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Minireview

Mechanisms by which pesticides affect insect immunity

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ABSTRACT

The current state of knowledge regarding the effect of pesticides on insect immunity is reviewed here. A basic understanding of these interactions is needed for several reasons, including to improve methods for controlling pest insects in agricultural settings, for controlling insect vectors of human diseases, and for reducing mortality in beneficial insects. Bees are particularly vulnerable to sublethal pesticide exposures because they gather nectar and pollen, concentrating environmental toxins in their nests in the process. Pesticides do have effects on immunity. Organophosphates and some botanicals have been found to impact hemocyte number, differentiation, and thus affect phagocytosis. The phenoloxidase cascade and malanization have also been shown to be affected by several insecticides. Many synthetic insecticides increase oxidative stress, and this could have severe impacts on the production of some antimicrobial peptides in insects, but research is needed to determine the actual effects. Pesticides can also affect grooming behaviors, rendering insects more susceptible to disease. Despite laboratory data documenting pesticide/pathogen interactions, little field data is available at the population level.

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Contents

1. Introduction

It is unquestionable that both pathogens and insecticides significantly affect insect populations, but questions often arise as to whether these two sources of mortality and poor health have interactive effects on each other. In particular, do pesticides affect insect immunity and the susceptibility of insects to infectious disease? The answer to this question is yes, sometimes, and the manner of this interaction is the topic of this review. Interactions between insecticides and pathogens has previously been investigated primarily on two fronts. On the one front, pest control strategies have been tested to determine whether the activity of microbial pesticides can be enhanced with certain insecticides (especially those chemicals least likely to cause environmental harm). On the other front, concerns have been raised as to whether sublethal doses of pesticides might render beneficial, non-target insects more

Abbreviations: AMPs, antimicrobial peptides; Imd, immune deficiency pathway; Jak-STAT, Janus kinase/signal transducers and activators of transcription; JH, juvenile hormone; PO, phenoloxidase; RNAi, ribonucleic acid interference; ROS, reactive oxygen species; SOD, superoxide dismutase; Toll, Toll pathway.

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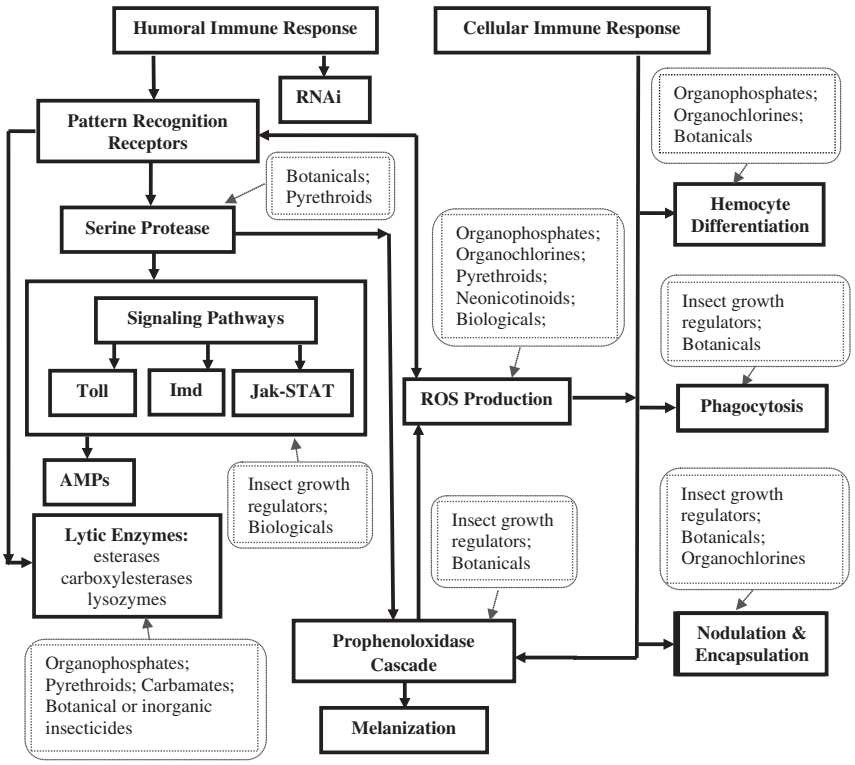

Fig. 1. The effect of insecticides on insect immunity. Solid boxes and arrows represent a schematic of the insect immune system. Stippled boxes and arrows identify where pesticides have been documented to affect particular immune responses.

susceptible to disease, and this concern has most often been raised with regard to bees.

