Apidologie (2018) 49:112–119

DOI: 10.1007/s13592-017-0533-3

Original article

From field to food—will pesticide-contaminated pollen diet lead to a contamination of royal jelly?

Franziska Böhme[1], Gabriela Bischoff[2], Claus P. W. Zebitz[3], Peter Rosenkranz[1], Klaus Wallner[1]

[1]Apicultural State Institute, University of Hohenheim, August-von-Hartmann-Str. 13, 70599, Stuttgart, Germany
[2]Julius Kühn-Institut, Institute of Bee Protection, 14195, Berlin, Germany
[3]Institute of Phytomedicine, Applied Entomology, University of Hohenheim, 70599, Stuttgart, Germany

Received 30 March 2017 – Revised 1 July 2017 – Accepted 24 July 2017

Abstract – The contamination of bee products, e.g., bee bread, by pesticides is an increasing problem of beekeeping in rural areas. Bee bread is used by nurse bees to produce larval food. However, the fate of pesticides originating from the pollen during this process is unknown. Over the entire period of queen rearing, adult honeybees in queenless mini-hives were fed with a pollen-honey diet containing a cocktail of 13 commonly used pesticides in high concentrations (34–920 μg/kg). Royal jelly (RJ) harvested from queen cells was subjected to a multi-residue analysis. Seven substances were rediscovered in traces (76.5% of all detections are below 1 μg/kg) with at most 0.016% of the original pesticide concentrations of the fed diet. Considering this extraordinary low contamination of RJ, it seems unlikely that pesticides, if used according to the approved application instructions, would impair the development and health of honeybee queens. Possible reasons for the low residue levels in RJ are discussed.

Apis mellifera **/ sublethal pesticide concentrations / contamination flow / pesticide mixture**

1. INTRODUCTION

Royal jelly (RJ) is considered the "most attractive functional food" with potential health benefits in dietetics as well as in cosmetics, resulting in growing attention on the international market (Ramadan and Al-Ghamdi 2012). However, its original purpose is to nourish honeybee larvae. In addition, RJ is a crucial factor in honeybee caste dimorphism. Caste differentiation in honeybees is a gradual, continuous, and complex process that is dependent on different factors (Weaver 1957, Dietz and Lambremont 1970, Rembold et al. 1974, Asencot and Lensky 1984, Kamakura 2011, Shao et al. 2014, Buttstedt et al. 2016, Guo et al. 2016). RJ is responsible for the colonies well-being and physical fitness as it is fed to both larvae during the first few days after hatch and during the whole life of the queen (Haydak 1970).

As reviewed by Ramadan and Al-Ghamdi (2012), the main constituents of RJ are proteins (9–18%), carbohydrates (7–18%), as well as lipids (3–8%). Lipids and proteins are derived from pollen (Crailsheim 1992), although only traces of pollen grains have been found in RJ (Haydak 1970).

Studies have shown that residues of crop protection products can be found in corbicular pollen loads of honeybees (Mullin et al. 2010, Stoner and Eitzer 2013, Roszko et al. 2016). These pollen pellets are stored inside the hive as bee bread, containing sometimes mixtures of various pesticides also coming into contact with acaricides used by beekeepers (Johnson et al. 2010, Al

Electronic supplementary material The online version of this article (doi:10.1007/s13592-017-0533-3) contains supplementary material, which is available to authorized users.

Corresponding author: F. Böhme,
franziska.boehme@uni-hohenheim.de
Manuscript editor: Monique Gauthier

Naggar et al. 2015, Traynor et al. 2016). This stored pollen is consumed by nurse bees in order to produce larval food. However, there is hardly anything known about residues of these agricultural substances in royal jelly. Some authors detected residues of different veterinary drugs in RJ, used as in-hive treatments (Calvarese et al. 2006, Shen et al. 2011, Zhang et al. 2016), and only few scientists found residues of acaricides (Smodiš Škerl et al. 2010, Notardonato et al. 2014) and one insecticide used as greater wax moth (*Galleria mellonella* L.) –treatment (Tananaki et al. 2009).

