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Factors Affecting Global Bee Health

Honey Bee Health and Population Losses in Managed Bee Colonies

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Summary

Many countries around the world rely on the Western honey bee, *Apis mellifera* for commercial pollination of certain crops. Over recent years some regions of the world have been suffering from an increase in losses of their managed honey bee colonies. Colony losses are not unusual, but the increase in losses reported in the USA, some European countries, the Middle East and Japan have received considerable attention, not least because of the honey bees' role in pollination and the absence of an easily identifiable cause.

It has been difficult to establish a common pattern for the colony losses, but it does appear to be a phenomenon of the Western honey bee. A number of different factors are considered to be involved and are now being monitored and investigated further such as pests and diseases, bee management, including bee keeping practices and breeding, the environment, including weather, agricultural practices and the use of pesticides and the availability and quality of food sources.

The global picture identifies the honey bee parasitic mite, *Varroa destructor*, as the major factor in colony loss, with regions that have established mite populations, suffering consistently higher colony losses than those without. The role of the *Varroa* mite in colony losses is supported by a wealth of data. The mite itself contributes to weakening colony health and modifying bee behaviour, but it also spreads secondary infections within and between colonies. A general consensus is emerging that this mite in association with a range of honey bee viruses is a significant factor in the losses of managed honey bee colonies seen globally. The spread of the mite *V. destructor* to the Western honey bee, and its ability to act as a viral reservoir, incubator, activator and transmitter has resulted in levels of certain viruses that affect the survival of the colony.

Increased monitoring and research are starting to shed some light on the factors involved in recent honey bee colony losses. This report focuses on the emerging view that pest and diseases are a key underlying factor in the increase in managed honey bee colony losses globally.

1.0 Introduction

Over a third of global food production is dependent on animal pollination for reproduction and managed honey bees are the most important commercial pollinators of those crops. Many countries around the world, particularly in the northern hemisphere, rely on the Western honey bee, *Apis mellifera* for commercial pollination of certain crops, but over recent years some regions of the world have been suffering from an increase in losses in their managed honey bee colonies. There have been reports of Colony Collapse Disorder (CCD) in the US since 2006 and elevated over-winter colony loss occurs across the northern hemisphere (Neuman and Carrick, 2010). Although some bee losses have been reported in Japan, data published by the Ministry of Agriculture show that the number of bee

hives in Japan has been stable for the past ten years. Significantly there has also been no increase in colony losses reported from South America, Africa and Australia.

Bee colony losses are not a new phenomenon and historical records show that extensive losses are not unusual. Over recent years elevated colony losses have been reported from parts of Europe (Opera, 2012), the USA and the Middle East. Whilst this gives the impression that there is a massive decline, global stocks of honey bee colonies actually increased between 1961 and 2007 with large increases recorded for Asia (426%), Africa (130%) and South America (86%) and Oceania (39%) (FAO, 2009). Even in Europe, there is no consistent trend in bee populations. Numbers have been falling in Northern and Central European countries, but this is also linked to falling number of beekeepers, whereas the number of managed bee colonies is increasing in many Southern European Countries (Potts, 2010).

It has been difficult to establish a common pattern for the colony losses, but it does appear to be a phenomenon of the Western honey bee. CCD involves a weakening of the colony to the point that it can no longer survive and although symptoms include a rapid loss of adult worker bees, it is characteristic of CCD that very few dead bees are found in the hive. Immature bees and food stores of honey and pollen are still present in the hive together with a small cluster of bees around a live queen. In the case of elevated winter colony loss there is also a general weakening of the colony and a decline in the adult bee population. Under temperate climatic conditions symptoms tend to appear from autumn to early spring leading to complete loss of the bee colony during the overwintering phase.

The absence of a single obvious cause of these losses led to the conclusion that it must be due to the combination of a number of factors. This has led to a number of investigations from monitoring bees and their colonies to studying possible underlying effects on bees in the laboratory. This includes looking at the interaction between bee diseases, the role of vectors of the disease and bee immunity, genetic diversity, beekeeping practices and food resources plus environmental factors such as the general climate, weather, agricultural practice and the use of pesticides. Each of these may influence the behaviour of the bees and/or affect the availability and quality of pollen and nectar (Genersch, 2010; Guzman-Novoa et al., 2010; Neuman and Carreck, 2010).

