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Review article

Modulation of pesticide response in honeybees

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Abstract – Honeybee exposure to pesticides is widely accepted, but the role they play in impacting bee health remains controversial. The development of risk assessment procedures is notably a difficult task due to the variability of responses observed for a single pesticide at a specific dose. Indeed, honeybees, during most of their lifetime, are exposed to fluctuating environmental conditions (e.g., pathogen pressure, resource availability, climatic conditions) and can go through important physiological changes within a few days (e.g., behavioral maturation) or even a day (e.g., circadian clock), which are all factors that can affect the bee response to pesticides. Integrating the range of variability in conditions experienced by bees is relevant to honeybee toxicology and will contribute to a better assessment of their susceptibility to pesticides. The aim of this review is therefore to provide empirical evidence of how co-exposure to stressors, and environmental and endogenous factors modulate the honeybee response to pesticide.

Apis mellifera **/ pesticide toxicity / ecotoxicology / toxicodynamics / toxicokinetics / co-exposure / risk assessment**

1. INTRODUCTION

Due to their great efficacy, pesticides are used worldwide for plant protection against pests and thus for minimizing the loss of crop yield. However, the production of approximately 75 % of all crop species depends on insect pollination, including by honeybees (Klein et al. 2007). Honeybee colonies, whose development and maintenance are intimately associated with environmental floral resources, can therefore be frequently exposed to multiple pesticide compounds that are recovered in pollen and nectar (Charvet et al. 2004; Mullin et al. 2010; Johnson 2015). Contaminated dust and water can also contribute to increase the risk of exposure (Girolami et al. 2012; Samson-Robert et al. 2014). While foraging, bees can be exposed to some pesticides, but acute exposure can become chronic after frequent foraging trips. In addition, pesticides and corresponding metabolites are brought back to the hive and stored in several matrices, like honey, wax, and bee bread, thereby exposing all members of the colony to these compounds. The large number of bees in a colony combined with their wide-ranging foraging (generally up to 10 km (Beekman and Ratnieks 2000; Steffan-Dewenter and Kuhn 2003)) together increase the potential for centralizing environmental toxicants in a hive. Miticides originating from beekeeping practices, as well as veterinary drugs (e.g., farm uses), can also contribute to the composition of the xenobiotic complex. Such level of exposure has been confirmed by numerous studies using residue analysis of bees and hive matrices (for examples, see Bogdanov 2006; Chauzat et al. 2009; Mullin et al. 2010; Lambert et al. 2013; Ravoet et al. 2015).

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Reflecting this frequent exposure to pesticides, bee toxicology has become a fast-growing field of research for characterizing the detrimental effects of pesticides on bee health, as well as the underlying mechanisms (Desneux et al. 2007; van der Sluijs et al. 2013; Godfray et al. 2014; Johnson 2015). The large body of literature that has accumulated shows that the toxicant effects depend on the type, dose, mode, and frequency of exposure (Belzunces et al. 2012; Godfray et al. 2014; Johnson 2015) but, most importantly, that the effect of a given pesticide can be modulated by several endogenous and/or exogenous factors (Le Conte et al. 2011). Indeed, owing to the diversity of methods used by researchers, the complex nature of pesticide toxicity is being revealed. At a given dose, pesticides do not induce one rigid effect but a range of responses that can vary in intensity depending on others factors. Such a modulation of response might not be surprising given that phenotypic responses (e.g., morphology, development, and behavior) are commonly regulated by endogenous (genotype, physiology) and exogenous factors (environment). Ultimately, a comprehensive view of this response variability will be required for better understanding the phenomenon of colony decline and to develop a more detailed pesticide risk assessment in bees.

In this review, we describe two relevant scenarios affecting the modulation of pesticide response in honey bees: first, the co-occurrence of pesticides with other stressors, and second, the role of environmental and physiological variability (Figure 1). We then review the experimental evidence for such modulation and, finally, propose directions for future research in the field of bee toxicology.

2. SCENARIOS OF BEE RESPONSE TO PESTICIDE

2.1. Pesticide effect

The effects of xenobiotics (e.g., pesticides) on organisms classically depend on two main factors: toxicokinetics and toxicodynamics. Toxicokinetics refers to the fate of the molecule in the body of the organism and involves different mechanisms: its uptake, distribution, biotransformation, and elimination. Toxicodynamics correspond to the interaction between the toxic compound, and the target, and its action and effect on the organism (e.g., physiological impairment, mortality). How an organism will deal with the toxicant (toxicokinetics) and how the toxicant will affect the organism (toxicodynamics) will depend on the physiological background of the organism.

