, larvae and royal jelly were analysed using Gas Chromatography-Electron Capture Detection (GC-ECD). Amitraz was quantified using High Performance Liquid Chromatography (HPLC), and Gas Chromatography-Tandem Mass Spectrometry (GC/MS/MS) was used for quantification of diazinon. Sixth day after treatment, coumaphos was found in the royal jelly (250 ng/g) secreted by nurse workers and fluvalinate was found in both bee heads (105 ng/g, 8 days after treatment) and in larvae (110 ng/g, 4 days after treatment). Amitraz residues in all sampled material were below the level of detection of 10 ng/g. Diazinon was not detected in any of the analysed samples. The large quantities of fluvalinate found in bee heads and larvae, the coumaphos residues in royal jelly, and additional potential sub-lethal effects on individual honey bees or brood are discussed.

Keywords Pesticides · Residues · Honey bee colony · Brood · Royal jelly

Honey bees are exposed to a variety of factors that can influence the vitality of a colony and weaken its reproductive abilities. Agrochemicals are one of the external factors that can affect the colony. Foragers return to the hive bringing fresh pollen and nectar which is stored in cells in the comb and which can potentially contain residues of pesticides. To reduce the population of Varroa mites in the bee colonies, beekeepers use acaricides that can potentially be distributed in the entire colony, not only in the honey, bee bread or wax, but also in royal jelly, the vital diet for brood and the queen.

The acaricides can be referred to as external, non-pathogenic factors and are widely used in beekeeping. In Europe, beside organic substances, several commercial products are used to control Varroa mites such as: Apivar® (amitraz); Perizin® (coumaphos); and Apistan® (fluvalinate; Martel et al. 2007). The use of pesticides inside bee hives raises the risk of contamination of honey bee products. Amitraz, coumaphos, diazinon and fluvalinate are all lipophilic, so have the potential to mainly contaminate beeswax (Bogdanov et al. 1998; Wallner 1999). Brood combs can contain coumaphos and fluvalinate residues ranging from 1.8 to 43.4 mg/kg (Bogdanov et al. 1998). The widely used compound amitraz is metabolized in honey (Bogdanov 1988) and wax (Korta et al. 2001, 2002). Fluvalinate residues have also been found in propolis, in greater amounts than in wax (Bogdanov et al. 1998; Wallner 1999). Pollen collected by foragers can also contain residues of different agricultural pesticides. Diazinon, a broad-spectrum insecticide used to control a variety of agricultural and garden pests, has been found in fresh pollen collected by workers, and in bee bread stored in the comb of colonies located in pesticide treated apple orchard (Smodiš Škerl et al. 2009). Organophosphate compounds, coumaphos and diazinon, can thus potentially all be found in honey bee colonies.

In order to asses the distribution of pesticides inside a honey bee colony, we treated colonies with the acaricides

M. I. Smodiš Škerl (✉) · V. Kmecl · A. Gregorc
Agricultural Institute of Slovenia, Hacquetova 17, Ljubljana, Slovenia
e-mail: maja.smodis.skerl@kis.si

A. Gregorc
Honey Bee Research and Extension Lab, University of Florida
Entomology and Nematology Department, Florida, USA

amitraz, fluvalinate and coumaphos, in quantities in accordance with the treatment protocol against Varroa mites, or with the insecticide diazinon following a typical Protection Treatment Plan performed in an apple orchard. The aim of the work was to identify and quantify the potential residues in worker hypopharyngeal glands (bee heads), royal jelly and developing bee larvae in treated colonies. We also aimed to confirm the hypothesis that pesticides present in a colony might pass through the nurse bee's body through the hypopharyngeal glands located in its head and accumulate in larvae.

Materials and Methods

External doses of pesticides were applied to colonies of nine combs, occupied with 20,000–30,000 adult bees and brood, located at the Agricultural Institute of Slovenia. The first treatment on four colonies was performed on 8 August 2007 when each treated colony received one of four applied pesticides: coumaphos; diazinon; amitraz; or fluvalinate. The first treatment solution was prepared from Perizin® (Bayer), containing 32 g/L of the active ingredient, coumaphos. The formulation was diluted 1:50 in water and 15 g sugar was added. The solution was sprinkled onto the bees on the top bars of the frames. A total of 45 mL of solution was used (corresponding to 32 mg of active ingredient per hive). The second solution was prepared with Diazinon 20® (Pinus): 1 g diazinon was added to 1 L water and 330 g sugar. 50 mL of solution was sprinkled onto bees of the second experimental hive. The third hive was fumigated with amitraz (Hemovar®). Four drops of solution applied onto the fumigation strip impregnated with KNO_3 finally contained approximately 40 μL amitraz. The fumigation was repeated after the second and fourth day. The fourth hive received two wooden strips containing approx. 100 μL fluvalinate mixed with Vaseline. Strips were inserted between brood combs and remained there for 8 days, when they were removed.