Inside the bee hives, agro-chemicals are found frequently in sometimes high concentrations in pollen pellets and bee bread (Traynor et al. 2016), often coinciding with the period of queen rearing. RJ plays a crucial role in the honeybee's life, and putative residues in RJ can have not only sublethal effects on larvae but might have effects on the queen's vitality and viability as well.

The objective of this research was to trace possible pesticide residues in RJ back to the protein source. Thus, we designed a field-realistic, yet worst-case trial where we fed a mixture of commonly found pesticides (Böhme et al. 2017) mixed in high concentrations into a pollen-honey diet, providing proteins for RJ similar to bee bread. While feeding, we initiated a queen rearing within the colony to obtain RJ, presumably contaminated with the given pesticides, which was subjected to a multi-residue analysis.

2. MATERIALS AND METHODS

2.1. Bee colonies and experimental design

An artificial swarm of honeybees (*Apis mellifera carnica* Pollmann 1879) was made in the beginning of August 2014 in order to set up three colonies. This month was chosen, as known from earlier studies, that crop protection intensity in the field is low. For this purpose, bees of different age of a queenless colony were shaken into a bucket and sprayed with water to prevent the bees from escape and to keep them calm. About 3500 bees of the artificial swarm, two frames with open brood, and four frames with a residue-free wax foundation were put into one body of a polystyrene mini hive (29 × 29 × 32 cm LWH). Sugar syrup (Apiinvert®, Südzucker AG, Mannheim, Germany) was offered ad libitum to facilitate cell building. Hive entrances were closed and the hives were placed into a dark and cool room for one night.

On the next day, the colonies were positioned in the spacious garden of the Apicultural State Institute in Stuttgart-Hohenheim and hive entrances were opened again. Two of the colonies were assigned the pesticides group, which were offered 70-g packages of a pollen-honey diet enriched with a mixture of pesticides (commercial products in their respective formulations, Table I) that are commonly found in pollen pellets collected in intensively used agricultural areas in Southern Germany (Böhme et al. 2017).

The pollen-honey diet was prepared as a mixture of pesticide-free commercially available dried willow pollen pellets (Andreas Bock, Ökologische Imkerei, Mertingen, Germany), finely ground with a common hand blender, and mixed with a creamy honey with a commonly used hand mixer with a dough hook at a ratio of 40:60% *w*/*w*. The test pesticides were diluted in tap water 1:100, added to the honey in the necessary amounts, and thoroughly homogenized before mixing with the pollen to achieve the respective concentration in the pollen diet (Table II). To avoid dilution of the pesticides by pollen collected in the field, a pollen grid was attached at the entrance of the two colonies to scrape off the pollen loads of the foragers. The third colony served as an untreated control receiving no additional pollen-honey diet and no pollen grid.

On the same day, 30 neonate larvae were grafted into plastic cell cups on a cell bar frame in each hive. A frame with grafted larvae and one frame with wax foundation replaced the two initial brood frames. Packages of prepared pollen-honey diet were placed on top of the frames inside the hive. After 2 days, cell bar frames with queen larvae were removed and cells with RJ were harvested with a thin spoon and stored in an Eppendorf tube in the freezer (−20 °C) until residue analysis. During continuous feeding of the pollen-honey diet, grafting and harvesting was repeated twice in the same way (three repetitions).

Table I. Trade names of the substances used in the pollen-honey diet, their respective pesticide class, and octanol-water partition coefficient

Active ingredient	Class[a]	Trade name (a.i. g/l or g/kg)	Octanol-water partition coefficient (Log P_{ow})[b]
Acetamiprid	I	Mospilan® (200 g/kg)	0.8
Azoxystrobin	F	Ortiva® (250 g/l)	2.5
Boscalid	F	Cantus Gold® (200 g/l)	2.96
Dimethenamid-P	H	Spectrum® (720 g/l)	1.89
Dimoxystrobin	F	Cantus Gold® (200 g/l)	3.59
Methiocarb	I	Mesurol® (500 g/l)	3.18
Prosulfocarb	H	Boxer® (800 g/l)	4.48
Prothioconazole	F	Proline® (250 g/l)	3.82
Pyraclostrobin	F	F500® (250 g/l)	3.99
Tau-Fluvalinate	I	Mavrik® (240 g/l)	7.02
Tebuconazole	F	Matador® (225 g/l)	3.7
Thiacloprid	I	Biscaya® (240 g/l)	1.26
Triadimenol	F	Matador® (75 g/l)	3.18

[a] *I* insecticide, *F* fungicide, *H* herbicide

[b] PPDB (2017)

2.2. Pesticide residue analysis

Samples of RJ were stored under −20 °C in the Apicultural State Institute until shipping on carbon dioxide dry ice to the Institute of Bee Protection of the Julius Kühn-Institut, where the multi-pesticide residue analysis was performed (detailed method in the Online Resource).