There is little information on the toxicokinetics of pesticides in honeybees. However, the detoxification system involved in toxicokinetics is gaining greater attention in recent toxicological studies (Berenbaum and Johnson 2015). This physiological system reduces the number of toxic molecules before they reach the target and/or after being removed from the target. Detoxification generally reduces the toxicity of xenobiotics if all metabolites are less active than the original molecule, but that is not always the case (e.g., conversion of imidacloprid to the more toxic metabolite olefin (Suchail et al. 2001)). This process involves enzymes that degrade xenobiotics and membrane transporters that facilitate their elimination. Generally, there are three types of enzymes: enzymes located on the membranes of the endoplasmic reticulum that catalyze oxidation, reduction, and hydrolysis reactions (e.g., carboxyl/cholinesterase (CE) and cytochrome P450 monooxygenases (CYP450)), transfer enzymes localized in the cytosol that catalyze conjugation reactions (e.g., glutathione S-transferases (GST)), and transmembrane enzymes that use adenosine triphosphate (ATP) to transport diverse substrates (peptides, toxins, lipids, and hydrophobic molecules) across the cell membrane (e.g., ATP-binding cassette transporters) (Claudianos et al. 2006; Berenbaum and Johnson 2015).

Enzymes from the detoxification system are known to have other functions related to cell signaling, dietary detoxification, hormone and pheromone metabolism, and neurodevelopment (Oakeshott et al. 2005; Claudianos et al. 2006; Laborde 2010). Changes in the detoxification system (toxicokinetics) are therefore expected throughout bee development or as the adult bee ages, which would suggest that pesticide response can be modulated depending on bee physiology. However, we cannot exclude an interplay with other physiological mechanisms: For instance, a

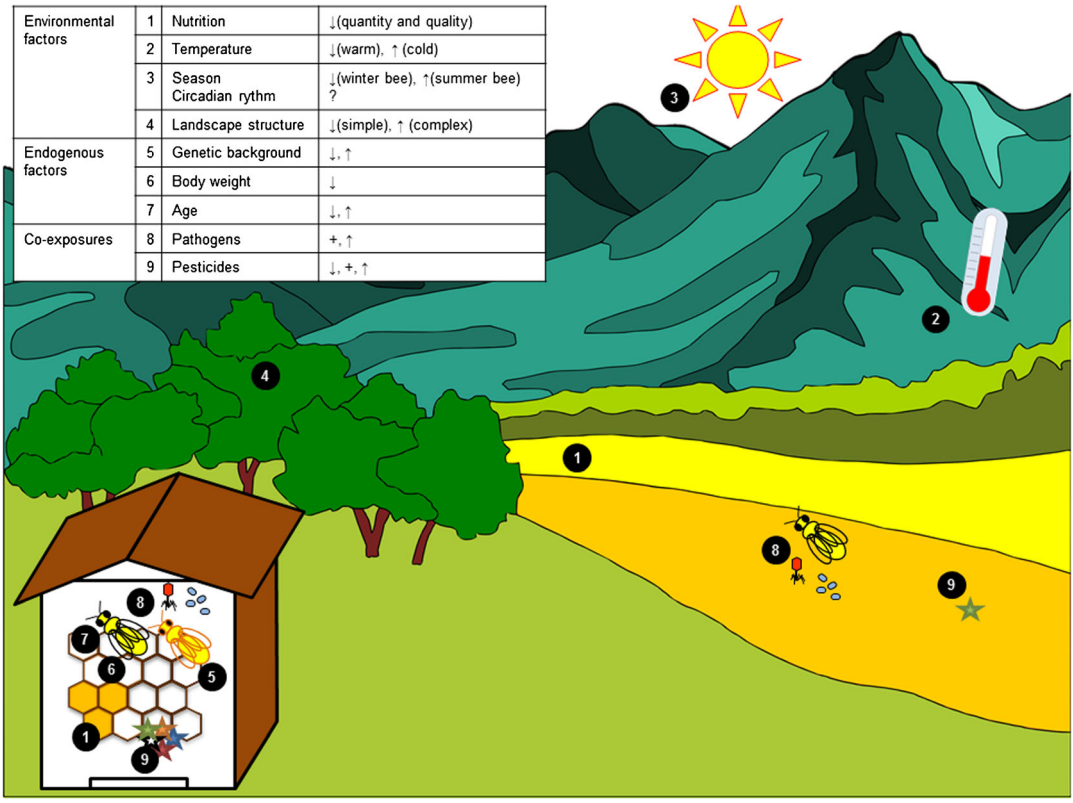

Figure 1. Factors involved in the modulation of pesticide response. *Downwards arrow* and *upwards arrow* indicate a decrease and increase in pesticide toxicity, respectively, and *plus sign* denotes an additive effect. *Question mark* means that the effect on pesticide toxicity is unknown.

toxicant might interfere with a specific physiological function at the time of exposure (e.g., energy storage, metabolism, thermoregulation) (Belzunces et al. 2012; Derecka et al. 2013) rendering the individual more or less responsive to the toxicant (toxicodynamics).

