Agricultural pesticides and veterinary substances in Uruguayan beeswax

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HIGHLIGHTS

- Lipophilic xenobiotics was accumulated in recycled beeswax (RB).
- Xenobiotic pollution presented a higher frequency and concentration in RB.
- Some fungicides and neonicotinoids could have synergistic effects.
- The use of honey cappings to make beeswax foundation will reduce the hive health risk.

GRAPHICAL ABSTRACT
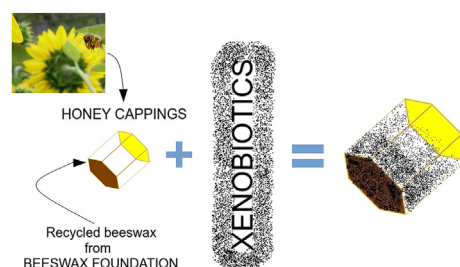
ABSTRACT

Over the last decade, Uruguay has expanded and intensified its rainfed crop production. This process has affected beekeeping in several ways: for example, by reducing the space available. This has increased the density of apiaries, the risk of varroosis and acaricide use. Additionally, the dominance of no-tillage crops has increased the frequencies of application and of loads of pesticides in regions where such crops share the land with beekeeping and honey production. Therefore, the exposure of bees to xenobiotics (agricultural pesticides and veterinary products) has increased in line with pollution of hives and their products. To document pollution from hive exposure to pesticides, we surveyed the presence of 30 xenobiotics normally used in Uruguay, in recycled beeswax (RB) and in honey cappings (HC) from the main Uruguayan beekeeping regions. There was contamination of all the analyzed samples (RB and HC) with the herbicide atrazine at a range of $1-2$ ng g^{-1}. At least three or four additional xenobiotics were detected: insecticides (chlorpyrifos-ethyl and thiacloprid); fungicides (azoxystrobin and tebuconazole); and veterinary products (coumaphos, ethion, and tau-fluvalinate). The frequency of detection of chlorpyrifos-ethyl and coumaphos in RB samples was higher than in those of HC. Moreover, the concentrations of azoxystrobin, coumaphos, and tebuconazole in RB samples were higher than in HC

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samples. Therefore, we suggest the use of HC to produce recycled printed beeswax films for use in hives to minimize pollution transfer.

1. Introduction

Agriculture on the Atlantic side of South America is tied to global commodity prices (Graesser et al., 2015). This form of agriculture is based on achieving maximal biomass production through reducing or eliminating natural constraints, with the use of pesticides as one of the main tools. In this regional framework, Uruguay has undergone recent intensification and expansion of its rainfed areas, with a land-use change by the introduction of glyphosate-resistant soybeans as the foremost crop grown in rotation systems (Céspedes-Payret et al., 2009). Thus, the Uruguayan rainfed crops increased from 278,000 ha (ha) in 2004 to 1.334 million ha in 2015 (DIEA, 2016). This land-use change led to the growth of annual imports of formulated pesticides from 14,326 Mg in 2004 to 24,160 Mg in 2015 (Servicios agrícolas, 2016) and increased the load and frequency of pesticide use. This has increased pesticide exposure and the risk of pollution and toxic effects on pollinators and other nontargeted organisms (Cárcamo, 2010; Carrasco-Letelier et al., 2006; Pareja et al., 2011). Additionally, zones free of pesticides were reduced in agricultural regions and thus restricted the pollen diversity available for beekeeping. These factors promoted increases in apiary density (Harriet and Campá, 2014), in the hive health risk of varroosis contagion (Anido et al., 2015) and in the frequency of acaricide application for controlling varroosis. Moreover, such xenobiotic exposure can be propagated and increased through the use of beeswax foundations. Xenobiotics can be transferred directly via beeswax by its application to new combs (e.g., coumaphos) (Van Buren et al., 1992) and residues can be retained by beeswax (Tremolada et al., 2004). Van Buren et al. (1992) described this pollution in beeswax obtained commercially in Europe. Since that report, such pollution has continued in different European countries (Bogdanov et al., 2003; Persano Oddo et al., 2003; Ravoet et al., 2015), USA (van Engelsdorp et al., 2009) and, recently, in Chile (Neira et al., 2011). Such beeswax pollution could cause problems for the immune system of bees (Prisco et al., 2013), reduce the life expectancy of newborn honey bees (Orantes-Bermejo et al., 2010), affect honey bee product quality (Bogdanov, 2006) and, perhaps, lead to colony collapse disorder (CCD) (van Engelsdorp et al., 2009).