These very diverse factors, may act individually or simultaneously but scientific studies investigating winter colony death have demonstrated the importance of infections in these losses (AFSSA, 2009). It is now, generally considered that the interaction between the bee, its parasites, and infections are the underlying cause of colony collapse and the increase in overwintering losses (Martin et al., 2012).

This report focuses on the pests and diseases affecting bee health and then summarises current thinking on how these relate to the causes of bee colony losses. The information summarised in this report is based on recent scientific publications listed in (Appendix IV).

2.0 Pest and Diseases Affecting Honey Bees

To gain a better view of what is causing the current increase seen in Western honey bee colony losses across the Northern hemisphere, it is important to understand the key pests and diseases affecting bee health. Honey bees are affected by a number of pests and diseases including mites, various viruses, bacterial infections and fungal diseases. Despite the diversity of infectious diseases and their agents that can cause bee mortality, surveillance has been fragmented and thus it has been difficult to gain a clear historical view of bee health (AFSSA, 2009). It is only more recently that the importance of the development and interactions of these pests and diseases is being better understood.

2.1 Parasitic Mites

Varroa, a parasitic mite, is commonly found in association with honey bees. The mite is mobile and moves between bees and within the hive. They are transported by the adult bees from colony to colony through the bees' natural processes of drifting, robbing, and swarming. Varroa can spread slowly over long distances in this way; mites can be found in almost every apiary in Europe and they have spread to all continents, where honey bees are managed with the exception of Australia.

2.1.1 Varroa Destructor

The most significant ectoparasitic honey bee mite is *Varroa destructor*. It originated from South-East Asia and was originally confined to the Eastern honey bee *Apis cerana*. After a shift to the new host the Western honey bee, *Apis mellifera*, during the first half of the last century, the parasite has become widespread across most continents.

There are two distinct phases in the life cycle of *V. destructor* females; a phase attached to the adult bees and a reproductive phase within the sealed drone and worker brood cells. The mite is spread by foraging and swarming bees and Varroa females are transported on adult bees to brood cells for reproduction. Shortly after leaving the brood cell on a young bee, the mites preferentially infest nurse bees for transport back to the brood cells. This may be an adaptive strategy for the Varroa females to increase their reproductive success (Rosenkranz et al. (2010).

The mite feeds on the bee by injuring the cuticle of the pupae and sucking substantial amounts of haemolymph. The haemolymph is an insect's equivalent to blood, distributing nutrients throughout the bee, including immune components which form one of the primary lines of defence against invading microorganisms (Genersch, 2010; Dainat et al., 2012).

2.1.2 The Role of V Destructor in Colony Losses

Although *V. destructor* has become widespread, it is notable that colony losses have not been reported in Australia where the mite has not been introduced. Also in Africa, the African honey bees survive despite the presence of *V. destructor*, as do the Africanized honey bees in South America. This increased resistance of the Africanised honey bees is thought likely to be because they are more aggressive than the Western honey bee and therefore more efficient at grooming and controlling

the Varroa mite populations (Rosenkranz et al., 2010). The association of *V. destructor* with the Western honey bee is seen as a significant factor in colony losses.

As indicated above, it is known that *V. destructor* injures the cuticle of the pupae to suck substantial amounts of haemolymph, and the loss of haemolymph during pupal development has been found to significantly reduce the size and the weight of the hatching bee (Genersch, 2010). For drones, it has been demonstrated that reduced weight has led to decreased flight performance and sperm production. It has also been reported that orientation and homing ability can be impaired in foraging bees with mite parasites, with infested bees needing more time to return to the colony or not returning at all (Genersch, 2010).

Therefore, even if the Varroa infestation is moderate, the reproductive capacity and, therefore, the fitness of honey bee colony is reduced, with the parasitized drones having a significantly lower chance to mate, and infested colonies producing less swarms. At low infestation rates obvious symptoms are not visible and the infestation often remains undetected. Moderate infestation rates may reduce the growth of the honey bee population and, therefore, the honey yield. However, the steps to irreversible colony damage are small, especially if during the autumn the mite population increases while the host population is decreasing (Rosenkranz et al., 2010). The damage threshold is not correlated with a fixed number of mites per colony, but is highly variable, depending on the bee and brood population, the season and the presence of bee viruses. That being said, bee colonies not effectively treated to control mites and which exceed an infestation rate of about 30% in the adult bees during the summer do not have a chance to survive the following winter (Rosenkranz et al., 2010).