2.2. Co-exposure to stressors

As described previously, honeybee colonies can be co-exposed to a complex web of pesticides and corresponding metabolites. In addition, honeybees represent a very attractive and valuable resource for pathogens due to the high concentration of individuals and stored food in the colony (Schmid-Hempel 1998). As a result, colonies are often infested by numerous pathogens covering several kingdoms, the most common being viruses, bacteria, fungi, and mites (Evans and Schwarz 2011; Nazzi and Pennacchio 2014; Runckel et al. 2011). Therefore, due to their social lifestyle and biology, honeybees can concentrate pesticides and pathogens in a single spot (hive) and thus are forced to cope with frequent co-exposure to stressors (pesticide/pesticide or pesticide/pathogen) (Chauzat et al. 2009; van Engelsdorp et al. 2009; Mullin et al. 2010; Cornman et al. 2012; Ravoet et al. 2013; Simon-Delso et al. 2014).

The pesticide response can be modified in several ways depending on the type of co-exposure: The response corresponds to the sum of expected individual responses (additive effects) or is different from the expected response (interactive effects). Two types of interactive effects can occur: synergism and antagonism, referring to greater or smaller effects than expected from the single exposure, respectively (Holmstrup et al. 2010). Classically, synergism is observed (i) when two

stressors interact in a way that generally enhance the effect of each other or (ii) when a nonharmful stressor enhances the harmfulness of another one, or when two nonharmful stressors induce harmful effects (potentiation). Antagonism occurs when the combination of two stressors decreases the response induced by a single one. However, while those interactive effects can be easily tested and classified, interpreting the underlying mechanisms requires knowing the mode of action of each stressor. Interaction between pesticides can occur at the pharmacological target sites and/or at the detoxification level, notably via competition. For example, detoxification of each toxicant can be undermined because certain mechanisms are required at the same time and toward several molecules (competitive inhibition). Pathogens can alter the physiology and metabolism of the host, impacting several important life-history traits (e.g., longevity and development) and modifying functions involved in pesticide toxicokinetics and toxicodynamics such as the detoxification system, as shown with *Varroa* mite and *Nosema* Microsporidia parasitism (Vidau et al. 2011; Dussaubat et al. 2012; Gregorc et al. 2012; Di Pasquale et al. 2013; Aufauvre et al. 2014). As for pesticides, there is evidence that toxicants can reduce immunocompetence of bees (Boncristiani et al. 2012; Di Prisco et al. 2013; Aufauvre et al. 2014) and energetic metabolism (Derecka et al. 2013), functions that are required to fight pathogens. Such data clearly show a great potential for interactive effects between pesticide and pathogens.

2.3. Variability of environmental and endogenous factors

Numerous studies have shown the incredible plasticity of endocrine and neurochemical functions of bees. For example, workers exhibit many changes in endocrine activity, metabolism, neural functioning, and circadian clock activity when shifting from nursing to foraging activity (Robinson 2002). This includes changes in body weight with foragers who can weigh half as much as nurses (Vance et al. 2009). Physiological changes can also be attributed to genotypic (Robinson 2002), seasonal (Castillo et al. 2012), and resource variation (Brodschneider and Crailsheim 2010). Whether the functions involved in pesticide toxicokinetics and toxicodynamics respond to this variability or can be altered by social, environmental, or genotypic conditions has not received much consideration. However, recent results indicate that pollen intake affects the level of CYP450 transcripts (Alaux et al. 2011; Corby-Harris et al. 2014) and that the activity of GST varies according to the quality of the ingested pollen (Di Pasquale et al. 2013). The expression level of genes coding for CYP450 enzymes can also oscillate with circadian rhythms but with a different phase between nurses and foragers (Rodriguez-Zas et al. 2012). In addition, foragers have been shown to exhibit a higher GST activity than nurses (Smirle and Robinson 1989). Differential expression in the type and level of target receptors also occurs (Jones et al. 2006), which potentially impacts pesticide toxicodynamics. Finally, a proteomic study revealed that the majority of detoxification enzymes are expressed at about the same level in the three castes (queen, drone, and worker) (Chan et al. 2013). However, queens exhibit a higher expression of the multidrug-resistant proteins as compared to drones and workers, and the enzymes belonging to the CYP450 family are significantly more highly expressed in workers than in queens and drones. Altogether, these results point to the fact that the bee response to a given pesticide will change as a function of its environment and proper physiological state.

