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Apidologie 41 (2010) 312–331
© INRA/DIB-AGIB/EDP Sciences, 2010
DOI: 10.1051/apido/2010018

Available online at:
www.apidologie.org

Review article

Pesticides and honey bee toxicity – USA*

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Received 12 October 2009 – Revised 26 January 2010 – Accepted 17 February 2010

Abstract – Until 1985 discussions of pesticides and honey bee toxicity in the USA were focused on pesticides applied to crops and the unintentional exposure of foraging bees to them. The recent introduction of arthropod pests of honey bees, *Acarapis woodi* (1984), *Varroa destructor* (1987), and *Aethina tumida* (1997), to the USA have resulted in the intentional introduction of pesticides into beehives to suppress these pests. Both the unintentional and the intentional exposure of honey bees to pesticides have resulted in residues in hive products, especially beeswax. This review examines pesticides applied to crops, pesticides used in apiculture and pesticide residues in hive products. We discuss the role that pesticides and their residues in hive products may play in colony collapse disorder and other colony problems. Although no single pesticide has been shown to cause colony collapse disorder, the additive and synergistic effects of multiple pesticide exposures may contribute to declining honey bee health.

pesticide / honey bee / toxicity / wax residue / CCD

1. PESTICIDES APPLIED TO CROPS

Despite the dependence on honey bees for the pollination of crops in the USA, colony numbers have declined by 45% over the past 60 years (NAS, 2007). Most honey bee losses from 1966–1979 were attributable to organochlorine, carbamate, organophosphorus, and pyrethroid pesticide exposure (Atkins, 1992). Efforts to restrict pesticide application during bloom provided some relief; however, the residual activity of some pesticides was never effectively addressed (Johansen and Mayer, 1990). Previous reviews and extension publications are available concerning the protection of honey bees from these 4 classes of pesticides (Johansen, 1977; Crane and Walker, 1983; Adey et al., 1986; Johansen and Mayer, 1990; Ellis et al., 1998).

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Colony losses were especially severe from 1981 to 2005 with a drop from 4.2 million to 2.4 million (NAS, 2007) although some of the decrease is attributable to changes in how colony numbers were estimated. The introduction of parasitic honey bee mites, *Acarapis woodi* (1984) and *Varroa destructor* (1987), contributed to dramatic bee losses. At the same time, the control of crop pests in USA agriculture was rapidly changing. Genetically engineered (GE) crops were developed and extensively deployed, and two new classes of systemic pesticides, neonicotinoids and phenylpyrazoles, replaced many of the older pesticides described above.

The rapid development and deployment of these 2 new insect control techniques distinguish USA agriculture from agriculture in other regions of the world. In Europe a more cautious approach to the adoption of new agricultural practicices has been taken. Since the registration and regulation of GE crops and neonicotinoid and phenylpyrazole pesticides

are major shifts in insect control in USA agriculture, they are emphasized in this section of our review.

The recent sequencing of the honey bee genome provides a possible explanation for the sensitivity of bees to pesticides; relative to other insect genomes, the honey bee genome is markedly deficient in the number of genes encoding detoxification enzymes, including cytochrome P450 monooxygenases (P450s), glutathione-S-transferases, and carboxylesterases (Claudianos et al., 2006).

1.1. GE plant varieties

GE (genetically engineered) plant varieties that have herbicide tolerance or insecticidal properties were first introduced into the USA in 1996. Soybeans and cotton are genetically engineered with herbicide-tolerant traits and have been the most widely and rapidly adopted GE crops in the USA, followed by insect-resistant cotton and corn. In 2007 these GE crops were planted on more than 113 million hectares worldwide (USDA-Biotech Crop Data, 2009), and the United States leads the world in acres planted with GE crops with most of the plantings on large farms (Lemaux, 2008). Insect resistance is conferred by incorporating genes coding for insecticidal proteins produced by *Bacillus thuringensis* (Bt), a widespread soil bacterium (ISB, 2007). While Bt is also delivered through traditional spray application, plants benefit from continuous production of Bt toxins through genetic engineering. Bt δ-endotoxins are activated in the insect gut where they bind to receptor sites on the midgut epithelium to form pores. These pores allow gut contents to leak out of the lumen and cause osmotic stress to midgut cells, leading to the eventual destruction of the midgut and the death of the insect (Soberon et al., 2009). To date, Bt genes have been incorporated into corn (*Zea mays*), cotton (*Gossypium hirsutum*), potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*), and GE seeds of these crops are available to producers (ISB, 2007). Precommercial field tests of 30 different plant species with Bt genes were conducted in 2008 including apples, cranberries, grapes, peanuts, poplar, rice, soybeans, sunflowers and walnuts (ISB, 2007).

Numerous studies have been conducted to determine the impact of GE crops on honey bees. Canadian scientists found no evidence that Bt sweet corn affected honey bee mortality (Bailey et al., 2005). Studies conducted in France found that feeding Cry1ab protein in syrup did not affect honey bee colonies (Ramirez-Romero et al., 2005). Likewise, exposing honey bee colonies to food containing Cry3b at concentrations 1000 times that found in pollen resulted in no effect on larval or pupal weights (Arpaia, 1996). Feeding honey bees pollen from Cry1ab maize did not affect larval survival, gut flora, or hypopharyngeal gland development (Babendreier et al., 2005–2007). A 2008 meta-analysis of 25 independent studies concluded that the Bt proteins used in GE crops to control lepidopteran and coleopteran pests do not negatively impact the survival of larval or adult honey bees (Duan et al., 2008).

There is no evidence that the switch to Bt crops has injured honey bee colonies in the USA. To the contrary, it has benefited beekeeping by reducing the frequency of pesticide applications on crops protected by Bt, especially corn and cotton. On the other hand, the switch to GE crops with herbicide resistance has eliminated many blooming plants from field borders and irrigation ditches as well as from the crop fields themselves. The reduction in floral diversity and abundance that has occurred due to the application of Round-UP® Herbicide (glyphosate) to GE crops with herbicide resistance is difficult to quantify. However, there is a growing body of evidence that poor nutrition is a primary factor in honey bee losses. Eischen and Graham (2008) clearly demonstrated that well-nourished honey bees are less susceptible to *Nosema ceranae* than poorly nourished bees. Because honey bees are polylectic, the adoption of agricultural practices that provide greater pollen diversity has been suggested, including the cultivation of small areas of other crops near monocultures or permitting weedy areas to grow along the edges of fields (Schmidt et al., 1995). A detailed review of management of uncropped farmland to benefit pollinators by Decourtye et al. (2010) is included in this special issue.

1.2. Neonicotinoid and phenylpyrazole pesticides

Another major shift in USA agriculture has been the development and extensive deployment of neonicotinoid and phenylpyrazole pesticides. These pesticides are extensively used in the USA on field, vegetable, turf, and ornamental crops, some of which are commercially pollinated by bees (Quarles, 2008). They can be applied as seed treatments, soil treatments and directly to plant foliage. Neonicotinoids are acetylcholine mimics and act as nicotinic acetychloline receptor agonists. Neonicotinoids cause persistent activation of cholinergic receptors which leads to hyperexcitation and death (Jeschke and Nauen, 2008). One neonicotinoid, imidacloprid, was applied to 788 254 acres in California in 2005 (CDPR, 2006), making it the 6th most commonly used insecticide in a state that grows many bee-pollinated crops. The phenylpyrazoles, including fipronil, bind to γ-amino butyric acid (GABA)-gated chloride ion channels and block their activation by endogenous GABA, leading to hyperexcitation and death (Gunasekara et al., 2007).

