IMPACTS OF HERBICIDES ON BEES: IMPLICATIONS OF GLYPHOSATE ON WILD BEE POPULATIONS

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ABSTRACT

Bees are an extremely valuable organism which contribute greatly to human life and wellbeing. The role they play in global food security and the applications of honey bee by-products within medicine have been recognised for many years. However, recently there has been an increase in concern over the status of bees and how losses in their populations can bring devastating ecological and economic consequences. With the complete absence of bees and their pollination services, human beings are at risk of developing many health issues. A study by Smith et al. estimated that approximately 71 million people, within low in-come countries, could become newly deficient in vitamin A, as well as an additional 2.2 billion (currently consuming less vitamin A than required) experiencing further declines in vitamin A supplies. Their population across the globe is experiencing major declines and some species have experienced extinction events. Habitat destruction, chemical-intensive agricultural practices and bee disease are all thought to be drivers behind such losses. An area in Southwest China currently has a complete absence of pollinators dues to its intensive agriculture practices and pollination is now carried out by hand. Numerous studies have been carried out which have looked at the impacts of pesticides on bee populations. In recent years many studies have focused on the effects of neonicotinoids – a systemic insecticide, on non-target organisms like bees. It has been found that bees exposure to neonicotinoids experiences reductions in learning ability and memory and is thought to be a major driver behind honey bee colony collapse disorder. The results from these studies have led to growing concern over the impacts of glyphosate on bee health and function. Recent studies carried out looking at glyphosates have given rise to potential impacts on human health and similar effects on bees as neonicotinoids.

INTRODUCTION

In recent years, campaigns which have focussed on pollinators, like bees, have gained a great momentum in terms of popularity. This is likely to be attributed to the ever-growing evidence which supports the importance of pollinators and the services they provide. Bees play a major role in global food security and crop production, as a result of the ecosystem service of pollination. The importance of crops which require animal mediated pollination has been recognised for many years and some have held great importance in the past. For example, in the 19th century, cocoa beans, a crop which is pollinated by bees, was used by the Aztecs as a method of currency (Einzig, 1966).

Currently there are approximately 20,000 different species of bee across the planet (Richardson et al., 2019), but this number could drop as agricultural practices, habitat destruction and climate change continue to alter the landscape. Since the late 1990's declines in the bee population have been observed. Habitat destruction, loss of flower resources and increased agricultural intensity are all drivers behind the reductions in species richness, geographical range and overall abundance (Potts et al, 2010; Breeze et al, 2011). Recent research has raised concern over pollinator declines and how their decreasing population can have both ecological and economic impacts.

A life without bees would look very different to the world we all know. It would be a world lacking in foods with vibrant colours, with the absence of many fruits and vegetables. The complete loss of pollinating insects however, is not a far-fetched idea and is a painful reality in an area in Southwest China. The chemical-heavy agriculture practices lead to a complete absence of pollinators and pollination is now carried out by humans. Increased agricultural intensity has been the focus of many studies conducted in the last few years (Potts et al, 2010), and the overuse of neonicotinoids (Van der Sluijs et al., 2013), in particular, has been a popular topic of research. Studies have found that neonicotinoids bring about many devastating impacts to the health and function of bees and it has been suggested that the systemic insecticide is a driver behind Colony Collapse Disorder (CCD) in honeybees and the loss of some wild bee species.

The impacts associated with the likes of neonicotinoids has led to growing concern over other pesticides that bees may encounter in the natural environment, and how they may impact them and the services they provide. The most recent topic of concern is the herbicide, glyphosate. I set out to focus on glyphosate and its impacts on wild bumblebee productivity and parasitology. This report will review literature which takes focus on the importance of pollinators and their role in global food security and products used as medicine, the drivers behind the declining bee population and the role played by pesticides as well as honing in on potential relationships between different stressors and how they may affect bee health and function.

