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A Locomotor Deficit Induced by Sublethal Doses of Pyrethroid and Neonicotinoid Insecticides in the Honeybee *Apis mellifera*

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Abstract

The toxicity of pesticides used in agriculture towards non-targeted organisms and especially pollinators has recently drawn the attention from a broad scientific community. Increased honeybee mortality observed worldwide certainly contributes to this interest. The potential role of several neurotoxic insecticides in triggering or potentiating honeybee mortality was considered, in particular phenylpyrazoles and neonicotinoids, given that they are widely used and highly toxic for insects. Along with their ability to kill insects at lethal doses, they can compromise survival at sublethal doses by producing subtle deleterious effects. In this study, we compared the bee's locomotor ability, which is crucial for many tasks within the hive (e.g. cleaning brood cells, feeding larvae. . .), before and after an acute sublethal exposure to one insecticide belonging to the two insecticide classes, fipronil and thiamethoxam. Additionally, we examined the locomotor ability after exposure to pyrethroids, an older chemical insecticide class still widely used and known to be highly toxic to bees as well. Our study focused on young bees (day 1 after emergence) since (i) few studies are available on locomotion at this stage and (ii) in recent years, pesticides have been reported to accumulate in different hive matrices, where young bees undergo their early development. At sublethal doses (SLD_{48h}, i.e. causing no mortality at 48h), three pyrethroids, namely cypermethrin (2.5 ng/bee), tetramethrin (70 ng/bee), tau-fluvalinate (33 ng/bee) and the neonicotinoid thiamethoxam (3.8 ng/bee) caused a locomotor deficit in honeybees. While the SLD_{48h} of fipronil (a phenylpyrazole, 0.5 ng/bee) had no measurable effect on locomotion, we observed high mortality several days after exposure, an effect that was not observed with the other insecticides. Although locomotor deficits observed in the sublethal range of pyrethroids and thiamethoxam would suggest deleterious effects in the field, the case of fipronil demonstrates that toxicity evaluation requires information on multiple endpoints (e.g. long term survival) to fully address pesticides risks for honeybees. Pyrethroid-induced locomotor deficits are discussed in light of recent advances regarding their mode of action on honeybee ion channels and current structure-function studies.

Introduction

Pollinators play a crucial role in maintaining vegetal biodiversity but also participate in improving agricultural production. Therefore, a number of managed honeybee colonies are periodically moved in the vicinity of agricultural fields, not only to increase honey production but to improve crop pollination as well. As a consequence, insecticide exposure of honeybee colonies does not only occur when individuals are foraging, but also happens directly in the hive, as demonstrated by measurements of in-hive pesticide residues [1, 2]. In the last few decades, an increase in honeybee colony mortalities has been reported around the world and focused the attention of a broad scientific community on the potential consequences of pesticide misuse on pollinator survival [3–5]. These studies have been especially focused on two families of insecticides, neonicotinoids and phenylpyrazoles, owing to their use as systemic insecticides in seed treatment [6]. Recently, the sublethal toxicity of neonicotinoids towards honeybees has been demonstrated in real-world environments and led the European Union to restrict the use of three members of this class for two years [7–9]. Similarly, fipronil, a phenylpyrazole highly toxic to bees even at sublethal levels (by impairing memory and synergistically enhancing sensitivity to the pathogen Nosema [10, 11]) has also been banned as an agrochemical product in France and more recently in other countries of the European Union, although it is still widely used elsewhere, like the neonicotinoids [12]. It is worth mentioning that these temporary restrictions apply for seed coating only, whereas other agrochemical formulations are still authorized (Official Journal of the European Union OJ L219/22–15.8.2013 and OJ L139/12–25.5.2013). Besides neonicotinoids and phenylpyrazoles, pyrethroid insecticides constitute a large insecticide family produced through chemical synthesis, with a limited number of compounds (e.g. deltamethrin, cypermethrin, λ-cyalothrin, permethrin) accounting for the majority of sales [13]. The restrictions imposed on neonicotinoids and phenylpyrazoles may lead to an increase in pyrethroid use. Many pyrethroids are also highly toxic towards honeybees [14], and very few studies have compared the sublethal toxicities of pyrethroids, neonicotinoids and phenylpyrazoles in honeybees [15, 16]. These insecticides all target ion channels involved in the function of a variety of tissues (including the nervous and the muscular systems), and it is known that their primary mode of action is to interfere with the normal function of voltage-gated sodium channels (for pyrethroids), nicotinic acetylcholine receptors (for neonicotinoids) and glutamate and GABA receptors (for phenylpyrazoles).

The effects of sublethal doses of insecticides on the neuromuscular system of honeybees are not easy to analyze. Methods for evaluating the ability of bees to fly back to the hive after exposure to a sublethal dose of insecticide (the 'homing flight assay') have been recently developed [7, 17, 18]. Besides the importance of flight for bees, efficient ambulation (walking) inside the hive is required for many tasks, including cell construction and cleaning, larval feeding and social interactions in general [19]. Muscle contraction, allowing physical movements, also produces heat [20] and thus participates in maintaining proper temperature levels around the brood. In feral colonies and in managed hives, the combs, built vertically, add an additional physical challenge by requiring vertical displacements. Experimentally, evaluation of locomotor abilities inside the hive is challenging and requires special observation hives with glass sides. Locomotion assays in laboratory conditions (open-field arena) are easier to set-up and produce a simple standardized and reproducible test to evaluate the effect of sublethal doses of insecticides [21]. Sublethal doses of neonicotinoids can (acetamiprid 0.1 μg/bee) or cannot (thiamethoxam 1 ng/bee) modify walking locomotion [22]. Whereas chronic exposure to thiamethoxam or imidacloprid sublethal concentrations (24h, 10 nM) did not modify the walking behavior, the righting reflex was affected [23]. Imidacloprid sublethal doses reduce waggle dancing 24 h after ingestion [24]. Low doses of phenylpyrazole (fipronil 1 ng/bee) modify