2.1.3 Control of Varroa Mites

In the original host, the Eastern honey bee, *Apis cerana*, there is sufficient control of the growth of the Varroa population to prevent any visible damage to the infested colonies. There is no reproduction in the worker brood at all, with reproduction being limited to the drone brood. In addition, grooming and hygienic behaviour help to control the mite on the bees and in the colony (Rosenkranz et al., 2010).

However, in the Western honey bee, *Apis mellifera*, if left untreated, or if treatment is ineffective, mite-infested colonies in temperate climates will eventually die within a few years (Genersch, 2010; Rosenkranz et al., 2010). As a result, Varroa control strategies have had to become an integral part of the beekeeping practice in order to keep infestation levels below the damage threshold for reducing colony losses.

Beekeepers have a wide range of different chemical substances, application techniques and methods to keep mite populations under control. The most effective synthetic chemicals against *V. destructor* are the organophosphates (coumaphos), the pyrethroids (tau-fluvalinate and flumethrin) and the amidines (formamidine and amitraz). Most of these chemicals are easy to apply, economical and convenient, however, as with any such treatments, resistance management strategies are

essential to maintain their effectiveness (Rosenkranz et al., 2010). This relies on bee keepers using different chemicals in strict rotation to minimise resistance.

The use of alternatives, such as organic acids and essential oils, give more variable control due to the specific conditions required for effective treatment. Therefore, it has been suggested that less effective control of the Varroa mite is a contributing factor in colony losses and more efficient treatment of honey bee colonies are urgently needed (OPERA, 2012; Rosenkranz et al., 2010). In 2009, there were only three medicinal products with a marketing authorisation in France available to control *V. destructor* and only one of these was considered to be effective against the parasite (AFSSA, 2009).

2.2 Viral infections

There are more than 18 different viruses that have been detected in association with the Western honey bee around the world. Many viruses are found in association with the honey bee and they generally persist as low level infections, causing no obvious signs of disease. Because bee viral infections were considered not to be a significant problem to honey bee health, there is very little known historically about the natural prevalence, viral load, and strain diversity of honey bee viruses. However, it was known that the viruses vary in virulence and infectivity and that some viruses could be pathogenic under certain favourable environmental circumstances.

The spread of viruses is influenced by the Varroa mite (Martin et al, 2012, Nazzi et al., 2012). *V. destructor* has been a parasite of the Western honey bee for at least 40 years, whereas increases in colony losses have only become more apparent recently, so it is not considered that the mite alone could have resulted in these losses. The mite is found to:

- (i) act as a viral reservoir, incubator, activator and transmitter;
- (ii) select out more pathogenic strains of the same virus, e.g. DWV;
- (iii) allow, through its feeding behaviour, the virus to be transmitted directly into the bees' haemolymph, thus by-passing conventional, established oral and sexual routes of transmission.

2.2.1 Key Viral Infections Implicated in Colony Loss

Although there are a number of different viruses that have been detected in association with the Western honey bee, identifying a definitive viral infection as the cause of bee colony losses globally has been difficult because of differing pathogen virulence and different host susceptibility in different regions.

Three viruses, in particular, have been associated with colony losses on a large scale; the Deformed Wing Virus (DWV) and the Acute Bee Paralysis Virus (ABPV) have been implicated in winter losses in Europe and the Israeli Acute Bee Paralysis Virus (IAPV) has been identified as a marker of CCD in the USA (Genersch, 2010; Rosenkranz et al., 2010).

(i) Deformed Wing Virus

The Deformed Wing Virus (DWV) is less virulent than some viruses, but it infects a large range of bee tissues and can produce high levels of infection in the bees. DWV infections are characterized by deformed wings, shortened and bloated abdomen and discoloring. Bees with deformed wings are not viable and die within 67 hours after emergence. If this virus spreads through a colony, it would cause considerable damage and threaten its survival (Genersch, 2010).