Neonicotinoid and phenylpyrazole insecticides differ from classic insecticides in that they become systemic (Trapp and Pussemier, 1991) in the plant, and can be detected in pollen and nectar throughout the blooming period (Cutler and Scott-Dupree, 2007). As a consequence, bees can experience chronic exposure to them over long periods of time. While some studies have shown no negative effects from seed-treated crops (Nguyen et al., 2009), acute mortality was the only response measured. Desneux and his colleagues (2007) examined methods that could be used to more accurately assess the risk of neonicotinoid and phenylpyrazole insecticides including a test on honey bee larvae reared in vitro to test for larval effects (Aupinel et al., 2005), a proboscis extension response (PER) assay to access associative learning disruption (Decourtye and Pham-Delegue, 2002), various behavioral effects (Thompson, 2003), and chronic exposure toxicity beyond a single acute dose exposure (Suchail et al., 2001; Decourtye et al., 2005; Ailouane et al., 2009). Pesticide exposure may interact with pathogens to harm honey bee health. Honey bees that were both treated with imidacoprid and fed *Nosema* spp. spores suffered reduced longevity and reduced glucose oxidase activity (Alaux et al., 2010).

1.3. Registration procedures and risk assessment

In the USA risk assessment related to agrochemical use and registration follow specific guidelines mandated by the Federal Insecticide Fungicide and Rodenticide Act (EPA, 2009a). Despite the importance of honey bees, the effect of pesticide exposure on colony health has not been systematically monitored, and the Environmental Protection Agency (EPA) does not require data on sublethal effects for pesticide registration (NAS, 2007).

For many years, the classical laboratory method for registering pesticides was to determine the median lethal dose (LD_{50}) of the pest insect. In a second step, the effects of pesticides on beneficial arthropods were examined by running LD_{50} tests on the beneficial species to identify products with the lowest non target activity (Croft, 1990; Robertson et al., 2007). The honey bee has often served as a representative for all pollinators in the registration process, though the toxicity of pesticides to non-Apis species may be different (Taséi, 2003; Devillers et al., 2003). In the USA this protocol remains the primary basis for risk assessment in pesticide registration. However, this approach to risk assessment only takes into account the survival of adult honey bees exposed to pesticides over a relatively short time frame (OEPP/EPPO, 1992). In Europe, where the standard procedures do not provide clear conclusions on the harmlessness of a pesticide, additional studies are recommended; however, no specific protocols are outlined (OEPP/EPPO, 1992). Acute toxicity tests on adult honey bees may be particularly ill-suited for the testing of systemic pesticides because of the different route of exposure bees are likely to experience in field applications. Chronic feeding tests using whole colonies may provide a better way to

quantify the effects of systemics (Colin et al., 2004).

Registration review is replacing the EPA's pesticide re-registration and tolerance reassessment programs. Unlike earlier review programs, registration review operates continuously, encompassing all registered pesticides. The registration review docket for imidacloprid opened in December 2008. To better ensure a "level playing field" for the neonicotinoid class as a whole and to best take advantage of new research as it becomes available, the EPA has moved the docket openings for the remaining neonicotinoids on the registration review schedule (acetamiprid, clothianidin, dinotefuran, thiacloprid, and thiamethoxam) to fiscal year 2012 (EPA, 2009b). The EPA's registration review document states that "some uncertainties have been identified since their initial registration regarding the potential environmental fate and effects of neonicotinoid pesticides, particularly as they relate to pollinators (EPA, 2009b)". Studies conducted in Europe in the late 1990s have suggested that neonicotinoid residues can accumulate in pollen and nectar of treated plants and represent a potential risk to pollinators (Laurent and Rathahao, 2003). Adverse effects on pollinators have also been reported in Europe that have further heightened concerns regarding the potential direct and/or indirect role that neonicotinioid pesticides may have in pollinator declines (Suchail et al., 2000). Recently published data from studies conducted in Europe support concerns regarding the persistence of neonicotinoids. While the translocation of neonicotinoids into pollen and nectar of treated plants has been demonstrated, the potential effect that levels of neonicotinoids found in pollen and nectar can have on bees remains less clear. Girolami et al. (2009) report high levels of neonicotinoids from coated seeds in leaf guttation water and high mortality in bees that consume it. While the frequency of guttation drop collection by bees under field conditions is not documented, the authors describe the prolonged availability of high concentrations of neonicotinoids in guttation water as "a threatening scenario that does not comply with an ecologically acceptable situation". The pending EPA review will consider the potential effects of the neonicotinoids on honey bees and other pollinating insects, evaluating acute risk at the time of application and the longer-term exposure to translocated neonicotinoids (EPA, 2009b; Mullin et al., 2010).

2. PESTICIDES USED IN APICULTURE

The *Varroa* mite, *Varroa destructor*, is one of the most serious pests of honey bees in the USA and worldwide. It injures both adult bees and brood, and beekeepers are frequently compelled to use varroacides to avoid colony death (Boecking and Genersch, 2008). Varroacides must be minimally harmful to the bees, while maintaining toxicity to mites, which is a challenge given the sensitivity of honey bees to many pesticides (Atkins, 1992). The varroacides used in the USA can be broadly divided into three categories: synthetic organic, natural product and organic acid pesticides.

2.1. Synthetic organic pesticides

The pyrethroid *tau*-fluvalinate, a subset of isomers of fluvalinate, was the first synthetic varroacide registered for use in honey bee colonies in the USA. It was first registered as a Section 18 (emergency use label, state by state approval) in 1987 (Ellis et al., 1988). The Section 18 label allowed plywood strips to be soaked in an agricultural spray formulation of *tau*-fluvalinate, (Mavrik®), and treatment was made by suspending the strips between brood frames. In 1990 plastic strips impregnated with *tau*-fluvalinate (Apistan®) replaced homemade plywood strips (PAN, 2009) with a Section 3 label (full registration for use in all states). According to the label, a single strip contains 0.7 g *tau*-fluvalinate, as much as 10% of which may diffuse from the plastic strip formulation into hive matrices over the course of an 8 week treatment (Bogdanov et al., 1998; Vita Europe Ltd., 2009). While the agricultural spray formulation of *tau*-fluvalinate (Mavrik®) is no longer legal to use in the USA, its low cost

and history of legal use in beehives make it vulnerable to misuse and may contribute to *tau*-fluvalinate residues detected in beeswax (Bogdanov, 2006; Wallner, 1999; Berry, 2009; Mullin et al., 2010).

As a pyrethroid, *tau*-fluvalinate kills mites by blocking the voltage-gated sodium and calcium channels (Davies et al., 2007). While most pyrethroids are highly toxic to honey bees, *tau*-fluvalinate is tolerated in high concentrations due in large part to rapid detoxification by cytochrome P450 monooxygenases (P450s) (Johnson et al., 2006). However, *tau*-fluvalinate is not harmless to bees and does affect the health of reproductive castes. Queens exposed to high doses of *tau*-fluvalinate were smaller than untreated queens (Haarmann et al., 2002). Drones exposed to *tau*-fluvalinate during development were less likely to survive to sexual maturity relative to unexposed drones, and they also had reduced weight and produced fewer sperm (Rinderer et al., 1999). However, the practical consequence of *tau*-fluvalinate exposure on drones may be limited, as drones exposed to *tau*-fluvalinate produced as many offspring as unexposed drones (Sylvester et al., 1999).