3. MODULATION OF PESTICIDE RESPONSE BY CO-EXPOSURE TO OTHER STRESS FACTORS

3.1. Interaction between pathogens and pesticides

Epidemiologic surveys conducted around the world have shown that honeybee colonies may host numerous pathogens. Their presence could be pathogenic, but most of the colonies overcome infestation and remain asymptomatic. Similarly, low doses of pesticides generally do not affect the survival of bees, but the same exposure can become lethal if bees are also exposed to

pathogens. For example, Vidau et al. (2011) tested the effects of sublethal exposure of the systemic insecticides thiacloprid and fipronil on bees experimentally infected by *Nosema ceranae*. The authors found that thiacloprid and fipronil exposures decrease the survival of *Nosema*-infected bees (synergistic interaction), whereas pesticide exposure alone had no effect. Comparable experimental settings using combinations of *Nosema* and pesticide exposure have reproduced this phenomenon (Aufauvre et al. 2012; Doublet et al. 2015). More importantly, it appears that this synergistic interaction is not specific to *Nosema* but ubiquitous, as it has been reported for intoxicated bees infected with the Black Queen Cell Virus (Doublet et al. 2015) or challenged with lipopolysaccharides from the bacteria *Escherichia coli* (Kohler et al. 2012). A decrease in the cypermethrin LD_{50} by a factor of 2.66 was also found in emerging bees infected with the chronic bee paralysis virus (Bendahou et al. 1997).

However, it seems that synergistic interactions between pathogens and pesticides are not highly reproducible and/or are dependent on others factors (environmental and endogenous factors, see below). For example, Aufauvre et al. (2014) did not find a synergistic interaction between *Nosema* and fipronil despite using a similar procedure to Vidau et al. (2011). This was likely due to a seasonal effect since experiments were performed in September (France) with a possible effect of *Varroa destructor* (Aufauvre et al. 2014). Exposure to *Nosema* and thiacloprid at the colony level did not produce the synergistic interaction (Retschnig et al. 2015) observed in laboratory conditions (Vidau et al. 2011; Retschnig et al. 2014). It is likely that the colony environment has a buffering effect on stress exposure and/or that control bees die faster in their natural environment than in laboratory conditions leading to no effect of treatments (Alaux et al. 2014). In addition, the type of interaction between pathogens and pesticides may be dose-dependent. For example, by exposing *Nosema*-infected bees to different concentrations of imidacloprid (0.7, 7, and 70 μg/kg), Alaux et al. (2010) reported additive effects on the mortality at the lowest and medium concentrations but synergistic effects at the highest concentration. Similar results were obtained with bees parasitized by *Nosema* and exposed to thiacloprid (35 and 70 mg/l); only the highest concentration led to a synergistic interaction affecting bee survival (Retschnig et al. 2014).

Another factor modulating pesticide response may be the type of pesticide applied. For example, recent work on bumble bees (*Bombus terrestris*) has shown exacerbated impacts of a combined exposure to the trypanosome parasite *Crithidia bombi* and neonicotinoid insecticides (clothianidin and thiamethoxam) (Fauser-Misslin et al. 2014), but similar effects were not observed when *B. terrestris* was exposed to *C. bombi* infection coupled with exposure to a pyrethroid pesticide (lambda-cyhalothrin) (Baron et al. 2014). The effect of this latter combination was no worse for these bumblebees than exposure to pyrethroid alone.

Mechanisms underlying these pathogen/pesticide interactions are unknown but a recent transcriptomic study showed that the level of expression of some genes is affected only in bees co-exposed to fipronil and *Nosema* (e.g., genes coding for cuticular related proteins and trehalose transporter) (Aufauvre et al. 2014). A decrease in glucose oxidase activity was also observed in bees exposed to the combination *Nosema*/imidacloprid, whereas the parasite and pesticide alone did not affect this enzymatic activity (Alaux et al. 2010). In the case of synergism, it is not clear yet whether the pesticide promotes the pathogenicity of the pathogen or, conversely, the pathogen increases the toxicity of the pesticide. Indeed, synergism occurs whether bees are exposed sequentially or simultaneously to the parasite and the pesticide (Aufauvre et al. 2012). However, pesticides can promote the replication of pathogens in the host, as observed with *N. ceranae* (Pettis et al. 2012; Wu et al. 2012) and some viruses (Black Queen Cell Virus and deformed wing virus) (DeGrandi-Hoffman et al. 2013; Di Prisco et al. 2013). Di Prisco et al. (2013) showed that the abrupt increase in viral loads was due to a reduction in immune activity by the pesticide; this response was specific to the neonicotinoid clothianidin and not observed with the organophosphate chlorpyriphos. Those latter results also suggest that pesticides have the potential to increase the probability of co-exposures within the

colony, and therefore, asymptomatic infection could become symptomatic (potentiation effect). Nevertheless, it seems that apart from the pathogenicity of the foreign agent, the cost of an immune response also affects the response to sublethal doses of pesticide. Indeed, the combined challenge of nonpathogenic immune-activators (lipopolysaccharides) and a toxicant triggers synergistic effect on bee survival (Kohler et al. 2012).