Soon after *V. destructor* arrived in the *Apis mellifera* population of the Western World, emerging bees with deformed or atrophied wings were increasingly observed. It then became apparent that the transmission of DWV to the bee through *V. destructor* can result in visible levels of infection. The more mites in a colony transmit the virus and the more of these mites support replication of the virus prior to transmission, the higher the chances that developing pupae will develop a fatal DWV infection and that the colony will eventually collapse (Genersch, 2010).

DWV is ubiquitous in areas where Varroa mites are well established and DWV has been associated with the collapse of Varroa-infested honey bee colonies. The global spread of Varroa appears to have favoured certain variants of DWV, allowing it to become one of the most widely distributed and contagious insect viruses. This is supported by a number of recent reports on how *V. destructor* has been shown to be an efficient vector of DWV:

- (i) **Martin et al., 2012.** The arrival of Varroa in Hawaii fundamentally altered the viral landscape in bee colonies. *V. destructor* accelerated the replication of latent viral infections by acting as a host and supporting replication of DWV. This also favoured more pathogenic strains, thereby increasing the presence, levels and effects of the virus in mite infested colonies. Further details can be found in Appendix I.
- (ii) **Dainat et al., 2012.** This study shows that the mite *V. destructor* is a key player in winter colony losses and provided the only predictive marker in summer of subsequent winter colony losses. The study found higher levels of DWV in collapsing colonies and provides supporting evidence that the virus is not efficiently transmitted in the absence of mites. The study also shows that the virus is able to replicate in various bee tissues, including the fat body, which is involved in bee immunity and ageing. Further details can be found in Appendix II.
- (iii) **Nazzi et al., 2012.** Some of the multi-factorial nature of bee colony losses starts to be understood in this study and shows that a combination of situations can result in intense viral replication, where previously the bees were harbouring harmless, low level viral infections. The de-stabilisation of a DWV infection can result from a widespread suppression of the bees' immunity. Further details can be found in Appendix III.

(ii) **Acute Bee Paralysis Virus**

The Acute Bee Paralysis Virus (ABPV) is highly virulent for bees and causes trembling, paralysis and black hairless bees. It is normally transmitted via faeces, but if injected directly into the haemolymph, it causes the death of an adult bee within 3–5 days (Genersch, 2010). The virus is believed to weaken the colony causing deaths of the worker bees and the brood. ABPV has a geographical distribution similar to that of *A. mellifera* and has been isolated from healthy adult bees from most regions of the world.

The apparent harmlessness of ABPV infections dramatically changed with the advent of *V. destructor* in Europe. In severely mite-infested colonies brood and adult bees were obviously dying from ABPV infection (Genersch, 2010). Considering the extreme virulence of ABPV when injected into the bee haemolymph, it is not surprising that this virus started to cause problems, when *V. destructor* established itself as a vector of ABPV, injecting the virus into the haemolymph of the bee. In studies in Germany, it has also been shown that there is a highly significant relationship between ABPV infections and winter losses (Genersch, 2010). The presence of this virus has also been observed in cases of CCD, which is being investigated further.

(iii) **Israeli Acute Bee Paralysis Virus**

The Israeli Acute Bee Paralysis Virus (IAPV) is closely related to ABPV and can cause rapid death with little or no symptoms. It is extremely virulent when injected into pupae or adult bees (Genersch, 2010). IAPV is prevalent in the Middle East, Australia and the USA, but less frequently found in Europe. The geographical spread of this virus could explain why IAPV is implicated in colony losses in the USA, but so far not in Europe.

The use of an IAPV-specific anti-viral treatment was able to reduce the symptoms of CCD and these results suggest that IAPV is at least in part responsible for the described symptoms and colony mortality of CCD (Genersch, 2010). Initially seen as an indicator for CCD, subsequent surveys suggest it is only one of many possible factors (Dainat et al., 2012).