POLLINATORS AND THEIR SERVICES

Pollination is a fundamental component to global food production and therefore the survival of humankind. Although there are several methods of pollination, seed production in the wild is considered pollination-limited (Ashman et al., 2004; Burd, 1994; Knight, Steets and Ashman, 2006). Therefore, it is animal mediated pollination which is of great importance. Birds, bats, bees and hoverflies are a few examples of animals which are considered 'pollinators' for the role that they play in the pollination of various plants and commercial crops. Just under 90% of angiosperms and 75% of agricultural crops, of which contribute to 35% of global food production, are somewhat dependent on pollinators for their production (Ollerton, Winfree and Tarrant, 2011; Klein et al, 2007).

Yet for centuries pollinators have been underappreciated and overlooked, but in recent years their services have become increasingly recognised within the agriculture setting (O'Toole, 1993; Cane, 1997; Kevan & Phillips, 2001; Klein, Steffan-Dewenter and Tscharntke 2003). The pollination services, carried out mostly by bees, now stands at an annual global value of around €153 billion per year (Gallai et al, 2009), an annual £603 million in the United Kingdom and at least €53 million in the Republic of Ireland each year. (Hanley, Ellis and Breeze, 2013; Bullock, 2008).

However, in terms of human health, the pollination services carried out by bees and other pollinators are of great importance. A 2015 study carried out by Smith et al., found that with the complete absence of pollinators, approximately 71 million people, within low income countries, could become newly deficient in vitamin A, as well as an additional 2.2 billion (currently consuming less vitamin A than required) experiencing further declines in vitamin A supplies. Not only this but the study estimated that global fruit, vegetable and nut supplies would experience reductions of 22.9%, 16.3% and 22.1%, respectively, following a complete loss in pollinator services. It was estimated that such dietary changes could bring increases of 1.42 million in global deaths caused from non-communicable and malnutrition-related diseases each year – a 2.7% increase in total annual deaths, and 27 million lost disability-adjusted life-years (DALYs), the equivalent to 1.1% increase in DALYs each year (Smith et al., 2015).

However, it is not only the role they play in global food security and crop production that serves to benefit human health and well-being, but those products produced directly by the pollinators, honey bees more specifically, that contribute to modern medicine. By-products of bees such as propolis and honey are a few examples of products that have been and continue to be used in medicine. Propolis is a non-toxic substance that possesses a vast range of applications and is used in the treatment of anemia, eczema, burns, immune system support and improvement, due to its anti-bacterial, anti-oxidant, anti-fungal, and anti-viral effects (Shruthi and S. Suma, 2012). In fact, Hippocrates, the founder of modern medicine, was aware of the medicinal applications of propolis and used it to heal sores and ulcers. Honey is another example of a by-product of bees which is frequently utilised for its medicinal properties. The use of honey as a medicine is not a modern development, in fact records as early as 350BC written by Aristotle illustrate that honey was used as a salve to treat wounds and sores (Aristotle, 350 BC). Honey has since been used for its antibacterial properties (Cooper and Molan, 1999) and was approved for wound dressing by the United States Food and Drug Administration in 2007.

BEE DECLINES

The fate of both domesticated and wild pollinators such as honey bees, bumble bees and solitary bees is of great concern as they have experienced declines in the last few years and their population continues to decrease. As briefly mentioned, research suggests anthropogenic stressors such as habitat destruction and chemical-intensive agricultural practices, as well as bee disease are all drivers behind such pollinator losses.

Long-term declines and annual losses in honey bee colonies have occurred throughout Europe. It has been suggested that such declines are a product of political and socioeconomic factors, where the European honey bee population experienced a 25% decrease since the mid 1980's. This is thought to have occurred due to the increased production costs, competition from cheaper honey and the increased ability to afford sugar-based products, all of which led to a 30% decrease in beekeepers and a 25% decrease in colony numbers since the 1980's. (Aizen and Harder, 2009; Potts et al., 2010)

