Chloronicotinyl insecticide imidacloprid: agricultural relevance, pitfalls and emerging opportunities

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Highlights

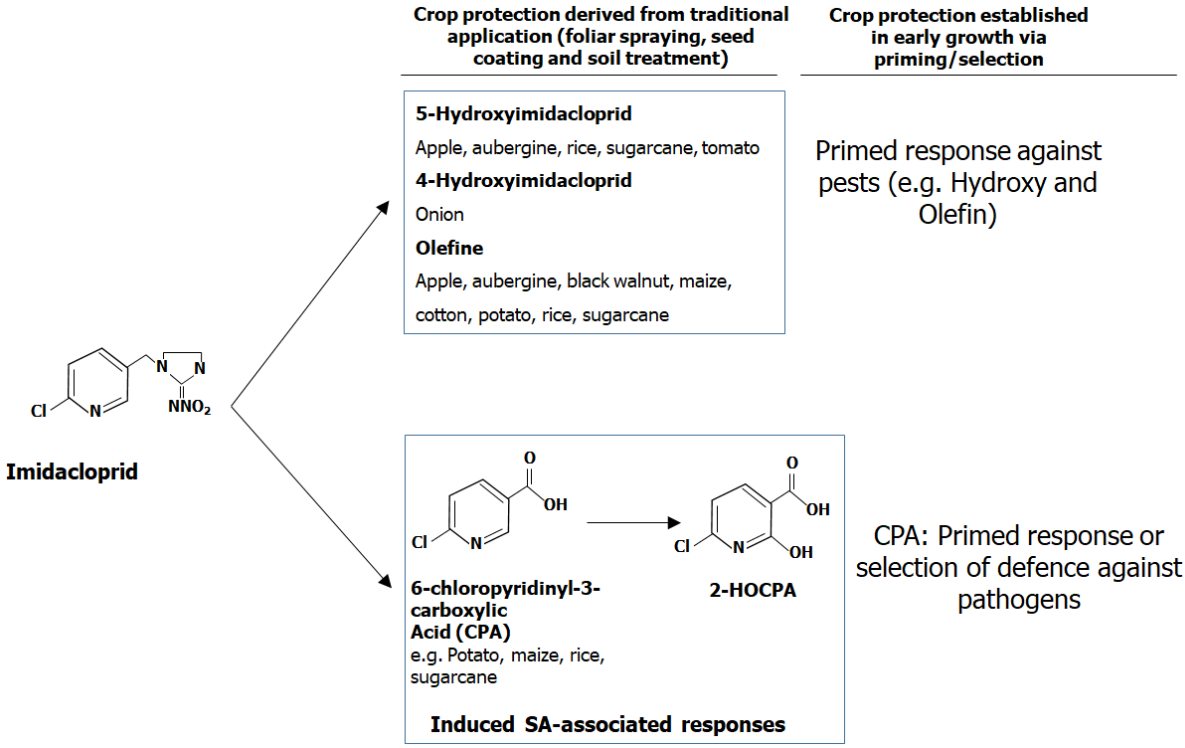
- Neonicotinoid imidacloprid targets and kills insects problematic to agriculture.
- However, it is potentially harmful to non-target beneficial insects.
- Priming or selection driven by imidacloprid might aid plant protection efforts.
- It might also challenge or supplement current application methods.

Abstract

Imidacloprid quickly gained popularity as the first commercial neonicotinoid following the invention of neonicotinoids by Bayer in 1985. Its relationship with agriculture is linked to protection of field crops against a range of insect pests. Beyond this, imidacloprid applications – foliar spraying, soil and seed treatment – have become intertwined with the negative impacts on ecosystem services, including killing of non-target organisms with great economic value such as pollinators, honey providers and beneficial insects which assist farmers with natural pest control. Because early plant growth (e.g. tissue, organ and seedling phase) forms an important part of a plant's life history, the current review draws attention to applying imidacloprid in priming or selection of physiologically important traits. And it reasons that neonicotinoid(imidacloprid)-driven priming/selection is enriched with the potential to integrate robust responses in, and for, plants to better deal with future hostile encounters from early life. Further discussed are some of the metabolites and synthetic compounds that are analogous to imidacloprid. Such compounds appear to greatly influence plant biochemical processes, and thus reflect the potential for imidacloprid to select traits important to safeguard plants against environmental stresses.

Keywords: Crop protection, neonicotinoids, pests, priming

Graphical abstract



1. Introduction

Imidacloprid was launched in 1991 by Bayer CropScience as the forerunner of neonics, and was since commercially sold in many different countries across the globe (Jeschke et al., 2011). This neurotoxic compound is often applied to control insect pests as an acetylcholine receptor agonist. It belongs to neonicotinoids (neonics), divided into *N*-nitroguanidines (imidacloprid, thiamethoxam, clothianidin and dinotefuran), nitromethylenes (nitenpyram) and *N*-cyanoamidines (acetamiprid and thiacloprid). Imidacloprid is commercially available under various brand names (e.g. Admire, Gaucho, Confidor, Premise, Prothor, and Winner; Table 1) although most brands popular in the pest management market are used in horticultural and agricultural crop production systems (Jeschke et al., 2011).

Table 1. Reported effects of Imidacloprid on non-target beneficial insects and plant growth properties after application.

Imidacloprid trade name	Species[a]	Amount[b]	Treatment[c]	Reported effect(s)[d]	Ref(s)[e]
Reported effects in bees and other beneficial insects					
Confidor	*Anagyrus pseudococci* and *Sphaerophoria rueppellii*	0.75 mL (soil) and 0.15 mL (foliar) of imidaclopri d/1 L	Soil and foliar application	Mortality recorded for pollinator hoverflies feeding on honeydew containing imidacloprid provided by hemipterans feeding on trees.	Calvo-Agudo et al., 2019
Pestanal™, analytical	*Apis mellifera*	0.25-0.50 ng	Administered orally or topically	Altered gustatory responsiveness and impairment of learning and memory observed in laboratory adults exposed to sublethal doses of imidacloprid.	Goñalons and Farina, 2015
Pestanal™, analytical	*Apis mellifera*	0.25-64 ng a.i./uL	Administered orally	Sublethal doses of imidacloprid caused alterations in bee midgut cells and regulated expression of proteins related to oxygen supply, neuronal degeneration, memory/learning, and nicotinic acetylcholine receptor alpha 1.	Catae et al., 2018
Imidacloprid (95% TG, Bayer)	*Apis mellifera*	1-500 µg/L	Feeding process	Sublethal doses of imidacloprid affected neural development of the honey bee brain, potentially resulting in olfaction and vision impairment.	Peng and Yang, 2016
Pestanal™, analytical	*Apis mellifera*	20 ppb	Feeding process	Study finds combined effect of imidacloprid and the parasitic mite *Varroa destructor* impairs immune response and thus compromises bee health.	Tesovnik et al., 2019
Pestanal™, analytical	*Melipona scutellaris* Latreille	0.3-64 ng a.i./µL	Administered orally and topically	The Brazilian native bee species *M. scutellaris* is more sensitive to imidacloprid relative to other bees such as *A. mellifera*.	Da Costa et al., 2015
Evidence® WG	*Podisus nigrispinus*	0.312-10 mg/L	Feeding process	Imidacloprid sublethal effects resulted in histological alterations in the midgut epithelium and certain cytotoxic features, which may impact predation in *P. nigrispinus*.	Martínez et al., 2019
Reported only as imidacloprid	*Bombus impatiens*	9.6 ppb	ad libitum feeding	Imidacloprid induces direct and rapid changes in nurse and caretaking behaviours, which affected productivity and harmed colony thermoregulation following exposure.	Crall et al., 2018
Reported only as imidacloprid	*Bombus terrestris*	6 µg kg/L (pollen) and 0.7 µg	ad libitum feeding	Bee colonies exposed to imidacloprid showed slow growth rate and an 85% reduction in	Whitehorn et al., 2012