There are two other viruses that have been identified as a threat to bee colonies in association with Varroa (OPERA, 2012):

The **Black Queen Cell Virus (BQCV)** kills the brood in queen cells and is involved in the death of worker bees. In the early stages signs of the disease are similar to Sacbrood Virus (SBV), which as the name suggests gives a sac-like appearance to the diseased larvae. Both BQCV and SBV are common and widespread in Europe. BQCV appears to be intimately associated with the fungus, *Nosema apis*, which infects honey bees, although its transmission appears to be related to Varroa infestation. It has been estimated that BQCV is the second most common virus after DWV in Europe (OPERA, 2012).

The **Kashmir bee virus (KBV)** is closely related to ABPV and IAPV and similarly can cause rapid death with little or no symptoms. It is prevalent in North America and New Zealand, but rarely found in Europe, although it has been detected in the UK and Spain (OPERA, 2012). It is extremely virulent when injected directly into adult bees causing death within 6 days, although the bees appear to be unaffected when the virus is ingested (Genersch and

Aubert, 2010). With the development and virulence of KBV being dependant on how it is transmitted, it has been identified as being a severe threat to bee colonies in association with Varroa.

2.2.2 Control of Viral Infections

Whilst a study has shown that the use of an IAPV-specific anti-viral treatment was able to reduce the symptoms of CCD (Genersch, 2010), this is a virus-specific treatment and not necessarily practical for widespread use.

The factors responsible for colony losses differ from continent to continent and from region to region, but the active role of *V. destructor* as a vector of bee viruses is a common factor. As such, control of the *V. destructor* to limit its role in the spread of disease as a viral reservoir, incubator, activator and transmitter is the most obvious and immediate option.

Research is ongoing into understanding the interplay and functional details between the parasites and viruses with the bees' immune system. This may also help in identifying bee traits that will assist breeding programmes to produce more robust Western honey bee strains.

2.3 Fungal Infections

There are two species of the microsporidian fungus, Nosema, associated with obvious signs of disease in honey bees: *Nosema apis* and *Nosema ceranae*. These fungi invade the digestive cells lining the mid-gut of the bee, they multiply rapidly and within a few days the cells are packed with spores. Nosema spores are transmitted by a variety of routes including honey, pollen, wax and royal jelly (Opera, 2013).

N. ceranae spread to the Western honey bee from the Eastern honey bee. It appears to be more virulent or certain strains of the Western honey bee are more susceptible to this fungus (Opera, 2012). *N. ceranae* is more stable at warmer temperatures. Although the impact of this parasite on colony health in Europe still remains controversial, it may be an increasing contributor to colony deaths in warmer drier climates (Genersch, 2010; Guzman-Novoa et al., 2010; OPERA, 2012).

The antibiotic, fumagillin, is the main treatment for Nosema. It is considered to be the only effective treatment for *N. apis*, but it only suppresses *N. ceranae*.

2.4 Bacterial Infections

There are two major bacterial infections affecting honey bee colonies: American foulbrood (AFB) caused by *Paenibacillus larvae* and European foulbrood (EFB) caused by *Melissococcus plutonius*.

In the case of AFB, larvae become infected by consuming the spores in food, which then germinate in the mid-gut, invading the tissues and killing the larvae, usually after pupation. Once a colony is infected with AFB, the disease will usually progress until the colony dies (Opera, 2013).

Similarly for EFB, it is the larvae that becomes infected by ingesting contaminated food and the bacteria multiply within the midgut of the infected larvae, competing with the larva for food. Infected larvae usually die due to starvation rather than invasion of the body tissues by the bacterium. In some cases the larvae survive to pupation, producing undersized adults (Opera, 2013).

AFB and EFB are notifiable diseases throughout Europe and the only method of control in Europe is destruction of infected colonies. Antibiotics are widely used outside Europe, but AFB cannot be eradicated with antibiotics, as they act merely to stop the bacteria from reproducing without killing them. Also antibiotics do not affect the bacterial spores which are the primary mode of transmission. Resistance to some antibiotics by *P. larvae* is believed to be developing, e.g. oxytetracycline in the USA (Opera, 2012).

3.0 Other factors Affecting Bee Health and Survival

There is an increasing understanding emerging of interaction between the diseases, the vectors of the disease and bee immunity and their role in colony loss. The developing association between the mite, *V. destructor* and a range of honey bee viruses is considered to be a major factor in the global collapse of honey bee colonies (Genersch, 2010).