The effects of pesticides on mammalian immune systems have been reviewed previously (Vial et al., 1996; Blakley et al., 1999; Holsapple, 2002; Salazar et al., 2008), but the effect of these compounds on insect immunity has not. The distinction between mammals and insects is important because insects lack an adaptive immune system, or at least they do not have antibodies and T-type memory cells that occur in vertebrates (Schmidt et al., 2008). Insects rely on innate immune responses that are generally non-specific (although not all mechanisms are non-specific). Prior infections can make individuals more resistant to new infections, but as a result of a prolonged non-specific immune response (Pham and Schneider, 2008). Insect immunity is basically composed of three parts: (1) the cuticle, which presents physical and chemical barriers to the outside world of microbes, (2) humoral responses, and (3) cellular responses. Little to no research has been conducted to determine if the cuticle is affected by pesticides in a way that affects its immune defense function, and so this organ is not covered in this review, except briefly as it relates to pesticide effects on behavioral defenses.

Pesticides are more broadly known to affect the insect humoral and cellular immune responses. In the initial humoral response, pattern recognition proteins identify invading microbes (or other internal non-self objects) and initiate the synthesis of various of antimicrobial proteins (AMPs). AMPs include such compounds as cecropins, defensins, attacins, and dipteracins (Hetru et al., 1998) (Fig. 1). AMP production is regulated through signaling pathways, mainly the Toll, Imd, and Jak-STAT pathways (Hoffmann, 2003; Boderick et al., 2009) (Fig. 1). The cellular immune response consists of pathogen recognition followed by phagocytosis (for invading bacteria and viruses), nodulation (for large microbial pathogens, such as fungi and clusters of bacteria), and encapsulation (for multicellular parasites) (Franssens et al., 2006) (Fig. 1). Phagocytosis is typically accompanied by melanin production and melanization of nodules and capsules (Fig. 1). Melanin production can occur more rapidly than the production of antimicrobial peptides (AMPs), can lead to the formation of reactive oxidative species (ROS) that can contribute to killing pathogens and are regulated through the phenoloxidase (PO) cascade (Ragan et al., 2009) (Fig. 1). Thus, the humoral and cellular responses are not separate entities, but are interdependent defensive forces. Furthermore, this complex system involves detoxification mechanisms that are also utilized by insects to prevent damage from environmental toxins such as plant secondary compounds and fungal toxins, providing another avenue for interactive effects between pesticides and immunity.

Since many readers may not be familiar with the functioning of the insect immune system, in this review we give short descriptions of the different immune responses, followed by a review of published reports regarding pesticide effects on each response. Melanization is technically a humoral response, but since it is most often involved with the cellular response, we have included it as such. In addition to the humoral and cellular immune responses, we also include sections on oxidative stress and behavior, as these responses are areas where pesticides and pathogens interact in a manner that affects insect health, both negatively and positively. In the end, we discuss the current state of knowledge, identify important areas of research needed, and the implications this knowledge has not only for pest control, but also for honey bees and other beneficial insects.