Sampling of adult workers from the treated and control colonies was conducted on days 1, 2, 3, 5 and 6. Larvae from all colonies were collected on the third day after treatments. We sampled newly emerged 1 day old workers, nurse workers collected whilst feeding young brood, and foragers at the entrance of the hive after returning from foraging flights. Eight workers were in each sampled group. Sampled larvae were 5–6 days old, and royal jelly was collected from 2 to 3 day old larvae using a plastic stick, and put into 1.5 mL plastic tubes (Eppendorf®). All samples were immediately frozen at −20°C and stored until analysis.

Analyses were performed at the Central Laboratory of the Agricultural Institute of Slovenia. For the extraction of the royal jelly and bee head samples, 0.5 g of royal jelly or 0.25 g of bee heads were dissolved in 20 mL of water. 90 mL of mixture of *n*-hexane and iso-propanol was added and neutralized with 0.28% NH_3 (pH 8). A combined extract was decanted into a separating funnel. After liquid–liquid extraction, the lower iso-propanol phase was discarded. The upper hexane phase was filtered into an evaporation flask and evaporated to dryness under vacuum at a temperature of 35–40°C. The residue was dissolved in 2 mL of acetone for GC and HPLC analyses (Martel and Zeggane 2002). Quantification of coumaphos and fluvalinate in royal jelly and bee head solutions was conducted using Gas Chromatography-Electron Capture Detection (GC-ECD; Bogdanov et al. 1998). Amitraz was quantified using High Performance Liquid Chromatography (HPLC; Martel and Zeggane 2002) and Gas Chromatography-tandem Mass Spectrometry (GC/MS/MS) (Fillion et al. 2000) was used for the quantification of diazinon.

Fortified sample recoveries in royal jelly samples were 98% for coumaphos, 97% for fluvalinate, 97% for amitraz and 92% for diazinon, and in bee head samples 80%, 95%, 94% and 86%, respectively.

Results and Discussion

Diazinon and coumaphos concentrations detected in bee heads, larvae or royal jelly, are reported in Table 1. Diazinon was not detected in any of the analysed samples. Coumaphos was found in the royal jelly secreted by nurse bees, but not in bee heads or larvae. Amitraz residues in all samples were below the level of detection of 10 ng/g, but fluvalinate was found in bee heads (1, 2, 4 and 8 days after treatment) and in larvae (2 and 4 days after treatment). Residues of amitraz and fluvalinate are shown in Table 2. All sample analyses were also conducted with the less sensitive GC-MS method which detected coumaphos and fluvalinate residues in royal jelly and larvae (Tables 1, 2).

Honey bees in the field are exposed to direct or indirect contamination. Large amounts of coumaphos were recorded in workers in a colony after the exposure and then dropped quickly below the detection limit (Tremolada et al. 2004). We found no coumaphos residues in bee heads, which could be explained by the fact that bees ingest most of the chemicals just after treatment, and then rapidly eliminate it by metabolism, advection and deposition (Tremolada et al. 2004) which is in accordance with our results. In younger larvae, we were not able to demonstrate residues of coumaphos, whereas large amounts were detected in royal jelly. Large amounts of fluvalinate in bee heads and larvae suggest that bees contaminated each other by trophallaxis in the colony, and then transmitted it to larvae trough the food. Diazinon and amitraz were below the level of detection in all samples.

Table 1 Concentrations of diazinon and coumaphos in indirectly contaminated matrices (honey bee heads, royal jelly and larvae) at different times after treatment

Samples	Date	Treatment	Time after first treatment (days)	Diazinon GC-MS (ng/g)	Coumaphos GC-ECD (ng/g)	Coumaphos GC-MS (ng/g)
	8. August	Coumaphos Diazinon	0			
Bee heads	9. August		1	< LOD*	< LOD*	< LOD**
Bee heads	10. August		2	< LOD*	< LOD*	< LOD**
Larvae	10. August		2	< LOD*	< LOD*	< LOD**
Bee heads	11. August		3	< LOD*	< LOD*	< LOD**
Larvae	11. August		3	< LOD*	< LOD*	< LOD**
Royal jelly	13. August		5	< LOD*	210	400
Bee heads	14. August		6	< LOD*	< LOD*	< LOD**
Royal jelly	14. August		6	< LOD*	170	250

< *LOD**, under level of detection 10 ng/g; < *LOD***, under level of detection 100 ng/g; *GC-MS*, Gas chromatography-mass spectrometry; *GC-ECD*, Gas chromatography-electron capture detector; *HPLC-UV/VIS*, High pressure liquid chromatography-Ultra violet/visible spectrum

Table 2 Concentrations of amitraz and fluvalinate in indirectly contaminated matrices (honey bee heads and larvae) at different times after treatment

