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Are bee diseases linked to pesticides? – A brief review



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ABSTRACT

The negative impacts of pesticides, in particular insecticides, on bees and other pollinators have never been disputed. Insecticides can directly kill these vital insects, whereas herbicides reduce the diversity of their food resources, thus indirectly affecting their survival and reproduction. At sub-lethal level (<LD50), neurotoxic insecticide molecules are known to influence the cognitive abilities of bees, impairing their performance and ultimately impacting on the viability of the colonies. In addition, widespread systemic insecticides appear to have introduced indirect side effects on both honey bees and wild bumblebees, by deeply affecting their health. Immune suppression of the natural defences by neonicotinoid and phenyl-pyrazole (fipronil) insecticides opens the way to parasite infections and viral diseases, fostering their spread among individuals and among bee colonies at higher rates than under conditions of no exposure to such insecticides. This causal link between diseases and/or parasites in bees and neonicotinoids and other pesticides has eluded researchers for years because both factors are concurrent: while the former are the immediate cause of colony collapses and bee declines, the latter are a key factor contributing to the increasing negative impact of parasitic infections observed in bees in recent decades.

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1. Introduction

Ever since pesticides were first used to control pests and weeds in agricultural production there was concern about the impact they could have on honey bees (*Apis* spp.), bumblebees (*Bombus* spp.) and other pollinators. On the one hand, since bees are insects, insecticides may harm them as much as they control the target pests. On the other, herbicides reduce the abundance and biodiversity of flowers in agricultural landscapes (Albrecht, 2005; Hald, 1999), and this also has a negative impact on the bees which find their food resources impoverished under intensive agricultural practices (Goulson et al., 2015). In recent years, however, the concern among beekeepers and scientists has shifted to the high prevalence and impact of parasites, viral and microbial diseases, which are blamed as the main culprits in the current worldwide high levels of colony losses in managed honey bees (vanEngelsdorp et al., 2010).

Diseases in bees are nothing new but their spread and adverse effects have been exacerbated by the accidental translocation of pathogens and parasites by man, exposing bees to natural antagonists to which they have no evolved resistance (Goulson and Hughes, 2015). The long history of beekeeping is documented with large losses of honey bee colonies in certain periods (vanEngelsdorp and Meixner, 2010). In this regard the genetic variability within the colony is important for disease resistance, homeostasis, thermoregulation, defence against parasites and overall colony fitness (Tarpy, 2003).

2. Increased prevalence of bee diseases

The question that must be asked is, why have diseases and parasites become more prevalent in recent decades? (Underwood and vanEngelsdorp, 2007; vanEngelsdorp and Meixner, 2010) Until now, the almost unique answer was to be found in the worldwide spread of the mite *Varroa destructor*, which was originally a parasite of the Asian honey bee (*Apis ceranae*), but jumped host to the European honey bee (*Apis mellifera*) in the far East in the middle of the last century, where both species are sympatric (Oldroyd, 1999). Only the Korean haplotype of this mite is of concern (Rosenkranz et al., 2010), and it has spread quickly due to trading practices among countries and continents, and

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also to the movement of hives for crop pollination. Because this parasite is a vector of several bee viruses, including acute bee paralysis virus (ABPV), Israeli paralysis virus (IAPV), Kashmir bee virus (KBV) and deformed wing virus (DWV), the rapid spread of the new parasite throughout the world was deemed sufficient to explain the rise in viral diseases in honey bees (Francis et al., 2013; Genersch et al., 2010; Janke and Rosenkranz, 2009; Rosenkranz et al., 2010).

Colony collapse disorder (CCD), which is characterised by large winter losses, and very low or no adult bee populations due to the disappearance of workers and/or queen failure (vanEngelsdorp et al., 2009), has been claimed to be caused by a combination of *V. destructor* and viral pathogens, since viruses alone do not cause colony failures unless the mites are present (Dainat et al., 2012; de Miranda et al., 2010; Hung et al., 1996; Siede et al., 2008). DWV and ABPV were known as honey bee viruses before the arrival of *Varroa* mites in the United Kingdom, but rarely caused clinical symptoms that led to colony death (Bailey and Gibbs, 1964). Viruses causing asymptomatic infections, unlike those inducing acute lethal infections, are expected to spread very easily in honey bee populations (Martin, 2001) and, indeed, this is the case for DWV, which is nearly always present in honey bee populations around the world (de Miranda and Genersch, 2010). This unique mite/virus association has strongly influenced the bee viral landscape and DWV virulence, through a synergism mediated by host immunity, which very often underpins the collapse of honey bee colonies (Martin et al., 2012; Nazzi et al., 2012; Ryabov et al., 2014). The widespread viral infection exposes colonies to the risk of explosive viral proliferation, that can be triggered by any stress factor further compromising the immune barriers of the host, in particular those under NF- κ B control, already negatively influenced by DWV infection (Nazzi et al., 2012; Nazzi and Pennacchio, 2014).

