Colony Collapse Disorder in relation to human-produced toxins: What's the buzz all about?

Available at: http://www.sawyoo.com/postpic/2013/09/honey-bee-hives_77452.jpg Last accessed: 17/04/2017

Abstract

In recent years, the global population of pollinating animals has been in decline. The honey bee in particular is one of the most important and well known pollinators and is no exception.The Western honey bee *Apis mellifera,* the most globally spread honey bee species suffers from one problem in particular. Colony Collapse Disorder (CCD), which causes the almost all the worker bees to abandon a seemingly healthy and food rich hive during the winter. One possible explanation for this disorder is that it is because of the several human produced toxins, such as insecticides, herbicides, fungicides and miticides. So the main question is: Are human-produced toxins the primary cause of CCD? It seems that insecticides and, in particular, neonicotinoid insecticides caused increased mortality and even recreated CCD-like symptoms by feeding the bees with neonicotinoids. Herbicides seem relatively safe for bees, though they do indirectly reduce the pollen diversity, which can cause the hive to suffer from malnutrition. Fungicides are more dangerous, causing several sublethal effects, including a reduced immune response and changing the bacterial gut community. The levels of one fungicide in particular, chlorothalonil, tends to be high in hives. Miticides levels tend to be high in treated hives and can cause result in bees having a reduced lifespan. Antimicrobial drugs are relatively safe, as long as they are not applied in a too low dose, as this will increase the chance of the parasites becoming immune. Adjuvants are supposedly biologically inactive ingredients that are added to pesticide mixtures in order to make them more effective without increasing the toxicity. However, recent studies suggest that they might be even more dangerous than the toxins themselves. Toxins can also cause synergistic effects when mixed, becoming even more dangerous than you would expect from the ingredients alone. In conclusion, although there are many negative effects that *A. mellifera* will suffer from due to exposure to pesticides, there is no significant evidence that pesticide exposure is the main factor for causing CCD, although it does seem that is an important exacerbating factor for triggering CCD.

In recent years, there has been an increased concern about the decline of pollinating animals. These species, which consist mostly of insects, but also include several mammals, birds and even some lizard species, have been negatively impacted by humanity (Hansen *et al.,* 2006, Fleming *et al.,* 2009, IPBES, 2016). In a recent report from IPBES (The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services), they found that worldwide at least 16.5% of all vertebrate pollinating species are currently in danger of going extinct (IPBES, 2016). While global data was not available for most invertebrate pollinators, more local surveys indicate that often the invertebrate pollinators are at high risk of dying out (IPBES, Potts *et al.,* 2010). These losses will, in turn, negatively affect any plant that is dependent on animals for pollination. It is estimated by IPBES (2016) that over 87% of flowering plants are at least partly dependant on pollination by animals worldwide, many of which are eaten or otherwise used by other organisms, forming the base of the food web (IPBES, 2016). When looking at it from an economic angle, Klein *et al.* (2007) found that over 80% of animal-pollinated plants that are consumed by humans will be negatively impacted by the loss of pollinators (Figure 1). As the current yearly market value of pollinated crops, such as coffee and tomatoes, is estimated at somewhere between 235 and 577 billion dollars, this would be a serious economic impact on the global market. This number does not even account for the economic damage that comes with the loss of the jobs dependent on either the pollinators or the pollinated crops or the intrinsic loss of cultural customs based on them.

Of all the different pollinating animals found in nature, none is more iconic than the honey bee. Consisting of several different species in

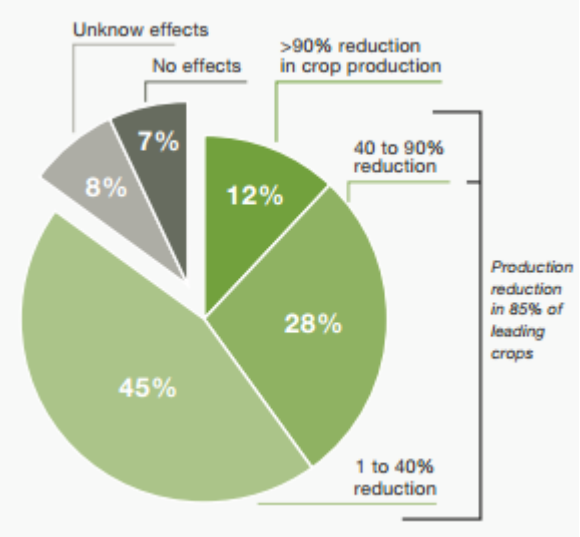

Figure 1: Percentage of human consumed crops affected by the absence of pollination and its impact on production. Source: IPBES, 2016 with data from Klein *et al.*, 2007

the genus of *Apis*, honey bees have been part of human society since prehistoric times. Archeologists have found 9000 year old evidence of beekeeping, where Neolithic farmers in Europe, the Middle East and North Africa kept honey bee colonies in ceramic pots (Roffet-Salque *et al.*, 2015), as well as even older cave paintings depicting humans harvesting honey from wild bees (Dams & Dams, 1977). In ancient Egypt, honey and beeswax were considered highly valuable, especially for medicinal and religious purposes (Chopra *et al.*, 2015). In Lower Egypt, the honey bee was important enough that they became the symbol of the country and eventually, the royal hieroglyph of the pharaoh of Lower Egypt (Chopra *et al.*, 2015). Other ancient cultures also featured bees in their mythology. From the Melissae bee priestesses and the tale of Aristaeus in Greek mythology to the Mayan bee god and goddess Ah-Muzen-Cab and Colel-Cab, bees were a part of human culture and mythology all around the globe. In the present, honey bees still exist as a symbol of hard work and cooperation, similar to the ant, wealth or on a colony level as a metaphor for human society.