3.2. Interaction between pesticides

Pesticide response in organisms can be influenced by previous or concomitant exposure to other chemicals. This is also true for honeybees (Thompson 2012; Johnson 2015). By far the majority of studies have reported synergistic interactions involving the inhibition of insecticide-metabolizing enzymes; such is the case of piperonyl butoxyde (PBO), one of the most commonly registered synergists used in plant protection formulations to enhance insecticide efficiency. In honeybees, this compound inhibits CYP450s and increases the toxicity of a wild range of insecticides, especially pyrethroids, organophosphates, carbamates, and neonicotinoids. However, depending on the relative importance of CYP450 activity in insecticide degradation, the scale of the increase in toxicity may differ widely, even within the same chemical family. As an example, Iwasa et al. (2004) showed that bee exposure to PBO increases the toxicity of acetamiprid and thiacloprid 6- and 154-fold, respectively, whereas toxicity of imidacloprid is unaffected. Similarly, toxicity of acaricides is modified in bees previously exposed to PBO as shown for coumaphos, fenpyroximate, and tau-fluvalinate (980-fold), but not for amitraz (Johnson et al. 2006; Johnson et al. 2013). Inhibitors of GST (diethyl maleate) and CE (*S*,*S*,*S*-tributyl phosphorotrithioate) can also modulate toxicity of insecticides and acaricides but to a lesser extent than PBO (Iwasa et al. 2004; Johnson et al. 2013).

Modulation of insecticide toxicity by fungicides has long been known (Pilling 1992), and that is why the use of some mixtures of active ingredients for protecting crops is regulated in some country. In particular, ergosterol biosynthesis-inhibiting (EBI) fungicides can modify the toxicity of pyrethroid insecticides. As an example, prochloraz, tebuconazole, flusilazole, and difenoconazole elevate the mortality rate of bees exposed to deltamethrin, alpha-cypermethrin, or lambda-cyhalothrin (Belzunces and Colin 1993; Pilling and Jepson 1993; Thompson and Wilkins 2003). In addition, the toxicity of the acaricides tau-fluvalinate and flumethrin (pyrethroid) can be enhanced by prochloraz and flusilazole, respectively (Thompson 2012). Data from Pilling et al. (1995) indicates that the mechanism underlying these interactions is an inhibition of CYP450 activity by EBI fungicides, slowing detoxification of pyrethroid in bees. However, an effect of the combination of EBI-fungicide and pyrethroids on bees thermoregulation cannot be excluded (Vandame and Belzunces 1998). Likewise, a synergistic effect of EBI fungicides occurs with some neonicotinoids as indicated by the 244- and 1141-fold increase in toxicity of acetamiprid and thiacloprid, respectively, in bees previously exposed to triflumizole (Iwasa et al. 2004). Finally, non-EBI fungicides may also influence the bee response to insecticide, but the underlying mechanisms have not yet been investigated (Schmuck et al. 2003; Thompson and Wilkins 2003).

In some cases, bees can be exposed to two pesticides that are both substrates of the same metabolizing enzymes. This has the potential to modify the pesticide toxicity as suggested by the synergistic interaction between the acaricides tau-fluvalinate and coumaphos (Johnson et al. 2009; Johnson et al. 2013), both metabolized by CYP9Q enzymes (Mao et al. 2011). A modulation of pesticide response can also occur independently of action on the detoxification system. For example, the mitochondrial inhibitors pyraclostrobin and boscalid used as fungicides increase the toxicity of tau-fluvalinate and fenpyroximate (Johnson et al. 2013), and some antibiotics affect the susceptibility of bees to coumaphos and tau-fluvalinate via an inhibition of efflux transporter (Hawthorne and Dively 2011).