The spread of viral infections is, therefore, a key issue, and not only for honey bees. Within a bee species, the vector mites can disperse and invade other colonies via “drifting” and “robbing” bees that move into non-natal colonies (Frey and Rosenkranz, 2014). Although some of these viruses affect bumblebees as well, it appears that the interspecies pathogen transmission originates primarily in the managed honey bee colonies, with transmission between species occurring via shared use of flowers (Furst et al., 2014; Graystock et al., 2015).

Varroa and viruses, however, are not the only causes of the bee demise. Infections by the gut microsporidian *Nosema ceranae*, another pathogen that jumped host from the Asian to the European honey bee (Fries, 2010), have also had an impact in managed honey bees, with some authors claiming unusual high mortalities in Spanish colonies due to this pathogen alone (Higes et al., 2009). In addition, it can also infect wild bumblebees (Cameron et al., 2011; Furst et al., 2014). *N. ceranae* can significantly suppress the immune response in honey bees (Antúnez et al., 2009), alters the behaviour of workers in the hives, shortens the lifespan of the adult honey bees after larvae are infected (Eiri et al., 2015; Goblirsch et al., 2013) and reduces the homing ability of foragers (Wolf et al., 2014), which may lead to colony failure if bees lose flexibility in their response to colony demands. In bumblebees this parasite causes high mortality of infected workers (Graystock et al., 2013). As with the mites, it appears that failing bee colonies contain not only *Nosema* but also viruses (Bromenshenk et al., 2010; Doublet et al., 2014), whereas the presence of either pathogen alone results in covert effects or no pathological symptoms (albeit see Higes et al., 2009).

3. Pesticides and diseases

Since *N. ceranae* was first found in honey bee colonies in the United States in 1995 (Chen et al., 2008) and Europe in 1998 (Paxton et al., 2007), about the same time as neonicotinoids (potent agonists of the acetylcholine receptors) were introduced in those countries, questions were asked about the possible association between the novel insecticides and the pathogen. It was found that when bees infected with *Nosema* are exposed to the neonicotinoid imidacloprid, they are unable

to sterilize the colony and brood food using glucose oxidase, thus facilitating the spread of this pathogen within the colonies (Alaux et al., 2010). Furthermore, exposure of honey bees infected with *N. ceranae* to sublethal doses of fipronil (phenyl-pyrazole that disrupts GABA regulated chloride channels) or the neonicotinoid thiacloprid resulted in higher bee mortality than in non-exposed bees, but surprisingly this synergistic effect was not due to inhibition of the insect detoxification system (Aufauvre et al., 2012; Vidau et al., 2011). Because the increase in pathogen growth within individual bees reared in colonies exposed to sublethal doses of imidacloprid is dose-dependent (Pettis et al., 2012), it is clear that the insecticide can promote *Nosema* infection. To explain this, Aufauvre et al. found that fipronil and imidacloprid suppress the immunity-related genes in honey bees, thus leading to higher mortality rates in *Nosema*-infected hives (Aufauvre et al., 2014). A recent study also indicates that *Nosema* infections appear to be more than twice as likely in bees that consumed sublethal doses of certain fungicide residues (i.e. chlorothalonil and pyraclostrobin present in pollen from field flowers and crops) than in bees that did not (Pettis et al., 2013). Even if the pathogen is present in healthy colonies, honey bees can usually cope with it through their natural immune system; it is only when the bees are exposed to pesticide stressors – including residues of products used for *Varroa* treatment found in the combs (Pettis et al., 2013; Wu et al., 2012) – that they are unable to contain the infection and may succumb. Moreover, a recent study found a significant correlation between the presence of fungicide residues in hives and honeybee colony viruses (Simon-Delso et al., 2014).

While the insecticides appear to suppress bees' immune system, the mechanism whereby fungicides exert their sublethal effect on *Nosema* infections is not known. Previous laboratory studies suggest that ergosterol inhibiting fungicides (EIF) such as prochloraz, triflumizole and propiconazole may inhibit the cytochrome P450 monooxygenase detoxification system in bees, thus increasing several hundred-fold the acute toxicity of acetamiprid and thiacloprid (Iwasa et al., 2004), and several-fold that of tau-fluvalinate, coumaphos and fenpyroximate (Johnson et al., 2013). The latter compounds are used to treat *Varroa* in the hives, so their synergism with fungicide residues presents a real dilemma. These acaricides also have negative effects on the mobility and foraging behaviour of bees, whether alone or in combination with insecticides such as imidacloprid (Williamson and Wright, 2013). It seems that coumaphos, thymol and formic acid are able to alter some metabolic responses that could interfere with the health of individual honey bees or entire colonies. These include detoxification pathways, components of the immune system responsible for cellular response and developmental genes (Boncristiani et al., 2012).