In this way, both the increased exposure to xenobiotics in Uruguay, and beeswax pollution in similar agricultural conditions allow us to propose as a first hypothesis that Uruguayan hives might be contaminated along with their beeswax. As a second hypothesis, it could be argued that pollution in beeswax will increase in frequency and magnitude by the production of beeswax foundation. This could lead to higher levels of xenobiotics in recycled beeswax (RB) than in the non-recycled waxes such as honey cappings (HC). To test these hypotheses, we assessed the presence of 30 different xenobiotics (pesticides and veterinary products) in HC and RB samples from the main Uruguayan regions of honey bee production. Based on these results, we compared the frequencies and levels of pollutants in each kind of wax to assess the consequences of each hypothesis. We also estimated whether the concentrations might become a risk for bees.

2. Materials and methods

2.1. Study zone

The expansion and intensification of Uruguayan crop cultivation has mainly been in the Western Departments (a Department is an administrative division of our national territory). Historically, the same regions have been used for beekeeping and honey production; thus, in some areas, there are more than five hives per km^2 (Harriet and Campá, 2014). For these reasons, the beeswax used in this study was collected from Departments with prominent agricultural and honey production (Fig. 1).

The beeswax samples were obtained in 2014 from eight beekeepers and two Uruguayan beeswax companies (Apícola Integral Las Piedras and TELGAR). Each beekeeper supplied one sample of HC and another of RB. The beeswax recycling companies provided four samples of HC and eight samples of RB. In this last case, the RB was made with a mixture of beeswax from different beekeepers.

2.2. Determination of pesticides and veterinary products

The xenobiotics (pesticides and veterinary products) were extracted using published protocols (Niell et al., 2014). Briefly, this procedure consists of liquid−liquid partitioning (acetonitrile:melted wax) followed by freeze-out and primary−secondary amine with C18 dispersive solid phase extraction cleanup. The extracted xenobiotics were determined using liquid chromatography−tandem mass spectrometry (LC−MS/MS) and gas chromatography−mass spectrometry (GC−MS).

LC−MS/MS was performed with an Agilent 1200 LC system (Agilent, Quantum Analytics Inc., Foster City, CA, USA) and insert coupled to a 4000 105 QTRAP® LC/MS/MS System from AB SCIEX (Foster City, CA, USA) run in the scheduled MS/MS-mode. LC separation was performed as described by Niell et al. (2015) on a ZORBAX Eclipse XDB-C18 (150 mm × 4.6 mm, 5 m) column (Agilent Technologies). The operation of the LC gradient involved the following elution program: A, water/HCOOH 0.1% (v/v); and B, MeCN. This was run at 600 μL min^{-1} starting with 10% component B at injection time for 1 min and gradually changing to 100% B over 15 min. This mobile phase was held for 10 min and then shifted back to the starting conditions (10% component B) and kept there until 35 min after the initial injection with a volume of 5 μL. 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 and 50 psi). The optimal MRM transitions, collision energies, and declustering potentials for each investigated compound were determined by infusing standard solutions with a syringe directly the to the instrument at a constant flow rate.

GC−MS analyses were performed using an HP 6890 GC system coupled with a HP 5973 MS supported by reference libraries, equipped with an HP-5 (5% diphenyl 95% dimethylsiloxane) bonded fused-silica capillary column (30 × 0.25 mm i.d. × 0.25 m film thickness; Hewlett Packard, Wilmington, DE, USA). Electron impact