To identify any nonparametric correlation between substances detected in RJ and the octanol-water partition coefficient (Log P_{ow}, Table I), a Spearman correlation analysis was conducted with the computer software JMP® 11.1.1 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS

The residue analysis of RJ from control colonies showed no detectable amounts of pesticides except for traces of pyraclostrobin and thiacloprid (< 0.5 and 0.23 μg/kg, resp.). These two pesticides are frequently used in many crops and thus represent an inevitable background contamination in free-flying colonies.

Boscalid, dimethenamid-P, methiocarb, tebuconazole, and triadimenol are pesticides with comparatively higher limits of detection (LOD; 0.67–2.67 μg/kg, Online Resource) and were not detected in any of the RJ samples of the experimental colonies fed with the pollen-honey diet (Table II). The complex matrix of RJ, containing sugars, proteins, fatty acids, etc., not only influences the extraction procedure but also the chromatographic measurements. Due to matrix-related interferences, prothioconazole (determined as its metabolite prothioconazole-desthio, Liu et al. 2017) could not be properly evaluated, because two of the three selected MRM transitions were superimposed by the MRM of a co-eluating interfering substance.

Thus, only seven of the 13 substances fed to the bees could be detected above their LOD. In both colonies and all three repetitions, pesticides could be detected above the limit of quantification (LOQ) in 17 cases. Concentrations of pesticides range between 0.42 and 2.16 μg/kg and vary in number and composition of the substances. 76.5% of all pesticide concentrations were below 1 μg/kg. Only in one sample (3rd repetition colony 1) could all seven pesticides be found. The insecticide thiacloprid was found in every sample, yet only once in a concentration > 2 μg/kg (Table II).

Table II. Pesticide concentrations (μg/kg) in the pollen-honey diet used in the feeding experiment, amount of consumed pollen-honey diet (g), and calculated amount of pesticide intake (μg) by adult honeybees for repetitions 1, 2, and 3, respectively (mean ± SD). Concentrations (μg/kg) of pesticide residues found in RJ samples (mean ± SD) for each repetition

Total amount of consumed pollen-honey diet (g; mean ± SD)		Repetition 1 69.3 (± 0.5)	Repetition 2 66.9 (± 3.2)	Repetition 3 74.49	Repetition 1	Repetition 2	Repetition 3[a]
Active ingredient (a.i.)	Concentration of a.i. in pollen-honey diet (μg/kg)	Calculated amount of a.i. incorporated into colonies via pollen-honey diet (μg; mean ± SD)			Concentration of a.i. detected in RJ samples (μg/kg; mean ± SD)		
Acetamiprid	787.89	54.6 (± 0.4)	52.7 (± 2.5)	58.7	< LOQ[b]	0.67[c]	1.92
Azoxystrobin	674.17	46.7 (± 0.3)	45.1 (± 2.1)	50.2	0.56 (± 0.12)	< LOQ	0.91
Boscalid	578.37	40.1 (± 0.3)	38.7 (± 1.8)	43.1	n.d.	n.d.	n.d.
Dimethenamid-P	632.10	43.8 (± 0.3)	42.3 (± 2.0)	47.1	n.d.	n.d.	n.d.
Dimoxystrobin	581.51	40.3 (± 0.3)	38.9 (± 1.8)	43.3	0.42 (± 0.07)	0.47 (± 0.19)	0.68
Methiocarb	34.73	2.4 (± 0.0)	2.3 (± 0.1)	2.6	n.d.	n.d.	n.d.
Prosulfocarb	634.40	44.0 (± 0.3)	42.4 (± 2.0)	47.3	0.55 (± 0.11)	0.52[c]	0.90
Prothioconazole-desthio[d]	43.69	3.0 (± 0.0)	2.9 (± 0.1)	3.3	n.d.	n.d.	n.d.
Pyraclostrobin	730.44	50.6 (± 0.4)	48.9 (± 2.3)	54.4	0.71 (± 0.09)	0.52 (± 0.21)	1.07
Tebuconazole	920.63	63.8 (± 0.5)	61.6 (± 2.9)	68.6	n.d.	n.d.	n.d.
Thiacloprid	480.38	33.3 (± 0.2)	32.1 (± 1.5)	35.8	0.99 (± 0.06)	0.93 (± 0.50)	2.16
Triadimenol	240.94	16.7 (± 0.1)	16.1 (± 0.8)	17.9	n.d.	n.d.	n.d.
Tau-Fluvalinate	721.02	50.0 (± 0.4)	48.2 (± 2.3)	53.7	n.d.	n.d.	1.56