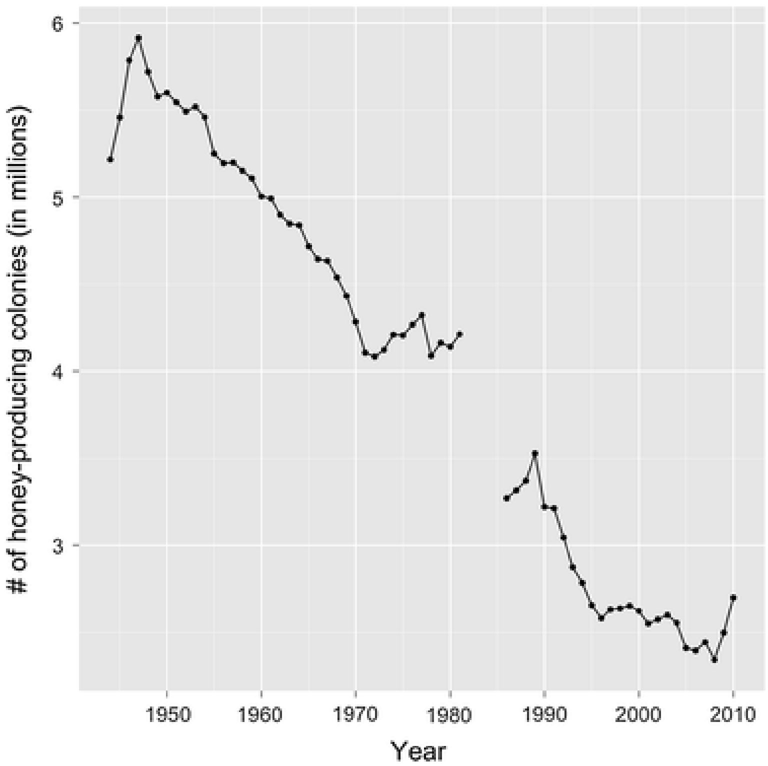

Fig.1.0 Graph showing the long-term declines in honey-producing honey bee colonies from, 5.9 million in 1947 to 2.3 million in 2008 – a loss of 3.6 million colonies (Potts et al., 2010)

However, growing evidence suggests that pests and pathogens have played a role in annual colony losses and could potentially be linked to long-term declines. Before the 1970's brood diseases such as European Foulbrood caused by *Melissococcus plutonius* were thought to be the most economically important threat to the bee population and to this day still plays a significant role in colony losses (vanEngelsdorp and Meixner, 2010).

The wild bee population in Europe has also experienced declines in the last few decades, in fact, it was found that three of the twenty-five British species of bumble bee has become nationally extinct, with a further eight experiencing major declines in their population. The declining bumble bee populations poses detrimental ecological and economic consequences as many wild plant species rely heavily or solely on bumblebees for their pollination. Professor Dave Goulson outlines that agricultural intensification is the primary driver behind wild bumblebee declines in Britain (Goulson et al., 2008)

With the increased awareness on the importance of the pollination services carried out by bees, growing numbers of studies have focused on factors which affect bee health and function. The impacts of pathogens and parasites and how they affect the bee population, as well as their productivity and function. The diagram seen in Fig.2.0 outlines common diseases experienced by honey bees, bumble bees, mason bees and stingless bees. *P.larvae, M.plutonis* and *Spiroplasma* spp. will be expanded upon.