It is now clear that systemic insecticides and fungicides play a role in the spread and virulence of *Nosema* infections among honey bees (Fig. 1). But, apart from that, is there any evidence to link pesticides with the other diseases and parasites mentioned above? Pesticide residue data in beebread or honey collected from managed honey bee hives does not correlate well with the prevalence of colony failures (vanEngelsdorp et al., 2010). Neonicotinoid or fipronil residues are not always found in the hives and consequently are thought to be unrelated to the colony losses (Bernal et al., 2011; Janke and Rosenkranz, 2009; Nguyen et al., 2009). Only the organophosphate coumaphos and other acaricides appear consistently (Higes et al., 2010; Johnson et al., 2010), but they can be readily metabolised by the P450 detoxification system of the bees, which appears to be enhanced by the flavonoid quercetin present in honey (Mao et al., 2011); as a result, these compounds are supposed to pose low risk of direct mortality in bees in comparison to neonicotinoids and pyrethroids (Sánchez-Bayo and Goka, 2014), but the presence of fungicide residues in pollen and honey may enhance their toxicity (Johnson et al., 2013), and consequently their risk, to moderate levels of concern. By contrast, Bacandritsos et al. (Bacandritsos et al., 2010) and Gregorc & Bozic (Gregorc and Bozic, 2004) found residues of imidacloprid in tissues (5–39 ppb) of bees from *Varroa* infected

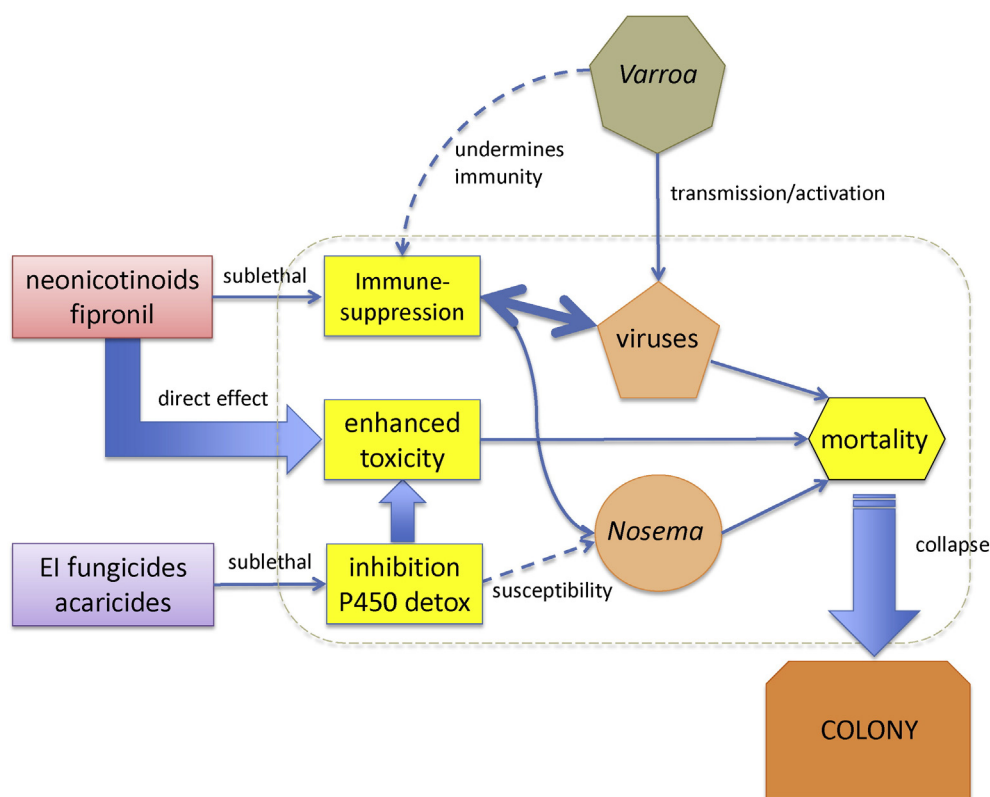


Fig. 1. Interactions between pesticides, parasites and pathogen stressors in relation to honey bee colony collapses. Individual bees (dashed rectangle) are exposed to interacting stressors. Infestation by *Varroa* mites promotes viral replication and an escalating immune-suppression, which may facilitate infections by other pathogens such as *Nosema*. This adverse effect on honey bee health of parasite/pathogen associations is reinforced by sub-lethal doses of insecticides, such as neonicotinoids and fipronil, which significantly contribute to the immune-suppression and exacerbate the resulting deadly effects. Moreover, ergosterol-inhibiting fungicides (EIF) and some acaricides inhibit the detoxification system mediated by cytochrome P450 monooxygenases, enhancing the toxicity of most insecticides, while apparently increasing the susceptibility to *Nosema* infections. With the bee defences down and the toxicity of pesticides increased, the combined deadly toll weakens the colony until it eventually collapses.

colonies, but could not tell what relationship might exist between the two stressors. Based on residue data alone, any suggestion that pesticides – be they neonicotinoids or others – have anything to do with colony losses is often downplayed, relegated to a secondary role as contributing factors (Genersch et al., 2010; Staveley et al., 2014) or dismissed altogether (Schmuck, 1999).