n.d. not detectable

[a] Grafted cells of repetition 3 in the second colony were not nourished by the bees; hence, no RJ was harvested then

[b] For LOD and LOQ (μg/kg) see details of the RJ analysis in the Online Resource

[c] There was only one detection above LOQ in these samples

[d] Metabolite of prothioconazole

The percentage of pesticides potentially transferred from the offered food to RJ ranged between 0.001 (pyraclostrobin) and 0.016% (thiacloprid).

The uptake of substances into RJ does not correlate with the octanol-water partition coefficient ($p = 0.2064$, $R = -0.1063$).

4. DISCUSSION

Our study shows that uptake of multiple pesticides at once by nurse bees from a pollen source resulted solely in a minor contamination of RJ. Not more than 0.016% of the average amount of an active ingredient (a.i.) that has been consumed by adult bees was found in the RJ.

For our test, we used substances that are commonly used in agricultural practice and have been found in bee-collected pollen (Genersch et al. 2010, Böhme et al. 2017). The concentrations chosen are higher than average concentrations regularly found in pollen and bee bread collected from honeybees under field conditions, although there are sometimes reports on peak values from field studies that are in the range of our applied pesticide concentrations (Pettis et al. 2013, Rosenkranz et al. 2014, Traynor et al. 2016). Only traces of these considerable high amounts of pesticides that have been consumed by the bees could be retrieved in the RJ. At first, this low contamination of RJ indicates that the risk of damaging queen larvae and young worker larvae by toxic concentrations of pesticides is lower than expected. Secondly, our results raise the question, how the difference of pesticide concentrations between contaminated food and RJ can be explained.

A first possibility to reduce the pesticide concentration is the degradation of active ingredients in the contaminated pollen-honey diet. Due to the low pH of the pollen or bee bread, some pH-instable substances may be decomposed (Herbert and Shimanuki 1978, Johnson and Percel 2013). The uptake and digestion of pollen by the nurse bees offers further possibilities for the reduction of the pesticide concentration. In the alimentary canal, different osmotic pressures between honeysac, midgut lumen, and pollen let the pollen grains burst and release the nutrients and other components (Kroon et al. 1974, Peng et al. 1986). Whereas nutrients are subjected to enzymatic digestion, secondary plant compounds and other xenobiotics, including synthetic pesticides, are at least in part metabolized by detoxifying enzymes, such as multi-function oxidases, glutathione-S-transferases, esterases, or epoxide hydrolases (Gilbert and Wilkinson 1974, Yu et al. 1984, Moritz and Crailsheim 1987, Crailsheim et al. 1992, Wang et al. 2014). Metabolization might have been also the reason for the low radioactivity found in food glands of honeybees fed with radiolabelled carbaryl and diflubenzurone (Wittmann 1982) or carbofuran and dimethoate (Davis and Shuel 1988). The nutrients and proteins used for the production of RJ may therefore be widely free of contamination. This is probably also the explanation why in some other studies, no residues of pesticides have been found in RJ after both field-applications (Pilling et al. 2013) and feeding on artificially contaminated pollen (DeGrandi-Hoffman et al. 2013). Also, commercially available RJ seems to be generally free of contamination by agricultural pesticides (Martínez-Domínguez et al. 2014). As a conclusion, pesticide-contaminated pollen loads seem to be a negligible route of contaminating RJ.