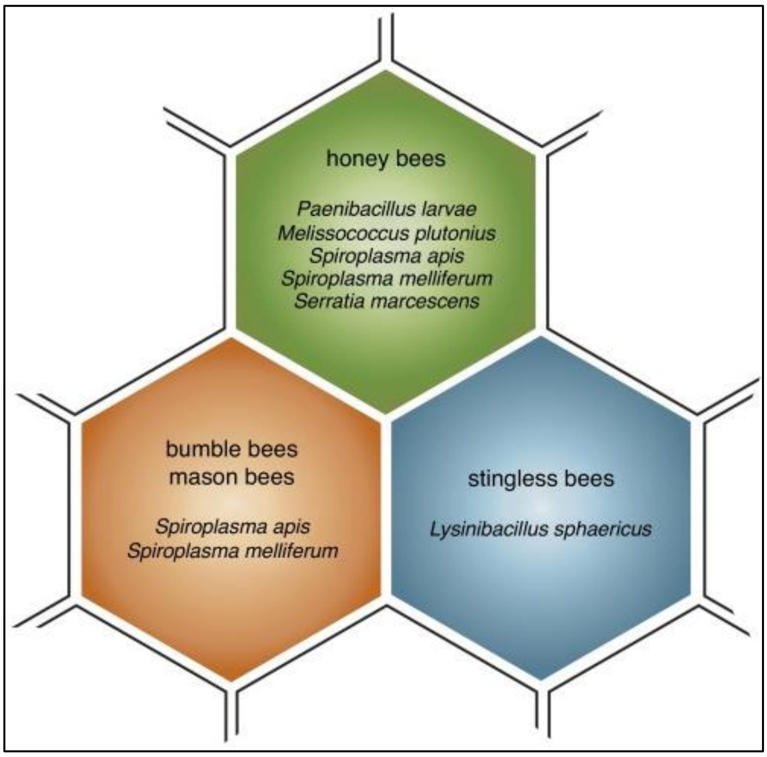

Fig 2.0 A image showing various bacterial pathogens which infect honey bees, bumble bees, mason bees and stingless bees.

Table.1.0 showing common pathogens of bees, their related dieases, common hosts and associate symptoms (Fünfhaus, Ebeling and Genersch, 2018).

Pathogen	Disease	Known host	Signs and Symptoms
Paenibacillus larvae	American Foulbrood	Honey bee	Ropy mass; foulbrood scale in the lower grove of the brood cell
Melissococcus plutonius	European Foulbrood	Honey bee	Dead larvae can be seen lying twisted along the cell wall; along with the presence of removable dark flakes
Spiroplasma apis ***Spiroplasma melliferum***	May disease (?)	Honey bee Bumble bee Mason bee	Crawling and quivering bees; an abdomen which is hard and swollen; undigested pollen within the intestines intestine; colonies recover spontaneously

P.larvae is a bacterium that brings about the development of American Foulbrood (AFB). This particular bacterium has been the focus of many studies and is thought to be the most destructive bacterial disease upon the honey bee population. The only known host for this pathogen is young honey bee larvae (Hoage and Rothenbuhler, 1966; Brødsgaard, Ritter and Hansen, 1998).

M.plutonis is a globally distributed bacterium which is the causative agent of European Foulbrood (EFB). This bacterium possesses a range of hosts including the larvae of the *Apis mellifera* the Western honey bee, *A. cerana* the Eastern honey bee (Bailey, 1974) and *A. laboriosa* the Himalayan honey bee (Allen, Ball and Underwood, 1990). The larvae become infected upon consumption of contaminated food (Fünfhaus, Ebeling and Genersch, 2018).

Spiroplasma spp. consists of a group of bacteria which can infect not only honey bees but bumble bees and mason bees. Originally Spiroplasmas were isolated as pathogens from plants (Gasparich., 2010). Bees which become infected with spiroplasma spp. are no longer able to fly and instead can be found on the ground crawling and quivering (Mouches et al., 1982).

Honey bees and bumble bees can also suffer from parasitic infections. Some of the common parasites seen to infect honey bees include *Varroa destructor* (the Varroa mite) and *Crithidia bombi* in bumble bees.

Varroa destructor is a parasitic mite, previously known as *Varroa jacobsoni* and is thought to be the most devastating parasite that infects honey bee species, in fact, it is considered the greatest single driver behind global honey bee declines. In the past it has been seen to cause vast colony losses (Finley, Camazine and Frazier, 1996) and if left untreated, will cause the collapse of the colony. Bees infected with *V. destructor* will experience reductions in immune function, reduced pesticide tolerance and impaired pupal development, as well as a shortened lifespan (Rosenkranz, Aumeier and Ziegelmann, 2010).

