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Across the other side of the river, over a pedestrian only bridge, and in the Expo Plaza was an Apimondia fringe event entitled "Bee! Bee!" with many stands selling honey and much bee themed entertainment for children (Figure 7). By Saturday part of it had become a dog show, which seemed a slightly bizarre combination, but it was very clear that the congress was fully embraced by the local population.

After much campaigning throughout the congress, at the Closing Ceremony, it was decided that the 2019 Congress will be held in Montréal, Canada, beating off close completion from Minneapolis, USA.

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Neonicotinoids and the prevalence of parasites and disease in bees

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The controversy regarding causes of worldwide bee declines observed in recent decades has focused on three main fronts: (1) the availability of food resources and habitat alteration, (2) biological agents such as parasites and disease, and (3) pesticide contaminants, particularly the neonicotinoid insecticides. In regard to pesticides, the use of herbicides has caused a reduction in floral diversity and the concomitant poor diet for bees that forage on monoculture crops. Other factors such as climate change have exacerbated the problems bees face, but cannot be considered the primary cause of their demise (Goulson, Nicholls, Botías, & Rotheray, 2015). Recent research shows, however, that there is a link between neonicotinoids and the prevalence of parasites and disease in honey bee colonies.

Here we review the current literature regarding bees and neonicotinoids, but let's see first how pesticides in general and biological agents in particular are implicated in the colony losses.

Pesticides and bees

Central European countries lost 25% of their honey bee colonies between 1985 and 2005, along with a decrease in the number of beekeepers, which dropped 38% (Potts et al., 2010). In the 1990s, many beekeepers in Western Europe complained about unusual honey bee losses, presumed to be due to the increasing use of imidacloprid-treated seeds (Bonmatin et al., 2003; Schmuck, 1999). In the US, average colony losses are around 40% per year, of which some 30% occur during winter. This high rate of loss is well above the 10–15% losses deemed acceptable as normal, natural losses. Overall, in North America 59% of colonies were lost over a 60-year period (vanEngelsdorp et al., 2012), although colony numbers have risen since 2008. Not only are beekeepers losing colonies, but the associated decline in productivity per colony is also worrying. Thus, to help alleviate the shortfall in productivity, beekeepers have increased their number of hives. For example, in Spain the average yearly yield was 20 kg of honey per colony from 1960–75, but currently it is only about 13 kg of honey per colony (MARM, 2014). So, in order to meet increasing market demands, Spanish beekeepers had no choice but to quadruple the number of managed colonies in the same period of time (Pajuelo, 2014).

It is well known that colony declines are correlated with the increasing use of insecticides in agriculture in the past six decades (Ellis, 2012). Inevitably, those chemicals had a toll on bees and other pollinators, such as butterflies and hoverflies, because these insects forage on the flowers of crops, which we know are contaminated with multiple pesticides residues (Krupke, Hunt, Eitzer, Andino, & Given, 2012). While there is no doubt that all insecticides affect bees and pollinators in one way or another, some are more dangerous than others. Until the mid-1990s, some 98% of the bee incidents reported in the UK were attributed to organophosphates, carbamates, pyrethroids, and organochlorine insecticides. However, the proportion of incidents linked to these chemicals started to decline from 1999 onwards (Barnett, Charlton, & Fletcher, 2007), as the old insecticides were replaced with a new class of chemicals: the neonicotinoids. Reports from Canada show that neonicotinoids account now for 72% of current bee incidents in that country, whereas other pesticides such as organophosphates are implicated only in 18% of the cases. Nevertheless, incidents that caused greater than 10% bee losses in the colony involved neonics 26% of the time, while 74% involved other pesticides (Cutler, Scott-Dupree, & Drexler, 2014). Wherever agriculture has switched from the old to the new insecticides, similar trends may be expected.

Neonicotinoids currently comprise a quarter of the world market for insecticides (Jeschke & Nauen, 2008), making them the leading insecticidal class of chemicals applied in agriculture. As it turns out, the three most common neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) also pose the highest toxicity risk to bees,

even more so than the organophosphates phosmet and chlorpyrifos (Sánchez-Bayo & Goka, 2014). The acute dose that kills 50% of adult bees in a cage test, known as an LD_{50}, is in the range of 0.004–0.05 μg/bee for these three neonicotinoids, while the organophosphates require bees to ingest higher doses in the range of 0.15–0.44 μg/bee.