		kg/L (sugar)		production of new queens compared with control colonies.	
Reported effects in plants and crop species					
Admire	Melon	Not reported	Drip irrigation injection	Insect pressure was reduced in the field, and there was a 20% more yield relative to the competitor compound.	Thielert, 2006
Confidor	Tobacco	Not reported	Floating box application	Improved growth compared to untreated plants.	Thielert, 2006
Gaucho	Cotton	Not reported	Not reported	High photosynthetic Quantum Efficiency under water deficit conditions and improved tolerance to short-term heat stress.	Thielert, 2006
Gaucho 70 WS	Bt cotton	3.2 g/Kg seeds	Seed treatment	Heightened level of Bt protein, peroxidase, and superoxide dismutase, as well as phenols.	Kaur and Sohal, 2015
Imidacloprid dacel 17.8 SL	Bt cotton	40 ml/acre	Foliar spraying	Heightened level of Bt protein, peroxidase enzyme activity, total phenols, height, number of bolls retained on plants and yield.	Kaur et al., 2011
Pestanal™, analytical	Arabidopsis	Not reported	Soil application	Improved survival and growth rate of drought stressed plants	Thielert, 2006
Pestanal™, analytical	Barley	Not reported	Not reported	Significantly increased leaf growth under drought stress conditions. Defence related genes (e.g. TLP7) were highly expressed.	Thielert, 2006
Pestanal™, analytical	Tomato	Not reported	Not reported	Significant root growth under hypoxic conditions.	Thielert, 2006
Pestanal™, analytical	Arabidopsis	4 mM	Soil application	Induced salicylic acid-associated responses and systemic acquired resistance, resulting in resistance to pathogen colonization.	Ford et al., 2010
Pestanal™, analytical	Soybean	100 ppm	Hydroponic treatment of seedlings	Induced foliar lesions and oxidative damage.	Ford et al., 2011
Pestanal™, analytical	Spinarch	50 – 100 ppm	Hydroponic treatment of seedlings	Induced foliar lesions (minor).	Ford et al., 2011
Pestanal™, analytical	Sugarcane	0.0120 g/pot	Spraying of single-bud cuttings	Increased the total weight of plants resulting in greater height, leaf area, stem diameter, and tillers.	Endres et al., 2016
Pestanal™, analytical	Sugarcane	100 µM	Incorporation into tissue culture medium at 40 °C	Enhanced growth in terms of shoots (plantlets) regenerated per 0.2g of callus.	PTC, SASRI
Pestanal™, analytical	Sugarcane	100 µM	Incorporation into low ψw	Enhanced growth in terms of root length after recovery.	PTC, SASRI

			tissue culture medium at 40 °C		
Pestanal™, analytical	Sugarcane	25-200 µM	Incorporation into tissue culture medium.	Enhanced growth in terms of callus fresh mass.	PTC, SASRI
Pestanal™, analytical	Sugarcane	25-200 µM	Incorporation into tissue culture medium.	Enhanced growth in terms of %callus with shoots.	PTC, SASRI
Pestanal™, analytical	Sugarcane	25-200 µM	Incorporation into tissue culture medium.	Enhanced growth in terms of shoots (plantlets) regenerated per 0.2g of callus.	PTC, SASRI
Merit® 2F	Poplar	1.44 g.a.i.	Plant fertility treatment; 6 mL/31 cm of plant height	Increased growth and total biomass (due to increase in total leaf area). No effect on percent root mass.	Chiriboga, 2009
Trimax™	Cotton	Not reported	Foliar spray	Increase in maturing leaves and earlier flowering	Thielert, 2006
Trimax™	Cotton	52.3 g.a.i./ha	Foliar application at the pinhead square growth stage	Increased levels of photosynthesis and higher values of chlorophyll fluorescence yield. Improved tolerance to heat stress and reduced glutathione reductase.	Gonias et al., 2008
Ultimo™ 200 SL	Mustard	0 – 40 g.a.i./ha	Foliar spraying	Malondialdehyde, and proline levels increased. Dose-dependent increase of superoxide dismutase, ascorbate peroxidase and glutathione reductase. Other enzymes were active only at lower concentrations.	Dar et al., 2015

[a, d]Bt, *Bacillus thuringiensis*

[b]a.i., active ingredient; ppm, parts per million; ppb, parts per billion

[c]Water potential (ψ) was reduced using 20-26% Polyethylene glycol (average Mn 6000)

[e]PTC, SASRI, plant tissue culture facility of SA Sugarcane Research Institute

Several key attributes such as predicted lower mammalian toxicity due selective targeting of insects' central nervous system (CNS), and systemic properties, ensure that imidacloprid is an admirable insecticide. Also augmenting this are a range of factors such as imidacloprid coming off patent that caused a major rise in generics and combined formulations, including in binary mixtures with other pesticides from different classes (Jeschke et al., 2011). To date, the global market of imidacloprid has been on an upward

trend ever since the invention of neonics, attaining massive use increases in the acreage of cropland for more than 140 crops globally. As a result, the agricultural application of traditional insecticides (e.g. methylcarbamates, organophosphates, and pyrethroids) has plummeted over the years due to the market introduction of neonics (Jeschke et al., 2011).

To date, several research groups have placed a great deal of emphasis in better understanding the potential negative consequences of imidacloprid and related neonics – these are discussed in more detail in section 4. Most of the studies have arrived at the conclusion that widespread use of neonics negatively alter agricultural farmlands and other environments, and have ultimately made it relatively easy to recognize the potential undesirable impacts of these compounds. However, there are additional hallmarks enabling imidacloprid to impact both crops and insects, and these also complicate its relationship with agriculture. Such a complicated relationship is decoupled in this review in terms of the dual effects (beneficial to crop protection and production, and toxic to non-target organisms) of this compound. These are highlighted along with a focus on the mechanisms of action, target selectivity, insect resistance mechanisms, and various applications.

2. The interaction of imidacloprid with insect nAChR

Imidacloprid contains a 6-chloro-3-pyridyl moiety that resembles compounds (e.g nicotine) (Fig. 1A) typically involved in selective targeting of the insect CNS nicotinic acetylcholine receptor (nAChR). A typical nAChR is a ligand-gated ion channel receptor organized into different arrangements of α and non-α subunits. For instance, a pentameric nAChRs are arranged into two α and three non-α subunits around a central

ion channel (Jones and Sattelle, 2010). Other forms of nAChRs (e.g. monomeric or multimeric with more or less than five subunits) have been identified but these are not thoroughly studied in insects as in mammalian species.