Natural wild bumble bees are also under threat of infection by numerous parasites. *Crithidia bombi* is a common parasite that infects the intestinal tract within bumble bees. It is frequently found within workers during the summer months. Bumble bees become infected upon ingestion and studies have found correlation between infection prevalence and the age of workers. The overall impact of these parasites are reductions in worker bee reproduction (Shykoff and Schmid-Hempel, 1991).

However, growing evidence suggests that parasites, once known to infect either honey bees or bumble bees are now experiencing spillover events. Currently an emerging pathogen problem in wild pollinators is occurring and it is thought to originate from honey bees (*Apis*). This is a cause for concern as the spillover of emerging infectious diseases between honey bees and bumble bees has the potential to become a major driver of mortality of wild pollinators and have the potential to lead to already vulnerable or unmanaged populations to become extinct (Dobson, 2004).

CHEMICAL-INTENSIVE AGRICULTURE

The latter part of the 20th century marked the start of modern agricultural practices with the introduction of powerful machinery and synthetic pesticides (made up of; herbicides, insecticides, fungicides). The growing population during the 20th century put global food production under greater levels of stress, and so give rise to industrial agriculture. Industrial agriculture involves the use of chemical treatments such as pesticides and fertilisers to protect crops against pests and to control weeds, whilst improving yield and quality. (Sánchez-Bayo et al., 2016) A study carried out by Tilman et al., in 2001 looked at the increased use of pesticides following World War II, where it saw an increase in global pesticide production and trade (Tilman et al, 2001) (see Fig.3.0).

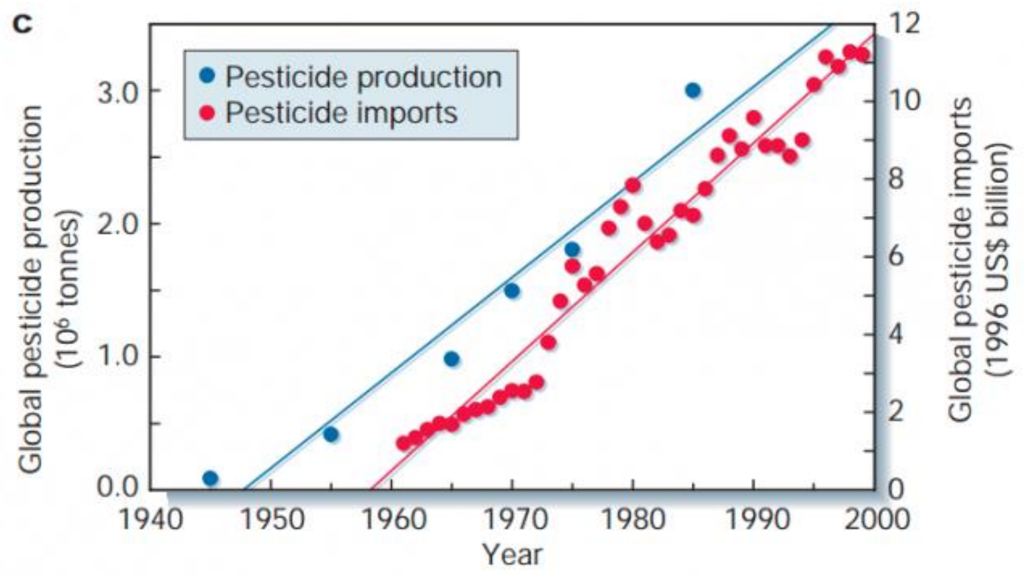

Fig.3.0 *A graph illustrating the rapid growth in global pesticide production and importation between the 1940's and 2000's (Tilman et al., 2002).*

The major changes associated with the movement to modern agricultural practices, have given rise to concern over its impacts upon the environment, biodiversity and human health. In modern day approximately 3 million tons of pesticide is used across the globe each year (Horrigan, Lawrence and Walker, 2002). However, the use of pesticides does not come without negative impacts upon non-target organisms like bees and other pollinators, and there is growing evidence to support just how harmful chemical-intensive agricultural systems can have devastating impacts on bee health at both individual and colony level**.** Insecticides have been directly linked to the death of bees and other pollinating insects (Sánchez-Bayo et al., 2016), and herbicides such as glyphosate indirectly cause declines in the bee population, as it reduced the diversity of their feeding resources (Sánchez-Bayo et al., 2016). Although, recent research suggests that the effects of glyphosate on bee health and function is more than once believed (Balbuena et al., 2015).