Irrespective of the route of uptake, the physicochemical characteristics of the a.i. can influence the rate of incorporation of pesticides into RJ. Considering the acidic pH of the RJ, lipophilic and basic substances are more likely to permeate through biomembranes and accumulate due to ion-trapping mechanisms in the acidic milieu (Nakajima et al. 1997, 1998). However, taking into account the octanol-water partition coefficient (P_{ow}, Table I), even low lipophilic substances (like the highly water-soluble neonicotinoids), such as thiacloprid or acetamiprid, were found in similar concentrations as more lipophilic substances such as prosulfocarb or pyraclostrobin in our experiment. However, there is no statistical correlation between the substances and the P_{ow}, which may be explained, by the low amount of lipids in the RJ.

A further point of consideration is the jelly allocation inside the hive to the brood and also to other nest members such as the queen, workers, other nurse bees, or drones, which may dilute the amount of pesticides reaching the individual (Crailsheim 1991, 1992).

However, we still found traces of the applied pesticides in RJ. Under the natural conditions of free-flying colonies, there are additional routes that can contribute to a contamination of RJ. The collection of contaminated nectar has been suggested by several authors and seems to be the most important pathway to contaminate RJ with pesticides (Wittmann 1982, Davis and Shuel 1988, Matsuka and Nakamura 1990). Accordingly, a contamination of RJ is accompanied by contaminated honey-sac content. As a source for carbohydrates, nectar is mixed with the secretion of the hypopharyngeal glands (HPG) and fed to the larvae (Simpson 1955, Haydak 1970). Additionally, the crop of a honeybee not only contains nectar but also pollen grains at the same time (Soehngen and Jay 1973, 1974). If nectar is added to the HPG secretion, the pollen grains are added likewise (Haydak 1970). These pollen grains could substantially increase the level of pesticide residues in RJ, if the pesticide concentrations are high.

Our results indicate that only very low concentrations of pesticides are delivered to the queen larvae via RJ which may also be true for worker larvae during the first 2 days of development. This should be considered in experiments aiming towards calculation of LD_{50} of bee larvae and for risk assessments of plant protection products in in vitro experiments (Czoppelt and Rembold 1988, Aupinel et al. 2007, 2009, Hendriksma et al. 2011, Gregorc et al. 2012, Human et al. 2014).

Under field realistic conditions, queen larvae may be less exposed to pesticides that are introduced into the hive compared to older worker larvae which receive not only RJ but also pollen and nectar as substantial part of their diet (Haydak 1970). Unfortunately, the majority of the in vitro rearing studies on honeybee larvae are conducted with worker brood. Johnson and Percel (2013) observed no effects on queen development when low concentrations of a fungicide were measured in RJ. In contrast, DeGrandi-Hoffman et al. (2013)) found no residues in RJ after feeding pesticides, yet they observed a reduction in queen emergence. In agreement with assumptions of Milchreit et al. (2016), they suspected a suppressed immunity and/or vitality of nurse bees that might have led to an insufficient care for brood. However, a recent study in the stingless bee (*Plebeia droryana* (Friese)) confirmed a significant disturbance of the caste differentiation process in queen larvae that were reared on food contaminated with chlorpyrifos (dos Santos et al. 2016).

Considering the facts that (i) the concentrations of pesticides in pollen collected in agricultural areas are usually lower than in our experiment and that (ii) only traces of these residues reach the RJ, we do not expect direct negative effects onto queen development in the field. However, as queens receive RJ during their whole life, generalizations must be taken with care. Thus, more long-term experiments on sublethal side effects on caste differentiation and imaginal development of honeybee queens are necessary.

ACKNOWLEDGEMENTS

The authors are grateful to Kerstin Jänicke and Hartmut Nowak for their contribution to the residue analysis of the pollen-honey diet and royal jelly samples at the Julius Kühn-Institut, Berlin.

AUTHOR'S CONTRIBUTIONS

FB conceived research and writing. GB did the residue analysis. CPWZ and PR participated in writing. KW participated in the experimental design.

Du champ à la nourriture – un régime composé de pollen contaminé par un pesticide peut-il conduire à une contamination de la gelée royale ?

