Honey Bee Mortality Crisis

One Big Sticky Mess?

Photo: Wikimedia Commons/Louise Docker

Is this bee only taking a nap or has it shuffled off its mortal coil?

Over the last decade, beekeepers, scientists, environmentalists and politicians have been lamenting the alarming unexplained decline in honey bee populations in Europe and North America. Jeremy Garwood reports on the scientific battle to save the bees … if only we could finally agree on what's actually killing them.

In Europe and North America, there has been a steady decline in the number of honey bee (*Apis mellifera*) colonies during the last half century. Colony numbers in Europe decreased from over 21 million hives in 1970 to about 15 million in 2007. In the US, the number of honey-producing colonies dropped from a 1947 high of 5.9 million hives to 2.3 million in 2008.

Bee numbers and decline

Up until 1980, most US honey bee losses were attributed to the combined toxicity of pesticides (e.g. organochlorine and organophosphorus), then came dramatic losses due to the parasitic bee mites, *Acarapis woodi* (in 1984) and *Varroa destructor* (in 1987). However, following the winter of 2006-07, many US beekeepers reopened their hives in the spring to discover a new problem – the adult bees had quite simply disappeared from the hive, abandoning their food and brood (young bees). Overall, US beekeepers reported losing 38% of their bee colonies. Unable to explain this strange new phenomenon, scientists named it "Colony Collapse Disorder" (CCD). There is still no clear explanation or cure for CCD but huge losses of US bees have continued – 36% during winter 2007-08, 29% in 2008-09 and another 33% last winter.

European beekeepers have experienced similar losses that retrospectively display characteristics similar to CCD. These losses have now attained alarming proportions. For example, from 2007 to 2008, they were estimated at 29% in France, up to 40% in Italy, 33% in Denmark, 33% in the UK and even 89% in parts of Spain!

Although beekeepers can continue to replace hives and found new bee colonies, it is a costly business and many apiculturists have simply given up. How long can such losses continue? In 2009, Apimondia, the international beekeeping organisation, warned that at this rate the entire European beekeeping industry could disappear in less than ten years!

Researchers around the world have been trying to work out what's killing the bee colonies. In general, CCD is characterised by the rapid loss from a colony of its adult bee population. No dead adult bees are found inside or in close proximity to the col-

ony. At the final stages of collapse, a queen is only attended by a few newly emerged adult bees. These collapsed colonies often have considerable capped brood and food reserves. But in the absence of large numbers of dead bees, analysis of the causes of CCD has proved difficult.

Disappearing bees and Colony Collapse Disorder

Many factors have been proposed including viruses, bacteria, fungi, parasitic mites, chemical toxins, electromagnetic radiation from mobile phones, genetically modified crop plants, poor nutrition and the general stress of modern bee life. Certain Americans have even suggested a mass bee kidnapping by UFOs.

However, one surprising feature of the scientific explanations for global bee deaths is that they tend to follow national boundaries. This means that while Spanish scientists say their research clearly shows that a novel fungi from Asia (*Nosema cerranae*) is responsible for the dramatic collapse in Spanish honey bee colonies, their neighbours in France have found their rise in bee mortality is correlated with the use of certain pesticides. Further north, Belgian bees are thought to be dying off due to infestation of their hives by parasitic mites (*Varroa destructor*). In Germany, researchers also point to mites as the major cause of bee decline but claim that this is accentuated because the mites serve as vectors for the spread of bee pathogenic viruses, like ABPV (acute bee paralysis virus) and DWV (deformed wing virus). Meanwhile, in Britain and Switzerland, there has been a dramatic increase in levels of the bacterial infection, European foulbrood, that might account for the weakening and demise of their honey bee colonies.

Photo: Wikimedia Commons/Scott Bauer, USDA

Imagine a world without honey...

In the United States, scientists took a forensic approach to their CCD-ravaged hives, systematically analysing the 'crime scene'. In particular, in 2007, Diana Cox-Foster found that healthy bee colonies could only be reintroduced into CCD-affected hives after these hives had first been irradiated, indicating that an infectious agent was at work. Her extensive analysis of RNA samples from affected hives identified a virus, the Israeli Acute Paralysis Virus (*Science* 318(5848):283-7). However, subsequent analyses have failed to confirm this hypothesis.