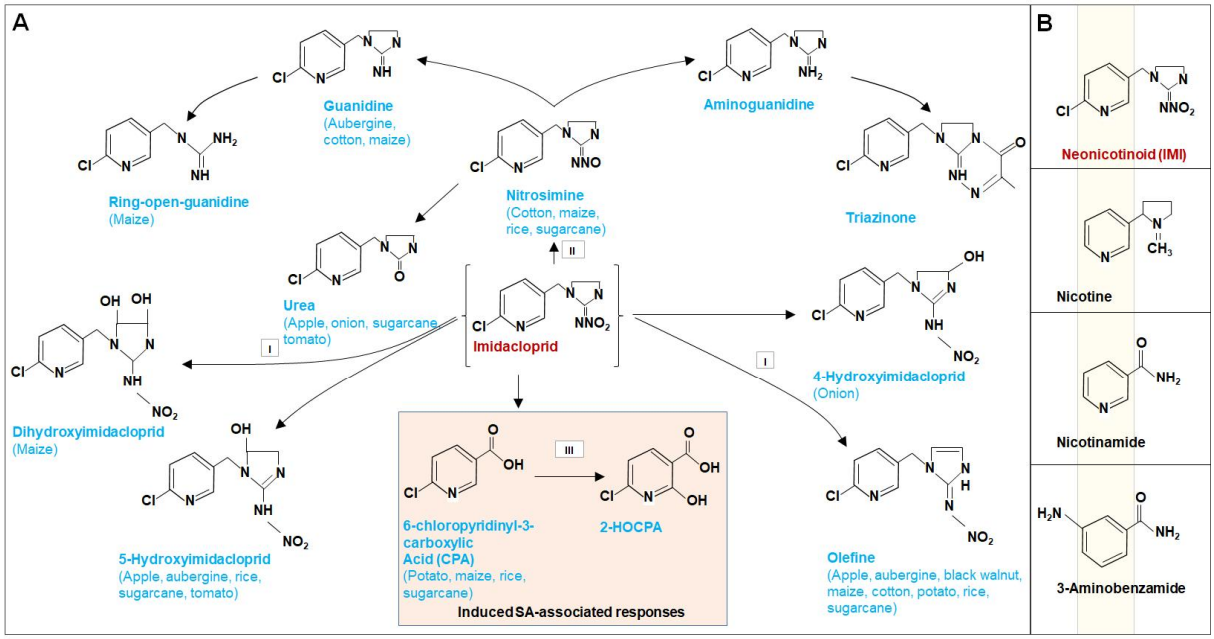


Fig.1 A. A suit of imidacloprid metabolites detected in different crop plants. **B.** Structural similarities between imidacloprid, nicotine and compounds commonly used as inhibitors of poly (ADP-ribose) polymerases.

nAChRs constitute a crucial component of the CNS as it regulates acetylcholine (ACh) neurotransmission in neuron synapses and key ion efflux channels both in vertebrates and invertebrates (Casida, 2018; Jones and Sattelle, 2010). Imidacloprid is one of the most commonly used nAChR agonists in field applications to control insect pests, and it acts on this receptor by inducing a conformational change and causing channel opening and, respectively, influx and efflux of extracellular Na^+ and intracellular K^+ (Casida, 2018). Unlike in mammals and other vertebrates, in insects nAChRs have the smallest gene families, although these can be diversified through a number of key mechanisms. Alternative splicing and mRNA editing seem to the most instrumental towards this diversity (Jones et al., 2007), and likely confer a magnitude of physiological responses

related to nAChRs. For instance, in nAChRs α subunits of the oriental migratory locust (*Locusta migratoria manilensis*), alternative splicing alters receptor function and pharmacological properties, as well as nAChRs diversity (Zhang et al., 2017).

Given the potency features of neonics, the interaction between nAChRs and imidacloprid can result in nervous stimulation at low concentrations of imidacloprid or receptor blockage, paralysis and death at higher concentrations of this compound (Casida, 2018). In general, potency of neonics can rely on the number of carbons in the ring – five-carbon ring neonics may be less potent than those with a six-carbon ring – and on the presence of non-carbon atoms in the following potency order: nitrogen > carbon > sulfur > oxygen, present in the ring (Matsuda et al., 2001). These features contribute to varying insect neurotoxicity and the efficacy of neonics to inhibit ACh neurotransmission.

Imidacloprid toxicity binding and receptor activation is affected by its 6-chloro-3-pyridyl and 2-nitroimino-imidazolidine group while target selectivity is specifically affected by the 2-nitroimino-imidazolidine group (Matsuda et al., 2001). Target selectivity has been confirmed by selective inhibition of neurotransmission for both native and recombinant insect nAChR, and high- and low-affinity binding to nAChR for insects and mammals, respectively (Casida, 2018; Matsuda et al., 2001).

3. Old and emerging players in resistance

Despite insecticides having desirable potency features against a wide range of insect species, insect pests have become notoriously resistant towards multiple classes of insecticides including neonicotinoids (Hawkins et al., 2019), potentially leading to increased use of these compounds. This has led to calls for strict adherence to guidelines for correct use of these compounds to manage risks of resistance to common destructive

pests of agriculture (Jeschke et al., 2011). To date, the success of some of the most agriculturally important insect pests, including the sweetpotato whitefly *Bemisia tabaci* Gennadius (B and Q type), *Myzus persicae* Sulzer (the green peach aphid), *Aphis gossypii* Glover (the cotton-melon aphid), *Nilaparvata lugens* Stål (the brown planthopper), *Musca domestica* Linn (housefly), *Leptinotarsa decemlineata* Say (the Colorado potato beetle), *Laodelphax striatellus* Fallén (small brown planthopper), and *Trialeurodes vaporariorum* Westwood (the glasshouse whitefly) can be ascribed to enhanced potential to defeat neonic toxicity (Bass et al., 2015).

Insect resistance to pesticides is a nuisance to agricultural production, and the expression of elaborate resistance mechanisms including metabolic and target-site resistance are common routes leading to it. Metabolic resistance entails an overexpression of metabolic enzymes while target-side resistance entails the introduction of point mutations in nAChR subunits (Bass and Field, 2018). Often these processes are studied independently although they may have an additive or alternative effect in nature. Moreover, less commonly reported mechanisms may be emerging as role players in neonic resistance, and together with traditional routes of resistance, these are briefly discussed below.

3.1. Metabolic resistance

Notably, cytochrome P450-dependent monooxygenases (P450s) are frequently identified in the detoxification or sequestration of neonics before target site interaction can take effect. P450s, a diverse group of enzymes that belong to a large family of proteins, are responsible for an array of catabolic and anabolic reactions (Scott, 1999). It comes as no surprise that imidacloprid resistant insect strains can over express P450s, and this is evident in a large number of successful insect species including *B. tabaci* (CYP6CM1), *M.*

persicae (CYP6CY3), *N. lugens* (CYP6ER1), *L. striatellus* (CYP353D1v2) and *M. domestica* (CYP6D1 and CYP6D3), to name a few (Elzaki et al., 2017; Ilias et al., 2015; Karunker et al., 2008; Markussen et al., 2010; Pang et al., 2016; Puinean et al., 2010). Glutathione *S*-transferases and UDP-Glycosyltransferases, both prevalently expressed in eukaryotes and involved in metabolising a broad spectrum of exogenous chemicals, may also contribute to imidacloprid insect resistance. However, the genes for these enzymes have only been identified as candidates and implicated in promoting resistant phenotypes in the house fly and Asian citrus psyllid (Reid et al., 2019; Tian et al., 2019).

3.2. Target-site resistance

Target-site resistance to imidacloprid has been reported in several important economic insects such as the resistant strains of *N. lugens* and *M. persicae* (Bass et al., 2014; Liu et al., 2005, 2006). In many of the insect species affected, resistance is typified by point mutations in the nAChR gene involving amino acid polymorphisms in nAChR subunits and resulting in reduced sensitivity to neonics. One notable and earliest described target-site mutation responsible for resistance phenotypes is Y151S, located within nAChR α subunits (Liu et al., 2005, 2006). However, the common occurrence of the Y151S mutation is in the lab strains of *N. lugens*, and is rarely identified in field populations (Liu et al., 2005). Furthermore, molecular studies performed on nAChR subunits of these strains reveal that this polymorphism occurs in close proximity to nAChR agonist binding site, and impose very little direct influence on binding of neonics, and largely influence resistance by inducing a conformational change within the nAChR binding site (Liu et al., 2006). A nicotinic acetylcholine receptor mutation Y176 in *M. persicae* is said to correspond to Y151S and may also have a hand in decreased sensitivity of nAChR to neonics

(Crossthwaite et al., 2014). In addition, polymorphisms in the β subunits of insect nAChRs have been identified, including the K264E, L80S, R81T and V61I mutations associated with resistant phenotypes in *A. gossypii* (Chen et al., 2017; Kim et al., 2015).