Tau-fluvalinate was initially very effective at controlling *Varroa* mites, but many *Varroa* populations now exhibit resistance (Lodesani et al., 1995). Resistance to *tau*-fluvalinate in *Varroa* is due, at least in part, to a mutation in the voltage-gated sodium channel which confers reduced binding affinity for *tau*-fluvalinate (Wang et al., 2002). Despite diminished effectiveness, *tau*-fluvalinate continues to be used for *Varroa* control in the USA (Elzen et al., 1999; Macedo et al., 2002).

As the efficacy of *tau*-fluvalinate against *Varroa* was beginning to wane, coumaphos, an organophosphate pesticide, was granted Section 18 approval in the USA in 1999 as varroacide (Federal Register, 2000), and as a treatment for the small hive beetle, *Aethina tumida* Murray. Coumaphos is administered as Checkmite+® strips, each containing approximately 1.4 g coumaphos, which are hung between brood frames for 6 weeks. Coumaphos, or its bioactivated oxon metabolite, kills through the inactivation of acetylcholinesterase, thereby interfering with nerve signaling and function. While coumaphos initially proved effective at killing *tau*-fluvalinate resistant *Varroa* populations (Elzen et al., 2000), coumaphos resistant mite populations were found as early as 2001 (Elzen and Westervelt, 2002). The mechanism of resistance to coumaphos in *Varroa* is unknown, though esterase-mediated detoxification may be involved (Sammataro et al., 2005). Resistance likely follows the mechanisms of coumaphos resistance observed in the southern cattle tick, *Boophilus microplus*, which include acetylcholinesterase insensitivity and enhanced metabolic detoxification (Li et al., 2005). Honey bees tolerate therapeutic doses of coumaphos, at least in part, as a consequence of detoxicative P450 activity (Johnson et al., 2009). However, honey bees can suffer negative effects from coumaphos exposure; queens exposed to coumaphos were smaller, suffered higher mortality and were more likely to be rejected when introduced to a colony (Haarmann et al., 2002; Collins et al., 2004; Pettis et al., 2004). Drone sperm viability was lower in stored sperm collected from drones treated with coumaphos (Burley et al., 2008).

Amitraz, a formamidine pesticide, was once registered (1992–Section 18 label) in the USA under the trade name Miticur® with the active ingredient incorporated in a plastic strip that was suspended between brood frames (PAN, 2009). However, the product was withdrawn from the market in 1994 when some beekeepers reported colony losses following treatment (PAN, 2009). While no conclusive evidence was presented that the product had harmed bees, the registrant decided to withdraw the product from the market (PAN, 2009). Amitraz is available in the USA as a veterinary miticide (Taktic®), but the label does not allow for use in honey bee colonies; however, the frequency with which amitraz metabolites are found in beeswax suggests that it continues to be used (Mullin et al., 2010; Berry, 2009). Amitraz strips (Apivar®) were granted an emergency registration for *Varroa* control by the Canadian PMRA for 2009 (PMRA, 2009), but they are not available to beekeepers in the USA.

Amitraz is an octopaminergic agonist in arthropods (Evans and Gee, 1980) and as such has the potential to influence honey

bee behavior. High levels of octopamine in the honey bee brain are associated with increased foraging behavior and young bees which are fed octopamine are more likely to begin foraging than untreated bees (Schulz and Robinson, 2001). Forager honey bees treated with octopamine increased the reported resource value when communicating via the dance language (Barron et al., 2007). Amitraz has also shown acute toxicity, with larvae showing increased apoptotic cell death in the midgut when exposed to an amitraz solution (Gregorc and Bowen, 2000).

Despite the status of amitraz as an unregistered varroacide, *Varroa* mite populations in the USA exhibit resistance to amitraz, possibly through elevated esterase-mediated detoxification (Sammataro et al., 2005). The mechanism of *Varroa* resistance may be similar to the detoxicative resistance to amitraz that has been observed in some populations of Southern cattle ticks (Li et al., 2004).

Fenpyroximate is a pyrazole acaricide that was introduced for use in the USA in 2007 as Hivastan® under Section 18 registration (Wellmark, 2009). Hivistan® is formulated as a patty containing 675 mg of fenproximate. Fenpyroximate presumably kills mites through the inhibition of electron transport in the mitochondria at complex I, thereby interfering with energy metabolism (Motoba et al., 1992). While resistance to fenpyroximate in *Varroa* has not yet been observed, the eventual emergence of resistance is likely as it has been observed in other mites including the 2-spotted spider mite (*Tetranychus urticae*) which achieved resistance through elevated detoxicative P450 and esterase activity (Kim et al., 2004). The mechanism of tolerance to fenpyroximate in honey bees is unknown, but it is likely through the same detoxicative mechanisms, P450-mediated hydroxylation followed by transesterification, that occurs in vertebrates and other insects (Motoba et al., 2000). One potential consequence of chronic exposure to fenpyroximate, as an inhibitor of complex I mitochondrial activity, is the increased generation of reactive oxygen species (Sherer et al., 2007). Increased adult mortality has been observed with fenpyroximate use during the first days after application (CDPR, 2008).

2.2. Natural product pesticides

Natural product based varroacides have come into widespread use as synthetic pesticides have dwindled in effectiveness. Thymol and menthol, monoterpenoid constituents of plant-derived essential oils, are used for control of *Varroa* and tracheal mites, respectively. Thymol is the chief constituents in the fumigants Apilife Var® (tablets) and Apiguard® (gel), both of which are registered under Section 3. Essential oil-based varroacides were exempted from extensive testing for EPA registration because they are common food additives and "generally recognized as safe" for human consumption (Quarles, 1996). However, monoterpenoids such as thymol and menthol may not necessarily be safe for honey bees, since these compounds play a role in plants as broad spectrum pesticides (Isman, 2006). Indeed, thymol and menthol were found to be among the most toxic of all terpenoids tested when applied to honey bees as a fumigant (Ellis and Baxendale, 1997). These monoterpenoids likely kill *Varroa* by binding to octopamine (Enan, 2001) or GABA receptors (Priestley et al., 2003). Despite being naturally derived, these compounds may harm honey bees: thymol treatment can induce brood removal (Marchetti and Barbattini, 1984; Floris et al., 2004) and may result in increased queen mortality (Whittington et al., 2000).

2.3. Organic acid pesticides

Two organic acids, formic acid and oxalic acid, are attractive options as varroacides because both are naturally present in honey (Bogdanov, 2006; Rademacher and Harz, 2006). Formic acid is registered with Section 3 approval in the USA under the trade name MiteAway II® (NOD, 2009). MiteAway II® is a fumigant varroacide that is packaged in a slow release pad. Formic acid likely kills *Varroa* by inhibiting electron transport in the mitochondria through binding of cytochrome c

oxidase, thereby inhibiting energy metabolism (Keyhani and Keyhani, 1980) and may produce a neuroexcitatory effect on arthropod neurons (Song and Scharf, 2008). Formic acid can harm honey bees by reducing worker longevity (Underwood and Currie, 2003) and harming brood survival (Fries, 1991).