2. The humoral immune response in insects

2.1. Overview

Humoral immunity can be either non-specific (i.e. the same compounds are released to control a variety of different pathogens) or

pathogen specific, and can be localized or systemic. The response to bacterial pathogens in insects has been well studied, especially in the fruit fly, *Drosophila melanogaster*. A localized response can occur in the gut, probably the most common site of invasion for bacterial pathogen. The localized response in the gut depends on two mechanisms, ROS and AMP production. ROS synthesis is a very rapid response and serves as the initial barrier to invasion. Peptidoglycans are produced on the cell walls of many bacteria, and these compounds can stimulate the synthesis of the second level of defense, the AMPs. If a bacterial or fungal pathogen invades the hemocoel, its presence may be detected by pattern recognition receptors, and these in turn induce a more systemic response and up-regulation of AMPs in the fatbody and hemolymph (Boderick et al., 2009) (Fig. 1). Over 20 AMPs in seven classes have been identified from insects. Some lytic enzymes can also serve as AMPs, such as lysozyme and esterases, although generally these function to produce ROS as a result of melanin formation (Fig. 1, and discussed further in Section 3.1). Gram positive bacteria and fungi mainly trigger the Toll pathway, which induces the production of AMPs related to control of these microbes. However, most insect AMPs are not effective against entomopathogenic fungi (Gliński and Buczek, 2003). The Toll and Jak-STAT signaling pathways play a role in viral defense (Dostert et al., 2005; Zambon et al., 2005), but inducible RNA interference (RNAi) is probably the primary defense insects have against viruses (Wang et al., 2006; Zambon et al., 2006). RNAi are small RNAs generated by insects to bind and inactivate viral RNA (Imler and Eleftherianos, 2009). The RNAi must bind to viral RNA, and thus have highly specific nucleic acid sequences; that is, the response is highly specific, unlike the bacterial defenses.

2.2. Impact of pesticides

Interestingly, we found no direct evidence to document any interaction between insecticides and the humoral immune response. Insect growth hormones such as ecdysone can affect signaling pathways and AMP production (Dimarcq et al., 1997), but whether this effect carries over to insecticides that are based on insect growth regulators has not been evaluated. *Bacillus thuringiensis* (Bt), a bacterium-based insecticide, has been demonstrated to increase gene expression for AMPs, and this effect is greatest for insects that have previously been exposed to Bt (Tamez-Guerra et al., 2008; Ericsson et al., 2009). These tests were conducted with the bacterium and not with purified Bt toxins, so the results are not surprising because bacterial pathogens are already known to elicit AMPs. Also, these tests do not give any basis for making predictions about how transgenic plants that express Bt toxins might affect insect immunity. Thus, in our current state of knowledge, chemical pesticides do not appear to affect AMP production, and nothing is known about pesticidal effects on RNAi.

3. The cellular immune response and melanization

3.1. Overview

Cellular immune responses include phagocytosis, nodulation, and encapsulation. These responses are carried out by hemocytes of various types, often followed by melanization. Hemocytes have several forms, but the function and nomenclature varies for different insect taxons. For practical purposes of comparison among different insects, we will use the cell type names defined for Lepidoptera (as described by Strand (2008a)), although different names may have been used by the cited authors. Prohemocytes are undifferentiated hemocytes that commonly occur in the hemolymph, and probably serve as a source of differentiated hemocytes. Phagocytosis occurs when hemocytes called granulocytes engulf small pathogens such as bacteria and viruses (Kurihara et al., 1992; Ling et al., 2005; Strand, 2008a). The aggregates can become larger and larger with the addition hemocytes, and eventually become melanized and dark, attaching to the insect body wall or various internal organs (Strand, 2008b). Nodulation is probably the primary defense again entomopathogenic fungi, in combination with the cuticular barrier and denaturation of fungal toxins (Vey and Götz, 1986; Gliński and Buczek, 2003). Encapsulation is a cellular immune response to very large parasites such as nematodes and parasitoids. The response is similar to nodulation (Rantala et al., 2003; Strand, 2008b).

Melanin production is usually triggered by these cellular responses, and is deposited on the nodules and sheaths (Jiang et al., 1998; Goldsworthy et al., 2003) (Fig. 1). The melanization pathway is activated by hemocytes, where serine proteases are released, triggering the PO cascade (Kurihara et al., 1992; Gajewski et al., 2007). Serine proteases are involved in other physiological activities as well, including protein digestion and hemolymph coagulation (in response to wounding), thus factors that affect serine proteases could have broader physiological effects.