Colony collapse due to parasites and disease

A concurrent phenomenon has taken place in the countries that switched to neonicotinoids: a marked increase in parasite infections, particularly *Varroa destructor* and *Nosema ceranae*, and a higher prevalence of viral diseases such as the deformed-wing virus (DWV) and others (de Miranda & Genersch, 2010; Higes et al., 2008; Schmuck, 1999). Global transport of bees and goods between countries explains the rapid spread of *Varroa* from Asia to Europe in the 1960s and then to the Americas in the mid-1980s and most recently to New Zealand – it has not yet reached Australia, though it has reached neighboring Papua New Guinea. Parasitic *Varroa* acts as a vector of the viruses commonly found at elevated levels in collapsed colonies (Chen, Pettis, Evans, Kramer, & Feldlaufer, 2004; Di Prisco et al., 2011; Francis, Nielsen, & Kryger, 2013; Shen, Yang, Cox-Foster, & Cui, 2005) and this parasite seems to be the main cause of the winter losses in Canada (Guzmán-Novoa et al., 2010). The microsporidian *N. ceranae,* originally a pathogen of *Apis cerana* that has now also infected *A. mellifera,* spread to Europe and the Americas in the last two decades (Fries, 2010; Klee et al., 2007). *N. ceranae* infections can be lethal (Higes et al., 2009) and are often associated with pesticide residues found in the hives (Alaux et al., 2010; Pettis, vanEngelsdorp, Johnson, & Dively, 2012; Wu, Smart, Anelli, & Sheppard, 2012). In Ontario, it was estimated that 76% of honey bee colonies are infected with *Varroa* during the fall, and 26% had *Nosema* (Guzmán-Novoa et al., 2010). With such widespread parasitic infections, it is not surprising that colony losses in Europe and America have largely been blamed on these parasites (Bacandritsos et al., 2010; Higes et al., 2010; VanEngelsdorp et al., 2010).

Due to the prevalence of parasites and diseases associated with honey bee hives that died over the winter (Underwood & vanEngelsdorp, 2007), most scientists accept they are one of the main causes behind the colony's collapse. High levels of *Varroa* are also frequently associated with colonies suffering from the symptoms of Colony Collapse Disorder (CCD), which display very low or no adult bee populations, typically with the queen still present and no dead honey bees in or around the hive. Scientific consensus indicates that no single biological agent is sufficient for the colony's collapse, but rather a combination of factors impact colony health, including perhaps the negative impacts of insecticides (Maini, Medrzycki, & Porrini, 2010; VanEngelsdorp et al., 2010). Thus, when French beekeepers blamed their unusual colony losses on the introduction of imidacloprid in France in 1994, the chemical manufacturer (Bayer) responded by blaming the parasites for the losses (Schmuck, 1999).

Neonicotinoids and bees

While the debate about the causes of this unusual disorder in honey bee colonies appears to be resolved (Cresswell, Desneux, & vanEngelsdorp, 2012; Godfray et al., 2014), some scientists and beekeepers have a different view. They suspect and implicate the neonicotinoid imidacloprid, since it was first introduced in the market about the same time as the parasites became hard to control – an epidemiological clue suggesting that perhaps the pesticides were amplifying the negative impacts of the parasites.

Although the acute toxicity of imidacloprid to bees was not publicly known at the time Bayer launched it on the market, it was later revealed to be extremely toxic to honey bees either by contact or chronic ingestion of contaminated nectar (Iwasa, Motoyama, Ambrose, & Roe, 2004; Suchail, Guez, & Belzunces, 2001). In fact, together with the pesticide fipronil, neonicotinoids are the most toxic agrochemicals to bees, and so pose the highest risk through ingestion of residues in both pollen and nectar (Sánchez-Bayo & Goka, 2014).