3.3. Emerging role players in neonic resistance

Least commonly characterized resistance mechanisms include amplification and differential expression of genes coding for proteins involved in promoting resistance, and drug transporters. Gene amplification has been implicated in reduced sensitivity to imidacloprid and clothianidin, and host shifts in *M. persicae* that led to the adaptation of this pest to tobacco (Bass et al., 2013). As seen in the field strains of brown planthopper, the amplification of the P450 CYP6ER1 into sequence variant CYP6ER1vA and CYP6ER1vB seems to have conferred an imidacloprid metabolizing function in this protein (Zimmer et al., 2018). This is heralded by the evolution of resistant phenotypes across various Asian regions. Nevertheless, CYP6ER1vA is the predominantly expressed variant in resistant individuals and this is because its increased expression is largely made possible by *cis*-acting elements upstream the sequence variant (Zimmer et al., 2018). Reduction in expression levels of α1 and β1 subunit of nAChR have been reported to cause resistance to imidacloprid in some insect species (Chen et al., 2017; Qu et al., 2016; Wang et al., 2018).

ATP Binding Cassette (ABC) transporters, which are part of a large superfamily of integral membrane proteins that hydrolyse ATP to shuttle molecules across lipid membranes, have recently emerged as important in imidacloprid resistance. In two independent reports, one research group has identified a number of ABC proteins in *B. tabaci* that were found

to be differentially expressed in response, and to induce resistance to imidacloprid (Her et al., 2019; Tian et al., 2017).

Insects can develop resistance to insecticides through effects that cannot be explained within the context of genetic variations. Examples include the populations of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), which, despite historic genetic bottlenecks, have evolved resistance to major classes of insecticides (Brevik et al., 2018). One possible explanation to this paradox is epigenetic variations, typically induced via processes such as DNA/histone methylation, histone acetylation/deacetylation, and small RNAs. Epigenetic processes may stimulate resistant phenotypes by operating at the genotype-to-phenotype interface without interrupting the DNA sequence. Noncoding small RNAs may also contribute to insecticide resistance by regulating the expression of genes involved in metabolic- or target-site resistance. This can take effect epigenetically when these noncoding RNAs influence target gene loci by altering DNA methylation signatures that are associated with insecticide resistance. However, the knowledge of how epigenetic modifications can influence resistant properties lags far behind our knowledge of insect resistance through metabolic- and target-site insect resistance, and to date, there's no clear evidence that these modifications contribute to neonic resistance. Nevertheless, implications on agroecosystems with indirect and indirect consequences have recently been explored (Brevik et al., 2018).

4. Applications with agroecological implications

The traditional uses of imidacloprid include foliar spraying, seed dressing and soil treatment. These methods form a vital component of protection for many crop species. Foliar spraying effectively controls destructive defoliating insects such as butterflies,

moths, leaf beetles, grasshoppers and sawflies. Seed coating and soil treatment can be applied directly at the site of action where they can more accurately target the crop.

Approximately 60% of all neonics treatments occur via seed and soil applications (Jeschke et al., 2011). However, in 2008 the neonic seed treatment market reached 80% of the global market share, while in the USA, it was estimated that from 2000 to 2012 nearly all neonics were applied as seed treatments for crops such as maize, soybean and wheat, and in 2011 between 79 and 100% of maize hectares were planted with seed-treated neonics (Douglas et al., 2015; Jeschke et al., 2011). Based on these numbers, neonics appear to be predominantly used in seed treatments, and the remainder of applications (e.g. irrigation water in drench and drip application) are probably used frequently in farming and glass house settings.

4.1. Concerns over impacts on beneficial insects

The application of imidacloprid has long been scrutinized because of potential to become a pernicious threat to pollinators and the natural enemies of agricultural pests (Baron et al., 2017; Bortolotti et al., 2003; Calvo-Agudo et al., 2019; Crall et al., 2018; Decourtye et al., 2003; Mitchell et al., 2017; Pisa et al., 2015; Prabhaker et al., 2011; Rundlöf et al., 2015; Schnier et al., 2003; Woodcock et al., 2017; Whitehorn et al., 2012; Yang et al., 2008; Zhu et al., 2015). Primarily underpinning this is the systemic nature of imidacloprid, which allows its metabolites to be distributed anyway within the plant, including in pollen, nectar and guttation fluids. Indeed, at various application rates imidacloprid concentrations were detected by many studies in the pollen and nectar of various plants (Wood et al., 2017). Many of the studies published are looking into the consequence of this largely using honeybees (*Apis mellifera* Linn), bumble bees (*Bombus terrestris* Linn),

and to a lesser extent, solitary bees (*Osmia bicornis* Linn); studies reporting on these and other insects are indicated in Table 1.

Some of the analyses have indicated side effects relating to loss of fitness in bumble bee colonies briefly exposed to imidacloprid treatments representing seed-treated *Brassica napus* Linn (oilseed rape) (Whitehorn et al., 2012). The bee colonies analysed by Whitehorn et al. (2012) experienced sluggish weight gain, colony growth and queen production when exposed to imidacloprid, while the unexposed bees behaved normally. Other groups have discovered a strong relationship between exposure to nectar containing field-realistic levels of imidacloprid and impaired nesting behaviour, social networks, and thermoregulation in the wild bees, and some of those adverse effects were similarly observed with other neonics (e.g. thiacloprid) on honeybees (Crall et al., 2018).

Other studies have reported impacts on hoverflies and parasitic wasps feeding on honeydew (a substance typically excreted by tree-eating mealybugs) provided by mealybugs feeding on imidacloprid-treated trees (Calvo-Agudo et al., 2019). According to this new study, these natural enemies may be in grave danger of toxic effects of imidacloprid. For one, pollinator hoverflies appear to be more susceptible to imidacloprid-contaminated honeydew than parasitic wasps, and higher mortality rates are observed when these insects feed on thiamethoxam-contaminated honeydew provided by tree-feeding mealybugs. Based on these findings, neonics can have dire consequences if found in excretion products of insects feeding on plants treated with these compounds, but the degree of toxicity may depend on which type neonic compound is being used.

4.2. Impact on the environment

The concentrations of neonics are also detectable in the wider environment including wetlands, aquatic environments, streams, and a host of other environments (Goulson, 2013; Hladik et al., 2016; Main et al., 2014; Morrissey et al., 2015; Wood et al., 2017). Reminiscent with this are numerous studies raising concerns over exposure of mammals to neonics from various contaminated sources (Han et al., 2018; Zhang et al., 2018). One of the routes to unintended spreading of neonics to the environment may be the dust generated from drilling of neonics-treated seeds (Wood et al., 2017). This may be lethal to flying insects, and enable neonics to reach broader environments, thus affecting other organisms such as vertebrates and aquatic invertebrates (e.g. certain members of Crustacea and Insecta) in addition to non-target beneficial insects.

4.3. Impacts arising from interaction with other agents

The advent of studies investigating combined effects, as opposed to effects of neonics alone, reflects a critical step in understanding the complex interactions that these compounds have with beneficial insects. Several of these studies indicate that insects do in fact encounter further toxicity effects when neonics, including imidacloprid, combine with other stressors such as parasites, fungicides and pesticides from other classes (Abbo et al., 2017; Dussaubat et al., 2016; Gregorc et al., 2018; Tesvnik et al., 2019; Zhu et al., 2017; van der Sluijs et al., 2013). Recent studies support this notion as they have uncovered that imidacloprid in combination with the ectoparasitic mite *Varroa destrcutor* can affect key biological processes (e.g. development and behaviour) and the health of bees (Abbo et al., 2017; Tesvnik et al., 2019). As noted by Van der Sluijs et al. (2013), neonics toxicity may increase in the presence of other compounds depending on whether

it is a pesticide–pesticide or pesticide–infectious agents combination. This important observation offers an opportunity for researchers to build profiles of neonics with other chemical or infectious agents present in the environment in relation to the level of toxicity detected.