4. MODULATION OF PESTICIDE RESPONSE BY THE ENVIRONMENT

Due to their perennial life cycle and generalist-feeding regime, honeybee colonies can experience major changes in climatic conditions and

nutrient intake, which provides additional sources of variability in the bee's response to pesticides. Indeed, it is well-established that resource quantity and/or quality provide specific nutrients that modulate stress resistance in insects (Simpson et al. 2015). As an example, young African bees (*A. mellifera scutellata*) provided with a protein-rich diet better withstand a dual stress (temperature and nicotine exposure) than individuals fed a poor-protein diet (Archer et al. 2014). More specifically, a protective role of honey and pollen against xenobiotic exposures has been revealed. One study reported that in young bees (8–9 days old), pesticide resistance increased with the amount and quality of pollen (protein rate) (Wahl and Ulm 1983). It was later found that pollen intake actually stimulates the expression of several genes encoding detoxification enzymes, which might be linked to an increase of the survival rate of bees exposed to pesticides (Alaux et al. 2011; Corby-Harris et al. 2014; Schmehl et al. 2014). The compound *p* -coumaric acid, a ubiquitous component of the pollen, was described as an upregulator of all classes of detoxification genes (Mao et al. 2013). Differences in protective effects between pollen types might also be due to diverse levels of detoxification enzymes. Indeed, Di Pasquale et al. (2013) found that bees fed with *Erica* pollen developed a higher GST activity in the head than control bees (fed no pollen), but *Castanea* pollen failed in promoting a higher GST activity. In addition, honey and nectar contain compounds that exert a positive effect on bee detoxification capacity (Mao et al. 2011; Johnson et al. 2012; Mao et al. 2013). Ethyl acetate and methanol subfractions of honey modulate the transcription of CYP9Q2 and CYP9Q3, belonging to the CYP450 gene subfamily (Mao et al. 2011). It also appears that quercetin, a natural component of plant nectar, administered through the food, increases the median lethal dose (LD_{50}) of tau-fluvalinate (Johnson et al. 2012). Therefore, those studies concluded that diets of sugar, often provided as honey substitute by beekeepers, might compromise bee capacities to cope with pesticides. Altogether, these results suggest that pesticide toxicokinetics might change according to availability of floral resources and thus the season.

Because honeybees are ectotherms, their body temperature and many of their biological functions are dependent on the environmental temperature. Several studies have shown that the response to pesticides is actually temperature dependant. In laboratory conditions, worker bees are less sensitive to some pesticides when the temperature increases (Ladas 1972). As an example, when exposed to dichlorodiphenyltrichloroethane (DDT), bees maintained at 27 °C tended to present a higher LD_{50} than bees kept at 21 °C (Graves and Mackensen 1965). A similar trend was found in the other castes (queen and drone). It was also found that a decrease in the brood rearing temperature from 35 to 33 °C modifies the susceptibility of larvae (higher LD_{50}) but also emerging adults to dimethoate (higher susceptibility) (Medrzycki et al. 2010), highlighting the potential long-term effect of thermal conditions on pesticide sensitivity. Metabolic rate, and therefore, flight activity are also tightly linked to the ambient temperature (Harrison and Fewell 2002; Woyke et al. 2003). A field experiment demonstrated that below 28 °C, thiamethoxam significantly decreased the homing rate, but effects were minor above this temperature (Henry et al. 2014).

Temporal variations in pesticide response have been observed with significant differences from season to season. Indeed, greater pesticide sensitivity has been observed in summer bees compared to winter bees (Wahl and Ulm 1983; Smirle and Winston 1987; Decourtye et al. 2003). The synergy that exists between prochloraz and deltamethrin was reported in summer bees but not in winter bees (Meled et al. 1998). The lower sensitivity to pesticides of winter bees seems to be a general phenomenon and might be linked to their unique physiology of long-lived bees as compared to summer bees, but whether they have an enhanced detoxification system is not known. Temporal variation in pesticide sensitivity could also occur within a day as suggested by the circadian oscillation in CYP450 gene expression of nurses and foragers (Rodriguez-Zas et al. 2012) that might lead to chronotoxicity, as found in the fruit fly *Drosophila melanogaster* (Hooven et al. 2009). Time of the day might then be a factor to consider regarding pesticide exposure.

Finally, because most of the pesticides targeting insect pests in agroecosystems are neurotoxicants, impairment of brain functions involved in learning and memory has been observed after sublethal exposure (Belzunces et al. 2012; Palmer et al. 2013). However, pesticide-induced impairment can vary depending on the complexity of the cognitive task; studies by Henry et al. (2012, 2014) showed that in bees exposed to thiamethoxam, part of the variation in homing failure was due to previous experience in the landscape and its structure. Forager bees use path integration and landmarks to navigate and collect food in their environment (Collett and Collett 2002), and thus, it was more difficult for intoxicated bees to perform homing flight in a challenging landscape (Henry et al. 2012, 2014). Navigation failure was later confirmed within a simplified landscape: Bees treated with neonicotinoids (imidacloprid, clothianidin, and thiacloprid) failed to use landmarks for returning to their colony when released in an unfamiliar site (Fischer et al. 2014).