Neonicotinoids

Various studies in the past have looked at neonicotinoids, a systemic insecticide. Neonicotinoids (neonicotinoids) have been used within agricultural settings since their introduction in the late 1990's and since became the most widely used class of insecticide (Van der Sluijs et al., 2013). They have received much attention, frequently appearing in the headlines for their impacts on non-target organisms like bees.

Neonicotinoids, used for the control of herbivorous insect pests, can be applied to crops following various methods including; soil drench, trunk injection, foliar spray and seed treatment – each of which gives rise to its own concerns. Seed treatment, a method which involves applying the chemical to the seedlings of a plant has been used on commercial crops such as corn and oil seed rape (Goulson, 2013). This method of application causes the pesticide to be taken up systemically by the growing plant where it is then distributed the across the plants surface area, and within the nectar and pollen. However, it has been found that only 1.6-20% of the amount of active substance applied enters the crop for its protection, the remaining 80-98.4% pollutes the environment (Sur & Stork, 2003). This is a cause for concern, as visiting bees encounter the pesticide while foraging.

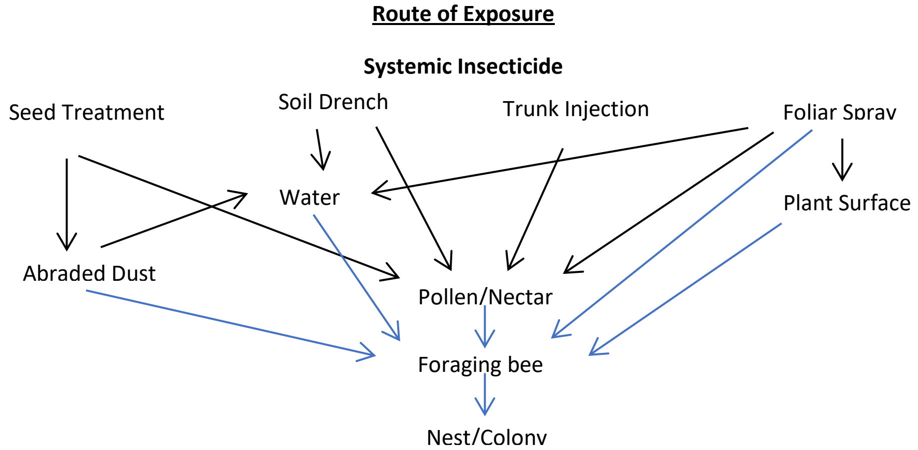

Fig.4.0 A flow diagram illustrating the methods of exposure which bees may experience when foraging

Neonicotinoids work by interacting with the nicotinic acetylcholine receptors (nAChRs) of the bee's central nervous system and due to its ability to mimic the natural neurotransmitter acetylcholine neuronal hyperexcitation, paralysis and even death can be induced (Belzunces, Tchamitchian and Brunet, 2012; Tomizawa and Casida, 2005). In fact, an extensive study carried out in 2007 by Desneux et al. found that sub lethal effects of neonicotinoids can be seen in the following;

- neurophysiology
- larval development
- moulting
- adult longevity
- immunology
- fecundity
- sex ratio
- mobility
- navigation orientation
- feeding behaviour
- oviposition behaviour
- learning

all of which were reported for bees and have potential to have; colony level, population level and community level impacts (Desneux, Decourtye and Delpuech, 2007). In 2013 the European Union introduced a moratorium on three neonic seed coatings (clothianidin, imidacloprid and thiamethoxam) in flowering crops that attract bees, but with increasing evidence have since established a Europe-wide ban of the three main neonicotinoids in 2018.