It is generally accepted that there are seven different species of honey bees worldwide. While some undomesticated species of honey bees, such as the dwarf honey bee *Apis florae,* are still harvested from nature, humans have domesticated two species of honey bee. The Western or European honey bee *Apis mellifera* and the Eastern or Asiatic honey bee *Apis cerana*. *A. cerana* and its subspecies are found in Asia, and are one of the native species of honey bees there. Meanwhile, the different *A. mellifera* subspecies trace their origin back to Africa, but are currently found on every continent, barring Antarctica, having spread across the Eurasia and having been exported to Oceania, North- and South America by human travelers in the 17th century (Figure 2) (Whitfield *et al,* 2006, van Engelsdorp & Meixner, 2009). *A. mellifera* in particular is responsible for the majority of honey and beeswax production in the world, with an estimated value of respectively 1.6 billion and 57.5 million American dollars in 2013 (FAO, 2017). Domesticated bees also provide pollination services for agriculture, which, while difficult to put a price on, provides valuable services by supplementing wild pollinators and, in some cases, being the only source of pollination in some areas. This is taken to the extreme in the USA, where beekeepers tend transport their hives across large distances to supplement farms in regions with few wild pollinators (Klein et al., 2007, Brutscher *et al.,* 2016, Dolezal *et al.,* 2016).

However, as mentioned before, it isn't going well with most pollinators and the honey bee is no exception. Since the 1970's, both wild and managed *A. mellifera* populations have been declining locally, mainly in Europe and the Americas, though worldwide the total amount of honey bee hives were increasing (Potts *et al.,* 2010). However, in 2006, the rate of decline of *A. mellifera* became even worse, largely because of a newly emerged mysterious colony disease. After winter, hives were suddenly found with almost no adult worker bees, containing only the queen, the brood and a small number of worker bees caring for them, even though there was still more than enough honey left to feed the entire hive (van Engelsdorp & Meixner, 2009, Dainat *et al.*, 2011). Furthermore, there were almost no dead worker bees found either within the hive or around it and there was an increased delay in the time it takes before scavengers, hive pests and other honey bees begin to invade the practically undefended hive (van Engelsdorp & Meixner, 2009, Dainat *et al.*, 2011). The remaining number of worker bees was too little to be able to provide for the remaining hive, resulting in the

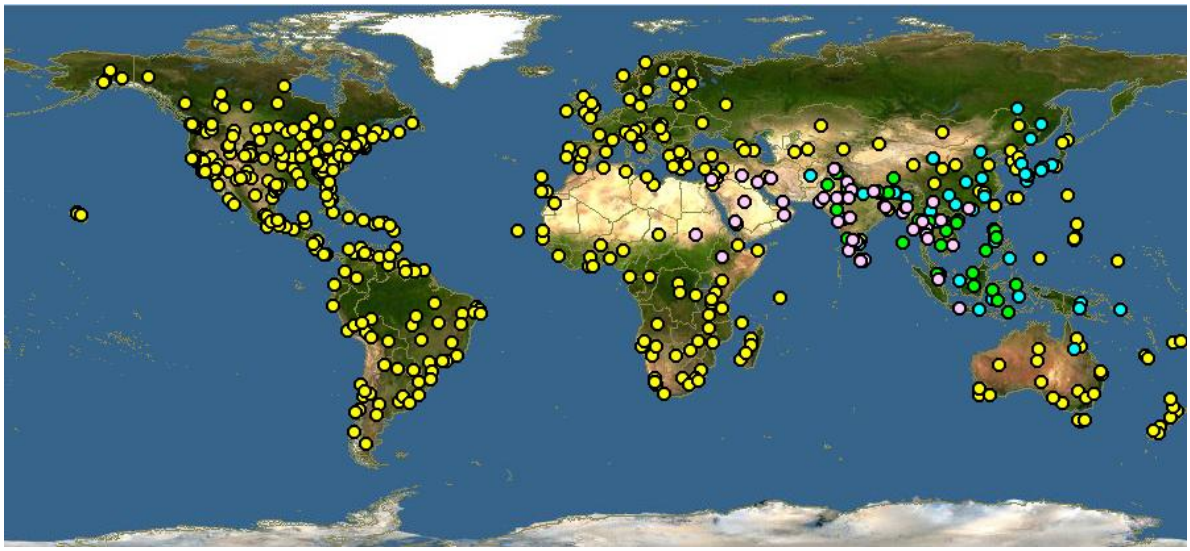

Figure 2: Distribution of four most widely distributed honey bee species across the world.
Legend: Yellow: *A. mellifera* Blue: *A. cerana* Green: *A. dorsata* Pink: *A. florae*
Source: http://www.discoverlife.org/mp/20m?act=make_map&kind=Apis_&map=SATW
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entire hive dying. Eventually, this became known as Colony Collapse Disorder (CCD) (van Engelsdorp & Meixner, 2009). While there were similar cases in the past, such 1890's in California, they were not as abundant or widespread as CCD is today (van Engelsdorp & Meixner, 2009).