4.4 Factors complicating the evaluation of evidence on impacts

The reports of neonics' side effects on beneficial insects may have been a trigger for the temporary restrictions of these compounds in regions such as the EU (European Food Safety Authority, 2013a, b, c), and mounting fears that other countries may want to implement such restrictions as well. However, most of the studies are yet to truly reflect the situation at the farm level. And thus, currently it may be challenging to single out imidacloprid (or neonics) effects as solely responsible for bee decline as the results that have been generated so far largely focused on bee research. Although such results reflect greater implications regarding the health of species affected by neonics, it should be kept in mind when interpreting this research for other organisms that bee research is only one of the main case studies, and that research on potential side effects from neonics is still a long way to go to establish conclusive proof that pertains to the situation in the field and on whole bee population level. Further complicating the answers is the plethora of interacting environmental factors such as bee related viruses, nutrition, immunity, and beekeeper practices (DeGrandi-Hoffman and Chen, 2015; McMenamin and Flenniken, 2018; Steinhauer et al., 2018), as well as contrasting findings where the results may be influenced by the bee and crop species analysed. Therefore, regulatory authorities should consider all aspects known to be involved, not only bee research, when evaluating evidence relating to the impact of neonics on pollinating and other beneficial insects.

5. Imidacloprid-driven priming

A multitude of imidacloprid applications described in the previous section lead to protection of crops against biotic stress factors. However, protection relies heavily on a number of factors such as the concentrations of imidacloprid applied, which can be high and often toxic to non-target organisms. But it is also dependent on how long imidacloprid or its metabolites can remain within or around the plant. It is important to note that imidacloprid can also be effective at low levels (e.g. micrograms), and therefore, be efficacious at lower concentrations. This is particularly applicable in rewiring responses from early growth in plants, such as in the case of selecting for specific traits from the parental cell lines for coping with stress or in priming, where a plant can develop a rapid response to stress due to treatment with a necrotizing agent (or in this case imidacloprid) as an initial stimulus.

5.1. Effects on insects

Although induced priming using imidacloprid may be useful for plant improvement, most evaluations around chemically-driven priming using imidacloprid in insects have focused largely on insect hormetic preconditioning, a phenomenon entailing low-dose stimulation and high-dose inhibition after exposure to a stimulus. In several studies analysing insect pests including the green peach aphid, housefly and Neotropical brown stink bug, the insect offspring exposed to sub-lethal doses of imidacloprid were usually observed to develop various primed responses including enhanced mating frequency, reduced fecundity and longevity, tolerance to stress and altered DNA methylation patterns (Ayyanath et al., 2014; Haddi et al., 2016; Kang et al., 2018; Rix et al., 2016; Sial et al., 2018; Yusmalinar et al., 2017). Furthermore, hormesis has been associated with

insecticide resistance, pest resurgence or outbreaks, all of which with great implications for integrated pest management.

5.2. Priming in plants informed by metabolites and synthetic compounds

While on the one hand imidacloprid applications have been reported to impact non-target organisms on land and in aquatic environments, on the other hand, very few studies recognize that this neonic can be repurposed for plant improvement. This is of particular importance because numerous neonic-derived metabolites including certain compounds with structural similarities and comparable potency features and after effects to neonics (e.g. salicylic acid (SA), 3-aminobenzamide (3-AB) and nicotinamide) have already been reported to exhibit physiologically relevant effects that may counter the impacts of stress from early plant development (De Block et al., 2005; Conrath et al., 2006; Hunt et al., 2007; Ford et al., 2010; Mauch-Mani et al., 2017). The potential for these compounds to induce physiologically relevant effects is a point of discussion in this section along with the prospect to use imidacloprid in priming or selection in plant improvement programs, such as in large scale plant multiplication during plant tissue culture. This is strategy may be key in reducing the application of insecticides when imidacloprid primed or selected plants are growing in the field.

5.2.1. Enhanced response against pests

Even though not much research is being done in terms imidacloprid-driven priming or selection for enhanced plant properties, studying metabolites can set the stage to define the plant improvement prospect of this approach. Three main pathways generate a suit of imidacloprid plant metabolites (Fig. 1A), i.e. ethylene-bridge hydroxylation of the imidazolidine ring and elimination of water (I), nitro-group reduction to nitrosamine and

loss of NO to form guanidine (II), and oxidative cleavage of the methylene bridge to form 6-chloropicolyl alcohol and subsequent oxidation to 6-chloronicotinic acid (III) (Sur and Stork, 2003). These pathways are present in many crops including maize, potato and rice, and in each of these, the metabolic pathways lead to the spatiotemporal distribution of metabolites within the plant (Benton et al., 2015; Coots et al., 2013; Erban et al., 2019; Ford and Casida, 2008; Ford et al., 2010; Nix et al., 2014; Seifrtova et al., 2017; Thurman et al., 2019). This indicates the potential for imidacloprid to induce "systemic priming" in plants i.e. priming of virtually any part of the plant.

Additional imidacloprid metabolites identified in onion, although expanding a list of metabolites with unclear physiological functions in plants, provide more alternatives to further understand imidacloprid-driven priming and selection of traits (Thurman et al., 2013). So far, a few imidacloprid metabolites play an important role in pest and pathogen control and insect physiology (Erban et al., 2019; Ford et al., 2010; Nix et al., 2014), and therefore, can help clarify the role that imidacloprid plays in plant environmental responses to biotic and abiotic stress.

Imidacloprid metabolites have also been found to persist in the plant for many years for the prolonged control of pest pollutions. This has been particularly so for metabolites 5-hydroxy and olefin, shown to be persistent in the Eastern Hemlock for 1-7 years' post-treatment to protect against infestation by hemlock woolly adelgid, *Adelges tsugae* Annand (Benton et al., 2015; Coots et al., 2013). The findings of these studies are consistent with the previous reporting of a 4-year long protection following a single application of red maple cultivars with imidacloprid treatments to discourage flatheaded

appletree borer (Oliver et al., 2010). These findings reflect the potential to use imidacloprid for long-term priming of response against pests.

5.2.2. Enhanced response against pathogens

Augmented defence against pathogens has been documented in parsley cell cultures pre-treated with SA (Conrath et al., 2006). The SA-induced primed response in the suspension cultures was shown to stimulated resistance to pests and abiotic stress conditions after SA-induced priming, consistent with the protective effect of SA against biotic and abiotic stress factors. Imidacloprid metabolite 6-chloropyridinyl-3-carboxylic acid, and that of clothianidin 2-chlorothiazolyl-5-carboxylic acid, are functional analogues of SA (Ford et al., 2010). In addition, both metabolites can activate a global transcriptional response that is similar to that of SA, and can activate defence against pathogens via SA-associated systemic resistance (Ford et al., 2010). Therefore, imidacloprid carboxylic metabolites may be useful to reflect the role of imidacloprid in priming a rapid response for plants to retaliate against future pathogen attacks.

5.2.3. Enhanced response against abiotic stress

In plants, various synthetic compounds that are structurally similar to imidacloprid can be used to drive priming or selection of enhanced stress response. For instance, Bayer scientists have regenerated oxidative stress-tolerant plants from *B. napus* callus cultures pre-treated with 3-AB, a pharmacological inhibitor of poly (ADP-ribose) polymerase (PARP) activity and protein ADP-ribosylation (De Block et al., 2005). Protein ADP-ribosylation is a post-translational modification process primarily catalysed by PARP, and it is usually activated in response to stress-induced DNA damage. However, increased PARP activity and subsequent ribosylation of recipient proteins by PARP can lead to

hypersensitivity of plants to a broad range of stresses. Hence, the pharmacological inhibition of this pathway using 3-AB can lead to tolerance of regenerated plants to stress (De Block et al., 2005). Noteworthy, 3-AB is structurally analogous to imidacloprid (e.g. the latter contains a benzamide-like structure similar to the benzamide structure in the former) (Fig. 1B). Other compounds that are structurally analogous to imidacloprid include nicotinamide (Fig. 1B), a product of NAD^+ catalysis generated during protein ADP-ribosylation. Nicotinamide can also inhibit ADP-ribosylation (Hunt et al., 2007). Therefore, there are prospects to use imidacloprid or its metabolites to stimulate plant environmental response to stress at genotypic and phenotypic levels by altering the trajectory of ADP-ribosylation.