Some studies conducted in the past have found that honeybees which have been exposed to neonicotinoids present impaired olfactory memory and learning capacity. The chemicals effects on the bees flying behaviour and navigation ability has been tested by running homing flight tests. Bees can travel within a several kilometres around their hive and can share information, about a flight vector relating to a feeding area or nest, with other individuals using the waggle dance. However, both communication and navigation involve various cognitive processes such as; visual distance estimation, recognition of the sun compass and the learning of multisensory cues both inside and outside of the hive (Fischer et al., 2014).

A 2014 study by Fischer et al. looked at the interference of neonicotinoids on honeybee navigation ability. This catch-and-release experiment consisted of training a group of 15-20 bees to forage at a feeder 250m east of the hive, before entering the feeder the bees were caught and transferred into small dark containers where they were fed 49µl of sucrose as well as 1µl of the neonicotinoids; clothianidin, imidacloprid or thiacloprid. The bees were kept in the small container for 90 minutes and were then released at 15-minute intervals where release time, start time of flying, time of arrival at the hive and the flight trace record with the harmonic radar, were measured. The study found that the application of each of the neonicotinoids (at sub-lethal doses) had an impact on the honeybee's navigation ability. The bees that were treated with thiacloprid experienced slower speed than those treated with clothianidin and imidacloprid. It was also found that sub-lethal doses of; clothianidin, imidacloprid and thiacloprid, disabled the retrieval of a remote memory or altered navigation memory (Fischer et al., 2014).

Similarly, a 2016 study carried out by Stanley et al. looked at the impacts of field-realistic exposure to neonicotinoids and how it affected bumblebee foraging and homing ability. Commercial *Bombus terrestris* audax were used and were treated with 40% sucrose solution containing approximately 2.4ppb thiamethoxam. To determine foraging ability, after 5 days of treatment, the number of individuals returning with pollen was measured over a 90 minute period (repeated twice a week for each colony, for a total of 11 observations for each colony, while fluctuating between morning and afternoon). Using RFID tags the mean number of times they entered the colony, the daily mean foraging visits, the mean time of foraging visits each day and the total number of days an individual left the colony to forage were measured. Homing ability was performed after 2 weeks of treatment. The distances used alternated between 1-2km, each of which were both placed in the same compass direction. This study found that bees that were exposed to levels of thiamethoxam that a bee would encounter in the wild, presented changes in foraging patterns, as well as the amount of bees that returned to their hive from 1km away. It was found that those treated with the pesticide spent more time foraging and collected less pollen, but travelled back to their hive from 1km away, more frequently during homing tests than bees from the control colonies (Stanley et al., 2016).

Glyphosate

Glyphosate is an active ingredient in one of the most widely used herbicides. It is considered a non-selective systemic herbicide and since 1974 it has been available for purchase by farmers. Since the latter part of the 1970's the use of glyphosate-based herbicides has increased approximately 100-fold, this figure is expected to keep rising. The pesticide is primarily used for the control of weeds, but due to the development of glyphosate resistant genetically modified crops, it is frequently applied to commercial crops which are consumed by many mammals and humans. Crops such as oilseed rape and soybeans are examples of crops which come into contact with glyphosate-based herbicide. The pesticide is sprayed in the fields where these crops are grown to prevent the growth of weeds, reducing competition between weed and crop growth. However, an increasing number of weeds are becoming glyphosate resistant, thus causing its use to rise substantially. This increased use has led to detection of glyphosate in the water, air and rain. Its use on consumable crops in particular has led to concern of the impacts upon the health on animals and humans.