The difficulty in relating pesticide with colony collapses is that the exposure of bees to the chemical residues is usually at sublethal levels and the induced effects can be variable, depending on the presence and intensity of different stress agents affecting this multifactorial syndrome. Moreover, honey bees, due to their complex colony structure, are not the best model to study the effects of neonicotinoids (Rundlof et al., 2015). Even if lethal chronic toxicity of imidacloprid to individual bees can be demonstrated (Rondeau et al., 2014), the implications this has for the survival of the colony cannot be easily assessed in field trials (Wehling et al., 2009), unless the data are analysed using fully crossed factorial designs and mathematical models that consider the effect of multiple stressors together. Using such models, Bryden et al. could demonstrate how exposure of individual bumblebees (*Bombus terrestris*) to imidacloprid impaired colony function to the point of failure (Bryden et al., 2013). It is not just worker bees that are affected: a significant interaction between thiamethoxam or clothianidin exposure and infection by a trypanosome gut parasite (*Crithidia bombi*) on bumblebee queen survival affected directly the colony success (Fauser-Misslin et al., 2014). However, the pyrethroid lambda-cyhalothrin had no significant impact on the susceptibility of bumblebee workers to *C. bombi*, or intensity of parasitic infection (Baron et al., 2014), indicating that this synergism may be a particular feature of neonicotinoids. Synergistic interactions that amplify the effects of a single stressor are also found among pathogens of honey

bees (Doublet et al., 2014), but while mortality due to pathogens is self-explanatory, the effects of field-realistic sublethal doses of neonicotinoid insecticides are more difficult to assess on larvae and adults.

4. Mechanism of immune suppression by neonicotinoids

Despite all the above, a mechanistic link between neonicotinoid insecticide exposure and the possible immune alteration of bees remained elusive until recently, when Di Prisco et al. demonstrated that sublethal doses of clothianidin negatively modulate NF- κ B immune signalling in insects, and that both clothianidin and imidacloprid adversely affect the honey bee antiviral defences controlled by this transcription factor (Di Prisco et al., 2013). By enhancing the transcription of the gene encoding a protein that inhibits NF- κ B activation, the neonicotinoid insecticides reduce immune defences and promote the replication of DWV in honey bees bearing covert viral infections (Fig. 1). By contrast, the organophosphate chlorpyrifos did not affect that signalling. This finding is crucial, as it uncovers a novel role of neonicotinoids in the regulation of the immune response in bees and probably other insects. Therefore, neonicotinoids can modulate the virulence of bee pathogens, and may more generally modulate interactions between insects and their natural antagonists, as indicated by the increased virulence of entomopathogenic fungi against *Aedes aegypti* induced by exposure to imidacloprid (Paula et al., 2011).

These synergistic interactions seem to be relevant under field conditions, where a significant positive correlation has been found between neonicotinoid treatment and *Varroa* infestation, as well as viral pathogens, suggesting that proximity of colonies to treated areas leads to subtle increases of these pathogens (Dively et al., 2015). This trend is

further corroborated by a separate field study (Alburaki et al., 2015). Conversely, a thorough field work, showing severe effects of neonicotinoids on wild bees, was unable to detect significant effects on honey bees in a single season (Rundlof et al., 2015). However, a careful inspection of the data on parasite/pathogen occurrence in the experimental populations reveals a low starting presence of *Varroa* infestation and viral loads (Goss, 2014). Collectively, these field studies can be interpreted in the framework of the immune model recently proposed (Nazzi and Pennacchio, 2014), where the “Achilles heel” of the honey bee colony is the escalating immune-suppression determined by increasing levels of mite-virus populations, that can be aggravated by different stress factors, among which neonicotinoid can play an important role. In this context, one would predict that the health status of the colony can significantly affect the adverse impact of any stress factor, including neonicotinoids (Nazzi and Pennacchio, 2014).

The contribution of neonicotinoids under realistic field conditions is therefore difficult to measure and is unlikely to generate unequivocal results, being part of a changing combination, over space and time, of stress agents underlying a multifactorial syndrome. However, a large-scale correlation study, over an 11-year period, has revealed a significant correlation between honey bee colony losses and national scale imidacloprid usage patterns across England and Wales (Budge et al., 2015). This study clearly supports the important role of neonicotinoids in contributing to the health decay of honey bees and to the eventual collapse of their colonies. Records of honey bee colony losses in the short period of the EU ban of neonicotinoids seem to further corroborate this hypothesis (Laurent et al., 2015), but more accurate data over a much longer time interval are necessary to confirm this trend.