6. Concluding remarks

The literature is clear on the role of imidacloprid in, and its implications for agriculture, but also provides useful information on how researchers can relook at the current usage of this compound to benefit crop improvement. Due to the widely reported toxic impacts emanating from increased use of this compound, an eco-friendly way of using it is urgently needed.

Imidacloprid-driven priming and selection of plant beneficial traits can serve as an alternative or a supplement to traditional application methods. In addition, as plants respond positively to imidacloprid application, research should be dedicated to understanding how this might manifest into agriculturally desirable physiological and genetic traits, how imidacloprid application can influence genotypic- and phenotypic-level variations, and to investigating the possibility that these variations can persist for future

generations and manifest into improved defence against ecological stress for plants to better counter environmental challenges.

Thorough research is indeed needed to fully understand the overall plant improvement potential of imidacloprid in priming and selection of enhanced traits. Future studies could, for instance, address the key areas including translating genetic- and phenotypic-level variations emanating from this approach into durable effects, and finding ways of maintaining these throughout the plant's life cycle and across generations, or determining if such primed or selected effects can indeed manifest as capability for plants to cope under stress. Therefore, imidacloprid-driven priming and selection may in future contribute to a significant reduction to the use of neonics in crop and pest management, and this in return will lower farmer input costs. Importantly, this approach will be applicable to nearly all plants and thus occupy an important role in improved plant yield and quality of the harvest.

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The author has no conflict of interest to declare.

References

Abbo, P.M., Kawasaki, J.K., Hamilton, M., Cook, S.C., DeGrandi-Hoffman, G., Li, W.F., Liu, J., Chen, Y.P., 2017. Effects of Imidacloprid and *Varroa destructor* on survival and health of European honey bees, *Apis mellifera*. *Insect Sci.* 24, 467–477.

Ayyanath, M.M., Cutler, G.C., Scott-Dupree, C.D., Prithiviraj, B., Kandasamy, S., Prithiviraj, K., 2014. Gene expression during imidacloprid-induced hormesis in green peach aphid. *Dose Response* 12, 480–497.

Baron, G.L., Jansen, V.A., Brown, M.J., Raine, N.E., 2017. Pesticide reduces bumblebee colony initiation and increases probability of population extinction. *Nat. Ecol. Evol.* 1, 1308– 1316.

Bass, C., Denholm, I., Williamson, M.S. Nauen, R., 2015. The global status of insect resistance to neonicotinoid insecticides. *Pestic. Biochem. Phys.* 121, 78–87.

Bass, C., Field, L.M, 2018. Neonicotinoids. *Current Biol.* 28, R772–R773.

Bass, C., Puinean, A.M., Zimmer, C.T., Denholm, I., Field, L.M., Foster, S.P., Gutbrod, O., Nauen, R., Slater, R., Williamson, M.S., 2014. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. *Insect. Biochem. Mol. Biol.* 51, 41–51.

Bass, C., Zimmer, C.T., Riveron, J.M., Wilding, C.S., Wondji, C.S., Kaussmann, M., Field, L.M., Williamson, M.S., Nauen, R., 2013. Gene amplification and microsatellite polymorphism underlie a recent insect host shift. *Proc. Natl. Acad. Sci. USA* 110, 19460–19465.

Benton, E.P., Grant, J.F., Webster, R.J., Nichols, R.J., Cowles, R.S., Lagalante, A.F., Coots, C.I., 2015. Assessment of imidacloprid and its metabolites in foliage of eastern hemlock

multiple years following treatment for hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in forested conditions. *J. Econ. Entomol.* 108, 2672–2682.

Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., Porrini, C., 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bull. Insectol.* 56, 63–68.

Brevik, K., Lindström, L., McKay, S.D., Chen, Y.H., 2018. Transgenerational effects of insecticides—implications for rapid pest evolution in agroecosystems. *Curr. Opin. Insect Sci.* 26, 34–40.

Calvo-Agudo, M., González-Cabrera, J., Picó, Y., Calatayud-Vernich, P., Urbaneja, A., Dicke, M., Tena, A., 2019. Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. *Proc. Natl. Acad. Sci. USA* 116, 16817–16822.

Casida, J.E., 2018. Neonicotinoids and other insect nicotinic receptor competitive modulators: progress and prospects. *Annu. Rev. Entomol.* 63, 125–144.

Catae, A.F., Roat, T.C., Pratavieira, M., da Silva Menegasso, A.R., Palma, M.S., Malaspina, O., 2018. Exposure to a sublethal concentration of imidacloprid and the side effects on target and nontarget organs of *Apis mellifera* (Hymenoptera, Apidae). *Ecotoxicology* 27, 109–121.

Chen, X., Li, F., Chen, A., Ma, K., Liang, P., Liu, Y., Song, D., Gao, X., 2017. Both point mutations and low expression levels of the nicotinic acetylcholine receptor β1 subunit are associated with imidacloprid resistance in an *Aphis gossypii* (Glover) population from a Bt cotton field in China. *Pestic. Biochem. Physiol.* 141,1–8.

Chiriboga, C.A., 2009. Physiological responses of woody plants to imidacloprid formulations (Doctoral dissertation, The Ohio State University).

Coots, C., Lambdin, P., Grant, J., Rhea, R., 2013. Spatial and temporal distribution of residues of imidacloprid and its insecticidal 5-hydroxy and olefin and metabolites in eastern hemlock (Pinales: Pinaceae) in the southern Appalachians. *J. Econ. Entomol.* 106, 2399–2406.

Crall, J.D., Switzer, C.M., Oppenheimer, R.L., Versypt, A.N.F., Dey, B., Brown, A., Eyster, M., Guérin, C., Pierce, N.E., Combes, S.A., de Bivort, B.L., 2018. Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. *Science* 362, 683–686.

Crossthwaite, A.J., Rendine, S., Stenta, M., Slater, R., 2014. Target-site resistance to neonicotinoids. *J. Chem. Biol.* 7, 125–128.

Da Costa, L.M., Grella, T.C., Barbosa, R.A., Malaspina, O., Nocelli, R.C.F., 2015. Determination of acute lethal doses (LD50 and LC50) of imidacloprid for the native bee *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae). *Sociobiology* 62, 578–582.

Dar, M.I., Khan, F.A., Rehman, F., 2015. Responses of antioxidative defense system and composition of photosynthetic pigments in Brassica juncea L. upon imidacloprid treatments. Abiotic Biotic Stress J. 1, 3–15.

De Block, M., Verduyn, C., Brouwer, D.D., Cornelissen, M., 2005. Poly (ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *Plant J.* 41, 95–106.

Decourtye, A., Lacassie, E., Pham-Delègue, M.H., 2003. Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. *Pest Manag. Sci.* 59, 269–278.

DeGrandi-Hoffman, G., Chen, Y., 2015. Nutrition, immunity and viral infections in honey bees. *Curr. Opin. Insect. Sci.* 10, 170–176.

Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environ. Sci. Technol.* 49,5088–5097.

Dussaubat, C., Maisonnasse, A., Crauser, D., Tchamitchian, S., Bonnet, M., Cousin, M., Kretzschmar, A., Brunet, J.L., Le Conte, Y., 2016. Combined neonicotinoid pesticide and parasite stress alter honeybee queens' physiology and survival. *Sci. Rep.* 6, 31430.

Elzaki, M.E.A., Miah, M.A., Wu, M., Zhang, H., Pu, J., Jiang, L., Han, Z., 2017. Imidacloprid is degraded by CYP353D1v2, a cytochrome P450 overexpressed in a resistant strain of *Laodelphax striatellus*. *Pest Manag. Sci.* 73,1358–1363.