Neonicotinoids comprise two different groups based on their chemical structure: the N-nitroguanidine neonicotinoids, which include imidacloprid, thiamethoxam, clothianidin, dinetofuran, and nitenpyram are very toxic to bees with an LD_{50} range of 3.5–60 ng/bee. The closely related group of N-cyanoamidine neonicotinoids, which include thiacloprid and acetamiprid are about a thousand times less toxic, with an LD_{50} range of 8,000–36,000 ng/bee. However, the latter group can become as toxic as the former when combined with ergosterol-inhibiting fungicides (Iwasa et al., 2004). Despite these synergistic effects, the chemical manufacturers do not accept that tiny residues of neonicotinoids found in pollen (5–10 ppb) and nectar (1–6 ppb), which are below the honey bee's acute LD_{50s}, could be a problem for pollinators, let alone cause any mortality (Schmuck, 2004).

Part of the problem lies in our poor understanding of the exposure of bees to systemic insecticides and the chronic toxicity of neonicotinoids. Unlike older insecticides, which are typically fat-soluble and kill insects by contact, neonicotinoids are water-soluble chemicals that are taken up by the plant and translocated to all its tissues. Thus, their residues appear in the pollen and nectar of the crops (Bonmatin et al., 2005) and other surrounding plants (Krupke et al., 2012), to be either consumed directly by the bees or stored in the hive as bee-bread and honey (Chauzat et al., 2006; Mullin et al., 2010). Even if not all the pollen and nectar collected by the bees contain such residues, the daily ingestion of 0.25 ppb imidacloprid is sufficient to cause mortality in a large proportion of winter bees toward the end of their life (Rondeau et al., 2014). This is because neonicotinoids bind strongly to nicotinic receptors in the brain's neurons, causing continuous electrical discharges that drain the organism's energy and eventually kill the neuron. Since neurons are not replaced, their death toll accumulates over time until the level of neuronal damage reached is unsustainable and the insect dies (Tennekes & Sánchez-Bayo, 2013). It is literally a slow death by small but constant inputs of poison.

This chronic lethality is not evident in standard laboratory tests used to evaluate the potency of pesticides, because it may take more than 10 days for the lethal effects to be observed (Rondeau et al., 2014). Additionally, there is ample experimental evidence that sublethal doses of imidacloprid cause abnormal foraging behaviors in both honey bees and bumble bees such as disrupting their navigational abilities (Desneux, Decourtye, & Delpuech, 2007). It is true that some laboratory data were obtained using high exposures to the insecticide in order to observe dose–response relationships, so they cannot be extrapolated to field situations (Carreck & Ratnieks, 2014). Many negative impacts that impair colony performance have been shown, including memory impairment, disorientation that leads bees to go astray, delays in returning to the hive, and loss of appetite (Decourtye & Devillers, 2009; Desneux et al., 2007; Laycock, Lenthall, Barratt, & Cresswell, 2012).

Despite the evidence, the chemical manufacturers have repeatedly challenged these laboratory findings and funded field experiments in several countries to demonstrate that under normal pesticide application, neither imidacloprid nor clothianidin interfere with the productivity of honey bee colonies (Bailey, Scott-Dupree, Harris, Tolman, & Harris, 2005; Cutler & Scott-Dupree, 2007; Nguyen et al., 2009; Schnier, Wenig, Laubert, Simon, & Schmuck, 2003; Stadler, Martinez Gines, & Buteler, 2003). Those field experiments, however, lacked sufficient statistical power to demonstrate that imidacloprid had no effect (Cresswell, 2011). Moreover, while some of the criteria used, such as honey and brood production can be easily assessed under field conditions, others like foraging efficiency and low, but steady mortality are not easily quantified. Foragers usually die after 10–15 days of prolonged consumption of imidacloprid at 4–8 ppb sublethal levels (Dechaume-Moncharmont, Decourtye, Hennequet-Hantier, Pons, & Pham-Delègue, 2003), but this mortality goes undetected in large colony experiments because of brood compensation. We know that honey bee queens can produce approximately 1000 eggs daily, so small and slow mortality rates cannot be detected unless all bees are counted regularly – an impossible task using a full-size hive under field conditions. Using radio frequency identification (RFID) has allowed researchers to investigate these important issues (Ohashi, D'Souza, & Thomson, 2010), and they have shown that foraging activity is lower in colonies treated with imidacloprid or clothianidin than in untreated colonies (Schneider, Tautz, Grünewald, & Fuchs, 2012). Similar findings were obtained with the neonicotinoid thiamethoxam (Henry et al., 2012) and with bumble bees *Bombus terrestris* (Feltham, Park, & Goulson, 2014).

