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Detection and quantification of boscalid and its metabolites in honeybees

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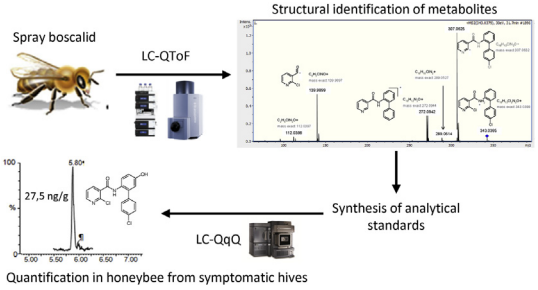
HIGHLIGHTS

- Methodology based on the use of both high- and low-resolution mass spectrometry.
- Identification for the first time of boscalid metabolites in honeybees.
- Quantification of boscalid and three metabolites in honeybees collected in hives.

GRAPHICAL ABSTRACT



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ABSTRACT

Boscalid is a new-generation fungicide that has been detected in several bee matrices. The objective of this work was to characterize boscalid metabolites in honeybees based on *in vivo* experimentation, and next to verify the presence of theses metabolites into honeybees from colonies presenting troubles. A methodology based on complementary mass spectrometric tools, namely ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-QToF) or triple quadrupole mass spectrometry (UHPLC-QqQ) was implemented. Honeybees were sprayed with boscalid, at field rate (to induce the metabolization process) and the parent compound with its generated metabolites were then extracted using modified EU-QuEChERS method. The mass characteristics including exact mass, isotopic profile and mass fragments allowed assuming the structure of several metabolites. Some of them were unambiguously identified by comparison with synthesized analytical standards. The metabolites were resulted from hydroxylation and dechlorination of the parent compound as well as the substitution of a chlorine atom with an hydroxyl group. The metabolites were then quantified in bee samples collected from various beehives located in France. Boscalid and three of its metabolites were present in some samples at a level ranged between 0.2 and 36.3 ng/g.

1. Introduction

Bees have been affected worldwide for twenty years by excess mortality. A combination of factors may explain this phenomenon as the decline in floral diversity, chronic exposures to pesticides or

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stress induced in particular by viruses or parasites (Stokstad, 2007; Ratnieks and Carreck, 2010; Goulson et al., 2015). The role of plant protection products in the weakening of pollinating insects is a proven fact. However it is difficult to demonstrate a relationship between the deleterious effects induced by pesticides and exposure to them.

One difficulty lies in the fact that the search for pesticides in bees involves parent target substances. However these may have been degraded by abiotic processes or metabolized by bees especially in case of delayed mortality findings; thus, the parent compound may no longer be detectable in the bee or at very low levels. The very few toxicokinetic available data make difficult the interpretation of results. However, these data are necessary to conclude both on the presence of the suspected parent molecule and on the initial contents that have induced detrimental effects. Evidence of contact between bee and pesticide may, nevertheless, be obtained by detecting metabolites or markers of exposure in bees. On the other hand, the study of metabolites and their toxicity to the honeybee, which may be greater than that of the parent molecule, is still undeveloped.

To date, there is a limited number of studies on the metabolism of pesticides in bees (Suchail et al., 2004; Brunet et al., 2005), although several types of enzymes have been identified as capable of metabolizing xenobiotics in the bee. Indeed phase I enzymes catalyze functionalization reactions whereas phase II enzymes catalyze conjugation reactions (Suchail et al., 2004; Xu et al., 2013). However, given the levels of exposure to which bees are subject, methods of analysis are neither, in many cases, adapted nor sensitive enough to detect and quantify the metabolites. Other challenges are related to the lack of information on the nature of the metabolites and moreover to the lack of analytical standards, which are essential for conducting the relevant pesticide analysis. It is, therefore, necessary to undertake studies on the degradation of pesticides in bees to enable diagnoses of bee poisoning consecutive to plant protection treatments.