Endres, L., Oliveira, N.G., Ferreira, V.M., Silva, J.V., Barbosa, G.V., Junior, S.O.M., 2016. Morphological and physiological response of sugarcane under abiotic stress to neonicotinoid insecticides. *Theor. Exp. Plant Physiol. 28*, 347–355.

Erban, T., Sopko, B., Talacko, P., Harant, K., Kadlikova, K., Halesova, T., Riddellova, K., Pekas, A., 2019. Chronic exposure of bumblebees to neonicotinoid imidacloprid suppresses the entire mevalonate pathway and fatty acid synthesis. *J. Proteomics.* 196, 69–80.

European Food Safety Authority, 2013a. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. *EFSA J.* 11, 3066.

European Food Safety Authority, 2013b. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance thiamethoxam. *EFSA J.* 11, 3067.

European Food Safety Authority, 2013c. Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid. *EFSA J.* 11, 3068.

Ford, K.A., Casida, J.E., 2008. Comparative metabolism and pharmacokinetics of seven neonicotinoid insecticides in spinach. *J. Agric. Food Chem.* 56, 10168–10175.

Ford, K.A., Casida, J.E., Chandran, D., Gulevich, A.G., Okrent, R.A., Durkin, K.A., Sarpong, R., Bunnelle, E.M., Wildermuth, M.C., 2010. Neonicotinoid insecticides induce salicylate-associated plant defense responses. *Proc. Natl. Acad. Sci.* USA 107, 17527–17532.

Goñalons, C.M., Farina, W.M., 2015. Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. *PloS One* 10, e0140814.

Gonias, E.D., Oosterhuis, D.M., Bibi, A.C., 2008. Physiologic response of cotton to the insecticide imidacloprid under high-temperature stress. *J. Plant Growth Reg.* 27, 77–82.

Goulson, D., 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *J. App. Ecology* 50, 977–987.

Gregorc, A., Alburaki, M., Rinderer, N., Sampson, B., Knight, P.R., Karim, S., Adamczyk, J., 2018. Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: Apidae) lifespan and antioxidant gene regulations in laboratory experiments. *Sci. Rep.* 8, 15003.

Haddi, K., Mendes, M.V., Barcellos, M.S., Lino-Neto, J., Freitas, H.L., Guedes, R.N.C., Oliveira, E.E., 2016. Sexual success after stress? Imidacloprid-induced hormesis in males of the neotropical stink bug *Euschistus heros*. *PLoS One* 11, e0156616.

Han, W., Tian, Y., Shen, X., 2018. Human exposure to neonicotinoid insecticides and the evaluation of their potential toxicity: An overview. *Chemosphere* 192, 59–65.

Hawkins, N.J., Bass, C., Dixon, A., Neve, P., 2019. The evolutionary origins of pesticide resistance. *Biol. Rev. Camb. Philos. Soc.* 94, 135–155.

He, C., Liang, J., Liu, S., Wang, S., Wu, Q., Xie, W., Zhang, Y., 2019. Changes in the expression of four ABC transporter genes in response to imidacloprid in *Bemisia tabaci* Q (Hemiptera: Aleyrodidae). *Pestic. Biochem. Physiol.* 153, 136–143.

Hladik, M.L., Kolpin, D.W., 2016. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environ. Chem.* 13,12–20.

Hunt, L., Holdsworth, M.J., Gray, J.E., 2007. Nicotinamidase activity is important for germination. *Plant J.* 51, 341–351.

Ilias, A., Lagnel, J., Kapantaidaki, D.E., Roditakis, E., Tsigenopoulos, C.S., Vontas, J., Tsagkarakou, A., 2015. Transcription analysis of neonicotinoid resistance in Mediterranean (MED) populations of *B. tabaci* reveal novel cytochrome P450s, but no nAChR mutations associated with the phenotype. *BMC Genomics* 16, 939.

Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. *J. Agric Food Chem.* 59, 2897–2908.

Jones, A.K., Brown, L.A., Sattelle, D.B., 2007. Insect nicotinic acetylcholine receptor gene families: from genetic model organism to vector, pest and beneficial species. *Invert. Neurosci.* 7, 67–73.

Jones, A.K., Sattelle, D.B., 2010. Diversity of insect nicotinic acetylcholine receptor subunits, ed. by Thany SH, Springer, New York, pp. 25–43.

Kang, Z.W., Pang, R.P., Liu, F.H., Tian, H.G., Liu, T.X., 2018. Effect of sublethal doses of imidacloprid on the biological performance of aphid endoparasitoid *Aphidius gifuensis* (Hymenoptera: Aphidiidae) and influence on its related gene expression. *Front. Physiol.* 9, 1729.

Karunker, I., Benting, J., Lueke, B., Ponge, T., Nauen, R., Roditakis, E., Vontas, J., Gorman, K., Denholm, I., Morin, S., 2008. Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 38, 634–644.

Kaur, N., Sohal, B.S. and Singh, K., 2011. Biochemical and physiological changes on *Bacillus thuringiensis* cotton after imidacloprid foliar spray. *Pestic. Biochem. Physiol.* 99, 280–284.

Kaur, N., Sohal, B.S., 2015. effect of imidacloprid seed treatment on the defense system of *Bacillus thuringiensis* cotton seedlings. Int. J. Rec. Sci. Res. 6, 2684–2689.

Kim, J.I., Kwon, M., Kim, G.H., Kim, S.Y., Lee, S.H., 2015. Two mutations in nAChR beta subunit is associated with imidacloprid resistance in the *Aphis gossypii*. *J. Asia-Pacific Entomol.* 18, 291–296.

Liu, Z., Williamson, M.S., Lansdell, S.J., Denholm, I., Han, Z., Millar, N.S., 2005. A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). *Proc. Natl. Acad. Sci. USA* 102, 8420–8425.

Liu, Z., Williamson, M.S., Lansdell, S.J., Han, Z., Denholm, I., Millar, N.S., 2006. A nicotinic acetylcholine receptor mutation (Y151S) causes reduced agonist potency to a range of neonicotinoid insecticides. *J. Neurochem.* 99, 1273–1281.

Main, A.R., Headley, J.V., Peru, K.M., Michel, N.L., Cessna, A.J., Morrissey, C.A., 2014. Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pothole Region. *PloS One* 9, e92821.

Markussen, M.D., Kristensen, M., 2010. Cytochrome P450 monooxygenase-mediated neonicotinoid resistance in the house fly *Musca domestica* L. Pestic. *Biochem. Phys.* 98, 50–58.

Martínez, L.C., Plata-Rueda, A., Gonçalves, W.G., Freire, A.F.P.A., Zanuncio, J.C., Bozdoğan, H., Serrão, J.E., 2019. Toxicity and cytotoxicity of the insecticide imidacloprid in the midgut of the predatory bug, Podisus nigrispinus. *Ecotoxicol. Environ. Saf.* 167, 69– 75.

Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grauso, M., Sattelle, D.B., 2001 Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharm, Sci.* 22, 573–580.

McMenamin, A.J., Flenniken, M.L., 2018. Recently identified bee viruses and their impact on bee pollinators. *Curr. Opin. Insect. Sci.* 26, 120–129.

Mitchell, E.A., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., Aebi, A., 2017. A worldwide survey of neonicotinoids in honey. *Science* 358, 109–111.

Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. *Environ. Int.* 74, 291–303.

Nix, K., Coots, C., Lambdin, P., Grant, J., Paulsen, D., Wiggins, G., Merten, P., 2014. Concentrations of imidacloprid and olefin-imidacloprid metabolite in the walnut husk maggot (Diptera: Tephritidae). *Florida Entomol.* 97, 1848–1851.

Oliver, J.B., Fare, D.C., Youssef, N., Scholl, S.S., Reding, M.E., Ranger, C.M., Moyseenko, J.J., Halcomb, M.A., 2010. Evaluation of a single application of neonicotinoid and multi-application contact insecticides for flatheaded borer management in field grown red maple cultivars. *J. Environ. Hort.* 28, 135–149.