"Bee Mortality in Europe"

Faced with such conflicting research reports, a big rethink was needed. In 2008, the European Food Safety Authority called for a systematic, Europe-wide analysis of the existing bee surveillance systems, the known data and all scientific publications related to honey bee colony mortality. Coordinated by the French Food Safety Agency (AFSSA), a consortium of seven European bee research institutes presented their findings in 2009 *(http://www.efsa.europa.eu/en/scdocs/scdoc/27e.htm*).

In the 24 European countries investigated, most of the bee surveillance systems were inadequate and poor with a lack of representative data for colony losses at both the country and EU levels and little standardisation of data, "Concerning surveillance procedures and protocols, of the 18 systems stating that they have in place active surveillance procedures, only 6 can be considered as valid active systems able to produce representative figures of the true colony loss situation for the countries in question."

Moreover, the only commonly used indicator was the "global colony loss rate" during the over-wintering period, which meant that not all aspects of colony losses, for example, those during the summer, could be addressed. Although temporal and geographical analyses indicated an important variability in colony losses, "such trends are difficult to interpret considering the wide variation in the quality of the systems that produce these data".

Based on such dodgy data, it's perhaps not so surprising to learn that they also found the existing scientific literature to be confusing, "There are many inconsistencies in the ways in which 'colony losses' are defined. Up to 17 different definitions for CCD exist in the literature (!). This means that reports may not always be referring to the same phenomenon and this creates confusion when trying to explain the origin of what has been identified in the field. The described pathology is varied, with authors using the same descriptions for different sets of circumstances."

Impossible to verify after the event...

Some researchers even have strong doubts that anyone can make a valid assessment of colony loss after it has occurred. In their 'Historical review of managed honey bee populations in Europe and the United States and the factors that may affect them' (*J. Invert. Pathology* 103, Suppl. 1: S80-95), Dennis vanEngelsdorp (Pennsylvania State University) and Doris Meixner (Bieneninstitut, Germany) state, "With few exceptions, it is nearly impossible to determine the cause of a honey bee colony death after the fact. If a colony dies during winter, a considerable amount of time may pass before it is noticed by the beekeeper and clues to the cause are usually lost. To definitively determine the cause or causes of mortality in colonies, a priori sampling and analysis of a representative portion of colonies is needed."

One possible cause of bee mortality that has generated considerable controversy is the use of agricultural pesticides.

In France, a mysterious disease that decimated bee hives was first reported in 1994. The affected bees had been foraging on sunflowers treated with a new insecticide, 'Gaucho' (manufactured by Bayer AG) whose active ingredient is imidacloprid, a neonicotinoid that causes insect paralysis and death by activating the postsynaptic nicotinic acetylcholine receptor.

However, Gaucho is not sprayed on growing plants; it is coated on the seeds. As a systemic pesticide, it is absorbed from the seed coating by the germinating plants and remains in the growing plant tissues, providing pest protection throughout the entire

growing season. But if the insecticide remains active that long, then it's probably present in the nectar and pollen the bees are feeding on.

Pest versus Pesticides

Bayer's researchers claimed that imidacloprid had no effects on bee health below a concentration of 20 parts per billion (ppb) and that its concentration in leaves was below 1.5 ppb. Eventually, they admitted it was also present in nectar and pollen but at such low concentrations as to pose no threat to bees.

In 2001, Luc Belezunces, a bee researcher at INRA (the French agricultural research institute) in Avignon published "Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*" (*Environ Toxicol Chem. 20(11):2482-6.*). He found an acute lethal dose of imidacloprid of only 40 ng per bee, much less than most other insecticides. However, his big discovery was that the lethal dose from chronic exposure to imidacloprid was 4,000 times less, "Ingesting 1 pg a day was enough to kill a bee within 10 days", he told *INRA magazine* (June 2009). "Moreover, imidacloprid degrades into 6 metabolites, some of which are even more toxic." He said that the capacity to measure very small traces of imidacloprid in pollen now shows that the concentration is in the range of microgrammes per kg of pollen and that this constitutes a risk for bees. "These results shifted classical conceptions and our first publication in 2001, initially commissioned by the Bayer company, was not well received."

In 2008, in S.W. Germany, there was a reminder that bees are nevertheless susceptible to insecticides: 11,000 honey bee colonies (around 400 million bees) died due to acute poisoning by another Bayer neonicotinoid insecticide, clothianidin. German authorities immediately suspended use of eight neonicotinoid pesticide seed treatment products for oilseed rape and maize. Bayer CropScience blamed defective seed corn batches, arguing that their insecticide was not at fault if used correctly – it had been incorrectly glued onto maize seeds such that the coating came off as the seeds were sown, generating toxic dust clouds. Yes, the bees were directly poisoned by clothianidin but it should never have been there if Bayer's handling procedures had been respected.