***Apis mellifera* / concentrations sublétales de pesticide/ flux de la contamination/ mélange de pesticides**

Vom Feld ins Futter - Führt eine Kontamination mit Pflanzenschutzmitteln im Pollen zu Rückständen im Gelée Royale?

***Apis mellifera* / subletale Pflanzenschutzmittel Konzentrationen / Wirkstoff-Fluss / Pestizid Mischungen**

REFERENCES

Al Naggar, Y., Vogt, A., Codling, G., Naiem, E., Mona, M., et al. (2015) Exposure of honeybees (*Apis mellifera*) in Saskatchewan, Canada to organophosphorus insecticides. Apidologie **46** , 667–678

Asencot, M., Lensky, Y. (1984) Juvenile hormone induction of "queenliness" on female honey bee (*Apis mellifera* L.) larvae reared on worker jelly and on stored royal jelly. Comp. Biochem. Physiol. B Biochem. **78B**, 109–117

Aupinel, P., Fortini, D., Michaud, B., Marolleau, F., Tasei, JN., Odoux, JF. (2007) Toxicity of dimethoate and fenoxycarb to honey bee brood (*Apis mellifera*), using a new *in vitro* standardized feeding method. Pest Manag. Sci. **63,** 1090–1094

Aupinel, P., Fortini, D., Michaud, B., Medrzycki, P., Padovani, E. et al. (2009) Honey bee brood ring-test: Method for testing pesticide toxicity on honeybee brood in laboratory conditions. Hazards of Pesticides to Bees – 10th International Symposium of the ICP-BR Bee Protection Group, Julius-Kühn-Archiv. **423**, 96–102

Böhme, F., Bischoff, G., Zebitz, CP., Rosenkranz, P., Wallner, K. (2017) Chronic exposure of honeybees, *Apis mellifera* (Hymenoptera: Apidae), to a pesticide mixture in realistic field exposure rates. Apidologie **48**, 353–363

Buttstedt, A., Ihling, CH., Pietzsch, M., Moritz, RFA. (2016) Royalactin is not a royal making of a queen. Nature **537** (7621), E10–E12

Calvarese, S., Forti, AF., Scortichini, G., Diletti, G. (2006) Chloramphenicol in royal jelly: Analytical aspects and occurrence in Italian imports. Apidologie **37**, 673–678

Crailsheim, K. (1991) Interadult feeding of jelly in honeybee (*Apis mellifera* L.) colonies. J. Comp. Physiol. B. **161**, 55–60

Crailsheim, K. (1992) The flow of jelly within a honeybee colony. J. Comp. Physiol. B **162**, 681–689

Crailsheim, K., Schneider, LHW., Hrassnigg, N., Bühlmann, G., Brosch, U. et al. (1992) Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): Dependence on individual age and function. J. Insect Physiol. **38**, 409–419

Czoppelt, C., Rembold, H. (1988) Effect of parathion on honey bee larvae reared *in vitro*. Anz. Schadlingskde. Pflanzenschutz Umweltschutz **61,** 95–100

Davis, AR., Shuel, RW. (1988) Distribution of ^{14}C-labelled carbofuran and dimethoate in royal jelly, queen larvae and nurse honeybees. Apidologie **19**, 37–50

DeGrandi-Hoffman, G., Chen, Y., Simonds, R. (2013) The effects of pesticides on queen rearing and virus titers in honey bees (*Apis mellifera* L.). Insects **4**, 71–89

Dietz, A., Lambremont, EN. (1970) Caste determination in honey bees. II. Food consumption of individual honey bee larvae, determined with P-labeled royal jelly. Ann. Entomol. Soc. Am. **63**, 1342–1345

Dos Santos, CF., Acosta, AL., Dorneles, AL., dos Santos, PDS., Blochtein, B. (2016) Queens become workers: Pesticides alter caste differentiation in bees. Sci. Rep. **6** (31605), 1–9

Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., et al. (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie **41**, 332–352

Gilbert, MD., Wilkinson, CF. (1974) Microsomal oxidases in the honeybee, *Apis mellifera* (L.). Pestic. Biochem. Physiol. **4**, 56–66

Gregorc, A., Evans, JD., Scharf, M., Ellis, JD. (2012) Gene expression in honey bee (*Apis mellifera*) larvae exposed to pesticides and Varroa mites (*Varroa destructor*). J. Insect Physiol. **58**, 1042–1049

