5.8 Results of a monitoring program of pesticide residues in Beebread in Spain. Using Toxic unit approach to identify scenarios of risk for management programs

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DOI 10.5073/jka.2018.462.061

Abstract

In this work we present the results of a monitoring program of apiaries conducted in spring 2014 in Spain The aim of the study was to identify the main pathogens and residues in beebread as chronic exposure source to managed honey bees.

Beebread and worker bee and samples from 71 and 51 apiaries, respectively were obtained. Beebread from the brood chamber combs were extracted aseptically from each honey bee colony as described previously1-3 Samples were stored at -80°C until further use. All honey bee worker samples were analyzed for the main pathogens related to the weakening and death of bee colonies in Spain. PCR was performed for *Nosema apis*, *Nosema ceranae* Trypanosomatids, Neogregarines, Lake Sinai Virus complex (LSV complex), and Acute Bee Paralysis Virus-Kashmir Bee Virus-Israeli Acute Paralisis Virus complex (AKI complex) Specific primers and probes for the amplification of Black Queen Cell Virus (BQCV) and Deformed Wing Virus (DWV) were used.

A Screening analysis of chemical residues was conducted with a modified QuEChERS protocol and under ISO 17025 standard and guidance document SANCO/12571/2013

The most prevalent pathogens were *Nosema ceranae* (69%), *Varroa destructor* mite (49%), with a mean percentage of parasitization around 1.7%, and Trypanosomatids (40.7%). Neogregarines (6%), *Acarapis woodi* (7%) and *Nosema apis* (7%) were detected a lower prevalence. Of the six screening viruses, the more prevalent were BQCV (57%) and DWV (54%). LSV complex was detected in the 14% of the samples.

The pesticides most commonly found in the samples were miticides typically used for Varroa mite control: coumaphos (98.6%), chlorfenvinphos (72.86%); *tau*-fluvalinate (70%) and secondly, carbendazim (40%) chlorpyriphos (45.71%), acrinathrin (24.9%) and imidacloprid (22.6%) were also detected.

Based on these results, we discuss the suitability of different methodologies proposed in the literature to assess the effect of honey bees chronically exposed to multiple residue and nosogenic agents found in hive.

Acknowledgement

This work has been funded by INIA Project "Holistic evaluation of risk factors in honey bees and wild pollinators. The situation in Spain" RTA2013-00042-C10. The authors gratefully acknowledge to subprojects RTA2013-00042-C10-2 and RTA2013-00042-C10-6 for their support.

5.9 Residues of plant protection products in honey – pilot study for a method to define maximum residue levels in honey (MRLs)

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DOI 10.5073/jka.2018.462.062

Abstract

Honey produced by honeybees exposed to plant protection products (PPPs) can contain residues of the applied active substances. A final decision of the residue definition (RD) in honey and on suitable test designs has not yet been made for MRL settings in honey according to Regulation (EC) No. 396/2005, and the discussion is still ongoing.

The concentration of residues in honey is influenced by many factors, such as the extent of filtration and metabolism by the honeybees, the characteristics of the PPP and its active substance(s) (a.s.), respectively, the use pattern of the PPP and, of course, by the amount of stored nectar containing residues of the active substance. Under realistic field conditions the amount of nectar containing residues depends on the

availability of treated and untreated crops, other plants in the surroundings of the respective colonies and also on the weather conditions after the application. Each of the addressed points will lead to a high variability in residue concentrations found in honey and potentially inhibit reproducible results.

To avoid these problems resulting from field conditions, the method of Oomen *et al.* 1992 was adapted and used as a worst case scenario to quantify the residues of active substances in freshly produced "artificial honey" under semi-field conditions. For this purpose, artificial swarms were placed in tunnels without any crop. To simulate an entry of an active substance into a hive via nectar after a PPP application in the field, bees were fed with a sugar solution (50% w/w) under tunnel conditions for 4 to 6 days. The sugar solution was spiked with realistic concentrations of active substances. The colonies were kept inside the tunnels and continuously fed with unspiked sugar solution until the cells with the "artificial honey" were capped. The sugar solution stored in the colonies, the "artificial honey" and wax were sampled and analysed for residues using solid phase extraction and GC-ECD or QuEChERs-extraction and LC-MS/MS, depending on the active substance.

The same approach was tested under lab conditions. Caged forager bees were fed with sugar solutions (50% w/w) mixed with PPP/active substances via plastic syringes. The bees were kept in groups under climatically controlled conditions for 0, 1, 3 and 5 hours and subsequently frozen. Pooled contents of honey sacs were analysed for residues (see above).

For both purposes two lipophilic (log Pow > 3) and one hydrophilic (log Pow < -3) substance were tested to investigate their behaviour in the stored sugar solution and freshly produced "artificial honey". Hydrophilic substances are soluble in aqueous solutions such as nectar and honey. Conversely, lipophilic substances are readily adsorbed by wax.

In the tunnel trial, during the feeding period with spiked sugar solution, an increase of the active substance concentration was observed in the stored sugar solution samples for both the hydrophilic and the lipophilic substances. However, the lipophilic substances were on a much lower level compared to the hydrophilic substance. As soon as feeding started with the pure sugar solution, the active substance concentrations decreased. In "artificial honey" lower concentrations than in the spiked sugar solution were found for all three active substances, especially for the lipophilic substances. When compared to the hydrophilic substance, the lipophilic substances were transferred from the spiked sugar solution to the honey sac content, stored sugar solution and "artificial honey" to a lesser extent. This was found to be the case in both the lab and the tunnel trial.

The lab trial showed that the residue concentrations of both lipophilic substances decreased markedly in the honey sacs over time. Only very low residue levels of the lipophilic substances were found in wax indicating that the reduction of the active substance was not based on sorption processes.

The current pilot study shows that the combination of lab and tunnel trials could provide a low cost first step during the ongoing discussions to set MRLs in honey. However, further investigations are needed, such as how the feed consumption can be improved.

References

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