Samples	Date	Treatment	Time after first treatment (days)	Amitraz HPLC-UV/VIS (ng/g)	Fluvalinate GC-ECD (ng/g)	Fluvalinate GC-MS (ng/g)
	21. August	Amitraz Fluvalinate	0			
Bee heads	22. August		1	< LOD*	228	< LOD**
Larvae	22. August		1	< LOD*	< LOD*	< LOD**
	23. August	Amitraz	2			
Bee heads	23. August		2	< LOD*	258	< LOD**
Larvae	23. August		2	< LOD*	1,038	1,170
	24. August	Amitraz	3			
Bee heads	25. August		4	< LOD*	204	< LOD**
Larvae	25. August		4	< LOD*	98	110
Bee heads	29. August		8	< LOD*	105	< LOD**
Larvae	29. August		8	< LOD*	< LOD*	< LOD**

< *LOD**, under level of detection 10 ng/g; <*LOD***, under level of detection 100 ng/g; *GC-MS*, Gas Chromatography-mass spectrometry; *GC-ECD*, Gas chromatography-electron capture detector; *HPLC-UV/VIS*, High pressure liquid chromatography-ultra violet/visible spectrum

Pesticides can potentially have sub-lethal effects on individual honey bees or brood. In our previous research, we have found that oxalic and formic acids applied to bees as acaricides in a colony, induced cell death in the larval midgut (Gregorc et al. 2004; Gregorc and Smodiš Škerl 2007) and salivary glands of larvae (Silva-Zacarin et al. 2006). Imidacloprid treatment in adult bees also induced cell death and decreased acinal diameter in hypopharyngeal glands in comparison to control untreated bees (Smodiš Škerl and Gregorc 2010). It is evident that pesticides have adverse, sublethal effects on individual bees and their tissues (Gregorc and Bowen 2000). Agricultural pesticides (Smodiš Škerl and Gregorc 2010), coumaphos and fluvalinate used in bee hives in order to control Varoa mites could be found in bees, brood and royal jelly. Further studies would be needed to compare pesticides effects in combination with other environmental stressors.

Acknowledgments We would like to acknowledge the assistance provided by Marjan Kokalj for helping with treatments and Vesna Lokar for collecting the samples. This research was financially supported by the Slovenian Ministry of Higher Education, Science and Technology and the Ministry of Agriculture, Forestry and Food, Research programme P4-133; Project Number L7-7602 and V4-0484.

References

Bogdanov S (1988) Bestimmung von Amitraz und seine Metaboliten in Honig durch HPLC, Mitt Schweiz Zentrum Bienenforsch 1–9

Bogdanov S, Kilchenmann V, Imdorf A (1998) Acaricide residues in some bee products. J Apic Res 37:57–67

Fillion J, Sauve F, Selwyn J (2000) Multiresidue method for the determination of residues of 251 pesticides in fruit and vegetables by gas chromatography/mass spectrometry and liquid chromatography with fluorescence detection. J AOAC Int 83: 698–712

Gregorc A, Bowen ID (2000) Histochemical characterization of cell death in honey bee larvae midgut after treatment with *Paenibacillus larvae*, Amitraz and Oxytetracycline. Cell Biol Int 24:319–324

Gregorc A, Smodiš Škerl MI (2007) Toxicological and immunohistochemical testing of honey bees after oxalic acid and rotenone treatments. Apidologie 38:296–305

Gregorc A, Pogačnik A, Bowen ID (2004) Cell death in honey bee (*Apis mellifera*) larvae treated with oxalic or formic acid. Apidologie 35:453–460

Korta E, Bakkali A, Berrueta LA, Gallo B, Vicente F, Kilchemann V, Bogdanov S (2001) Characterisation and monitoring of acaricide degradation in honey and beeswax. J Agric Food Chem 49: 5835–5842

Korta E, Bakkali A, Berrueta LA, Gallo B, Vicente F, Bogdanov S (2002) Determination of amitraz and of other residues in beeswax. Anal Chim Acta 475:97–103

Martel AC, Zeggane S (2002) Determination of acaricides in honey by high-performance liquid chromatography with photodiode array detection. J Chromatogr A 954:173–180

Martel AC, Zeggane S, Aurieres C, Drajnudel P, Faucon JP, Aubert M (2007) Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® or Asuntol®50. Apidologie 38:534–544

Silva-Zacarin ECM, Gregorc A, Silva de Moraes RLM (2006) In situ localization of heat-shock proteins and cell death labelling in the salivary gland of acaricide-treated honey bee larvae. Apidologie 37:1–9

Smodiš Škerl MI, Gregorc A (2010) Heat shock proteins and cell death in situ localisation in hypopharyngeal glands of honey bee (*Apis mellifera carnica*) workers after imidacloprid or coumaphos treatment. Apidologie 41:73–86

Smodiš Škerl MI, Velikonja Bolta S, Baša Česnik H, Gregorc A (2009) Residues of pesticides in honey bee (*Apis mellifera carnica*) bee bread and in pollen loads from treated apple orchards. Bull Environ Contamin Toxicol 83:374–377

Tremolada P, Bernardinelli I, Colombo M, Spreafico M, Vighi M (2004) Coumaphos distribution in the hive ecosystem: case study for modelling applications. Ecotoxicol 13:589–601

Wallner K (1999) Varroacides and their residues in bee products. Apidologie 30:235–248