...or apples...

Commercial interests vs. environment?

Pesticides are big business. In 2007, sales of Bayer's imidacloprid and clothianidin were €587 million and €110 million, respectively. Due to possible toxic effects on honey bees, their use has been heavily restricted in France (imidacloprid since 1999), Germany, Slovenia and Italy since 2008. Therefore, it should come as no surprise to discover that the agrochemical companies are busily trying to convince decision-makers that their products are not to blame.

But could the commercial interests of pesticide companies be affecting the impartiality of researchers? Bee researchers at Italy's University of Bologna certainly think so.

"Despite the fact that CCD is unanimously considered by scientists to depend on several causes, two camps are now in conflict," they write in "The puzzle of honey bee losses" (Maini, *Bulletin of Insectology* 63:153-60). "On the one side are the environmentalists/beekeepers and on the other pesticide companies and the scientists sponsored by them."

These researchers have spent years studying the possibility of adopting the honey bee as a bioindicator of environmental pollution. But their experience suggests that non-scientific factors are influencing the progress of research on honey bee mortality, "We believe that papers published in scientific journals influence politicians and legislators preparing rules regarding prohibition and limitation of pesticide use. Scientific papers that indicate no hazard of pesticides and refuse to discuss data offering contrary opinions on the effect of pesticides on honey bees and other beneficial insects, may cause an underestimation of the real damages that agrochemicals inflict on ecosystems."

Anything but pesticides!

Although they concede, "It is impossible to 'demonstrate scientifically' the direct influence that the pesticide corporations, seed companies and some farm lobbies have on research teams that conduct research on honey bees," they do provide examples where other researchers have been surprisingly certain that pesticides could not possibly be involved in CCD. In a recent article, "Clarity on honey bee collapse?" (*Science* 327: 152-3), Sussex University's Francis Ratnieks and Norman Carreck state, "The consensus seems to be that pests and pathogens are the single most important cause of colony losses" and that imidacloprid, implicated in French bee losses, now "seems unlikely" to be responsible for French bee deaths.

Maini wrote a letter to *Science*, objecting that "many other scientists are concerned about the inappropriate use or even misuse of insecticides" and that by stating the "main cause of bee losses are 'diseases' ", Ratnieks and Carreck "may give the false impression that insecticides can be sprayed" without due attention. Furthermore, their conclusion about imidacloprid and French bee mortality "appears to be a biased opinion and a conflict of interest", given that it relied on a citation by "a researcher employed by the producer of imidacloprid" (Bayer AG) and it had been chosen from a special issue of the Bulletin of Insectology that presented several other articles with different conclusions concerning imidacloprid. Maini's letter was swiftly rejected by *Science* without explanation.

Lethal and sub-lethal effects

There had been previous conflict with Carreck who, as senior editor of the *Journal of Apicultural Research*, took six months to reject their critical manuscript, written in response to a 2009 paper by Belgian researchers, "Does imidacloprid seed-treated

maize have an impact on honey bee mortality?" (*J. Econ. Entomol.* 102: 616-23). The latter study had concluded that imidacloprid has no negative impact on honey bees, at least in Belgium (where mites are uniquely to blame). Again, Maini *et al.* pointed out that Nguyen had selectively cited work by Bayer researchers, while simultaneously ignoring numerous scientific publications reporting lethal and sub-lethal effects of pesticides. Furthermore, the methodology of the Belgian study only looked for effects late in the maize-growing season, despite evidence that complex effects had been detected both before and after this period.

Other examples of agrochemical influence have come to light elsewhere. For example, in 2009, it was discovered that the British Beekeepers' Association was receiving money from Bayer CropScience in return for endorsing its products as "bee-friendly". It has also been shown that UK publicly-funded bee research projects co-financed by pesticide companies tend to avoid looking at any negative pesticide effects. For example, Syngenta, manufacturers of the neonicotinoid pesticide, thiamexotham, contributed ten per cent towards a £1 million study at Warwick University to investigate the "parasitic diseases caused by the varroa mite" and the "link between these diseases and the quality of pollen and nectar that the bees are feeding on". When asked by the Guardian newspaper, researcher David Chandler confirmed that this study will not look at any role pesticides might play in affecting the quality of bee food or the bee's resistance to these parasitic diseases.

