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Effects of Pesticides and Adjuvants on the Honey Bee, *Apis mellifera*: An Updated Bibliographic Review

Raymond A. Cloyd

Abstract

The European or western honey bee, *Apis mellifera*, pollinates approximately 75% of crop species in agricultural and horticultural production systems worldwide at a value of \$170–\$200 billion per year. While foraging for pollen and nectar in flowering plants, honey bees may be exposed to insecticides; however, they may also be exposed to a multitude of other pesticides and compounds including: fungicides, insect growth regulators, herbicides, and adjuvants. Previous and recent studies show that these pesticides and compounds are directly or indirectly harmful to honey bees, which could negatively impact pollination and colony health. Fungicides can directly and indirectly affect honey bees, and enhance the toxicity (synergize) of certain insecticides, thus increasing their toxic effects to honey bees. Insect growth regulators negatively affect larvae, which impacts brood production in honey bee colonies. Herbicides can indirectly affect honey bee populations by reducing the availability of flowering plants, which decreases pollen and nectar sources during foraging, and consequently reduces colony survival during the winter. Adjuvants, especially surfactants, are a component of pesticide formulations, and are indirectly harmful to honey bees. This book chapter provides a detailed discussion of the effects of fungicides, insect growth regulators, herbicides, and adjuvants on honey bees.

Keywords: fungicides, insect growth regulators, herbicides, adjuvants, surfactants, synergism

1. Introduction

The European or western honey bee, *Apis mellifera* L., is relied upon extensively worldwide for pollinating approximately 75% of crop species in agricultural and horticultural cropping systems at a value of \$15–\$17 billion per year in the USA and \$170–\$200 billion per year globally [1–3]. When foraging for pollen and nectar in flowering plants, honey bees can be exposed to a diverse array of pesticides, including: insecticides, fungicides, and herbicides [4–8] that can cause direct or indirect toxic effects to honey bees [9]. Direct toxicity occurs when honey bees are immediately killed when exposed to wet sprays or dried pesticide residues on leaves or flowers [10, 11]. Indirect toxicity is associated with sublethal effects on foraging behavior, development, orientation, reproduction, learning and memory retention, immune system functionality, longevity, and overwintering survival. Indirect

effects may also be related to social interactions resulting from sharing a contaminated food source [11–13]. However, any direct or indirect effects depend on the age of honey bees, because larvae or brood tend to be more susceptible to pesticides than adults [14].

Insecticides are known to be directly or indirectly harmful to honey bees [15–18] with recent research focusing primarily on the direct or indirect effects of neonicotinoid insecticides (imidacloprid, thiamethoxam, dinotefuran, clothianidin, acetamiprid, and thiacloprid) on honey bees, which has resulted in some neonicotinoids, such as: imidacloprid, thiamethoxam, and clothianidin being banned in the European Union and other countries [19–26]. However, although the initial focus has been on insecticides, research demonstrates that other pesticides and compounds can have direct or indirect effects on honey bees, such as; fungicides, insect growth regulators, herbicides, and adjuvants. Therefore, this chapter discusses the issues regarding the effects of fungicides, insect growth regulators, herbicides, and adjuvants on honey bee health.

2. Fungicides

Fungicides are pesticides used to manage fungal plant pathogens of agricultural and horticultural crops [27] and are commonly applied to fruit tree crops during the blooming period when honey bees are most active [9, 18, 28]. Therefore, honey bees are more likely to encounter fungicides than insecticides in agricultural or horticultural settings when foraging for pollen and nectar [9, 29]. Although fungicides are generally considered less toxic to honey bees than insecticides, fungicides may in fact negatively compromise honey bee health [30, 31].