3.2. Impact of insecticides on the cellular immune response

In general, granulocytes and total hemocyte counts are known to increase in association with both detoxification and immune defense (Kurihara et al., 1992), so it is not surprising that both organophosphate and organochlorine insecticides have been found to affect hemocyte abundance and variation, except that the effects of these two pesticide classes are the opposite of each other (George and Ambrose, 2004) (Table 1). When the predatory reduviid, *Rhynocoris kumarii*, is exposed to an organophosphate, the total hemocyte count increases, with an increase in granulocytes but a decrease in prohemocytes and plasmatocytes. Organochlorines cause the total number of hemocytes to decrease, with a decrease in the granulocytes and an increase in the prohemocytes and plasmatocytes (George and Ambrose, 2004). How these cellular changes actually affected the immune response, or immunity in general, in this insect has not been studied.

Some botanical insecticides also affect hemocyte number. Azadirachtin significantly reduces the number of hemocytes in the reduviid, *Rhodnius prolixus* (Azambuja et al., 1991). Another botanical insecticide that has been studied for its effects on the insect immune system is the aqueous extract of the plant *Artemisia annua*. The insecticidal properties of this extract are partially due to artemisinin, a compound produced by the plant that also has anti-malarial activity (Klayman, 1989). However, these extracts contain a complex of many toxic secondary compounds (Maggi et al., 2005). This extract decreases the number of plasmatocytes and granulocytes in the corn pest, *Eurygaster integriceps* (Heteroptera: Scutelleridae), an effect that increases with dose (Zibaee and Bandani, 2010). In addition, this extract significantly reduced phagocytosis of the spores of *Beauveria bassiana* (an entomopathogenic fungus) in the hemolymph of *E. integriceps* (Zibaee and Bandani, 2010). Azadirachtin and the *A. annua* extract, have also been reported to reduce the initiation of nodulation (Azambuja et al., 1991; Zibaee and Bandani, 2010). However, *A. annua* extracts have anti-fungal and anti-bacterial properties, as well as being insecticidal (Liu et al., 2001; Stermitz et al., 2002), thus the interactive effects between disease development and exposure to this pesticide may not be due entirely to changes in insect immune responses.

The cellular response to pathogens and parasites is known to be affected by insect growth hormones. For example, when the cockroach, *Periplaneta americana*, is treated with octopamine, 5-hydroxytryptamine, or dopamine, phagocytic activity increases in the hemolymph (Baines et al., 1992). Similarly, ecdysone

Table 1
Pesticides with documented effects on insect immunity.

Pesticide class	Pesticide mode of activity	Pesticide examples	Immune functions affected
Botanical insecticides	Varied	*Acacia senega* extract	Serine protease activity (Babu and Subrahmanyam, 2010)
		Artemisia annua extract	Cellular response (Zibaee and Bandani, 2010)
			Nodulation (Zibaee and Bandani, 2010)
			PO cascade (Babu and Subrahmanyam, 2010)
		Azadirachtin	Hemocyte abundance (Azambuja et al., 1991)
			Nodulation (Azambuja et al., 1991)
		Quercetin	PO cascade (Luo et al., 2005)
		Terpinen-4-ol	PO activity (Ma et al., 2008)
Inorganic insecticides	Metabolic processes, feeding inhibition	Sodium tetraborate	Lysozyme activity (Durmuş and Büyükgüzel, 2008)
Insect growth regulators	Insect growth and development	Buprofezin	PO cascade (Nasr et al., 2010)
		Fenoxycarb	Nodulation (Franssens et al., 2006)
		Flufenoxuron	Heat shock protein production (Salokhe et al., 2006)
		Pyriproxyfen	Nodulation (Franssens et al., 2006)
			PO cascade (Nasr et al., 2010)
Neonicotinoids	Nervous system, acetylcholine agonist	Imidacloprid	Glucose oxidase production (Alaux et al., 2010)
			Grooming behavior (Boucias et al., 1996; Quintella and McCoy, 1997; Koppenhöfer et al., 2000)
Organochlorines	Nervous system, GABA-gated chloride channel antagonists	Endosulfan	Encapsulation (Delpuech et al., 1996)
			Hemocyte abundance (George and Ambrose, 2004)
		Dieldrin	Encapsulation (Delpuech et al., 1996)
Organophosphates	Nervous system, cholinesterase inhibitors	Dimethoate	Hemocyte abundance (George and Ambrose, 2004)
		Malathion	SOD activity (Büyükgüzel, 2009)
		Quinalphos	Hemocyte abundance (George and Ambrose, 2004)