Oxalic acid is registered for use as a varroacide in Canada and Europe, but not in the USA. In Canada it is trickled over honey bees in a sugar syrup solution (Canadian Honey Council, 2005) or sublimated using a vaporizer (Varrox, 2007). Research has shown it to be highly effective against *Varroa* in cool climates when brood is not present (Aliano and Ellis, 2008). The mode of action of oxalic acid against *Varroa* is unknown, but direct contact between *Varroa* and oxalic acid is required (Aliano and Ellis, 2008). Oxalic acid treatments administered in water are ineffective (Charrière and Imdorf, 2002), but administration in sugar water improves efficacy by adhering the active ingredient to the bees (Aliano and Ellis, 2008). In mammals, oxalic acid interferes with mitochondrial electron transport, probably through interaction with complex II or IV, leading to increased production of reactive oxygen species and to kidney toxicity (Cao et al., 2004; Meimaridou et al., 2005). Repeated treatment of colonies with oxalic acid can result in higher queen mortality and a reduction in the amount of sealed brood (Higes et al., 1999). The midguts of honey bees fed oxalic acid in sugar water exhibited an elevated level of cell death (Gregorc and Smodisskerl, 2007), though in field conditions bees will generally avoid consuming syrup with oxalic acid (Aliano and Ellis, 2008).

Oxalic acid is readily available and inexpensive in the USA for use as a wood bleach, but it is not labeled for use in controlling *Varroa*. Its easy availability from many sources has limited the willingness of suppliers to undergo the expensive and time-consuming registration process.

2.4. Interactions

With the large number of varroacides available to beekeepers in the USA, there is potential for interactions between compounds, a problem compounded by the fact that many synthetic varroacides are lipophilic and may remain in the wax component of hives for years following treatment (Bogdanov et al., 1997; Wallner, 1999; Mullin et al., 2010). The overlapping modes of action and mechanisms of tolerance in honey bees are also cause for concern. Interactions have been observed between *tau*-fluvalinate and coumaphos at the level of P450 detoxification (Johnson et al., 2009), and it seems likely that all varroacides depending on P450-mediated detoxification will display similar interactions. Fenpyroximate and the organic acids all interact with components of the mitochondrial electron transport chain (Keyhani and Keyhani, 1980; Motoba et al., 1992), where interactions could also be possible. Synergistic interactions between formamadines and pyethroids occur in other insects (Plapp, 1979) and may occur in honey bees between amitraz and *tau*-fluvalinate.

Interactions between in-hive varroacides and out-of-hive insecticides and fungicides are also of concern, particularly interactions between the P450-detoxified varroacides and the P450-inhibiting ergosterol biosynthesis inhibiting fungicides (Pilling et al., 1995).

3. PESTICIDE RESIDUES IN HIVE PRODUCTS

3.1. Need for sensitive pesticide analysis

Pesticide contamination of hive products is expected when honey bee colonies perish due to pesticide exposure. Colony mortality is often accompanied by part-per-million (ppm) residues in wax, beebread, honey and dead bee samples. However, part-per-billion (ppb) and occasionally ppm residues levels can be detected in hive matrices when honey bees forage in any conventional agricultural or urban setting. Since honey or pollen contaminated at ppb levels with newer classes of insecticides such as neonicotinoids (e.g. imidacloprid) or phenylpyrazoles (e.g. fipronil) are known to impair honey bee health (Decourtye et al., 2004; Halm et al., 2006; Desneux et al.,

2007), it is important to use sensitive analytical technologies. Many pesticide contaminants, such as lipophilic pyrethroids and organophosphates, can be monitored in the hive using gas chromatography-mass spectrometry (GC-MS). The more recently developed liquid chromatography-tandem mass spectrometry (LC/MS-MS) analytical capability is essential for newer systemic pesticides, particularly the neonicotinoids (Bonmatin et al., 2005; Chauzat et al., 2006). Older systemic residues, like the toxic sulfur-oxidation metabolites of aldicarb, many modern fungicides and herbicides, and the polar degradates of newer fungicides and herbicides cannot be analyzed at ppb limits of detection without LC/MS-MS (Alder et al., 2006).

During the last decade, some European beekeepers have reported heavy losses of honey bee colonies located near crops treated with the neonicotinoid imidacloprid (Rortais et al., 2005). Although Bonmatin et al. (2005) and Chauzat et al. (2006) found low ppb levels of the imidacloprid in a high percentage of pollen samples collected from maize, sunflower and canola, when pesticide residues from all matrices were pooled together, analysis did not show a significant relationship between the presence of pesticide residues and the abundance of brood and adults, and no statistical relationship was found between colony mortality and pesticide residues (Chauzat et al., 2009). Another way to associate pesticides and honey bee mortality is to examine dead bees, but obtaining samples can be difficult if bees die away from the hive as it is necessary to use recently dead or dying bees (Johansen and Mayer, 1990).

3.2. Major incidences of pesticide residues in the beehive

This review will focus on pesticide residues studies done during the past 20 years. Smith and Wilcox (1990) report residues found in beehives in the USA prior to the period covered by this review. Over 150 different pesticides have been found in colony samples (Mullin et al., 2010). In recent years, the highest residues of pesticides in colonies are from varroacides that accumulate in the wax (Mullin et al., 2010). Varroacides found in beeswax, pollen and bee bread include amitraz, bromopropylate, coumaphos, flumethrin and *tau*-fluvalinate. Residue levels of these varroacides generally increase from honey to pollen to beeswax (Lodesani et al., 1992; Wallner, 1995; Bogdanov et al., 1998; Bogdanov, 2004; Tremolada et al., 2004; Martel et al., 2007; Frazier et al., 2008).

Varroacide residues in honey have been found to reach as high as 2.4 ppm acrinathrin, based on its 3-phenoxybenzaldehyde degradate (Bernal et al., 2000), 0.6 ppm of amitraz (Mullin et al., 2010), 2 ppm of coumaphos (Martel et al., 2007), and 0.75 ppm fluvalinate (Fernandez et al., 2002). Bee bread was also found to be contaminated with up to 1.1, 0.01, 5.8 and 2.7 ppm, respectively of amitraz, bromopropylate, coumaphos and fluvalinate (vanEngelsdorp et al., 2009b; Mullin et al., 2010). Levels in brood and adult bees can be higher than in the food, with 14 ppm amitraz, 5.9 ppm fluvalinate (vanEngelsdorp et al., 2009b; Mullin et al., 2010), 2.8 ppm of coumaphos (Ghini et al., 2004), and 2.2 ppm bromopropylate (Lodesani et al., 1992) being reported. Nevertheless, wax remains the ultimate sink for these varroacides reaching 46, 94 and 204 ppm, respectively, of amitraz, coumaphos and fluvalinate (vanEngelsdorp et al., 2009b; Mullin et al., 2010), 135 ppm of bromopropylate (Bogdanov et al., 1998), and 7.6 and 0.6 ppm, respectively, of the miticides chlorfenvinophos and acrinathrin (Jimenez et al., 2005).