Also, whilst foragers from control colonies improve their pollen foraging performance as they gain experience, the performance of bees exposed to imidacloprid becomes worse: a condition known as chronic behavioral impairment (Gill & Raine, 2014). Both delayed mortality and impaired foraging activity eventually take a toll on the colony. Mathematical models based on similar observations demonstrate that small but continuous stresses on bees, as those produced by chronic exposure to pesticides, eventually cause the demise of the colony (Khoury, Myerscough, & Barron, 2013; Perry, Søvik, Myerscough, & Barron, 2015).

Chronic intoxication also affects the queen. Whilst no data exist about the neonicotinoid residue levels in royal jelly, the food consumed by the queen, they are expected to be no different from those in bee-bread and honey. Field experiments have shown that imidacloprid hampered the growth of *B. terrestris* colonies, causing an 85% reduction in the production of new queens (Whitehorn, O'Connor, Wackers, & Goulson, 2012). Furthermore, honey bee colonies chronically exposed to thiamethoxam and clothianidin over two brood cycles experienced declining numbers of adult bees (-28%) and brood (-13%) in the short term, while queen supersedure was observed in 60% of the neonicotinoid-exposed colonies within a one-year period compared to zero in the control colonies (Sandrock et al., 2014).

Neonicotinoid's immune suppression effects

Increases in parasites and disease due to intoxication with imidacloprid was suspected by Gregorc in 2004 (Gregorc & Bozic, 2004) and was pointed out by Desneux et al. (2007). Indeed, while *Varroa* has recently become a parasite of *A. mellifera* (see above), viral diseases of bees have presumably occurred for time immemorial, so the fundamental question that must be asked is: why have they become so much more prevalent in the last two decades?

Cornman et al. (2012) found high levels of pathogens associated with CCD, whereas a higher prevalence of *Nosema* infections appeared in colonies that had pesticide residues (Alaux et al., 2010), pointing to the latter factor as the underlying cause of the pathogenic infections (Mason, Tennekes, Sánchez-Bayo, & Jepsen, 2013). This suspicion was confirmed later when other researchers found that imidacloprid promoted *Nosema* infections (Pettis et al., 2012). Other systemic pesticides such as fipronil also produced similar synergistic effects (Aufauvre et al., 2012; Vidau et al., 2011), so neonicotinoids were not alone in fostering the spread of this parasite. On the other hand, surveys of CCD could not find a good correlation between the levels of *Nosema* infection in hives and exposure to neonicotinoids (VanEngelsdorp et al., 2010). Thus, most bee researchers still thought that the main cause of the collapses was *Varroa* and the diseases this parasite vectored, with systemic insecticides and other pesticides regarded as additional confounding factors (Staveley, Law, Fairbrother, & Menzie, 2014). The fact that Australia has not experienced any CCD, because *Varroa* has not yet reached its shores, supported this theory. However, bees in Australia prefer to gather pollen and nectar from the abundant sources of native flowers, such as *Acacia* spp. and *Eucalyptus* spp. (Figure 1), and Australian beekeepers only take their hives to agricultural crops for pollination purposes (Gibbs, 2013), so exposure of their bees to pesticides is not widespread. Nevertheless, feral honey bees in Australia are less abundant in agricultural land treated with pesticides than in forests and other untreated

Figure 1. Feral honey bees in Australia collect pollen and nectar preferably from native plants as this *Acacia* spp. While parasites such as *Varroa* have not reached this continent yet, bee populations are larger in undisturbed areas compared to agricultural land that is treated with pesticides (Hinson et al., 2015).

areas (Hinson, Duncan, Lim, Arundel, & Oldroyd, 2015).