5. MODULATION OF PESTICIDE RESPONSE BY ENDOGENOUS FACTORS

In honeybees, the queen mates with many males creating genetic diversity (multiple patrilines) within the colony. This contributes to the production of bees which exhibit different sensitivities to social and environmental conditions (Robinson and Page 1989). One would thus expect that bees from different patrilines differ in their pesticide sensitivity or detoxification capacities, but this remains to be investigated. However, several studies indicate that the genetic background might influence the response to pesticides, given that the toxicity of a chemical varies between bees originating from different subspecies (Ladas 1972; Suchail et al. 2000; Laurino et al. 2013; Sandrock et al. 2014; Rinkevich et al. 2015) or colonies from the same strain (Tahori et al. 1969; Smirle and Winston 1987). Indeed, one CYP450 gene was found to vary in expression between colonies originating from a hybrid population (*A. m. mellifera*/*carnica*) (Derecka et al. 2013). This topic requires dedicated experiments because the expression of genes, biological functions, and thus phenotypic response is also largely dependent on environmental conditions (see above).

In toxicology, the effect of a dose is often related to the body weight of the organism; it is therefore recommended to express the dose of exposure as the ratio of chemical weight by individual body weight. For example, in bumblebees, which exhibit large differences in body size and weight within a colony, smaller individuals tend to have a lower LD_{50} than larger individuals (Thompson and Hunt 1999). Within honeybee colonies, differences in body size between workers are very minor, but nurses are heavier than foragers and a large variability in body weight is observed between nurse bees as compared to foragers (Vance et al. 2009). Accordingly, it was found that heavier bees are less sensitive to toxicants than lighter bees (Tahori et al. 1969; Gerig 1975; Nogueira-Couto et al. 1996).

Bees exhibit tremendous physiological changes when aging and switching behavioral functions. It is therefore not surprising to observe age-dependent response to toxicants. For example, young bees were found to be more sensitive to some toxicants but less to others as compared to older bees (Mayland and Burkardt 1970; Ladas 1972; Bendahou et al. 1997; Rinkevich et al. 2015). Wahl and Ulm (1983) found an age-related increase in sensitivity to an herbicide and fungicide, as well as a higher level of *Nosema* spore loads in old bees. In this particular case, age and parasitic loads seemed to be confounded where the higher sensitivity of older bees might simply be a consequence of *Nosema* and pesticide co-exposure. More surprising was the contrasting effect of a pesticide (imidacloprid) on a cognitive task (habituation of the proboscis extension reflex) between bees of 1 day of difference (7- vs 8-day-old bees) (Guez et al. 2001). In a follow-up of their study, the authors suggested that this effect is associated to toxicodynamics factors with a differential expression of two subtypes of nicotinic acetylcholine receptors during the bee behavioral maturation (Guez et al. 2003). A significant change occurring within such a short time-window was further confirmed by the work of Whitfield et al. (2006), who found that during

behavioral maturation, young bees exhibit massive change in brain gene expression and those changes are essentially completed by 8 days of age.

Finally, despite clear physiological differences between the three castes (queen, drone, worker), few studies investigated their differential susceptibly to pesticides. By adjusting the body weight difference and comparing the LD_{50} values of queen and workers exposed to widely used acaricides, Dahlgren et al. (2012) found that queens were more tolerant than workers to four (tau-fluvalinate, fenpyroximate, thymol, and coumaphos) of the five tested acaricides; no difference was found for amitraz. Similarly, a higher LD_{50} to DDT was previously found for queens as compared to workers (Graves and Mackensen 1965). The underlying mechanisms have yet to be elucidated, but differences might arise from a caste-specific toxicokinetics of pesticides, because queens have different expression profiles of CYP450 family proteins and multidrug-resistant proteins than workers (Chan et al. 2013). Both types of proteins are involved in pesticide resistance in insects (Buss et al. 2002; Srinivas et al. 2004; Li et al. 2007). Not mutually exclusive is the hypothesis that queens and workers exhibit different pesticide toxicodynamics as suggested by the higher tolerance of queens to the oxidative stress generated by a xenobiotic (Corona et al. 2007). Since the glycoprotein vitellogenin has a protective role against oxidative stress (Seehuus et al. 2006) and is expressed at a higher level in queens compared to workers (Corona et al. 2007) and winter bees compared to summer bees (Fluri et al. 1982), it is reasonable to assume that the greater tolerance to toxicants of queens and winter bees is mediated by this protein.

6. CONCLUSIONS AND PERSPECTIVES

The major recent finding in honeybee toxicology is that pesticides may interact with other stress factors and that their toxic effects are modulated by the environment or the physiological background of individuals (Figure 1). The body of evidence is growing and clearly shows that pesticide response in honeybees is variable. However, the amount of data is still limited, and thus, much remains to be investigated in this particular domain of bee toxicology. Studying the modulation of pesticide responses by other factors will be a crucial step to understand the bee responses observed in the field, notably whether and how exposure below the LD_{50} can cause high bee mortality.