Taken together, the findings reported here partly account for the rising prevalence of viral and other pathogenic diseases in bees in recent times, which parallel the worldwide growth in neonicotinoid contamination. Indeed, neonicotinoid residues are found not only in the pollen and nectar of treated crops (Dively and Kamel, 2012; Stoner and Eitzer, 2013), but also in the adjacent vegetation (Krupke et al., 2012), in puddles and other surface waters that are consumed by the bees (Samson-Robert et al., 2014), and in over 50% of rivers in agricultural areas of the US (Hladik et al., 2014) and many other countries (Morrissey et al., 2015).

Since the immune suppression caused by neonicotinoids likely acts upon a conserved cross-regulatory mechanism of the nervous system on immunity (Di Prisco et al., 2013; Tracey, 2009; Tracey, 2011), one could expect a general undermining of the body defences in other organisms upon exposure to sublethal doses of these insecticides. In fact, immune deficiency by exposure to imidacloprid has been observed in chickens (Kammon et al., 2012), partridges (López-Antia et al., 2015) and mussels (Dondero et al., 2010), and was suggested as the explanation for massive *Trichodina* ectoparasite infections in medaka fish in rice fields treated with this insecticide (Sánchez-Bayo and Goka, 2005). These correlations require to be corroborated by further mechanistically-oriented studies.

5. Conclusion

We can now summarize our current knowledge regarding colony collapses in honey bees caused by parasites, pathogens and pesticide stressors as shown in Fig. 1. Synergistic interactions among the parasitic mite *Varroa* and viral pathogens severely reduce host immune competence and favour the health decline of bees. The negative impact on immune barriers can be further exacerbated by concurrent stress agents that interfere with NF- κ B signalling (e.g. neonicotinoids), or activate competing stress responses (e.g. poor nutrition, extreme thermal conditions), thus favouring pathogens and parasites. The combined effect of two or more of these stressors can induce mortality of the individual bees, including the queen, and may eventually end with the collapse of the colony. Therefore, neonicotinoids and interacting pesticides (e.g. EIFs) are important stress factors

underpinning colony health decline and eventual collapse, significantly contributing to the spread and abundance of pathogens and parasites, which are the proximate mortality factors. Broadly similar patterns of synergy between parasites and pesticide exposure are evident in bumblebees and are likely to be found in many wild insects and other wildlife taxa.

Statement

The authors declare no conflict of interests.