increases phagocytosis in *D. melanogaster* (Dimarcq et al., 1997), and juvenile hormone (JH) suppresses both PO and the encapsulation response in mealworms, *Tenebrio molitor* (Rantala et al., 2003). Thus, insecticides based on growth regulators have the potential to affect phagocytosis, and this has been demonstrated a few times. For example, ecdysone agonists can enhance the formation of nodules, while the juvenile hormone analogs fenoxycarb and pyriproxyfen, impair the nodulation reaction (Franssens et al., 2006) (Table 1). The stimulation of nodule formation by ecdysone might be related to the fact that ecdysone induces cell differentiation into macrophages (Dimarcq et al., 1997).

Encapsulation has been shown to be affected by the insecticides dieldrin (a cyclodiene) and endosulfan (an organochlorine) (Table 1). These insecticides decrease the encapsulation response in larvae of the fly, *D. melanogaster*, to eggs of the hymenopteran endoparasite, *Leptopilina boulardi*, leading to an increase in the survival rate of the parasitoid eggs (Delpuech et al., 1996). Conversely, lindane (another organochlorine), propoxur (a carbamate), oxydemeton-methyl (an organophosphate), and chlordimeform (a formamidine) have no measurable effect on encapsulation of parasitoid eggs (Delpuech et al., 1996).

3.3. Impact of insecticides on melanization

Potentially, insecticides could interfere with the melanization process either by disrupting serine protease activity or by interfering with the PO cascade (Fig. 1). However, we have not found any reports of pesticides affecting serine protease activity. Pesticides that are known to be protease inhibitors potentially might also interfere with melanization, such as the extract from seeds of the tree legume, *Acacia senega* (Babu and Subrahmanyam, 2010). Conversely, insecticide resistant strains of the mosquito, *Culex pipiens*, possess high serine protease activity, expressing up to three times the levels of activities found in non-resistant strains (Gong et al., 2005; Yang et al., 2008). This over expression is probably related to a detoxification mechanism for the insecticide, but it likely has implications for immunity.

The PO cascade is probably more vulnerable to being affected by pesticides because some of the oxidizing, deoxidizing, and hydrolysis processes involved are also important detoxification mechanisms in insects. JH and JH-analogs increase PO activity in the cuticle of housefly larvae, *Musca domestica* (Ishaaya and Casida, 1974). However, injection of JH into male mealworm beetles, *T. molitor* suppresses PO activity (Rantala et al., 2003). This difference in response may be due to the fact that cuticular PO is produced in the epidermis, and hemolytic PPO is produced in the fatbody and hemocytes, and both have different activities and targets. Two insect growth regulator-based insecticides, buprofezin and pyriproxyfen, reduce PO activity in larvae of *Spodoptera littoralis* (Nasr et al., 2010) (Table 1).

Some botanical insecticides have been shown to reduce PO activity (Table 1). Terpinen-4-ol inhibits PO activity in fifth instars of the armyworm *Mythimna separate* (Ma et al., 2008), and *A. annua* extract reduces the normal PO response of *E. integriceps* to *B. bassiana* spores (Zibaee and Bandani, 2010). Quercetin (a plant derived flavonoid) inhibits the activity of two PO related enzymes, monophenolase and o-diphenolase (Luo et al., 2005). On the other hand, azadirachtin does not interfere with the PO cascade (Azambuja et al., 1991). It is interesting that most of the compounds tested are biological insecticides, and the effects of synthetic insecticides on PO activity are largely unstudied.