Pesticide co-exposure with other toxicants or pathogens in honeybees seems to be a common phenomenon. This web of exposure combined with high genetic variability within (patrilines) or between colonies (subspecies, ecotype) and a range of physiological states and environmental conditions experienced by bees opens many potential avenues for the modification of pesticide toxicity. Determining whether and in what way pesticide toxicokinetics and toxicodynamics are influenced by these factors is essential for predicting the honey bee response to pesticides. However, since bee exposure to some of these factors is age- or task-related, one needs to take into account the exposure relevance before developing such studies (Figure 2). For example, nurse bees are constantly exposed to hive matrices and might be more prone to cocktail effects than foragers in the case of colony contamination, but foragers might experience higher rates of pathogen/pesticide co-exposure than nurse bees due to the risk of infection accumulated through their life.

The risk assessment of pesticide toxicity is generally based on laboratory assays performed under optimal conditions for the bees (e.g., temperature and ad libitum nutrients). Such assays enable the isolation of the pesticide effect on specific biological parameters (e.g., behavior, development, and mortality). However, in their natural environment, honeybees rarely face such optimal conditions but are exposed to a large variability of environmental conditions from suboptimal to severe. For example, bees reared under optimal conditions in the laboratory clearly live longer than bees reared in colonies in the field (Alaux et al. 2014). The observed effects of potential stressors (parasitism and immune stimulation) on bee longevity under natural conditions are also reduced (parasitism) or not detected (immune stimulation) compared to laboratory conditions which is likely due to the fact that control bees

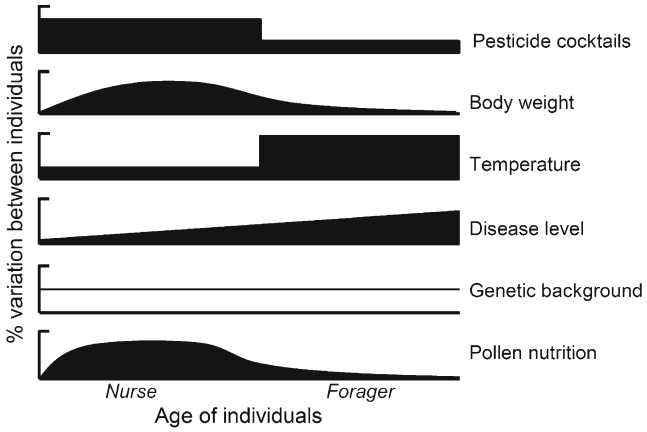

Figure 2. Age- or task-related variability of factors that can modulate pesticide response.

have a reduced longevity in nonoptimal field conditions (Alaux et al. 2014). It is therefore recommended to perform pesticide bioassays under a range of test conditions that reflect the range of variability in conditions experienced by bees in the field (Holmstrup et al. 2010). Standardization of pesticide assays is necessary for comparing results between laboratories and countries, but it should not overshadow the need to test a range of conditions for risk assessment.

Due to the large number of factors that can modulate individual response to pesticides, a great variability of responses is also expected at the colony level. In bumblebees, some studies have advanced into the investigation of the consequences of pesticide/pathogens or pesticide/pesticide combinations at the colony level (Gill et al. 2012; Baron et al. 2014; Fauser-Misslin et al. 2014). Testing such combinations of exposures in honeybee colonies is more complex when compared to the small-size and annual life cycle of bumblebee colonies. However, recent progress in modeling honeybee colony response to stress factors could solve a part of this problem (Bryden et al. 2013; Khoury et al. 2013; Becher et al. 2014; Perry et al. 2015). Efforts should then be made to supply models with accurate data on the diverse factors that can modulate pesticide response (e.g., genetic, pathogen, nutrition), which will reduce uncertainty factors in these models. Finally, in field toxicological surveys monitoring colony decline, some factors may be more relevant than others in shaping the colony response to toxicants. Indeed, within an apiary, colonies are exposed to the same microclimate, landscape, and environmental resources. The way each colony exploits its environment will determine its level of exposition to pesticides, but with similar exposure, genetic and disease backgrounds of the colony will be key contributors of the variability in the colony response.

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Modulation de la réponse aux pesticides chez les abeilles

***Apis mellifera* / toxicité des pesticides / écotoxicologie / toxicocinétique / toxicodynamique / co-exposition / évaluation des risques**

Modulation bei der Reaktion gegenüber Pestiziden bei Honigbienen

***Apis mellifera* / Pestizidtoxizität / Ökotoxikologie / Toxikodynamik / Toxikokinetik / Risikoabschätzung**

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