References

- Alaux, C., Brunet, J.-L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L.P., Le Conte, Y., 2010. Interactions between *Nosema microspores* and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ. Microbiol.* 12, 774–782.
- Albrecht, H., 2005. Development of arable weed seedbanks during the 6 years after the change from conventional to organic farming. *Weed Res.* 45, 339–350.
- Alburaki, M., Boutin, S., Mercier, P.-L., Loublier, Y., Chagnon, M., Derome, N., 2015. Neonicotinoid-coated *Zea mays* seeds indirectly affect honeybee performance and pathogen susceptibility in field trials. *PLoS One* 10, e0125790.
- Antúnez, K., Martín-Hernández, R., Prieto, L., Meana, A., Zunino, P., Higes, M., 2009. Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). *Environ. Microbiol.* 11, 2284–2290.
- Aufauvre, J., Biron, D.G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M., Vignes, B., Belzunces, L.P., Delbac, F., Blot, N., 2012. Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee. *Sci. Rep.* 2, 326.
- Aufauvre, J., Misme-Aucouturier, B., Viguès, B., Texier, C., Delbac, F., Blot, N., 2014. Transcriptome analyses of the honeybee response to *Nosema ceranae* and insecticides. *PLoS One* 9, e91686.
- Bacandritsos, N., Granato, A., Budge, G., Papanastasiou, I., Roinioti, E., Caldon, M., Falcara, C., Gallina, A., Mutinelli, F., 2010. Sudden deaths and colony population decline in Greek honey bee colonies. *J. Invertebr. Pathol.* 105, 335–340.
- Bailey, L., Gibbs, J., 1964. Acute infection of bees with paralysis virus. *J. Invertebr. Pathol.* 6, 395–407.
- Baron, G.L., Raine, N.E., Brown, M.J.F., 2014. Impact of chronic exposure to a pyrethroid pesticide on bumblebees and interactions with a trypanosome parasite. *J. Appl. Ecol.* 51, 460–469.
- Bernal, J., Martín-Hernández, R., Diego, J.C., Nozal, M.J., Gozalez-Porto, A.V., Bernal, J.L., Higes, M., 2011. An exposure study to assess the potential impact of fipronil in treated sunflower seeds on honey bee colony losses in Spain. *Pest Manag. Sci.* 67, 1320–1331.
- Boncrisiani, H., Underwood, R., Schwarz, R., Evans, J.D., Pettis, J., vanEngelsdorp, D., 2012. Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. *J. Insect Physiol.* 58, 613–620.
- Bromenshenk, J.J., Henderson, C.B., Wick, C.H., Stanford, M.F., Zulich, A.W., Jabbour, R.E., Deshpande, S.V., McCubbin, P.E., Seccomb, R.A., Welch, P.M., Williams, T., Firth, D.R., Skowronski, E., Lehmann, M.M., Bilimoria, S.L., Gress, J., Wanner, K.W., Cramer, R.A., 2010. Iridovirus and microsporidian linked to honey bee colony decline. *PLoS One* 5, e13181.
- Bryden, J., Gill, R.J., Mitton, R.A., Raine, N.E., Jansen, V.A.A., 2013. Chronic sublethal stress causes bee colony failure. *Ecol. Lett.* 16, 1463–1469.
- Budge, G.E., Garthwaite, D., Crowe, A., Boatman, N.D., Delaplane, K.S., Brown, M.A., Thygesen, H.H., Pietravalle, S., 2015. Evidence for pollinator cost and farming benefits of neonicotinoid seed coatings on oilseed rape. *Sci. Rep.* 5, 12574.
- Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L., 2011. Patterns of widespread decline in North American bumble bees. *PNAS* 108, 662–667.
- Chen, Y., Evans, J.D., Smith, I.B., Pettis, J.S., 2008. *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. *J. Invertebr. Pathol.* 97, 186–188.
- Dainat, B., Evans, J.D., Chen, Y.P., Gauthier, L., Neumann, P., 2012. Dead or alive: deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *Appl. Environ. Microbiol.* 78, 981–987.
- de Miranda, J.R., Genersch, E., 2010. Deformed wing virus. *J. Invertebr. Pathol.* 103, S48–S61 (Supplement).
- de Miranda, J.R., Cordoni, G., Budge, G., 2010. The acute bee paralysis virus—Kashmir bee virus—Israeli acute paralysis virus complex. *J. Invertebr. Pathol.* 103, S30–S47 (Supplement).
- Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F., Gargiulo, G., Pennacchio, F., 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *PNAS* 110, 18466–18471.
- Dively, G.P., Kamel, A., 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. *J. Agric. Food Chem.* 60, 4449–4456.
- Dively, G.P., Embrey, M.S., Kamel, A., Hawthorne, D.J., Pettis, J.S., 2015. Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. *PLoS One* 10, e0118748.
- Dondero, F., Negri, A., Boatti, L., Marsano, F., Mignone, F., Viarengo, A., 2010. Transcriptomic and proteomic effects of a neonicotinoid insecticide mixture in the marine mussel (*Mytilus galloprovincialis*, Lam.). *Sci. Total Environ.* 408, 3775–3786.