While people do agree on the existence of CCD, the exact causes are less clear and hotly debated. People have placed the blame on various causes, such as the parasitic *Varoa destructor* mite and microsporidian *Nosema ceranae*, which has changed hosts from *A. cerana* in Asia to *A. mellifera* worldwide (Higes *et al.*,2008, Rosenkranz *et al.*, 2009, Le Conte *et al.*, 2010, Williams *et al.*, 2011, Chantawannakul *et al.*, 2015). Others put the blame on an increased disease load caused by various bee viruses, such as the Black Queen Cell virus, Kashmir bee virus and the Israeli acute paralysis virus (Cox-Foster *et al.*, 2007, Maori *et al.*, 2009, McMenamin *et al.*, 2016). Another possible explanation is stress and nutrition deficiency caused by human agriculture. For example, due to long distance travel, replacement of honey with fructose or sucrose syrup and only pollinating monoculture crops with low quality pollen, such as maize (Alaux *et al.*, 2009, Brodschneider & Crailsheim, 2009, Di Pasquale *et al.*, 2016, Simone-Finstrom *et al.*, 2016). But the most hotly debated possible cause of CCD are the human produced toxins, such as the neonicotinoid pesticides. Not only is this a factor where humans have direct influence on, there are multiple sides with different interests to it. Most farmers and chemical companies would prefer to keep using the pesticides as their livelihood depends on it, while beekeeper and conservationist would prefer reducing or even stopping the use of pesticides. Then there is also the discussion about how much effect each possible cause has on triggering CCD, ranging from not important at all to being the main cause of CCD, as well as the possible synergistic effects they could have on each other, complicating the discussion even further (van Engelsdorp & Meixner, 2009).

Term	Explanation
CCD	Colony Collapse Disorder
LD50	Concentration of toxin at which half of the population dies
PPM	Parts toxin per million total parts

Table 1: List of abbreviations used in the essay and their meanings.

So, the main question of this essay is, are human produced toxins, the main cause in the collapse of *A. mellifera* hives? In order to research this in an efficient way, I divided the human produced toxins in 3 general categories based on their use and how they could act upon honey bees and cause CCD. Insecticides are arguably the largest and most researched group, containing all the toxins used by humans to protect their crops from insects. Herbicides and fungicides are similar, being about the toxins humans use to kill unwanted plants and fungi. The last category contains miticides and other preventative measures, toxins used by humans to protect honey bees from parasites such as *Varoa destructor* mite. While some of the parasites that are treated with these measures are also linked to CCD in some degree, this is not relevant for my question. So, when discussing miticides and other preventative measures the focus will be on the side effects of the medicine, not about the effects on the parasite and the benefits *A. mellifera* gets from it.

Insecticides

Due to modern agriculture, *A. mellifera* is exposed to many different kinds of pesticides. Surveys in North-America and Belgium have shown that up to 120 different pesticides can be found in *A. mellifera* hives, with up to 40% of those being insecticides (Mullin *et al.*, 2010,

Johnson *et al.,* 2013, Simon-Delso *et al.,* 2014). In this report I will mostly focus on the most commonly used pesticides, for two reasons. Because these are the most well researched pesticides, as well as being the most abundant in *A. mellifera* hives and thus more likely to have an influence.

In general, honey bees are exposed to pesticides in two ways. They can be directly exposed to the toxin in the form of spray mixtures or fine dust or they can be exposed to pesticide residue that has accumulated in pollen, beeswax, nectar, water or honey. These then can affect the honey bee topically (through exoskeleton contact) or orally (through eating). Furthermore, honey bees have evolved to be very efficient in gathering small particles, being covered in densely packed hairs. This also makes them very good at carrying small pesticide-contaminated particles and transporting them back to the hive, causing pesticides to accumulate in the hive, especially in the beeswax (Thorp, 1979, Mullin *et al.,* 2010, Johnson *et al.,* 2013, Simon-Delso *et al.,* 2014).

One class of insecticides in particular has been researched a lot for being a potential cause of CCD, the neonicotinoid pesticides. These insecticides affect the central nervous system of insects and bind to the same receptors as nicotine, eventually causing death while not being very toxic for mammalian life. They also spread very easily throughout the entire plant when it is absorbed by them, being either sprayed on it or applied as a seed coating before sprouting (Simon-Delso *et al.,* 2015). One of the most commonly used insecticides in the world is the neonicotinoid imadacloprid (Figure 3) (Simon-Delso *et al.,* 2015)). Besides that, it is also the neonicotinoid that is most toxic to bees (Blacquiére *et al.,* 2012).It was shown that a sublethal level of imadacloprid caused an increase in *Nosema* infections in A. *mellifera* (Pettis *et al*, 2011). Lu *et al.* (2012)