Guo, X., Su, S., Geir, S., Li, W., Li, Z., et al. (2016) Differential expression of miRNAs related to caste differentiation in the honey bee, *Apis mellifera*. Apidologie **47**, 495–508

Haydak, MH. (1970) Honey bee nutrition. Annu. Rev. Entomol. **15**, 143–156

Hendriksma, HP., Härtel, S., Steffan-Dewenter, I. (2011) Honey bee risk assessment: new approaches for *in vitro* larvae rearing and data analyses. Methods Ecol. Evol. **2**, 509–517

Herbert, EW., Shimanuki, H. (1978) Chemical composition and nutritive value of bee-collected and bee-stored pollen. Apidologie **9**, 33–40

Human, H., Archer, CR., du Rand, EE., Pirk, CWW., Nicolson, SW. (2014) Resistance of developing honeybee larvae during chronic exposure to dietary nicotine. J. Insect Physiol. **69**, 74–79

Johnson, RM., Percel, EG. (2013) Effect of a fungicide and spray adjuvant on queen-rearing success in honey bees (Hymenoptera: Apidae). J. Econ. Entomol. **106**, 1952–1957

Johnson, RM., Ellis, MD., Mullin, CA., Frazier, M. (2010) Pesticides and honey bee toxicity – USA. Apidologie **41**, 312–331

Kamakura, M. (2011) Royalactin induces queen differentiation in honeybees. Nature **473**, 478–483

Kroon, GH., van Praagh, JP., Veltuis, HHW. (1974) Osmotic shock as a prerequisite to pollen digestion in the alimentary tract of the worker honeybee. J. Apic. Res. **13**, 177–181

Liu, H., Yao, G., Liu, X., Liu, C., Zhan, J. et al. (2017) Approach for pesticide residue analysis for metabolite prothioconazole-desthio in animal origin food. J. Agric. Food Chem. **65**, 2481–2487

Martínez-Domínguez, G., Romero-González, R., Garrido-Frenich, A. (2014) Multi-class pesticide determination in royal jelly by gas chromatography coupled to triple quadrupole tandem mass spectrometry. Anal. Methods **6**, 5376–5386

Matsuka, M., Nakamura, J. (1990) Oxytetracycline residues in honey and royal jelly. J. Apic. Res. **29**, 112–117

Milchreit, K., Ruhnke, H., Wegener, J., Bienefeld, K. (2016) Effects of an insect growth regulator and a solvent on honeybee (*Apis mellifera* L.) brood development and queen viability. Ecotoxicology **25**, 530–537

Moritz, B., Crailsheim, K. (1987) Physiology of protein digestion in the midgut of the honeybee (*Apis mellifera* L.). J. Insect Physiol. **33**, 923–931

Mullin, CA., Frazier, M., Frazier, JL., Ashcraft, S., Simonds, R., et al. (2010) High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. PLoS One **5**, e9754

Nakajima, C., Okayama, A., Sakogawa, T., Nakamura, A., Hayama, T. (1997) Disposition of ampicillin in honeybees and hives. J. Vet. Med. Sci. **59**, 765–767

Nakajima, C., Sakogawa, T., Okayama, A., Nakamura, A., Hayama, T. (1998) Disposition of mirosamicin in honeybee hives. J. Vet. Pharmacol. Ther. **21**, 269–273

Notardonato, I., Avino, P., Cinelli, G., Russo, MV. (2014) RSC Advances using XAD-2 adsorbent and gas chromatography coupled with an ion trap mass spectrometer detector. RSC Adv. **4**, 42424–42431

Peng, YS., Nasr, ME., Marston, JM. (1986) Release of alfalfa, *Medicago sativa*, pollen cytoplasm in the gut of the honey bee, *Apis mellifera* (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. **79**, 804–807

Pettis, JS., Lichtenberg, EM., Andree, M., Stitzinger, J., Rose, R., vanEngelsdorp, D. (2013) Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae.* PLoS One **8** (7)

Pilling, E., Campbell, P., Coulson, M., Ruddle, N., Tornier, I. (2013) A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS One **8** (10)

PPDB (2017) Pesticide Properties Data Base, University of Hertfordshire. [online] http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm, (assessed on 2017 February 20)