Fungicides are widely detected in honey bee colonies [4], although the effects of fungicide exposure are primarily associated with brood or larvae and not adults [14, 28]. Nonetheless, foraging adults may transport fungicide residues back, along with pollen and nectar, to a hive where the residues are mixed into larval diets, which can result in inhibition of larval and pupal development [28]. In addition, fungicide residues may be present in pollen stores and wax combs, resulting in contamination of food for honey bees [30]. The widely used fungicide, chlorothalonil, which is applied to blooming crops when honey bees are active [14], has been detected at levels up to 300 ppm in bee-collected pollen and wax [4]. Moreover, high concentrations of chlorothalonil were found in bee bread (honey or pollen used as food by bees) samples collected from colonies that died during the beekeeping season [32].

In general, fungicides by themselves, demonstrate minimal direct or indirect effects on honey bee adults [6, 33–36]. However, fungicides are directly or indirectly harmful to honey bee larvae or brood, which can negatively impact colony health [37, 38]. Studies demonstrate that even fungicides alone can negatively affect honey bees, especially larvae. For instance, the fungicide, iprodione, affects the survival of larvae and causes malformations during development, although adults are not affected [28, 37]. Another study demonstrated that honey bee larvae are more sensitive to the fungicide, chlorothalonil, than adults and that dietary exposure to chlorothalonil resulted in a reduction in larval survival by more than 50% [14].

Fungicides may be affiliated with indirect (sublethal) effects on honey bees. The indirect effects of some fungicides can negatively affect honey bees in a way that resembles nutritional deficiencies or weakens honey bees by compromising the immune system, consequently increasing susceptibility to parasites (e.g., varroa mite, *Varroa destructor*) and/or pathogens (e.g., *Nosema ceranae*) [39]. In addition, exposure to the fungicide, myclobutanil, resulted in indirect effects by inhibiting the respiration rate of honey bee workers [31]. The fungicides, boscalid and

pyraclostrobin, were found to negatively impact nutrition and functionality of the immune system in honey bees. Also, a formulated pesticide mixture of pyraclostrobin and boscalid in combination with iprodione was shown to increase adult honey bee worker mortality [40]. Therefore, the indirect effects of fungicides not only can impact adults but can also contribute to colony losses [14, 41].

Another important factor is related to the common practice of tank mixing fungicides with insecticides into a single spray solution or the commercial availability of formulations that blend multiple pesticides into premixtures [7, 42, 43]. Studies report that fungicides can enhance the toxicity of insecticides to honey bees when mixed together [6, 36, 43]. This enhanced toxicity is called synergism or synergistic activity. Synergism is a reaction that occurs when one pesticide in a mixture enhances the toxicity of another pesticide or when the mortality induced by a pesticide combination is greater than the individual pesticides [44–46].

Pesticide mixtures can lead to high levels of toxicity to honey bees and even contribute to a reduction in overall colony health [47]. The ergosterol or sterol biosynthesis inhibiting class of fungicides enhances the toxicity of certain insecticide classes, including: organophosphates, neonicotinoids, and pyrethroids to honey bees [42, 48–51]. The reason for this may be associated with the fact that these fungicides decrease the ability of honey bees to metabolize insecticides [19]. The toxicity of pyrethroid insecticides to honey bees is enhanced—over a thousandfold—when mixed with ergosterol biosynthesis inhibitors [42, 43, 48]. The fungicide, propiconazole, increases the toxicity of the pyrethroid insecticide, lambda-cyhalothrin, to honey bees when the two are mixed together by inhibiting microsomal monooxygenase activity [42]. In addition, propiconazole, when mixed with the insecticide, chlorantraniliprole, resulted in an increase in toxicity to larvae and adult honey bees, which may be associated with propiconazole inhibiting P450 enzymes that are responsible for detoxifying insecticides [9, 50]. Ergosterol biosynthesis inhibitor fungicides also increase the toxicity of thiamethoxam (a neonicotinoid) up to eightfold [51].