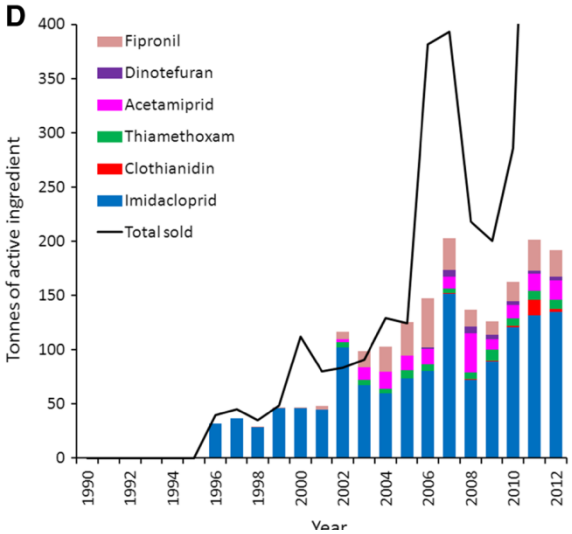

Figure 3: Tonnes of insecticticide used in California in recent years. Source: Simon-Delso *et al.,* 2015

showed evidence for a link between imadacloprid and CCD. They were able to replicate CCD in 15 out of 16 experimental hives by feeding the bees high-fructose corn syrup laced with a sublethal concentration of imadacloprid over 13 weeks. The hives survived for 12 more weeks before succumbing to CCD or CCD like behavior. Even more intriguing is that they did not find high levels of the other possible causes of CCD, *V. destructor* and *Nosema* infection, though they did have to guess the imadacloprid levels within the legally allowed limits and didn't account for the possible toxicity of the miticides used or the negative effects of a limited diet. A later experiment with the neonicotinoid imadacloprid and clothianidin showed similar results, with half of the treated hives showing CCD after overwintering (Lu *et al.* 2014). However, Faucon *et al.* (2005) found no such effects from feeding honey bee hive saccharose syrup laced with imadacloprid. Imadacloprid, along with clothianidin, was also found to increase infection rate of the deformed wing virus (McMenamin *et al.*, 2016). Another neonicotinoid, thiamethoxam, increases the chance that workers will suffer homing failure, never returning to the hive. This is particularly relevant as it seems similar to parts of CCD (Henry *et al.,* 2012).

Pesticide	Class
Imadacloprid	Neonicotinoid insecticide
Clothianidin	Neonicotinoid insecticide
Thiamethoxam	Neonicotinoid insecticide
Dinotefuran	Neonicotinoid insecticide
Fipronil	Phenylpyrazol insecticide
Chlorpyrifos	Organophosphate insecticide
Phosmet	Organophosphate insecticide
Glyphosate	Organophosphorus herbicide
Chlorothalonil	Organic fungicide
Captan	Pthalimide fungicide
Myclobutanil	Ergosterol inhibiting fungicide
Boscalid	Carboxamide fungicide
Tebuconazole	Fungicide
Iprodione	Fungicide
Fluvalinate	Pyrethroid miticide
Tau-fluvalinate	Pyrethroid miticide
Coumaphos	Organophosphate miticide & insecticide
Fumagillin	Anti-microbial agent
Thymol	Organic miticide & insecticide

Table 2: List of pesticides that are discussed in the essay and their classes.

Clothianidin and dinotefuran, another neonicotinoid, also show this effect (Matsumoto, 2013). Sandrock *et al.* (2014) also tested the effects of thiamethoxam and clothianidin on the hives viability. They found that exposure to thiamethoxam and clothianidin caused increased mortality in the workers and in the larvae, as well as reducing foraging success, pollen retrieval and honey production. They also found more long-term effects, with a reduced propensity to start swarming, while gaining an increased chance of replacing the old queen. Woodcock *et al.,* (2016) found that insect species (including *A. mellifera*) that preferred to forage on oilseed rape were negatively affected if they foraged on fields treated with neonicotinoids.

Of course, neonicotinoids aren't the only insecticides that could be affecting *A. mellifera*. Other commonly used insecticides are fipronil and chlorpyrifos. Fipronil and chlorpyrifos both work by blocking neuroreceptors in the insect nerve system, resulting in death (Simon-Delso *et al.,* 2015).

In lower concentrations, fipronil indirectly decreases the reproduction of the queen, by impacting the drones' ability to produce healthy spermatozoa, though drones are not usually exposed to high concentrations of pesticides (Kairo *et al.*, 2016). While fipronil wasn't often found inside hives, when it was found, it was usually in high amounts above the lethal dose at which 50% of the sample dies (LD50), which was shown by multiple dead bees (Mullin *et al.,* 2010). According to Sanchez-Bayo & Goka, (2014), thiamethoxam, clothianidin and imadacloprid, alongside non-neonicotinoids insecticides phosmet and chlorpyrifos, were considered to have some of the highest chances of negatively affecting *A. mellifera* through consumption of contaminated pollen, honey and nectar. Chlorpyrifos in particular has negative effects on queen emergence (McMenamin *et al.*, 2016)