Recent developments have demonstrated that sublethal levels of imidacloprid and clothianidin effectively cause immune suppression in bees (Di Prisco et al., 2013), leaving them defenseless against virus proliferation. These neonicotinoids mimic the role of leucine-rich proteins that regulate the expression of antibodies in bees and most animals by modulating NF-kB immune signaling. When an infective agent such as a virus enters a bee its immune system does not respond in the presence of trace amounts (21 ng/bee) of these two neonicotinoid insecticides. This suppression of the immune system by neonicotinoids allows viral replication until the bee succumbs to the disease. This same mechanism may explain the synergistic effects observed with *Nosema*, and may be responsible as well for the higher prevalence of *Varroa* among honey bee colonies. In fact, positive correlations between the incidence of *Varroa* and imidacloprid exposure have been observed in field experiments (Alburaki et al., 2015; Dively, Embrey, Kamel, Hawthorne, & Pettis, 2015). The immune system suppression in honey bees is not that surprising, since it was already known that imidacloprid caused immune deficiency in chickens (Kammon, Brar, Banga, & Sodhi, 2012), partridges (Lopez-Antia, Ortiz-Santaliestra, Mougeot, & Mateo, 2015), mussels (Dondero et al., 2010), and fostered parasite infections in medaka fish (Sánchez-Bayo & Goka, 2005). What is new, however, is the role that neonicotinoids play in colony collapses: these insecticides seem to be one of the main drivers behind the widespread propagation of parasites and diseases in the last two decades, not just another factor enhancing the demise of the colonies. It is not a coincidence, therefore, that the bee collapses are concurrent with the spread of *Varroa* and *Nosema* and are observed in countries that use neonicotinoids on a large scale.

Conclusion

After a decade of intense research on the problems affecting bees, we gain clarity over the once blurry picture of confounding factors that included parasites, pathogens, pesticides, lack of food, and others stressors. On the one hand is it obvious that all those factors contribute to the problems observed (Goulson et al., 2015). On the other, it has become evident that neonicotinoids (and insecticides like fipronil and perhaps others not yet identified) play a crucial role as the promoters of pathogen and parasite infections that effectively drive colony losses. In other words, these systemic insecticides are the ultimate cause of this complex crisis of honey bee health.

Because the cascade of effects prompted by neonicotinoids involves irreversible biological pathways that are not observable until after death (e.g. diseases), it has taken a long time to unravel the mechanisms involved in the chronic intoxication by these insecticides. What happens with honey bees and bumble bees however, may also be happening with other pollinators such as our butterflies and hoverflies, and likely impacts predatory insects and parasitoids too (e.g. see Desneux et al. 2007), as all of them are exposed to sublethal doses of neonicotinoids in agricultural environments. The impact that these systemic insecticides are having on the ecosystems built upon these myriad insects may thus compromise the sustainability of our agricultural production (Chagnon et al., 2015).

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On the Shelf: Bees Up-Close

Kirsten S. Traynor

In their new book "Bees: An up-close look at pollinators around the world," Sam Droege and Laurence Packer capture the beautiful diversity of nature's best flying machines. Bound as a large horizontal hardcover, this coffee table book begs the reader to dip in and flip the pages. Each page or spread introduces another eye-catching bee from around the world. The individual bees were photographed using a special image stacking process that combines multiple photos, creating one image in sharp focus over a large depth of field.

"You'll discover an entirely new part of nature that lives hidden in plain sight in everyone's backyards, neighborhoods, and parks. We are literally surrounded by bees, and beauty that we cannot readily see with the naked eye."

The reader revels in the vibrant purple metallic splendor of a *Euglossa* species Orchid Bee (Figure 1). One of 132 named species that "glint in almost all the colors of the rainbow," these gaudy bees search out orchids in the tropical jungles of the western hemisphere. The males perfume themselves with the orchid scents to court their mates.

Meet the fast flying Emerald Comb-Bearer, *Ctenocolletes smaragdinus*, from Western Australia (Figure 2). The territorial males guard clumps of flowering shrubs or herbs, until they hitch a ride on a foraging female. Hard to catch, these speedy bees often zip out of the way of a swooping insect net.

Figure 1. *Euglossa* species; Orchid Bee. *Collected by Sam Droege in Guyana, South America.*