However, there are a number of other factors such as land management and environmental factors that affect the health and survival of bee colonies through the availability and quality of food sources and by affecting production conditions in the hive. In some cases it is the interaction of a number of these factors that is considered to be the underlying cause of colony losses, a situation that can be exacerbated by the more recent unusual fluctuations in the weather.

3.1 Availability and Quality of the Food Supply (AFSSA, 2009)

A colony's life cycle and survival depend heavily upon the vegetation in the surrounding environment and more specifically on the available pollen and nectar sources. Honey bees need good-quality food to successfully complete their larva development and to survive through the winter. They require carbohydrates from flower nectar for energy to support temperature regulation, hive maintenance work, such as cell cleaning, brood feeding and foraging journeys. The bees also require proteins from pollen for growth and to carry out vital functions in the body and reproduction. There may be periods when sufficient suitable pollen or nectar producing plants are not available. In agricultural areas, simplified crop rotation practices may limit the diversity of crops or grazing/mowing may occur before flowering. Similarly a drought can reduce flowering and therefore access to sufficient food for the bees. Depending on the timing or the provision of alternative food sources the bee colony will be weakened and may not survive through the winter.

3.2 Climate and Weather (AFSSA, 2009)

The climate is a key factor affecting temperature and humidity. The humidity in the hives must be maintained as low as possible and the temperature of the brood must be maintained at 34°C and in winter the core temperature of the hive must not fall below 13°C. It is essential therefore that the colony have sufficient access to carbohydrate to maintain these temperatures to survive. Prolonged periods of cold or wet weather can also influence the development and behaviour of honey bee colonies. It can inhibit the flying activity and interrupt nectar and pollen supplies to the hive. Equally, if the brood temperature rises above 34.5°C, the bees display learning and memory difficulties. Worker bees reared at sub-optimum temperatures lost their sense of direction and were no longer able to dance properly.

The effect of weather on bee colonies as a key factor in CCD is reported in a survey of honey bee colony losses in the USA (van Engelsdorp et al., 2010). Whilst fluctuations in the weather cannot be controlled, mitigation of the effects is possible in terms of protecting the colony against the cold, ensuring adequate ventilation of hives. The provision of supplemental feed can improve colony survival.

3.3 Pesticides

A factor that has received a lot of attention has been the use of pesticides in agriculture, particularly insecticides. Historically, insecticide sprays were responsible for a number of fatal incidents with bees, but the introduction of new insecticides has reduced this significantly (Oliver, 2012). In France these are reported to be relatively rare, occurring in the summer season and usually due to agricultural misuse of certain pesticide products (AFSSA, 2009).

One family of insecticides, the neonicotinoids, were introduced in the late 1990s, and these are used widely as a seed treatment. This means the seed is coated by a film, which contains the product and is designed to protect the seed or seedling from being eaten by insects. As the approach has reduced the spray application to crops and the potential for direct exposure of bees, it has been welcomed by bee keepers (Oliver, 2012). However, there have been occasional bee kills due to seed planting dust (Oliver, 2012), which is now being addressed through improved formulation and application technology.

More recently, the concern around bee health has focussed attention on the potential for the neonicotinoids to have an adverse effect on bees at levels of exposure below that which causes immediate death. As the insecticide may be found throughout the treated plant, concerns have been raised about the potential for exposure via ingestion of ,or direct contact with, the nectar and pollen, or indeed, through contact with any residues in the soil or vegetation (AFSSA, 2009). The concern therefore, is that low levels of exposure may affect the normal functioning of bees such that it is a threat to the honey bee colony or bumble bee population. These concerns about the potential for sub-lethal effects are theoretically valid and the European Food Safety Agency concluded that sub-lethal effects could not be fully excluded in worst case situations (EFSA, 2012). However to date, there is no overwhelming evidence from

“real-life” situations that any of them cause serious problems. In France in 2009 an initial review of the evidence concluded:

“Factors identified responsible for high mortality in colonies have been mostly biological, particularly the varroasis agent. The group’s deliberations do not confirm the hypothesis of a predominant role attributed to pesticides by beekeeping professionals in French bee colony mortality” (AFSSA, 2009).