Herbicides & fungicides

Herbicides and fungicides are chemicals used by humans to protect their crops and other plants from unwanted weeds and fungi, primarily used in agriculture. Like insecticides, these are usually dissolved and sprayed across the fields, either by tractor, spray tower or in some cases, planes. Some fungicides get absorbed into the plant, which will them kill fungi that tries to absorb the plants nutrients. Other herbicides are instead absorbed into the soil and kill the plants which absorb it. However, it is the contact herbicides and pesticides, which get sprayed on top of the plants and stays there, that is the most likely to come in contact with *A. mellifera*. The effects of these pesticides on honey bees are twofold. The first way that herbicides and fungicides can affect *A. mellifera* is through direct poisoning, either by contact or oral intake. There hasn't been a lot of research done on the direct toxic effects herbicides have on *A. mellifera*, most of which is focused

on glyphosate, a herbicide that came into the news because of possible carcinogenic properties on humans. Whether or not it does have carcinogenic effects on humans, glyphosate seems safe for *A. mellifera* in the amount that is usually used on fields. Cheng Zhu *et al. (*2015) found it the least harmful to bees of the 42 different pesticides tested at recommended field level exposure, killing less than 1% of the bees, though there might be some sublethal effects in combination with metals on the vitamin A metabolic pathways (Jumarie *et al.,* 2016).

The other effect herbicides have on honey bees is that by killing and removing the unwanted plants, people remove other sources of pollen from bees, reducing pollen diversity (Sánchez-Bayo *et al.,* 2016). While bees can survive on the pollen of a single plant species, this is only healthy for a small amount of plants, such as sweet clover and mustard. Besides those plants, honey bees need a variety of different pollen to avoid nutrient deficiency in the colony (Brodschneider & Crailsheim, 2009). Adult *A. mellifera* who suffer from nutrient deficiency live shorter lives, have problems with the development of their ovaries and hypopharyngeal glands and they suffer a reduced immune response compared to *A. mellifera* from a well-fed hive (Alaux *et al.*, 2009 ,Brodschneider & Crailsheim, 2009). Larvae reared with a low diversity of pollen exhibit similar symptoms as adults, but to a larger degree (Brodschneider & Crailsheim, 2009, Scofield &Matilla, 2015). In addition to the shared problems, the larvae also were suffering from behavioral problems. As adults, they were less proficient foragers, foraging of a smaller amount of time, as well as being twice as likely to die (Scofield &Matilla, 2015). They were also less proficient waggle dancers, relaying the position of patches of flowers not as often, while also being not as accurate with relaying the position of the flowers (Scofield &Matilla, 2015).

There has been a bit more research done on the effects of different fungicides on *A. mellifera*. Because fungicides can be applied to blooming plants without damaging them, bees are more likely to come in contact with it than with an indiscriminate herbicide (Johnson *et al.,* 2013). One of the most commonly used fungicides in agriculture is chlorothalonil. Chlorothalonil works by blocking the fungal cellular detoxification process, which in turn causes cell death, eventually killing the fungus. Besides the intended effect, chlorothalonil also affects honey bees negatively, with even more severe effects on their brood. The usual concentration of chlorothalonil found in the pollen & beeswax in North-American hive is unusually high (respectively with an average of 1.6 & 0.5 ppm) compared to most other found pesticides, while the hive also contains high levels of other fungicides: captan, myclobutanil and boscalid (Mullin *et al.,* 2010). However, the concentration of chlorothalonil and the other fungicides are still well below the LC50 (1110 ppm) of adult honey bees, so the adult honey bees usually don't die. However, when testing a similar level of pollen chlorothalonil being fed to *A. mellifera* larvae in the form of royal jelly, Zhu *et al.,* (2014) found that over half of the brood died within 6 days. Furthermore, even though the *A. mellifera* adults don't die, there are several sublethal effects found linked to chlorothalonil exposure. The microbial gut biota of honey bees is shown to be associated with both the immune system and metabolism (Vásquez *et al*., 2012, Kakumanu *et al.,* 2016). Exposure to chlorothalonil changes the structure of the bacterial community in the honey bee gut, with the most severe change being a decline in *Lactobacillaceae*, overall beneficial symbionts

which are suspected be involved in several important processes including immunomodulation and nectar processing (Vásquez *et al*., 2012, Kakumanu *et al.,* 2016, Schwarz *et al.,* 2014). The effect on the immune system has further supported by Pettis *et al.* (2013), who found that bees that were eating pollen contaminated with a high concentration of fungicides, with the largest part being chlorothalonil, were more likely to be infected with parasitic *Nosema.* Lastly, in a survey conducted of Belgium *A. mellifera* hives, Simon-Delso *et al.* (2014) found a positive relationship between the amount fungicide residue found in beeswax and beebread (Preserved pollen mixed with nectar and digestive fluids) and CCD symptoms found, though the fungicide found was not chlorothalonil, but boscalid and iprodione (Pettis *et al.* 2013, Simon-Delso *et al.,* 2014). They also found a very clear positive relationship between the amount of farmland surrounding the hive and the probability if that hive displaying CCD symptoms, indicating that even if it is not caused directly by human pesticides, there is some factor related to human agriculture that increases the possibility of CCD (Figure 4)