Pesticide residues of agrochemicals acquired by foragers are equivalent or higher in pollen (stored and trapped at the hive entrance), adult bees and occasionally honey, than in wax. Major pollen detections include the insecticides aldicarb (1.3 ppm), azinphos methyl (0.6 ppm), chlorpyrifos (0.8 ppm), and imidacloprid (0.9 ppm); fungicides boscalid (1 ppm), captan (10 ppm), and myclobutanil (1 ppm), and herbicide pendimethalin (1.7 ppm; Tab. I). The carbamates carbofuran and carbaryl, and the organophosphate parathion methyl have been frequently found at up to 1.4 (Bailey et al., 2005), 94 (cited in Chauzat et al., 2006), and 26 ppm (Rhodes

et al., 1979), respectively. High levels of pyrethroids including cyhalothrin and cypermethrin at 1.7 and 1.9 ppm, respectively, have been reported in mustard pollen along with up to 2.2 and 2.1 ppm, respectively, of the cyclodiene endosulfan and the new lipid-synthesis inhibitor insecticide spiromesifen (Choudhary and Sharma, 2008b).

Fungicides often account for most of the pesticide content of pollen. Unprecedented levels (99 ppm) of the widely-used fungicide chlorothalonil were found in honey bee-collected pollen (Tab. I, Mullin et al., 2010). Chorothalonil, a contact and slightly volatile fungicide, was found to be a marker for entombing behavior in honey bee colonies associated with poor health (vanEngelsdorp et al., 2009a). Entombing may be a defensive behavior of honey bees faced with large amounts of potentially toxic food stores. Kubik et al. (1999) noted high residues of the fungicides vinclozolin and iprodione up to 32 and 5.5 ppm, respectively, in beebread.

High residues in the honey bees themselves (Tab. I) are often associated with direct kill from the respective pesticide application, such as with 19.6 ppm of permethrin (LD_{50} of 1.1 ppm) and 3.1 ppm of fipronil (LD_{50} 0.05 ppm) (Mullin et al., 2010). Anderson and Wojtas (1986) linked dead honey bees to high residues of the insecticides carbaryl (5.8 ppm), chlordane (0.7 ppm), diazinon (0.35 ppm), endosulfan (4.4 ppm), malathion (4.2 ppm), methomyl (3.4 ppm), methyl parathion (3.6 ppm), and fungicide captan (1.7 ppm). Walorczyk and Gnusowski (2009) found exceptional amounts of the organophosphates dimethoate (4.9 ppm), fenitrothion (1 ppm), and omethoate (1.2 ppm), and up to 1.2 ppm of the systemic fungicide tebuconazole in honey bees from other poisoning incidences (Tab. I). Similarly, elevated residues of the organophosphates bromophos methyl (1.7 ppm) and fenitrothion (10.3 ppm) were associated with high honey bee mortality (Ghini et al., 2004). In contrast to systemic fungicides, systemic neonicotinoid residues are generally absent from bee samples, although present in pollen and wax.

Notable honey residues in Europe include 0.65, 0.66 and 4.3 ppm, respectively, of carbofuran, DDT, and lindane (Blasco et al., 2003), and 0.6 ppm of methoxychlor (Fernandez-Muino et al., 1995). A recent broad sampling of US honey following reports of CCD (USDA-PDP, 2008) showed only a few detections of low ppb amounts of external pesticides like dicloran and dicofol, but also revealed more frequent low levels of coumaphos and fluvalinate up to 12 ppb. A standard treatment of synthetic piperonyl butoxide-synergized pyrethrum to kill managed and feral honey bees in a hive (Taylor et al., 2007) can leave high residues in both honey (up to 3, 0.6 ppm, respectively) and wax (470 237 ppm).

Very high amounts of the fungicide chlorothalonil (54 ppm) and substantial levels of chlorpyrifos (0.9 ppm), aldicarb (0.7 ppm), deltamethrin (0.6 ppm), and iprodione (0.6 ppm) were found in comb wax (vanEngelsdorp et al., 2009b; Mullin et al., 2010). Elevated levels of the acetylcholinesterase-inhibitors azinphos methyl (0.8 ppm), fenitrothion (0.5 ppm, Chauzat and Faucon, 2007), carbaryl (0.8 ppm), parathion methyl (3.1 ppm, Russell et al., 1998), and malathion (6 ppm, Thrasyvoulou and Pappas, 1988) have been reported. Bogdanov et al., (2004) detected up to 60 ppm of *p*-dichlorobenzene and Jimenez et al., (2005) up to 0.6 ppm of the miticide tetradifon in beeswax.

3.3. A recent survey found a high diversity of pesticides in beehive samples

The recent phenomenon of CCD triggered a close look at the role of pesticides as a possible contributing factor to honey bee decline in general and CCD specifically. Mullin and Frasier used LC/MS-MS and GC/MS and a modified QuEChERS method to analyze for pesticide residues in honey bees and hive matrices in the USA and Canada to examine colonies exhibiting CCD symptoms (vanEngelsdorp et al., 2009b; Frazier et al., 2008). One hundred twenty-one different pesticides and metabolites were found within 887 wax, pollen, bee and associated hive samples (average of 6.2 detections per sample)

Table I. Maximum pesticide incidence in apiary samples of wax, pollen, bee and honey.