Ramadan, MF., Al-Ghamdi, A. (2012) Bioactive compounds and health-promoting properties of royal jelly: A review. J. Funct. Foods **4**, 39–52

Rembold, H., Lackner, B., Geistbeck, I. (1974) The chemical basis of honeybee, *Apis mellifera*, caste formation. Partial purification of queen bee determinator from royal jelly. J. Insect Physiol. **20**, 307–314

Rosenkranz, P., von der Ohe, W., Schäfer, M., Genersch, E., Büchler, R., Berg, S., et al. (2014) Zwischenbericht Deutsches Bienenmonitoring, Projektzeitraum 01/2015-12/2015, Landesanstalt für Bienenkunde, Stuttgart [online] https://bienenmonitoring.uni-hohenheim.de/fileadmin/einrichtungen/bienenmonitoring/Dokumente/Zwischenbericht_DeBiMo_2015.pdf (assessed on 2017 June 22)

Roszko, MŁ., Kamińska, M., Szymczyk, K., Jędrzejczak, R. (2016) Levels of selected persistent organic pollutants (PCB, PBDE) and pesticides in honey bee pollen sampled in poland. PLoS One **11**, 1–22

Shao, XL., He, SY., Zhuang, XY., Fan, Y., Li, YH., Yao, YG. (2014) mRNA expression and DNA methylation in three key genes involved in caste differentiation in female honeybees (*Apis mellifera*). Zool. Res. **35**, 92–98

Shen, C., Shen, D., Ding, T., Xu, J., Jiang, Y. et al. (2011) Simultaneous determination of 14 quinolones in royal jelly by liquid chromatography-tandem mass spectrometry using anion-exchange solid-phase extraction. J. Liq. Chromatogr. Relat. Technol. **34**, 1415–1430

Simpson, J. (1955) The significance of the presence of pollen in the food of worker larvae of the honey-bee. Q. J. Microsc. Sci. **96**, 117–120

Smodiš Škerl, MI., Kmecl, V., Gregorc, A. (2010) Exposure to pesticides at sublethal level and their distribution within a honey bee (*Apis mellifera*) colony. Bull. Environ. Contam. Toxicol. **85**, 125–128

Soehngen, U., Jay, SC. (1973) Studies on the honey-sac contents and pollen loads of honeybees 1. Honey-sac contents of bees in the hive. J. Apic. Res. **12**, 65–73

Soehngen, U., Jay, SC. (1974) Studies on the honey-sac contents and pollen loads of honeybees 2. Honey-sac contents of foraging bees. J. Apic. Res. **13**, 199–206

Stoner, KA., Eitzer, BD. (2013) Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. PLoS One **8**, 1–10

Tananaki, C., Goras, G., Thrasyvoulou, A., Zotou, A. (2009) Determination of 1.4-dichlorobenzene in royal jelly. J. Apic. Res. Bee World **48**, 79–80

Traynor, KS., Pettis, JS., Tarpy, DR., Mullin, CA., Frazier, JL. et al. (2016). In-hive pesticide exposome: Assessing risks to migratory honey bees from inhive pesticide contamination in the eastern United States. Nat. Sci. Rep. **6** (33207), 1–16

Wang, Y., Ma, LT., Hang, XB., Yang, WR., Liu, F., Xu, BH. (2014) Digestion of protein of two pollen types in China by the honeybee (*Apis mellifera* L). Apidologie **45**, 590–600

Weaver, N. (1957) Effects of larval age on dimorphic differentiation of female honey bee. Ann. Entomol. Soc. Am. **50,** 283–294

Wittmann, D. (1982) Tracer-Versuche zur Passage von Insektiziden durch Ammenbienen als Basis für die Abschätzung von Intoxikationswegen der Bienenbrut. Apidologie **13**, 328–330

Yu, SJ., Robinson, FA., Nation, JL. (1984) Detoxication capacity in the honey bee, *Apis mellifera* L. Pestic. Biochem. Phys. **22,** 360–368

Zhang, Y., Liu, X., Li, X., Zhang, J., Cao, Y. et al. (2016) Rapid screening and quantification of multi-class multi-residue veterinary drugs in royal jelly by ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Food Control **60**, 667–676