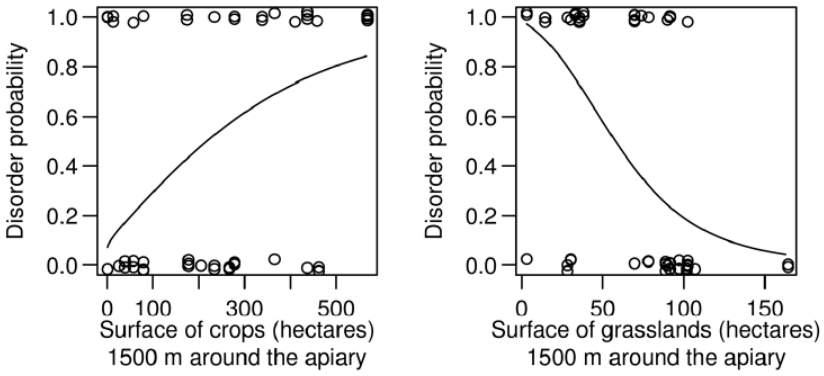

Figure 4: Surface of farmland & grassland surrounding hive vs. the probability of developing CCD symptoms (Simon-Delso *et al.,* 2014)

Miticides & other preventive substances

Miticides, also known as acaricides, are an interesting conundrum, as they are deliberately applied to bee hives in order to protect the bees from parasitic mites, with the hope that the reduction of parasitism offers enough advantage to the hive to offset any negative effects the bees suffer from the toxicity of the miticide itself, while at the same time preventing the miticide from contaminating the honey supply or getting accumulated in the honeycombs (Johnson *et al.,* 2009). Depending on the substance used, they are applied by placing either chemically impregnated strips or gel packets in the hive (Johnson *et al.,* 2009). Bees come in contact with the strips or the gel, hopefully killing the mites that are carried by said bee as well as spreading the toxin further into the hive. It is mostly used to combat the *Varoa destructor* mite, one of the commonly suspected causes of CCD.

Two of the most commonly used miticides are fluvalinate and coumaphos. Fluvalinate and its isomer tau-fluvalinate are pyrethroid pesticides affecting the voltage-gated sodium and calcium channels, while the organophosphate coumaphos interferes with nerve signaling and function (Johnson *et al.,* 2009, Johnson *et al.,* 2010). Beside its effect on mites, coumaphos is also used as an insecticide against parasitic flies, beetles and fleas. Mullin *et al.* (2010) found in their survey of North-American honey bee colonies that there was a large amount fluvalinate or coumaphos in 98% of their wax samples and at least 75% of their pollen samples and that over 47% of their samples contained both, making it the most detected pesticide they found. This is most likely due to its widespread use against parasitic mites. Though the quantities found were below the LD50 (15 and 46 ppm for fluvalinate and coumaphos respectively), they were still very high (6 and 3 ppm on average respectively) (Mullin *et al.,* 2010). Sanchez-Bayo & Goka's (2014) worldwide survey found a lower abundance of fluvalinate and coumaphos in their samples,

with North-American samples being more likely to contain the miticides, while they also reported a high average concentration of fluvalinate and coumaphos. Again, the effects of the pesticide concentrations in the hive are more pronounced on the larvae compared to adults. When Zhu *et al.,* (2014) tested these levels of fungicides in their food on larvae over 6 days, they reached a cumulative mortality rate of over 50%, with coumaphos being the less toxic than fluvalinate. Berry *et al.* (2013) tested sub-lethal effects of two miticides containing fluvalinate and coumaphos (Apistan TM and Check Mite + TM respectively), while accounting for the beneficial effects of reduced parasitism by *V. destructor*. They found that coumaphos accumulates more rapidly in the beeswax than expected and that neither fluvalinate nor coumaphos directly affects the survival of the adult bee population, but that they do affect brood survival. Coumaphos also decreases the lifespan of bees if they were exposed to it as larvae, while fluvalinate actually seems to improve it (Berry *et al.*, 2013). Wu *et al.* (2011) found that fluvalinate nor coumaphos were the most abundant pesticides in contaminated hives and that larvae raised in said hives took longer to grow into adults, had a higher chance to die and lived shorter as adult workers. Similar to the fungicides described above, fluvalinate and coumaphos also change the composition of the microbial gut biome of *A. mellifera*, though their effects are less pronounced than chlorothalonil. (Kakumanu *et al.,* 2016).

Fumagillin is an anti-microbial medicine, usually applied by dissolving it in syrup. It is the most commonly used medicine for combating the gut parasites *Nosema ceranae* and *Nosema apis* (Williams *et al.,* 2010, Huang *et al.,* 2013, Johnson, 2014). It is also relatively safe to use, with little to none apparent side effects (Williams *et al.,* 2010, Huang *et al.,* 2013, Johnson, 2014). The largest disadvantage that it has, which it shares with other anti microbial medicines and miticides is that they can't be used all the time or at a too low concentration, as this will cause the parasites to become resistant towards that particular medicine (Huang *et al.,* 2013, Johnson, 2014). Furthermore, at low concentrations, the more dangerous invasive *N. ceranae* seems to gain a competitive advantage over the less dangerous native *N. apis* (Huang *et al.,* 2013).This is even worse if the beeswax is impregnated with it, causing the fumagillin to remain in the hive even longer (Mullin *et al.,* 2010). Lastly, thymol is a natural insecticide that is produced by the common thyme *Thymus vulgaris* that is both effective against mites and *Nosema* infections depending on its method of distribution (Costa *et al.*, 2009, Tananaki *et al.,* 2013, Johnson, 2014). Compared to other chemical miticides, it is on average less effective than other manmade chemicals (Tananaki *et al.,* 2013). It can also cause the workers to abandon or kill the queen if it is applied in a too high dose, as well as reducing the survival chance of the larvae and affecting the phototactic behavior of the adult worker bees (Carayon *et al.,* 2013, Tananaki *et al.,* 2013, Johnson, 2014).