The pesticide selected for this study is the broad spectrum fungicide boscalid (Fig. 1), a new generation compound of the carboxamide family that acts by inhibiting fungal respiration by blocking the ubiquinone-binding sites in the mitochondrial complex II involved in the Krebs cycle (Aveno and Michailides, 2007). Since its placing on the market in 2002, boscalid has been intensively applied, mainly during the flowering period, for crop protection throughout the plant growth, especially for the control of white mold and foliar diseases in vineyards or in fruits and vegetables such as corn, carrots, cabbage, beans or peas. Therefore honeybee is likely to be in contact with this fungicide. Some recent works of David et al. (2015, 2016) have revealed the presence of boscalid in bumblebees (up to 9.8 ng/g) and pollens (up to 38 ng/g). Near μg/g levels of the fungicide were reported in wax samples or pollen (Mullin et al., 2010; Jabot et al., 2015). Ostiguy and Eitzer (2014) quantified the fungicide in an overwintered honey sample (2.3 ng/g).

The main objectives of our study were (1) the identification of the metabolites of boscalid in honeybees, based on an *in vivo* biotransformation following an experimental spraying of the fungicide and (2) the quantification of these metabolites in bees from various hives in France.

To achieve these objectives, we have implemented analytical strategies based on the use of the coupling of ultra-high performance liquid chromatography (UHPLC) with two different mass spectrometers: a time of flight (QqToF) and triple quadrupole (QqQ) analyzers. The QqToF technology was used for its ability to determine the accurate mass and the formulas of by-products. Moreover, the use of MS/MS fragmentation has helped in identifying new or hypothesized metabolites. The QqQ was used for its selectivity and sensitivity to quantify the identified metabolites, in a complex matrix such as bees.

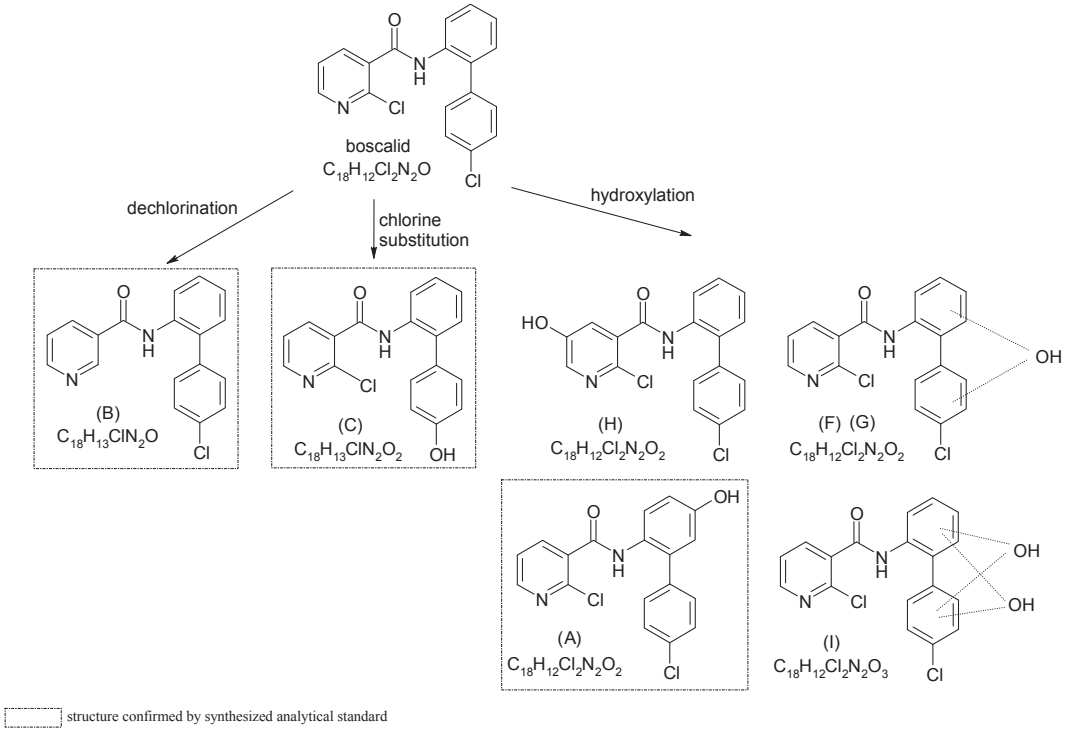

Fig. 1. Structure of boscalid and the identified or predicted metabolites.