Pesticides accumulate in US hives

Maryann Frazier (Penn State University) decided to take a closer look at the exposure of US bees to pesticide residues (*Am. Bee J.* 148: 521-3). She found 121 different pesticides and metabolites in 887 wax, pollen, bee and associated hive samples from migratory and stationary beekeepers. Over 40 pesticides were systemic. There were on average 6 pesticides per sample. Only one wax sample, three 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. Quite a chemical cocktail!

Bee genomic insights

The honey bee genome hints at other bee-specific problems (*Nature* 443: 931-49): "Given the predicted disease pressures in honeybee colonies, the honey bee genome encodes fewer proteins implicated in insect immune pathways when compared to other insect genomes," suggesting that the bee's immune system might be more vulnerable to extrinsic factors than other insects.

There have been reports that honey bees are having less success in resisting microbial infections and mite infestations but why are the bee's immune defenses weakening? A recent study by Yves Le Conte (INRA Avignon) suggests that pesticide exposure can interact with pathogens to harm honey bee health ("Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees," *Environ. Microbiol.* 12: 774-82). Bees treated with imidacloprid while being fed *Nosema* spores had higher individual mortality rates than those exposed to imidacloprid or *Nosema* alone. The activity of glucose oxidase, enabling bees to sterilise colony and brood food, was significantly decreased only by the combination of both factors compared with control, *Nosema* or imidacloprid groups, suggesting a synergistic interaction.

"In the long term this could lead to a higher susceptibility of the colony to pathogens."

Honey bees also have a smaller genetic repertoire for safely metabolising pesticides. "Contact pesticides affect the worker bees, whereas residual pesticides accumulate in lipophilic substances, such as wax or pollen lipids, and impact on the developing brood and queen fecundity." It seems that the size of the major detoxifying gene families is smaller in the honey bee, making it "unusually sensitive to certain pesticides". Compared with *Anopheles* and *Drosophila*, the honey bee has 30–50% fewer genes encoding the carboxylesterase, cytochrome P450 and glutathione S-transferase enzymes that are principally responsible for the metabolism of pesticides. These are the genes where the great majority of metabolic resistance mutations have been found in other species of invertebrates.

Sub-lethal effects are possible

In 2008, Maryann Frazier told the US Congress hearing into the plight of the honey bee, "We are becoming increasingly concerned that pesticides may affect bees at sub-lethal levels, not killing them outright, but rather impairing their behaviors and their abilities to fight off infections."

The dispute over the role of pesticides in bee colony loss is increasingly focussed on possible 'sub-lethal' effects that do not directly kill the bees but instead disrupt their highly organised social and foraging behaviour, contributing to a collapse of the colony's cohesion.

In CCD, adult bees disappear from the hive – what if the bees got lost while out foraging and simply couldn't find their way home?

...or bee beards.

Research into the impact of various substances at sub-lethal concentrations has already shown that bees have similarities to humans – they both get drunk on ethanol! Might bees also be getting "drunk" on pesticides?

Under current legislation, pesticide manufacturers are only required to determine the lethal effects of their products on pests and other creatures that might be affected. Only mortality tests are considered when making a choice between several pesticides in an integrated pest management context, ignoring studies that may document sub-lethal pesticide effects on the targeted 'natural enemies'.

Yet, in contrast to the easily observable direct poisoning of bees (i.e. they drop dead), sub-lethal effects are much more difficult to demonstrate. They may only become apparent after prolonged exposure and affect various life stages. And what's affected? The cell physiology or immune system of individual bees, or social organisation with consequences for the colony as a whole, such as learning, behaviour and communication?

The possibility that bees are experiencing sub-lethal behavioural perturbation due to certain neurotoxic insecticides has been tested.

The insecticides, imidacloprid, fipronil (a phenylpyrazol that targets GABA receptors) and deltamethrin (a very popular pyrethroid) have all been shown to affect the bees' ability to detect food and accurately return to the hive after foraging. When landing on a flower, each forager bee is subjected to a conditioning process, where floral cues (smell, colour, shape) are memorised after being associated with a food reward (nectar and pollen). Under laboratory conditions, olfactory learning can be studied using a bioassay based on the conditioning of the proboscis extension reflex (PER) applied to "restrained" individual bees. By stimulating the antennae with a sucrose solution, the bee extends its proboscis for feeding. This can be used to assess the 'gustatory threshold to sugary foods' – the lowest sugar concentration capable of eliciting a PER. But the application of some pesticides, e.g. fipronil at a dose of 1 ng per bee, strongly reduces bee sensitivity for low-sucrose concentrations suggesting that pesticide exposure could effectively reduce the capacity of honey bees to detect food sources (*Pharmacol. Biochem. Behav.* 82: 30-39).