4. Oxidative stress and metabolism, as they relate to immunity and pesticides

Oxygen is reduced to water during the normal anaerobic production of energy in the mitochondria of cells. Partially reduced oxygen species, such as hydrogen peroxide and superoxide, are produced in this process and are ROS (Turrens, 2003). ROS can cause cell damage, and so are rapidly inactivated in the cell with oxygen scavengers and reduction reactions, the activities of which

are facilitated by enzymes such as superoxide dismutase (SOD), catalase, cytochrome c, and glutathione S-transferases (GSTs) (Turrens, 2003). If the ROS are not sufficiently reduced, they cause damage to the organism by reacting with macromolecules of biological importance, such as lipids, proteins, nucleic acids, and carbohydrates, eventually leading to cell death. However, a balance between the radical-generating and radical-scavenging systems is imperative to homeostasis because the ROS also have beneficial effects, mainly in bacterial defense (they serve as anti-bacterial agents during phagocytosis) (Fig. 1) and are needed for the functioning of some hydrolytic enzymes. ROS can also play a role in apoptosis (programmed cell death) and denaturing toxins, including pesticides. Oxidative stress occurs when the radical-generating and radical-scavenging systems are out-of-balance.

A wide variety of synthetic insecticides are known to suppress the activity of key reduction enzymes, including SOD (Büyükgüzel, 2009; Adamski et al., 2003) and GST (Papadopoulos et al., 2004; Cossio-Bayugar et al., 2002; Wu et al., 2009). Many toxins are detoxified physiologically in insects via oxidation, and this response maybe be a result of a feedback mechanism to increase ROS production. However, the actual effect is not clear because up-regulation of many of these enzymes is known to increase insecticide resistance in a variety of insects, and in response to a variety of pesticides (e.g. Hemingway and Karunaratne, 1998; Vontas et al., 2001; Coleman et al., 2002). In any case, it is clear that these enzymes and ROS play an important role in both homeostasis, detoxification, and immunity—thus this physiological response is very likely to be a source of interactive effects between pesticides and infectious disease. For example, *Nosema cerana* infections increase GST production in both the midgut and fatbody of honey bees (Vidau et al., 2011). However, although insecticides have been shown to increase mortality in infected honey bees, it is not clear if this response is related to GST regulation (Vidau et al., 2011). Similarly, the activity of many entomopathogenic fungi is dependent on the production of toxins such as destruxin, and these toxins can induce catalase activity patterns that are similar to those seen for pesticide exposure, namely, increased catalase activity at low toxin exposure levels, and decrease catalase activity at high exposure levels (Adamski et al., 2003; Sowjanya Sree et al., 2010).

Likewise, heat shock proteins are produced in response to heat shock and oxidative stress, but are also involved in insect immunity (Tsan and Gao, 2004; Wojda and Jakubowicz, 2007). Some insect growth regulators affect the expression of heat shock proteins in insects (Table 1). For example, heatshock protein levels increase in the beetle *Tribolium castaneum* after exposure to the JH-analog, flufenoxuron, but the effect on immunity has not been tested (Salokhe et al., 2006).

Glucose oxidase is involved in the metabolism of glucose, but also has oxygen reduction properties and antibiotic activity, and is thought to be an important factor in social immunity in honey bees, possibly enabling these bees to disinfect the nest and brood food (Alaux et al., 2010; Sano et al., 2004). Imidacloprid has been shown to increase honey bee mortality from *N. cerana* infections, and it also significantly decreased the glucose oxidase activity, but not PO and total hemocyte counts (Alaux et al., 2010) (Table 1).