- Doublet, V., Labarussias, M., de Miranda, J.R., Moritz, R.F.A., Paxton, R.J., 2014. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. *Environ. Microbiol.* 17, 969–983.
- Eiri, D.M., Suwannapong, G., Enderler, M., Nieh, J.C., 2015. *Nosema ceranae* can infect honey bee larvae and reduces subsequent adult longevity. *PLoS One* 10, e0126330.
- Fausser-Misslin, A., Sadd, B.M., Neumann, P., Sandroock, C., 2014. Influence of combined pesticide and parasite exposure on bumblebee colony traits in the laboratory. *J. Appl. Ecol.* 51, 450–459.
- Francis, R.M., Nielsen, S.L., Kryger, P., 2013. *Varroa*-virus interaction in collapsing honey bee colonies. *PLoS One* 8, e57540.
- Frey, E., Rosenkranz, P., 2014. Autumn invasion rates of *Varroa destructor* (Mesostigmata: Varroidea) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. *J. Econ. Entomol.* 107, 508–515.
- Fries, I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). *J. Invertebr. Pathol.* 103, S73–S79 (Supplement).
- Furst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J., Brown, M.J.F., 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* 506, 364–366.
- Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., Berg, S., Ritter, W., Mühlen, W., Gisder, S., Meixner, M., Liebig, G., Rosenkranz, P., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41, 332–352.
- Goblirsch, M., Huang, Z.Y., Spivak, M., 2013. Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by *Nosema ceranae* infection. *PLoS One* 8, e58165.
- Goss, J., 2014. Neonicotinoids and Honeybee Health. Swedish University of Agricultural Sciences, Uppsala.
- Goulson, D., Hughes, W.O.H., 2015. Mitigating the anthropogenic spread of bee parasites to protect wild pollinators. *Biol. Conserv.* 191, 10–19.
- Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347, 1255957.
- Graystock, P., Yates, K., Darvill, B., Goulson, D., Hughes, W.O.H., 2013. Emerging dangers: deadly effects of an emergent parasite in a new pollinator host. *J. Invertebr. Pathol.* 114, 114–119.
- Graystock, P., Goulson, D., Hughes, W.O.H., 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. *Proc. R. Soc. B* 282, 20151371.
- Gregorc, A., Bozic, J., 2004. Is honey bee colonies mortality related to insecticide use in agriculture? (Ali cebelje družine odmirajo zaradi uporabe insekticida v kmetijstvu?) *Sodobno Kmetijstvo* 37, 29–32.
- Hald, A.B., 1999. Weed vegetation (wild flora) of long established organic versus conventional cereal fields in Denmark. *Ann. Appl. Biol.* 134, 307–314.
- Higes, M., Martín-Hernández, R., Garrido-Bailón, E., González-Porto, A.V., García-Palencia, P., Meana, A., Del Nozal, M.J., Mayo, R., Bernal, J.L., 2009. Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. *Environ. Microbiol. Rep.* 1, 110–113.
- Higes, M., Martín-Hernández, R., Martínez-Salvador, A., Garrido-Bailón, E., González-Porto, A.V., Meana, A., Bernal, J.L., Del Nozal, M.J., Bernal, J., 2010. A preliminary study of the epidemiological factors related to honey bee colony loss in Spain. *Environ. Microbiol. Rep.* 2, 243–250.
- Hladik, M.L., Kolpin, D.W., Kuvila, K.M., 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ. Pollut.* 193, 189–196.
- Hung, A.C., Shimanuki, H., Knox, D.A., 1996. The role of viruses in bee parasitic mite syndrome. *Am. Bee J.* 136, 731–732.
- Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R.M., 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Prot.* 23, 371–378.
- Janke, M., Rosenkranz, P., 2009. Periodical honey bee colony losses in Germany: preliminary results from a four years monitoring project. *Julius-Kuhn-Archiv* 108–117.
- Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M., 2010. Pesticides and honey bee toxicity – USA. *Apidologie* 41, 312–331.
- Johnson, R.M., Dahlgren, L., Siegfried, B.D., Ellis, M.D., 2013. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). *PLoS One* 8, e54092.
- Kammon, A.M., Brar, R.S., Banga, H.S., Sodhi, S., 2012. Ameliorating effects of vitamin E and selenium on immunological alterations induced by imidacloprid chronic toxicity in chickens. *J. Environ. Anal. Toxicol.* 54, S4–007.
- Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7, e29268.
- Laurent, M., Hendrix, P., Ribiere-Chabert, M., Chauzat, M.-P., 2015. A pan-European Epidemiological Study on Honeybee Colony Losses 2012–2014. European Union Reference Laboratory for Honeybee Health (EURL).
- López-Antía, A., Ortiz-Santaliestra, M.E., Mougeot, F., Mateo, R., 2015. Imidacloprid-treated seed ingestion has lethal effect on adult partridges and reduces both breeding investment and offspring immunity. *Environ. Res.* 136, 97–107.
- Mao, W., Schuler, M.A., Berenbaum, M.R., 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). *PNAS* 108, 12657–12662.
- Martin, S.J., 2001. The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: a modelling approach. *J. Appl. Ecol.* 38, 1082–1093.
- Martin, S.J., Highfield, A.C., Brettell, L., Villalobos, E.M., Budge, G.E., Powell, M., Nikaido, S., Schroeder, D.C., 2012. Global honey bee viral landscape altered by a parasitic mite. *Science* 336, 1304–1306.
- Morrissey, C.A., Mineau, P., Devries, J.H., Sánchez-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.