Total pesticide[a]	CLASS[b]	Maximum detection in ppb (*ref.*)[c]			
		Wax	Pollen	Bee	Honey
Acephate	S OP	n.d.	163	n.d.	52
Acetamiprid	S NEO	n.d.	134	n.d.	n.d.
Acrinathrin	PYR	590 (*1*)	–	–	2400 (*2*)
Aldicarb	S CARB	693	1342	n.d.	n.d.
Aldrin	CYC	5 (*3*)	–	–	150 (*4*)
Allethrin	PYR	139	11	24	n.d.
Amicarbazone	HERB	n.d.	98	n.d.	n.d.
Amitraz	FORM	46 060	1117	13780	555
Atrazine	S HERB	31	49	15	81 (*5*)
Azinphos ethyl	OP	–	–	94 (*6*)	–
Azinphos methyl	OP	817 (*7*)	643	91(*6*)	n.d.
Azoxystrobin	S FUNG	278	107	n.d.	4 (*27*)
Bendiocarb	S CARB	22	n.d.	n.d.	n.d.
Bifenthrin	PYR	56	13	12	3
Bitertanol	S FUNG	–	–	–	0.1 (*8*)
Boscalid	S FUNG	388	962	33 (*9*)	n.d.
Bromophos ethyl	OP	–	–	–	12 (*10*)
Bromophos methyl	OP	–	–	1733 (*6*)	–
Bromopropylate	MITI	135 000 (*11*)	11	2245 (*12*)	245 (*12*)
Captan	PS FUNG	400 (*13*)	10 000	1740 (*13*)	19 (*14*)
Carbaryl	PS CARB	820 (*13*)	94 000 (*15*)	5800 (*13*)	42
Carbendazim	S FUNG	133	149	14	27 (*27*)
Carbofuran	S CARB	22	1400 (*16*)	669 (*6*)	645 (*17*)
Carfentrazone ethyl	PS HERB	17	3	n.d.	n.d.
Chlordane	CYC	60 (*13*)	–	690 (*13*)	–
Chlorfenapyr	PS MITI	12	1	3	n.d.
Chlorfenvinphos	OP	7620 (*1*)	11	n.d.	0.2 (*18*)
Chlorothalonil	FUNG	53 700	98 900	878	10 (*19*)
Chlorpyrifos	OP	890	830	57 (*6*)	15 (*5*)
Chlorpyrifos methyl	OP	–	–	36 (*6*)	0.2 (*18*)
Clothianidin	S NEO	n.d.	2.6 (*20*)	n.d.	0.9 (*20*)
Coumaphos	OP	94 131	5828	2777 (*6*)	2020 (*21*)
Cyfluthrin	PYR	45	34	14	9 (*19*)
Cyhalothrin	PYR	17	1672 (*22*)	2	0.8 (*23*)
Cymiazole	MITI	–	–	–	17 (*24*)
Cypermethrin	PYR	131	1900 (*15*)	26	92 (*5*)
Cyproconazole	S FUNG	–	8 (*15*)	–	–
Cyprodinil	S FUNG	106	344	19	n.d.
DDT-p,p′	OC	>40	45	7	658 (*17*)
Deltamethrin	PYR	613	91	39	7 (*23*)
Dialifos	OP	–	–	–	92 (*4*)
Diazinon	OP	4	29	350 (*13*)	35 (*24*)
p-Dichlorobenzene	OC	60 000 (*25*)	n.d.	n.d.	112 (*25*)
Dichlofluanid	FUNG	–	–	–	11 (*26*)
Dichlorvos	OP	–	–	–	8 (*19*)
Dicloran	FUNG	–	–	–	2 (*27*)
Dicofol	OC	21	143	4	90 (*27*)
Dieldrin	CYC	35	n.d.	12	13 (*4*)
Difenoconazole	S FUNG	n.d.	411 (*14*)	n.d.	0.9 (*14*)
Diflubenzuron	IGR	n.d.	128	n.d.	n.d.
Dimethomorph	S FUNG	133	166	56	n.d.
Dimethoate	S OP	–	–	4864 (*9*)	9 (*23*)
Diphenamid	S FUNG	n.d.	1	n.d.	n.d.
Diphenylamine	FUNG	n.d.	32	n.d.	n.d.
Endosulfan	CYC	800 (*13*)	2224 (*22*)	4400 (*13*)	24 (*5*)
Endrin	CYC	–	–	–	7 (*4*)
Esfenvalerate	PYR	56	60	9	0.7 (*23*)
Ethion	OP	131	n.d.	n.d.	n.d.
Ethofumesate	S HERB	560	n.d.	n.d.	n.d.

Table I. Continued.

Total pesticide[a]	CLASS[b]	Maximum detection in ppb (*ref.*)[c]			
		Wax	Pollen	Bee	Honey
Etoxazole	MITI	n.d.	n.d.	n.d.	1
Famoxadone	FUNG	n.d.	141	n.d.	n.d.
Fenamidone	FUNG	138	74	n.d.	n.d.
Fenbuconazole	S FUNG	183	264	n.d.	n.d.
Fenhexamid	FUNG	9	129	n.d.	n.d.
Fenitrothion	OP	511 (*7*)	–	10 330 (*6*)	–
Fenoxaprop-ethyl	S HERB	n.d.	n.d.	15	n.d.
Fenoxycarb	IGR	–	–	157 (*6*)	–
Fenpropathrin	PYR	200	170	37	n.d.
Fenthion	S OP	–	–	38 (*6*)	–
Fipronil	INS	36	29	3060	n.d.
Fluoxastrobin	S FUNG	45	n.d.	n.d.	n.d.
Fluridone	S HERB	7	24	7	n.d.
Flusilazole	S FUNG	–	71 (*15*)	–	0.03 (*8*)
Flutolanil	S FUNG	105	n.d.	n.d.	n.d.
Flumethrin	PYR	50 (*28*)	–	–	1 (*28*)
Fluvalinate	PYR	204 000	2670	5860	750 (*24*)
Fonofos	OP	–	–	–	15 (*10*)
Heptachlor	CYC	31	2	n.d.	57 (*4*)
Heptenophos	S OP	–	–	162 (*6*)	230 (*17*)
Hexachlorobenzene	FUNG	1	1	n.d.	270 (*17*)
Hexaconazole	S FUNG	–	12 (*15*)	–	–
Imidacloprid	S NEO	14	912	n.d.	2 (*29*)
Indoxacarb	INS	n.d.	330	n.d.	n.d.
Iprodione	FUNG	636	5511 (*30*)	n.d.	266 (*30*)
Lindane	OC	290 (*1*)	7 (*29*)	11 (*29*)	4310 (*17*)
Malathion	OP	6000 (*31*)	61	4200 (*13*)	243 (*5*)
Metalaxyl	S FUNG	1	n.d.	n.d.	n.d.
Methamidophos	S OP	–	–	38 (*6*)	–
Methidathion	OP	79	33	32	68 (*17*)
Methiocarb	CARB	–	–	346 (*6*)	27 (*17*)
Methomyl	S CARB	140 (*13*)	–	3400 (*13*)	–
Methoxychlor	OC	–	–	–	593 (*4*)
Methoxyfenozide	IGR	495	128	21	3 (*27*)
Metolachlor	PS HERB	n.d.	103	n.d.	n.d.
Metribuzin	S HERB	8	44	n.d.	n.d.
Myclobutanil	S FUNG	n.d.	981	n.d.	n.d.
Norflurazon	S HERB	38	108	n.d.	n.d.
Omethoate	S OP	–	–	1156 (*9*)	–
Oxamyl	S CARB	n.d.	43	n.d.	n.d.
Oxyfluorfen	HERB	34	5	5	n.d.
Parathion ethyl	OP	99 (*7*)	19 (*15*)	5 (*6*)	–
Parathion methyl	OP	3085 (*32*)	26 000 (*33*)	3600 (*13*)	50 (*33*)
Penconazole	S FUNG	–	126 (*15*)	8 (*29*)	–
Pendimethalin	HERB	84	1730	28	n.d.
Permethrin	PYR	372	92	19 600	11 (*27*)
Phenothrin	PYR	n.d.	84	n.d.	n.d.
Phenthoate	OP	–	–	1 (*6*)	–
Phorate	S OP	–	–	–	0.9 (*18*)
Phosalone	OP	n.d.	31	66 (*9*)	n.d.
Phosmet	OP	209	418	96 (*6*)	n.d.
Phosphamidon	S OP	–	–	50 (*6*)	–
Phoxim	OP	–	–	355 (*6*)	–
Piperonyl butoxide	SYN	470 000 (*34*)	n.d.	3000 (*34*)	10 (*27*)
Pirimiphos ethyl	OP	–	–	30 (*6*)	–
Pirimiphos methyl	OP	57	n.d.	62	19 (*10*)
Prallethrin	PYR	7	8	9	n.d.
Prochloraz	FUNG	–	–	412 (*9*)	–
Procymidone	S FUNG	28 (*7*)	–	–	–

Table I. Continued.