Pang, R., Chen, M., Liang, Z., Yue, X., Ge, H., Zhang, W., 2016. Functional analysis of CYP6ER1, a P450 gene associated with imidacloprid resistance in *Nilaparvata lugens*. *Sci. Rep.* 6, 34992.

Peng, Y.C., Yang, E.C., 2016. Sublethal dosage of imidacloprid reduces the microglomerular density of honey bee mushroom bodies. *Sci. Rep.* 6, 19298.

Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M., Morrissey, C.A., Noome, D.A., Settele, J., Simon-Delso, N., Stark, J.D., Van der Sluijs, J.P., Van Dyck, H., Wiemers, M., 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. *Sci. Pollut. Res.* 22, 68–102.

Prabhaker, N., Castle, S.J., Naranjo, S.E., Toscano, N.C., Morse, J.G., 2011. Compatibility of two systemic neonicotinoids, imidacloprid and thiamethoxam, with various natural enemies of agricultural pests. *J. Econ. Entomol.* 104, 773–781.

Prime-A-Plant Group, Conrath, U., Beckers, G.J., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.A., Pieterse, C.M., Poinssot, B., Pozo, M.J., Pugin, Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., Mauch-Mani, B.A., 2006. Priming: getting ready for battle. *Mol. Plant Microbe Interact.* 19, 1062–1071.

Puinean, A.M., Foster, S.P., Oliphant, L., Denholm, I., Field, L.M., Millar, N.S., Williamson, M.S., Bass. C., 2010. Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *PLoS Genet.* 6, e1000999.

Qu, Y., Chen, J., Li, C., Wang, Q., Guo, W., Han, Z., Jiang, W., 2016. The subunit gene Ldα1 of nicotinic acetylcholine receptors plays important roles in the toxicity of imidacloprid and thiamethoxam against *Leptinotarsa decemlineata*. *Pestic. Biochem. Physiol.* 127,51–58.

Reid, W.R., Sun, H., Becnel, J.J., Clark, A.G., Scott, J.G., 2019. Overexpression of a glutathione S-transferase (Mdgst) and a galactosyltransferase-like gene (Mdgt1) is responsible for imidacloprid resistance in house flies. *Pest Manag. Sci.* 75, 37–44.

Rix, R.R., Ayyanath, M.M., Cutler, G.C., 2016. Sublethal concentrations of imidacloprid increase reproduction, alter expression of detoxification genes, and prime *Myzus persicae* for subsequent stress. *J. Pest Sci.* 89, 581–589.

Rundlöf, M., Andersson, G.K., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J., Smith, H.G., 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* 521, 77–80.

Schnier, H.F., Wenig, G., Laubert, F., Simon, V., Schmuck, R., 2003. Honey bee safety of imidacloprid corn seed treatment. *Bull. Insectol.* 56,73–76.

Scott, J.G., 1999. Cytochromes P450 and insecticide resistance. *Insect Biochem. Mol. Biol.* 29, 757–777.

Seifrtova, M., Halesova, T., Sulcova, K., Riddellova, K., Erban, T., 2017. Distributions of imidacloprid, imidacloprid-olefin and imidacloprid-urea in green plant tissues and roots of rapeseed (*Brassica napus*) from artificially contaminated potting soil. *Pest Manag. Sci.* 73, 1010–1016.

Sial, M.U., Zhao, Z., Zhang, L., Zhang, Y., Mao, L. and Jiang, H., 2018. Evaluation of Insecticides induced hormesis on the demographic parameters of *Myzus persicae* and expression changes of metabolic resistance detoxification genes. *Sci. Rep.* 8, 16601.

Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P., Chauzat, M.P., 2018. Drivers of colony losses. *Curr. Opin. Insect. Sci.* 26, 142–148.

Sur, R., Stork, A., 2003. Uptake, translocation and metabolism of imidacloprid in plants. *Bullet Insectol.* 56, 35–40.

Tesovnik, T., Zorc, M., Gregorc, A., Rinehart, T., Adamczyk, J., Narat, M., 2019. Immune gene expression in developing honey bees (*Apis mellifera* L.) simultaneously exposed to imidacloprid and Varroa destructor in laboratory conditions. *J. Apicult. Res.* 58, 730–739.

Thielert, W., 2006. A unique product: The story of the imidacloprid stress shield. *Pflanzenschutz-Nachrichten Bayer* 59, 73–86.

Thurman, E.M., Ferrer, I., Zavitsanos, P., Zweigenbaum, J.A., 2013. Identification of imidacloprid metabolites in onion (*Allium cepa* L.) using high-resolution mass spectrometry and accurate mass tools. *Rapid Commun. Mass Spectrom.* 27, 1891–1903.

Tian, F., Wang, Z., Li, C., Liu, J., Zeng, X., 2019. UDP-Glycosyltransferases are involved in imidacloprid resistance in the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Lividae). *Pestic. Biochem. Physiol.* 154, 23–31.

Tian, L., Song, T., He, R., Zeng, Y., Xie, W., Wu, Q., Wang, S., Zhou, X., Zhang, Y., 2017. Genome-wide analysis of ATP-binding cassette (ABC) transporters in the sweetpotato whitefly, *Bemisia tabaci*. *BMC Genomics* 18, 330.

Van der Sluijs, J.P., Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J.M., Belzunces, L.P., 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr. Opin. Environ. Sustainability* 5, 293–305.

Wang, K., Zhang, M., Huang, Y., Yang, Z., Su, S., Chen, M., 2018. Characterisation of imidacloprid resistance in the bird cherry-oat aphid, *Rhopalosiphum padi*, a serious pest on wheat crops. *Pest. Manag. Sci.* 74,1457–1465.

Whitehorn, P.R., O'connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336, 351–352.

Wood, T.J., Goulson, D., 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environ. Sci. Pollut. Res. Int.* 24, 17285–17325.

Woodcock, B.A., Bullock, J.M., Shore, R.F., Heard, M.S., Pereira, M.G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S., Pywell, R.F., 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science* 356, 1393–1395.

Yang, E.C., Chuang, Y.C., Chen, Y.L., Chang, L.H., 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 101, 1743–1748.

Yusmalinar, S., Anggraeni, T., Kustiati, K., Wibowo, I., Eka Putra, R., Ahmad, I., 2017. Reproductive ability enhancement of housefly (*Musca domestica* Linn) (Diptera: Muscidae) through hormesis by application of sublethal doses of imidacloprid and permethrin. *J. Entomol.* 14, 199–207.

Zhang, Q., Li, Z., Chang, C.H., Lou, J.L., Zhao, M.R., Lu, C., 2018. Potential human exposures to neonicotinoid insecticides: a review. *Environ. Pollut.* 236, 71–81.

Zhang, Y., Liu, Y., Bao, H., Sun, H., Liu, Z., 2017. Alternative splicing in nicotinic acetylcholine receptor subunits from *Locusta migratoria* and its influence on acetylcholine potencies. *Neurosci. Lett.* 638, 151–155.

Zhu, Y.C., Adamczyk, J., Rinderer, T., Yao, J., Danka, R., Luttrell, R., Gore, J., 2015. Spray toxicity and risk potential of 42 commonly used formulations of row crop pesticides to adult honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* 108, 2640–2647.

Zhu, Y.C., Yao, J., Adamczyk, J., Luttrell, R., 2017. Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*). *PloS One 12*, e0178421.

Zimmer, C.T., Garrood, W.T., Singh, K.S., Randall, E., Lueke, B., Gutbrod, O., Matthiesen, S., Kohler, M., Nauen, R., Davies, T.E., Bass, C., 2018. Neofunctionalization of duplicated P450 genes drives the evolution of insecticide resistance in the brown planthopper. *Curr. Biol.* 28, 268–274.