"Inactive" ingredients

Recent findings suggest that in some cases, the actual toxic substance might not even be the true danger. During the application of pesticides, depending on exactly what kind of pesticide is used and the delivery method, so called "inactive ingredients" are used to make the pesticide more effective or more easily applicable. Because these inactive ingredients are biologically inactive, they are not as thoroughly tested as the actual pesticide and are subject to less tight regulations (Mullin *et al.,* 2014). However, it was recently found that the inactive ingredients might form an even

greater danger to unintended targets such as *A. mellifera* than the actual pesticide itself, either by themselves or by changing the effects of the toxin (Mullin *et al.,* 2014, Mullin, 2015). For example, with the right adjuvants added, the fungicide tebuconazole can be as lethal to bees as enamectin benzoate, the most bee toxic insecticide known to man (Mullin, 2015). Even oral and topical application of only the adjuvants can cause death in adult bees (Goodwyn & McBrydie, 2000, Mullin, 2015). Organosilicone surfactants are often used to reduce the surface tension of the pesticide solution, increase the absorption of pesticides by plants and improve the mixing of pesticides with water, but they can cause learning impairments, decreased viral responses and increased mortality in *A. mellifera* (Ciarlo *et al.,* 2012, Mullin *et al.,* 2014, Fine *et al.*, 2017). Nonionic surfactants also cause learning impairments, but to a lesser degree than organosilicone surfactants (Ciarlo *et al.,* 2012, Mullin *et al.,* 2014). N-methyl-2-pyrrolidone, another solvent that is often added to pesticides, was found to be dangerous for larvae, with the lowest concentration killing half of the larvae within 4 days (Zhu *et al.,* 2014). Overall, adjuvants are still a relatively unstudied area, especially in relation to CCD.

Synergies between pesticides

While the sections above focused on different kinds of toxins, there is another factor which might contribute to CCD. Most of the studies that have been done tend to focus on a single toxin or sometimes two at a time. While this does give a clearer image of what a certain pesticide does, it is not reflective of reality, where bees are more likely to be exposed to multiple pesticides (Mullin *et al.,* 2010, Johnson *et al.,* 2013, Sanchez-Bayo & Goka, 2014, Simon-Delso *et al.,* 2014). It is possible, even at a dose at which the individual pesticides have little to no negative effects, that when the bees are exposed to multiple pesticides at the same time, the different pesticides will synergize, resulting in even greater negative consequences than you would expect from the individual components alone. Thiamethoxam, clothianidin and imadacloprid all become more dangerous to bees when they are also exposed to ergosterol inhibiting fungicides, such as myclobutanil. However, the latter are not often found in the vicinity of honey bees (Sanchez-Bayo & Goka, 2014). It is also not limited to just a few pesticides. Johnson *et al.* (2013) tested 53 different combinations of different kinds of miticides and fungicides. They found that 28 of those combination resulted in additional synergistic toxicity, with tau-fluvalinate in particular being more toxic in most combinations.

One possible explanation for the synergistic effects of multiple pesticides is the competition for the detoxicative cytochrome P450 monooxygenase process. This is one of the processes used by *A. mellifera* and other insects to remove the toxic influence of nicotine, neonicotinoids and other toxins (Johnson *et al.,* 2009, Johnson *et al.,* 2013). For example the miticides coumaphos & fluvalinate have an increased toxicity, more than you would expect from just adding the 2 together. This is presumably because detoxicative P450 enzymes will try to remove them both miticides, but there too much for it to be removed fast enough, even though they were applied in sublethal doses (Johnson *et al.,* 2009).

However, there is also the possibility that certain mixtures could negate each other's toxicity. For example, Zhu *et al.,* (2014) found, by feeding different mixtures of fluvalinate, coumaphos, chlorothalonil and chlorpyrifos to *A. mellifera* larvae that, while most mixtures didn't have synergistic effects, the combination of chlorothalonil and fluvalinate,

as well as the combination of chlorothalonil and coumaphos, synergized to be even deadlier to larvae. However, the combination of all three seemed to make the entire mixture less toxic. Overall, like adjuvants, this is still a relatively uncharted area, especially in relation with CCD.

Conclusions

So, can we conclude that human produced toxins are the main cause in triggering Colony Collapse Disorder in *A. mellifera?* While *A. mellifera* definitely experiences negative effects from the different kinds of pesticides they come in contact with, there is not enough evidence to conclude that pesticides are the main cause of triggering CCD. While two studies in particular (Lu *et al.* 2012 and Lu *et al.* 2014) showed a major possible link between CCD and neonicotinoid pesticides, these were done by the same person and they were the only ones to find such a direct link, with other studies not finding such a direct link. Furthermore, sublethal effects at low doses seem more important than lethal effects for CCD. This makes sense, as a high dose would just kill the bees immediately or after a relatively short while and, in doing so, would not show the characteristic disappearance during overwintering. So, in conclusion, pesticides are most likely an exacerbating factor for CCD. In other words, CCD can occur without exposure to human made toxins, it is just more likely to occur if the hive is exposed to one or more type of pesticides. Furthermore, neonicotinoid insecticides and fungicides seem to be the most dangerous of the different pesticides, because of the combination of the potential damage they can cause and their prevalence in *A. mellifera* hives. On the other side, herbicides seem the least harmful directly, though their effect through reducing pollen diversity might make them as dangerous as the neonicotinoids.