5. Behavioral immunity and pesticides

Insecticides often affect insect behavior, such as reducing movements and affecting feeding levels. These changes in behavior can affect disease susceptibility, even when the insecticide does not alter the internal immune response of the insect. Cases where insecticides have altered insect behavior in such a way that disease levels were also affected have only been reported for imidacloprid. Perhaps imidacloprid has been the most investigated insecticide because for a long time it was considered to be relatively safe to non-target organisms (especially mammals and birds), and thus was desired as a companion to biological control systems in integrated pest management systems.

The synergistic effects of imidacloprid have been seen for both entomopathogenic fungi and nematodes (Table 1), and both of these pathogen groups typically infect insects through the cuticle. Most entomopathogenic fungi infect after spores attach to and germinate on the cuticle, then penetrate directly into the hemocoel (Clarkson and Charnley, 1996). Termites that have been fed imidacloprid are significantly more susceptible to fungal diseases than those not exposed to this insecticide. The reason is that imidacloprid disrupts grooming behavior, where, normally, nest mates remove spores from each others bodies (Boucias et al., 1996). Interestingly, imidacloprid-exposed termites also are more susceptible to an undescribed Gram-negative bacterium, even though the gut biota and cellular immune responses were not disrupted by this insecticide (Boucias et al., 1996).

Behavioral effects have also been seen with non-social, soil insects. When the root weevil *Diaprepes abbreviatus* moves though the soil, the abrasive soil particles remove spores of entomopathogenic fungi, and for this reason, this pest is not very susceptible to fungal pathogens. However, sublethal doses of imidacloprid reduce the activity of these insects enough to significantly increase fungal infections (Quintella and McCoy, 1997). Imidacloprid has a similar synergistic reaction with entomopathogenic nematode parasitism rates in scarab grubs. Increased parasitism in the presence of imidacloprid is caused by an increase in nematode attachment and invasion as a result of decreased activity and grooming behaviors by larvae (Koppenhöfer et al., 2000). In other words, the larvae are no longer able to defend themselves against nematode invasion.

In addition to these effects of imidacloprid that have been verified, azadirachtin is known to inhibit feeding behavior in insects, and this behavioral change has been offered as a logical explanation for the antagonistic effects seen when this insecticide is used in combination with Bt against the beet armyworm (*Spodoptera exigua*) and the Colorado potato beetle (*Leptinotarsa decemlineata*) (Schmutterer, 1997).

6. Discussion and future research needs

Understanding the interactive effects of pesticides and pathogens on insect immunity is important for several reasons. This interaction could be used to improve methods for controlling pest insects in agricultural settings where both chemical and microbial pesticides are used, it has implications for controlling insect vectors of human pathogens, and it could improve our understand of mortality factors affecting beneficial insects such as bees. Combining pesticides and pathogens could lead to either an enhanced or inhibited effectiveness of pest control efforts. For example, combining imidacloprid with entomopathogenic fungi can greatly increase mortality over using either one alone, especially for soil dwelling pests (Boucias et al., 1996; Quintella and McCoy, 1997; Koppenhöfer et al., 2000). On the other hand, insecticides which decrease immunity in insect vectors could potentially increase disease transmission by increasing infections in the vector population, if environmental exposures are insufficient to kill the insects outright.

Empirical research documenting the effects of pesticides on insect immunity and susceptibility needs to be expanded, especially if the data are to be sufficient to provide scientific guidance to pesticide regulators and users. The current state of knowledge is insufficient for making reliable predictions about particular classes of pesticides. However, the current state of knowledge does reveal

some areas of research that might be particularly informative. For example, the potential effects of imidacloprid, and perhaps other insecticides, on social immunity should be further investigated. In particular, imidacloprid has already been shown to increase fungal pathogens in termites due to its affects on social grooming, and thus, it is plausible that it might also affect behaviors associated with social immunity in honey bees. Of particular interest should be fungal honey bee pathogens, such as *Nosema*, *Ascosphaera*, and *Aspergillus*. Current studies have focused on the physiological responses of honey bees to imidacloprid, but have not adequately addressed the potential behavioral responses.