However, the synergism of pyrethroid toxicity by certain fungicides is dependent on the proportion or dose of the fungicide in relation to the insecticide in the mixture. The higher the proportion or dose of the fungicide in the mixture compared to the insecticide, the greater the synergistic effects [51, 52]. Furthermore, studies have shown that mixing some neonicotinoid insecticides with certain fungicides can increase honey bee toxicity by as much as a thousandfold. Nevertheless, it is important to differentiate between laboratory and field studies, because synergism associated with honey bee toxicity under laboratory conditions may not predict what occurs under field conditions [43].

3. Insect growth regulators

Insect growth regulators are insecticides that disrupt insect growth and development, eventually leading to death [53]. Insect growth regulators are primarily active on the immature stages (larvae or nymphs) of certain insect pests [53, 54]. There are three categories of insect growth regulators: chitin synthesis inhibitors (diflubenzuron and novaluron), juvenile hormone mimics or analogs (fenoxycarb and pyriproxyfen), and ecdysone receptor agonists/antagonists (azadirachtin, methoxyfenozide, and tebufenozide) [27, 53, 55]. Initially, the effects of insect growth regulators on honey bees were not well known [56]. However, more recent studies show that insect growth regulators are, in fact, directly harmful to honey bees, especially the larvae or brood [12, 13, 57], and there may even be indirect effects on adult behavior [56, 58]. A number of insect growth regulators, associated

with the three categories, and commonly used in agricultural and horticultural cropping systems, directly or indirectly negatively affect honey bees [6].

3.1 Chitin synthesis inhibitors

Chitin synthesis inhibitors disrupt molting of insect larvae by interfering with enzymes responsible for stimulating the synthesis and formation of chitin, an important component of the insect exoskeleton [27, 53, 56, 59, 60]. Studies demonstrate that the chitin synthesis inhibitor, diflubenzuron, negatively affects learning behavior [56], decreases the number of adult honey bees [58], and reduces larval and queen survival [9, 57, 58]. Consequently, this impacts brood production in whole colonies [57, 61–63]. Another chitin synthesis inhibitor, novaluron, is directly toxic to honey bees and negatively affects brood production [64].

3.2 Juvenile hormone mimics

Juvenile hormone mimics (analogs) arrest development and cause insects to remain in an immature stage, which inhibits adult emergence and prevents insects from completing their life cycle [27, 53, 60, 65]. The juvenile hormone mimic, fenoxycarb, affects adult worker honey bees [66], causes adults to age prematurely [67], and, in whole colonies, causes extensive mortality of honey bee larvae, thus reducing the number of brood and size of over-wintering colonies in the subsequent year [58]. In addition, exposure to fenoxycarb affects the ability of colonies to overwinter, which reduces winter survival [58]. The juvenile hormone mimic, pyriproxyfen, affects synthesis and accumulation of vitellogenin (protein in hemolymph from which egg yolk is derived) in young worker bees [68] and negatively affects survival of honey bee foragers [69].

3.3 Ecdysone receptor antagonists/agonists

Ecdysone receptor antagonists/agonists are insect growth regulators that disrupt molting by inhibiting metabolism of the molting hormone, ecdysone, or they bind to ecdysone receptors, resulting in premature molting of larvae or nymphs, and eventually death [54, 60, 70, 71]. Methoxyfenozide does not exhibit any harmful effects on honey bee larvae or adults [9] although Fisher et al. [69] reported that methoxyfenozide negatively affected the survival of honey bee foragers. In general, tebufenozide has been shown to exhibit no direct or indirect harmful effects to honey bee colonies or queen development [58]; however, Abramson et al. [56] found that tebufenozide negatively affected the learning behavior of honey bee adults. Azadirachtin does not indirectly effect brood production, with only minimal harmful effects to honey bee colonies by negatively affecting overwintering survival [58].