Total pesticide[a]	CLASS[b]	Maximum detection in ppb (*ref.*)[c]			
		Wax	Pollen	Bee	Honey
Profenofos	OP	–	–	17 (*6*)	–
Pronamide	S HERB	23	378	2	n.d.
Propanil	HERB	n.d.	358	n.d.	n.d.
Propiconazole	S FUNG	227	361	n.d.	n.d.
Pyraclostrobin	FUNG	438	265	9	17
Pyrazophos	S OP	–	–	53 (*6*)	6 (*10*)
Pyrethrins	PYR	237 000 (*34*)	62	600 (*34*)	n.d.
Pyridaben	MITI	5	27	n.d.	n.d.
Pyrimethanil	FUNG	28	83	n.d.	4
Pyriproxyfen	IGR	8	n.d.	n.d.	n.d.
Quinalphos	OP	–	–	70 (*6*)	10 (*23*)
Quintozene = PCNB	FUNG	3	n.d.	n.d.	n.d.
Sethoxydim	S HERB	n.d.	173	n.d.	8
Simazine	S HERB	n.d.	54	n.d.	17 (*5*)
Spinosad	INS	–	320 (*16*)	–	–
Spirodiclofen	MITI	29	2	n.d.	n.d.
Spiromesifen	S INS	n.d.	2101 (*22*)	n.d.	n.d.
Tebuconazole	S FUNG	n.d.	34	1146 (*9*)	5 (*5*)
Tebufenozide	IGR	28	58	23	n.d.
Tebuthiuron	S HERB	22	48	n.d.	n.d.
Tefluthrin	PYR	3	n.d.	n.d.	n.d.
Temephos	OP	–	–	689 (*6*)	7 (*10*)
Tetradifon	MITI	580	n.d.	n.d.	19 (*19*)
Tetraconazole	S FUNG	–	–	17 (*29*)	–
Tetramethrin	PYR	n.d.	6	23	n.d.
Thiabendazole	S FUNG	76	6	n.d.	n.d.
Thiacloprid	S NEO	8	115	n.d.	33
Thiamethoxam	S NEO	n.d.	53	n.d.	n.d.
Triadimefon	S FUNG	2	n.d.	n.d.	n.d.
Triallate	HERB	–	–	–	4 (*26*)
Triazophos	OP	–	–	9 (*6*)	–
Tribufos = DEF	SYN	59	4	n.d.	n.d.
Trifloxystrobin	PS FUNG	22	264	n.d.	0.3 (*8*)
Trifluralin	HERB	36	14	n.d.	9 (*19*)
Vamidothion	S OP	–	–	24 (*6*)	–
Vinclozolin	FUNG	27	31 909 (*30*)	657 (*9*)	173 (*30*)

[a] Acrinathrin is based mostly on 3-phenoxybenzaldehyde degradate, Aldicarb based on sulfoxide and sulfone metabolites; Amitraz based on total DMA and DMPF metabolites; Bromopropylate based on 4,4-dibromobenzophenone; Captan includes THPI; Carbaryl includes 1-naphthol; Carbendazim is also a degradate of benomyl; Carbofuran based on parent plus 3-hydroxy metabolite; Coumaphos includes oxon, chlorferone and potasan; DDT includes DDD and DDE; Endosulfan includes isomers and sulfate; Heptachlor includes heptachlor epoxide; Imidacloprid includes 5-hydroxy and olefin metabolites; Thiabendazole is a degradate of thiophanate methyl.

[b] Class: CAR = carbamate, CYC = cyclodiene, FORM = formamidine, FUNG = fungicide, HERB = herbicide, IGR = insect growth regulator, INS = misc. insecticide, MITI = miticide, NEO = neonicotinoid, OC = organochlorine, OP = organophosphate, PS = partial systemic, PYR = pyrethroid, S = systemic, SYN = synergist.

[c] Data from Frazier et al. (2008); vanEngelsdorp et al. (2009b); or Mullin et al. (2010); unless otherwise referenced; n.d. = not detected.

1 Jimenez et al. (2005); *2* Bernal et al. (2000); *3* Estep et al. (1977); *4* Fernandez-Muino et al. (1995); *5* Rissato et al. (2007); *6* Ghini et al. (2004); *7* Chauzat and Faucon (2007); *8* Nguyen et al. (2009); *9* Walorczyk et al. (2009); *10* Blasco et al. (2008); *11* Bogdanov et al. (1998); *12* Lodesani et al. (1992); *13* Anderson and Wojtas (1986); *14* Kubik et al. (2000); *15* Chauzat et al. (2006); *16* Bailey et al. (2005); *17* Blasco et al. (2003); *18* Balayiannis and Balayiannis (2008); *19* Rissato et al. (2004); *20* Cutler and Scott-Dupree (2007); *21* Martel et al. (2007); *22* Choudhary and Sharma (2008b); *23* Choudhary and Sharma (2008a); *24* Fernandez et al. (2002); *25* Bogdanov et al. (2004); *26* Albero et al. (2004); *27* USDA-AMS (2009); *28* Bogdanov (2006); *29* Chauzat et al. (2009); *30* Kubik et al. (1999); *31* Thrasyvoulou and Pappas (1988); *32* Russell et al. (1998); *33* Rhodes et al. (1979); *34* Taylor et al. (2007).

from migratory and stationary beekeepers. These included 16 parent pyrethroids, 13 organophosphates, 4 carbamates, 4 neonicotinoids, 4 insect growth regulators, 3 chlorinated cyclodienes, 3 organochlorines, 1 formamidine, 8 miscellaneous miticides/insecticides, 2 synergists, 30 fungicides, and 17 herbicides. Over 40 of the pesticides detected are systemic (Tab. I). Only one of the wax samples, 3 pollen samples and 12 bee samples had no detectable pesticides.

Overall, pyrethroids and organophosphates dominated total wax and bee residues followed by fungicides, systemics, carbamates, and herbicides, whereas fungicides prevailed in pollen followed by organophosphates, systemics, pyrethroids, carbamates, and herbicides. By comparing these residue levels across the matrices, in-hive varroacides were more concentrated in wax than in pollen, whereas externally-derived pesticides were higher or equivalent in pollen compared to wax. This is consistent with chronic use and long-term accumulation of these lipophilic varroacides in the wax as a source to contaminate pollen.

All foundation, beeswax pressed into sheets and used as templates for comb construction, sampled from North America is uniformly contaminated with *tau*-fluvalinate, coumaphos and lower amounts of other pesticides and metabolites (Mullin et al., 2010). The broad contamination of European foundation, especially with varroacides, has been reviewed previously (Wallner, 1999; Bogdanov, 2004; Lodesani et al., 2008). The uniform presence of these acaricides in foundation is particularly disturbing since replacement of frames is the main avenue currently used to purge a colony of accumulated pesticide contaminants. Fluvalinate residues in beeswax were the best correlative with the French honey bee winter kill of 1999–2000 (Faucon et al., 2002), although disease factors were emphasized in the report.

High levels of the pyrethroid fluvalinate and the organophosphate coumaphos are co-occuring with lower but significant levels of 119 other insecticides, fungicides and herbicides in hive matrices. Fluvalinate and coumaphos, but not amitraz, are highly persistent in the hive with an estimated half-life in beeswax of 5 years (Bogdanov, 2004). Chronic exposure to high levels of these persistent neurotoxicants elicits both acute and sublethal reductions in honey bee fitness (Stoner et al., 1985; Lodesani and Costa, 2005). The direct association of any one of these varroacides with CCD remains unclear, although higher coumaphos levels may benefit the colony presumably via mite control (vanEngelsdorp et al., 2009b).