Based on what I have read, I would speculate that pesticides are a bit of an optional first step in causing CCD mostly by weakening the immune system and enhancing the symptoms caused by other factors. Furthermore, though I haven't read extensively about the other potential causes of CCD, I speculate that the most likely cause is a combination of poor nutrition and high viral load of recently discovered viruses. Likewise, the *V. destructor* mite and *Nosema* infection, especially of the invasive *N. ceranae*, are also exacerbating factors. Note that this is all speculation on my part based on limited reading done on these other factors. To make a real, more definitive conclusion about the relative importance of each possible factor is above the scope of this essay and would take more research and 3 or 4 more essays about the subject.

Discussion

First of all, the role of pesticides in relation to CCD and, more generally, in the increased honey bee mortality in recent years is a hotly debated topic with competing different sides and interests. Each of which place a different value on the survival of bees vs. the benefits of using pesticides, ranging from ideological (activists and consumers) to political (politicians), scientific (ecologists and other researchers) and commercial (beekeepers, farmers and chemical companies). Because it is such a hot topic, it might be a bit difficult to find unbiased sources. For example, what in one article is used as field relevant doses is decried in another as being an unreasonably high value that can't be found under field conditions. If I would judge the sources I used, I would say that some might be a bit biased towards blaming pesticides. Although I might be a bit biased against pesticides in that regard myself, especially regarding the more indiscriminate pesticides during the flowering season.

There already has been a political movement to ban some of the pesticides discussed here. Thiamethoxam, imadacloprid and another neonicotinoid pesticide clothianidin, as well as fipronil have been under a temporal, partial ban in Europe since 2013 and are currently under review to see if the ban should be renewed or changed. At the moment, the European Food Safety Authority (EFSA) has concluded that imadacloprid and clothianidin are harmful for bees in multiple forms (EFSA (1), 2015, EFSA (2), 2015), while no such decision could be reached about thiamethoxam, in part because there hasn't been enough studies done on its effects (EFSA, 2016). Whether or not the ban will extended hasn't been decided yet. In case of fipronil, the EU has decided to not ban it, but to allow the license to sell and use it in the EU to expire in 2017, effectively banning it (EU, 2016).

On that note, there are a lot of different kinds of pesticides. What I've discussed here is only a small part, though I have tried to go for the pesticides that bees are most likely to come in contact with. Furthermore, most work has been done on one kind of toxin at the same time, which does not entirely reflect reality. Especially the less well researched synergetic effects could be very important, making the research into the effects of multiple pesticides at once even more important. Another major drawback of some of the studies used here is that they only look at the effects on an individual level. In nature however, the loss of a single or even a small amount of workers is no problem. It is much more ecologically relevant to look at the effects on a colony level, although this is much harder. Individual studies do have the advantage as being easier to do, as well as being relatively good models for the possible effects on a colony level.

Should the honey bee population go extinct or be reduced enough that, for pollination purposes, they can no longer pollinate in any significant amount, not everything is lost. For example, honey bees are not the primary pollinators everywhere. In South America the stingless bees are much more important pollinators than the imported *A. mellifera* (Barbossa *et al.*, 2015). So for regulatory reasons, it would be important to also look at these species and not only honey bees for regulating pesticides in order to safe pollinators. Furthermore, in regions where *A. mellifera* is the primary pollinator and with significant wild populations, wild bee colonies could possible pick up some of the slack. Wild bee populations are less likely to suffer from CCD, so they could serve as a buffer for the losses agricultural bees suffer (Winfree *et al.*, 2007, Simon-Delso *et al.,* 2014). However, this backup should not mean that we shouldn't take any actions and hope that the wild and alternative pollinator populations are capable of picking up the slack. It is, at most, a fragile net that buys us some leeway if we do nothing.

Furthermore, according to Pettis *et al.* (2013), even bees that are supposed to be primarily pollinating certain agricultural crops got part of their pollen from wild plants outside the fields. While this has the advantage that bees might not be entirely dependent on only the sprayed pollen of most likely one crop, it does mean it is not only important to be careful on the fields, but also of be wary of possible pesticide runoff into the surrounding area. This would also be advantageous for the wild bee populations.

Note that CCD is mainly a problem in Europe and North America. If you look at South America and Asia, there are almost no cases of CCD. In case of Asia, this might be because two of the potential causes of CCD (*V. destructor* and *N. ceranae*) are native to that region, resulting in the native honey bees having a better resistance against them (Chantawannakul *et al.*, 2015, Lin *et al.*, 2016).

In case of South America, it might be due to a lower agricultural pressure, as well as a higher temperature year round (Vandame & Palacio, 2010, Maggi *et al.*, 2016).

Ultimately, to be able to draw a more elaborate conclusion, I would have to do more research into the other potential causes of CCD, something that is a bit out of the scope of the current essay. So, summarizing, it is unlikely that pesticides are the single prime cause in triggering Colony Collapse disorder. It is however, an important exacerbating factor.

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