4. Herbicides

Herbicides are the most widely used pesticides in agricultural and horticultural cropping systems for control of unwanted vegetation or plant material [27, 35, 72]. Therefore, herbicides should have minimal, if any, direct or indirect effects on honey bees [34, 73, 74]. The post-emergent herbicides, dicamba and picloram, were found not to be directly harmful to adult honey bees or brood [73, 74]. However, the contact, post-emergent herbicide, paraquat, was reported to be directly harmful to honey bees [75]. In addition, laboratory studies found that honey bee colonies

fed two herbicides, 2,4-D and 2,4-trichlorophenoxyacetic acid, resulted in negative effects on brood development, but there were no toxic effects to adult honey bees [33, 73, 74].

Furthermore, the herbicide, glyphosate (sold as Roundup[®]), which is a broad-spectrum, post-emergent herbicide [76], and is the most widely used pesticide worldwide [77–79], exhibits no direct harmful effects to honey bees [80]. However, research has shown that glyphosate may exhibit indirect effects on honey bees by influencing foraging behavior [79], navigation [81], or beneficial gut microbiota [82]. Nevertheless, it is important to differentiate the effects of laboratory and field studies to assess how glyphosate actually directly or indirectly affects honey bees. There are a host of factors that can influence the direct and indirect effects of herbicides on honey bees including: herbicide used application rate, method and timing of application, and location that honey bees are foraging for pollen, nectar, and water [83].

Herbicides, in general, are more likely to have indirect effects on honey bees by eliminating plants (weeds) that, when in flower, provide pollen and nectar for honey bees during foraging [18, 33, 84]. Consequently, any reduction in floral resource availability (pollen and nectar) could indirectly affect honey bee development, foraging, and survival of managed honey bees [85]. In addition, this could lead to starvation, resulting in a reduction in colony health and winter survival [13].

5. Adjuvants

Honey bees are exposed to a multitude of pesticides while foraging for pollen and nectar in flowering plants, and many formulated pesticides that are applied to control insect and mite pests, or diseases typically contain adjuvants [5, 7, 8]. Therefore, honey bees are likely being directly exposed to adjuvants when foraging [7]. Adjuvants are compounds that are a component of the pesticide formulation (as an “inert ingredient”) or are added as a tank-mix additive [86, 87]. Adjuvants are designed to enhance the effectiveness of pesticides, including insecticides and herbicides, by improving or altering deposition, increasing toxicity, improving mixing ability, and/or extending residual activity or persistence [86].

Some of the most widely used adjuvants are surfactants that increase pesticide efficacy by reducing the surface tension of spray droplets, which allows the spray solution to cover more leaf surface area—especially waxy or hairy leaf surfaces of certain plants [5, 7, 88]. In addition, surfactants have been shown to have insecticidal and miticidal properties [89–92]. Initially, surfactants were assumed to be biologically inert with no direct or indirect harmful effects to honey bees [7, 93]. However, studies show that certain surfactants may be toxic to honey bees [88, 94, 95], especially the organosilicone surfactants, which are reported to exhibit direct and indirect harmful effects to honey bees [5, 7, 94, 96]. Nonetheless, the mechanism by which organosilicone surfactants indirectly affect honey bees, such as, impairing learning ability, is not known [5].

6. Conclusion

The European or western honey bee, *Apis mellifera*, is exposed to a diverse array of pesticides when foraging on flowering plants for pollen and nectar. Although insecticides are commonly encountered, honey bees are also exposed to other pesticides (fungicides, insect growth regulators, and herbicides) and compounds (adjuvants) that can result in direct or indirect effects on individual honey bees,

thus affecting colony health. Therefore, it is important to understand the direct and indirect harmful effects of fungicides, insect growth regulators, herbicides, and adjuvants on honey bees, and implement measures that will reduce exposure of honey bees to these pesticides and compounds. These measures include: timing pesticide applications when honey bees are not present, avoid applying pesticides to flowering plants that are attractive to honey bees, select and apply pesticides that are less directly and indirectly harmful to honey bees, and follow specific requirements on pesticide labels regarding honey bee protection.

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