Externally-derived, highly toxic pyrethroids were the most frequent and dominant class of insecticides samples (Mullin et al., 2010). Contact pyrethroids, and systemic neonicotinoids and fungicides are often combined as pest control inputs, and many of the latter may synergize the already high toxicity of neonicotinoids and pyrethroids to honey bees (Pilling and Jepson, 1993; Iwasa et al., 2003). Pyrethroids frequently are associated with honey bee kills (Mineau et al., 2008), as has been the case with neonicotinoids (Halm et al., 2006), although the latter with less documentation of acute residues in bees. The effects of toxic chronic exposure to pyrethroids, organophosphates, neonicotinoids, fungicides and other pesticides can range from lethal and/or sub-lethal effects in the larvae and workers to reproductive effects on the queen (Thompson, 2003; Desneux et al., 2007). These chemicals may act alone or in concert, in ways currently unknown, to create a toxic environment for the honey bee.

4. CONCLUSIONS

The widespread planting of transgenic crops appears to be a net benefit for honey bees in the USA, since the pesticidal toxins produced by these plants do not appear to harm honey bees. Additionally, such crops do not require as many applications of traditional pesticides, most of which are known to be toxic to bees.

The systemic nature of the neonicotinoids and phenylpyrazoles present a trade-off from the standpoint of honey bee health. New methods of application help to minimize direct exposure of bees to these compounds during application. The downside is that honey bees

may instead be exposed to these pesticides, or their metabolites, in pollen, nectar, and plant exudates over extended periods of time. Further research is needed to assess the true dangers posed by extended low dose exposure to these systemic pesticides.

Beekeepers searching for the primary source of pesticides contaminating bee hives need only to look in a mirror. Unfortunately, the regulatory system governing the veterinary use of pesticides in bee hives in the USA may be perversely contributing to the problem. Two of the handful of pesticides registered for legal use in the USA, coumaphos (CheckMite+®) and *tau*-fluvalinate (Apistan®), both of which seriously contaminate wax, have become largely useless against the primary pest of honey bees, the *Varroa* mite. Another effective varroacide used in Europe and Canada, oxalic acid, is not registered in the USA because it is low in cost, readily available, and potential registrants are deterred by the cost of the registration process. There are 3 registered in-hive pesticides that provide effective *Varroa* control in the USA, fenpyroximate (Hivistan®), formic acid (Miteaway II®) and thymol (ApiGuard® and Api-Life Var®). Other effective pesticides, including amitraz and oxalic acid, are used by some beekeepers in the absence of any regulatory approval. A change in the regulatory system needs to occur to make effective and safe veterinary pesticides available to beekeepers and to spur research into the effects of candidate compounds on honey bee health. Likewise, beekeepers need to realize that honey bee pests and parasites are community problems, as well as individual problems, and that pesticide labels are crafted to protect the sustainability of pesticides. The use of unregistered products is a serious threat to the beekeeping community and should not occur.

Honey bees are being exposed to high levels of in-hive varroacides and agrochemical pesticides. Chronic exposures to neurotoxic insecticides and their combinations with other pesticides, in particular fungicides, are known to elicit reductions in honey bee fitness, but direct association with CCD and declining honey bee health remains to be resolved.

ACKNOWLEDGEMENTS

We gratefully recognize the major input of Dennis vanEngelsdorp, Pennsylvania Department of Agriculture, and Jeffery S. Pettis, USDA-ARS, for providing samples for the CCD analyses reviewed here; Roger Simonds, USDA-AMS National Science Laboratory, Gastonia, NC for conducting the pesticide analyses; and of Jim Frazier, Sara Ashcraft (PSU), Lizette Peters and Alex Heiden (UNL) for technical support. We thank the National Honey Board, the Florida State Beekeepers, the Tampa Bay Beekeepers, Penn State College of Agriculture Sciences, Preservation *Apis mellifera* (PAm), The Foundation for the Preservation of Honey Bees, the USDA Critical Issues Program, the USDA CAPS Program, and the EPA for the financial support that made some of the work included in this review possible.

Pesticides et toxicité chez l'abeille – USA.

pesticides / abeille / toxicité / résidus dans la cire / CCD / USA

Zusammenfassung – Pestizide und Toxizität für Honigbienen – USA. Neuere systemisch wirkende Pestizide, einschließlich der Neonikotinoide (z. B. Imidacloprid) und Phenylpyrazole (z. B. Fipronil) finden in den USA verbreitete Anwendung im Pflanzenschutz. Das Gefährdungspotenzial von Bienen durch diese Präparate unterscheidet sich von dem traditioneller Pestizidanwendungen, bei denen die hauptsächliche Sorge der akuten Giftigkeit galt. Im Hinblick auf die Verordnungen zu Pestiziden in den USA wurden die Folgen von chronischer und sublethaler Belastung durch systemische Mittel bisher nicht umfassend in Betracht gezogen, obwohl die Sachlage, was diese Präparate betrifft, gegenwärtig von der Umweltbehörde (EPA) begutachtet wird. Zahlreiche in den USA angebaute Pflanzen wurden genetisch verändert, um entweder insektizid wirkende Bt Toxine oder Herbizidresistenz zu exprimieren. Insektizid wirkende Bt Toxine scheinen jedoch spezifisch toxisch für Ertragsschädlinge zu sein und können daher den Bienen nützen, indem sie die Anwendung traditioneller Pestizide reduzieren.
Bis zur Einführung von arthropoden Bienenschädlingen in die USA in der Mitte der achtziger Jahre wurden Bienen den verschiedenen Pestiziden nur unbeabsichtigt ausgesetzt, während sie auf gespritzten Pflanzen sammelten. Die Notwendigkeit, Bienenschädlinge, besonders die Varroamilbe (*Varroa destructor*), zu bekämpfen, erfordert seitdem jedoch oft eine absichtliche Anwendung von Pestiziden in Bienenvölkern. Tau-Fluvalinat und

Coumaphos, jeweils in Streifenform angewendet, sind in den USA immer noch für die Anwendung in Bienenvölkern zugelassen, obwohl die Wirksamkeit dieser Substanzen gegen Varroamilben durch die Entwicklung von Resistenzen vermindert wurde. Ein neues Varroazid, Fenpyroximate, wurde in einigen Staaten zur Anwendung zugelassen. Essentielle Öle, einschließlich Thymol und Menthol, sind ebenso wie Ameisensäure zur Anwendung in der Verdampfung zugelassen. Oxalsäure ist nur in Kanada, jedoch nicht in den USA zugelassen.

Über 150 verschiedene Pestizide wurden in Proben aus Bienenständen in den USA gefunden. Von Imkern eingesetzte Pestizide werden tendenziell öfter im Wachs der Völker nachgewiesen, von wo aus Pollen, Bienenbrot und Honig damit kontaminiert werden. Auf der anderen Seite werden Pestizide, vor allem Fungizide, die nicht in Bienenvölkern eingesetzt werden, tendenziell am häufigsten in Pollen nachgewiesen und kontaminieren das Wachs nur dann, wenn sie eingelagert werden. Da Honigbienen den sublethalen Konzentrationen zahlreicher Pestizide gleichzeitig ausgesetzt sind, wird zusätzliche Forschung zur Aufklärung synergistischer Effekte bei chronischer sublethaler Belastung mit mehreren Pesitziden benötigt.

Pestizide / Honigbiene / Toxizität / Wachsrückstände / CCD

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