Another area of potential is the effects of synthetic insecticides, especially the organophosphates, organochlorines and pyrethroids on immunity via oxidative stress. Insects that are resistant to these insecticides have been shown several times to have greatly increased oxidative or hydrolytic enzyme production systems, and it has also been documented that these systems increase detoxification of the pesticides (e.g. Ishaaya and Casida, 1974; Vontas et al., 2001; Coleman et al., 2002). However, what this means for immunity in insects is still unclear, even though ROS production and regulation is an important element of the humoral immune response. A clever experiment might be to compare the disease susceptibility of pesticide resistant and pesticide susceptible strains of an insect, in the presence and absence of the pesticide. Such an experiment could be used to determine how pesticides affect immunity via oxidative stress.

It is unfortunate that more research has not been conducted to test the effects of pesticides on the humoral response. Especially lacking are experiments to determine whether pesticides affect AMP and RNAi production and activity. Honey bees contain many viral pathogens (Genersch et al., 2010), and most of these are both difficult to detect and to control. Thus, it would of great benefit to beekeepers to have a better understanding of how environmental factors, like pesticide exposure, affect bee susceptibility to viral pathogens. However, it is not clear that any mechanisms exist by which any of today's pesticides might affect the production or functioning of RNAi-based defenses, based on the research reviewed here. With our current state of knowledge, pesticides seem highly unlikely to affect viral immunity in bees, unless, again, they affect the behaviors associated with grooming, hive cleaning and social immunity.

We would also like to point out that the majority of research on insecticide/pathogen interactions in insects have focused on imidacloprid, azadirachtin, and other botanical extracts. Insecticides that are considered by many to have a potentially low environmental impact. Research is need to evaluate more synthetic pesticides. As already stated, many of these compounds are known to cause oxidative stress and alter insect behavior (many are neural toxins). In addition, insect growth regulators are known to affect many components of the humoral and cellular immune response, but whether the same response results from exposures to the synthetic insect growth regulators which are used as insecticides is still poorly studied.

Lastly, we would like to point out the special importance this topic has for bees. Bees are critical as pollinators in many agricultural ecosystems (James and Pitts-Singer, 2008), but their numbers have declined over large geographical areas (NRC, 2007). All bees are important, but honey bees have a particular economic importance because they provide the vast majority of commercial pollination services and produce other commodities, such as honey and wax. Unfortunately, honey bees have also declined world wide, despite a great deal of effort by beekeepers to maintain their managed populations, yet the causes of these declines have yet to be elucidated (IBRA, 2010). The reason bees are a special case with regard to pesticide exposures is that their habit of collecting pollen and nectar and storing it in the hive means they collect and concentrate environmental toxins, and in agriculture fields, this can mean the collection and concentration of pesticides. Mullin et al. (2010) found 121 different pesticides in bees wax, bee pollen, and honey bees sampled from throughout the US. Ninety-eight percent of the bees wax samples were contaminated with at least one pesticide, and 47% of the hives were contaminated with all three of these synthetic pesticides: coumaphos and fluvalinate (miticides used to control varroa mites in honey bee colonies) and chlorothalonil (an agricultural fungicide) (Mullin et al., 2010). Most often, these 121 pesticides were found at levels below concentrations expected to cause acute toxicity in honey bees, but little is known about sublethal, chronic, or combined effects of pesticides on bees (or other insects) (Desneux et al., 2007).

In addition to the risks of pesticide for bees, combinations of pathogens have been identified as being associated with the large colony losses called colony collapse disorder, in particular, the combination of viruses and a *N. cerana* (Cox-Foster et al., 2007; Bromenshenk et al., 2010). However, infectious diseases have not been sufficient to fully explain the symptoms and incidence of colony collapse disorder, and for this reason, speculations have been made regarding the possibility that sublethal pesticide exposures might make bees more susceptible to a variety of common infectious diseases. Unfortunately, little evidence to date exists to test this hypothesis, but it has served as the primary reason why we put together this review.

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