- Nazzi, F., Pennacchio, F., 2014. Disentangling multiple interactions in the hive ecosystem. *Trends Parasitol.* 30, 556–561.
- Nazzi, F., Brown, S.P., Annoscia, D., Piccolo, F.D., Prisco, G.D., Varricchio, P., Vedova, G.D., Cattonaro, F., Caprio, E., Pennacchio, F., 2012. Synergistic parasite–pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathog.* 8, e1002735.
- Nguyen, B.K., Saegerman, C., Pirard, C., Mignon, J., Widart, J., Thirionet, B., Verheggen, F.J., Berkvens, D., Pauw, E.D., Haubruge, E., 2009. Does imidacloprid seed-treated maize have an impact on honey bee mortality? *J. Econ. Entomol.* 102, 616–623.
- Oldroyd, B.P., 1999. Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Tree* 14, 312–315.
- Paula, A.R., Carolino, A.T., Paula, C.O., Samuels, R.I., 2011. The combination of the entomopathogenic fungus *Metarhizium anisopliae* with the insecticide imidacloprid increases virulence against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Parasites Vectors* 4, 8.
- Paxton, R., Klee, J., Korpela, S., Fries, I., 2007. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie* 38, 558–565.
- Pettis, J., vanEngelsdorp, D., Johnson, J., Dively, G., 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften* 99, 153–158.
- Pettis, J.S., Lichtenberg, E.M., Andree, M., Stitzinger, J., Rose, R., vanEngelsdorp, D., 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLoS One* 8, e70182.
- Rondeau, G., Sánchez-Bayo, F., Tennekes, H.A., Decourtye, A., Ramírez-Romero, R., Desneux, N., 2014. Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termite. *Sci. Rep.* 4, 5566.
- Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.* 103, S96–S119.
- Rundlof, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederstrom, 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.
- Ryabov, E.V., Wood, G.R., Fannon, J.M., Moore, J.D., Bull, J.C., Chandler, D., Mead, A., Burroughs, N., Evans, D.J., 2014. A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or *in vitro*, transmission. *PLoS Pathog.* 10, e1004230.
- Samson-Robert, O., Labrie, G., Chagnon, M., Fournier, V., 2014. Neonicotinoid-contaminated puddles of water represent a risk of intoxication for honey bees. *PLoS One* 9, e108443.
- Sánchez-Bayo, F., Goka, K., 2005. Unexpected effects of zinc pyrethione and imidacloprid on Japanese medaka fish (*Oryzias latipes*). *Aquat. Toxicol.* 74, 285–293.
- Sánchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees – A risk assessment. *PLoS One* 9, e94482.
- Schmuck, R., 1999. No causal relationship between Gaucho seed dressing in sunflowers and harm to bees in France (Kein Zusammenhang zwischen Saatgutbeizung mit Gaucho in Sonnenblumen und Bienenschaden in Frankreich). *Pflanzenschutz-Nachrichten Bayer* 52, 267–309.
- Siede, R., König, M., Büchler, R., Failing, K., Thiel, H.-J., 2008. A real-time PCR based survey on acute bee paralysis virus in German bee colonies. *Apidologie* 39, 650–661.
- Simon-Delso, N., Martin, G.S., Bruneau, E., Minsart, L.-A., Mouret, C., Hautier, L., 2014. Honeybee Colony Disorder in crop areas: the role of pesticides and viruses. *PLoS One* 9, e103073.
- Staveley, J.P., Law, S.A., Fairbrother, A., Menzie, C.A., 2014. A causal analysis of observed declines in managed honey bees (*Apis mellifera*). *Hum. Ecol. Risk Assess.* 20, 566–591.
- Stoner, K.A., Eitzer, B.D., 2013. Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. *PLoS One* 8, e77550.
- Tarpy, D.R., 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *Proc. Biol. Sci.* 270, 99–103.
- Tracey, K.J., 2009. Reflex control of immunity. *Nat. Rev. Immunol.* 9, 418–428.
- Tracey, K.J., 2011. Ancient neurons regulate immunity. *Science* 332, 673–674.
- Underwood, R.M., vanEngelsdorp, D., 2007. Colony Collapse Disorder: have we seen this before? *Bee Cult.* 135, 13–15.
- vanEngelsdorp, D., Meixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J. Invertebr. Pathol.* 103, S80–S95.
- vanEngelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D.R., Pettis, J.S., 2009. Colony Collapse Disorder: a descriptive study. *PLoS One* 4, e6481.
- vanEngelsdorp, D., Speybroeck, N., Evans, J.D., Nguyen, B.K., Mullin, C., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Tarpy, D.R., Haubruge, E., Pettis, J.S., Saegerman, C., 2010. Weighing risk factors associated with bee Colony Collapse Disorder by classification and regression tree analysis. *J. Econ. Entomol.* 103, 1517–1523.
- Vidau, C., Diogon, M., Aufaivre, J., Fontbonne, R., Viguès, B., Brunet, J.L., Texier, C., Biron, D.G., Blot, N., El-Alaoui, H., Belzuncos, L.P., Delbac, F., 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS One* 6, e21550.
- Wehling, M., Ohe, W.V.D., Brasse, D., Forster, R., 2009. Colony losses – interactions of plant protection products and other factors. *Julius-Kuhn-Archiv* 153–154.
- Williamson, S.M., Wright, G.A., 2013. Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. *J. Exp. Biol.* 216, 1799–1807.
- Wolf, S., McMahon, D.P., Lim, K.S., Pull, C.D., Clark, S.J., Paxton, R.J., Osborne, J.L., 2014. So near and yet so far: harmonic radar reveals reduced homing ability of *Nosema* infected honeybees. *PLoS One* 9, e103989.
- Wu, J.Y., Smart, M.D., Anelli, C.M., Sheppard, W.S., 2012. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection. *J. Invertebr. Pathol.* 109, 326–329.