Tunnels, mazes and food direction

Honey bees use visual landmarks to navigate to a food source as well as to accurately communicate to their hive mates the distance and flying directions for reaching it. A bee exposed to pesticide during a foraging trip may incorrectly acquire or integrate visual patterns, causing disorientation and loss. Aside from impairing the orientation behaviour of exposed foragers, insecticides could affect the accuracy of information relayed through the dances of the returning foragers.

To study the effects of deltamethrin, honey bees were trained to forage on an artificial feeder filled with sucrose solution and were then individually marked with coloured number tags. In an insect-proof tunnel with the feeder located eight metres from the hive, deltamethrin altered the homing flight in foragers treated topically with sub-lethal doses. Treated bees flew towards the sun and took significantly longer to fly back to the hive. This disorientation was attributed to an effect on the storage or retrieval of spatial information (*Environ. Toxicol. Chem.* 14: 855-60).

In 1998, Marc Colin's team at INRA-Avignon also observed effects of low ppb concentrations of imidacloprid on honey bees, finding short-term errors in the bees' flight plans and that, after a few days, exposed bees stopped feeding altogether – their numbers soon dropped sharply compared to the control groups.

The orientation of bees in a complex maze has been measured to simulate learning of complex routes under field conditions. This relies on associative learning to fly through a maze according to the presence or absence of a visual cue with the reward of sugar solution at the end. Using this experimental setup, researchers found that foragers receiving one ppb fipronil performed less well than those in control groups. In parallel, the percentage of bees that did not find the goal within five minutes of entering the maze increased dramatically when exposed to fipronil (Decourtye, Julius-Kühn-Archiv 423: 75-83).

Consequences for plant pollination

Although honey bees were originally cultivated for their honey, they have recently become an essential part of world agriculture. Insect pollination of flowering plants results in large increases in the yields of 56 of the world's leading 115 food crops,

including apples, citrus fruit, tomatoes, sunflowers, rapeseed and soya. Historically, pollination was left to the uncontrolled efforts of native bees but modern agricultural practices require managed pollination. Industrial-scale farming of agricultural crops means there are huge areas grown as monocultures. These crops have short blooming periods that require intensive pollination but cannot support wild bee populations – intensive farming makes the areas barren or even toxic for most of the year. The only solution is to use managed honey bee colonies. In very large numbers!

Professional beekeepers have become migratory, seasonally moving their colonies to the high-demand areas of pollination. In the US, they travel up to 40,000 km a year pollinating apples, pears and cherries in the NW, citrus and vegetables in Florida, blueberries in Maine, etc.). Pollination of large monocultures requires the concentration of very high populations of bees at bloom. The world's largest managed pollination event is in the Californian almond orchards, where 50% of all US honey bees (>1 million hives!) perform each spring.

What could happen if the honey bee population collapses? About 35% of the human diet benefits from pollination. A decline in pollination would result in lower yields of many fruits and seeds with changes in prices and dietary preferences. To compensate for lower yields, more land could be grown with pollinator-dependent crops (up to 42% more in the developing world) adding more pressure on existing ecosystems and biodiversity. Are we closer to understanding why honey bee colonies are collapsing? The recent consensus isn't very encouraging. The "first comprehensive survey" of CCD-affected bee populations concluded that CCD "involves an interaction between pathogens and other stress factors" (*PloS One* 4(8):e6481). It found that CCD must be multifactorial and complex since, "of 61 quantified variables (including adult bee physiology, pathogen loads, and pesticide levels), no single measure emerged as a most-likely cause of CCD". Bees in CCD colonies are subject to higher pathogen loads and are co-infected with a greater number of pathogens than control populations, which could mean either they have increased exposure to disease or that they aren't as disease-resistant.

Dramatic consequences

In other words, we still don't know. But the decline in bee populations is set to continue with potentially dramatic consequences for plant pollination and agriculture. And if bees are potential indicators of new forms of environmental pollution, what other organisms may be affected?

Jeremy Garwood

(A more comprehensive version of this article on our website – *www.lab-times.org* – features background information on the life cycle and susceptibility of bee colonies, a more complete description of research into bee diseases and the pesticide debate, as well as the global bee pollination problem.)