Once applied, the herbicide accumulates in fruits, grains and leaves. The residues left behind on the crops after treatment cannot be removed. The chemical cannot be washed away and is not broken down during the cooking process (Tudisco et al., 2006). Therefore, it is not surprising that the application of glyphosate-based herbicides to agricultural land, which is utilised for foraging, has led to the detection of the pesticide glyphosate residues within Danish dairy cow’s urine, faeces and milk, according to study by Krüger et al. carried out in 2014**.** In addition, the same study found traces of glyphosate in rabbits.

It has also recently been suggested that the consumption of crops treated with glyphosate shares potential links with the development of many health issues in humans, such as; gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer’s. Krüger et al., found that the concentration of glyphosate within the urine of individuals that consumed primarily organic food was longer than individuals that consumed conventional food (Krüger et al., 2014).

Given the potential risks of glyphosates on human and animal health, questions on the impacts of glyphosate on other non-target organisms like bees could be asked. One study has carried out experiments similar to those conducted using neonicotionoids, the systemic pesticide, to investigate the impacts of glyphosate on honey bee navigation. This study involved a capture-and-release experiment. Bees were first caught at the feeding site and were transferred to the release site. The time taken and the route of the homing flight were measured. The experiment involved the use of control honey bees which were fed with sucrose only and treated bees which were fed with sucrose containing 2.5mg, 5mg, and 10mg l^{-1} glyphosate, and the process was run twice. The study found that a single exposure to glyphosate within the range of 2.5mg – 10mg l^{-1} caused delays in the time taken for the honey bees to return to the hive as well as a change in flight trajectories. This suggests that exposure to glyphosate has an impact on spatial learning processes within honey bees and the impairment between navigation and exposure to increased concentrations of glyphosate were found to correlate. The results outlined that honey bees which were treated with glyphosate displayed more indirect homing flight routes compared to those that remained untreated. Not only this, but control bees displayed improvements in navigation upon second release, where they performed more direct flights compared to their flight trajectories displayed on first release. Whereas, those treated with glyphosate at a concentration of 10mg l^{-1} displayed no changes in navigation ability despite already performing the task. This suggests that bees that are exposed to high concentrations of glyphosate experience impaired navigation performance improvement (Balbuena et al., 2015).

RELATIONSHIPS BETWEEN STRESSORS

It's a reasonable to assume that each driver behind pollinator declines do not work independently of one another but possess a synergistic relationship. Meaning that the various stressors that bees are subject to work together to produce a combined effect on their population declines. It has been found that *V. destructor* and the numerous viruses it carries shares a synergistic interaction that causes increased mortality at both the individual and colony level (Nazzi et al., 2012; Francis et al., 2013). There has also been suggested links between pesticide exposure and honey bee mortality and development. Not only this, but is it suspected that exposure to pesticides increases pathogen burden in both larval and adult bees (Pilling and Jepson, 1993; Johnson et al., 2009a; 2013; Wu et al., 2011)

However, it should be noted that that the extent to which the interactions between stressors occur remains uncertain, as the studies associated which such statements carried out experiments which involved the use of pesticides at unrealistic levels that bees would not encounter in the natural environment. Therefore, there is a need for further research that utilises field-realistic levels of pesticides to confirm suggested relationships between pesticide exposure and increased vulnerability to bees diseases and reduced productivity.

CONCLUSION:

The importance of pollinators and the role they play in human life has been a topic of discussion for many years. However, the recent declines has led to concern over the state of their overall population and how it may impact the services they provide. Many studies which have set out to investigate the drivers behind such declines have found that habitat destruction, chemical-intensive agricultural practices and bee diseases are all contributing factors to the losses on their biodiversity and abundance. It has become increasingly clear that industrial agriculture plays a major role in pollinator losses. It has been found that pesticides like neonicotinoids have an impact on the learning and memory processes in bees. However, the majority of these studies can be criticised for the use of non-realistic concentrations of the chemical which bees would not encounter in the wild. Regardless, the findings of these studies has led to growing concern that other pesticides that bees may encounter while foraging, will have similar effects. More and more studies have set out to investigate the effect of glyphosate on bee health and function and some have already found potential links between glyphosate exposure and impaired learning and memory.

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