A subsequent review in the UK in 2012 concluded:

“Whilst there are studies that provide evidence of sub-lethal effects of neonicotinoids under the conditions applied in the research, none of the studies provided unequivocal evidence that sub-lethal effects with serious implications for colonies are likely to arise from current uses of neonicotinoids” (DEFRA, 2012).

There is a significant amount of field data that supports these conclusions. Reports from beekeepers in the USA corn-belt and on Canadian canola show good colony survival and honey production despite the bees foraging in landscapes with high neonicotinoid use (Oliver, 2012). Also the levels of neonicotinoid residues in pollen and nectar are generally at tolerable levels. This may be because the field doses from seed treatments are typically very low and actual measured amounts of neonicotinoid residues found in the nectar or pollen of treated plants are therefore also very low (0-3 ppb , rarely above 5 ppb) (Oliver, 2012; EFSA, 2012).

Bees also appear to find neonicotinoids unattractive and several surveys have found that bees in agricultural areas often avoid treated areas, if other food sources are available (Oliver, 2012).

In conclusion, using studies performed at realistic dose levels and appropriately conducted field studies, no independent investigatory body has been able to confirm that the current use of neonicotinoids are responsible for large-scale colony mortality nor that sub-lethal exposure has any serious implications for bee colonies (Oliver, 2012; Cresswell, 2012). This is supported by regulatory decisions, where the approval process looks at the potential for adverse effects to occur under normal conditions of use.

This review throughout has focussed on the honey bee as the most important commercial pollinators of crops. With regard to the bumble bee there is very limited data on the exposure and effect of neonicotinoids. However, widely cited experiments are flawed, because they used unrealistically high dosages. In a report to the UK government it was reported:

“Whilst declines in certain bumble bee species have been reported and these are coincident with the increasing use of neonicotinoids, the effects of infections and habitat degradation are also plausible culprits” (Cresswell, 2012).

A recent field study with bumble bees (FERA, 2013) reported:

“The study did not show that neonicotinoids used within a normal agricultural setting have a major effect on bumble bee colonies. Even when there was a large area of

oilseed rape present, it is clear that bumble bees have diverse foraging strategies. Exposure to treated crops was diluted by foraging on a range of sources of pollen.”

“The study underlines the importance of taking care in extrapolating laboratory toxicology studies to the field, as well as the great need of further studies under natural conditions”.

3.4 Beekeeping Practices (AFSSA, 2009)

It is the beekeeper's role to help colonies survive so they will produce honey every year. This includes maintaining the right food supply, the right balance of different bees and favourable conditions inside the hive. A lack of worker bees means the food supply will limit colony growth and reduce population size. In the winter, too few bees will not be able to maintain the necessary temperature for the bee cluster to survive.

To help keep the humidity in the hive as low as possible the placement and rain protection is an important factor. In the case of sedentary apiaries the availability of food sources all season, particularly before the winter period is critical. When foodstuffs are scarce, the number of colonies per site must be adapted, so that each one can be assured a continuous supply of pollen and nectar.

Monitoring the bees for pathogens and signs of disease is another key responsibility of the bee keeper. Treatment to control mites, particularly *V. destructor*, has become more difficult over the last few years as resistance to the available chemical treatments has emerged in Europe. The application by the beekeeper of a single treatment against the mite in the autumn may therefore no longer be sufficient, since the damage inflicted on the colony population is already too serious.

The selection of queens is also another important task. This selection has been largely based on the behaviour of bee colonies, specifically non-aggressiveness and honey yields. But these criteria may be insufficient to guarantee healthy, strong and performing colonies, as it overlooks the hygiene behaviour criterion of bees.

4.0 Concluding Remarks

The recent concerns in some regions over an increase in colony losses has prompted investment in more co-ordinated monitoring of bees and research into how pests and diseases, bee diversity, bee-keeping practices and their foraging environment is affecting bee vitality. The global picture shows that regions with established honey bee parasitic Varroa mite populations (*V. destructor*) have consistently higher colony losses than those without (Neuman and Carreck, 2010). There is no doubt that the Varroa mite itself contributes to weakening colony health, but in addition data shows that the mites role in facilitating secondary infections is an additional and significant factor.

The active role of *V. destructor* as a vector of bee viruses is emerging as a significant factor in the losses of honey bee colonies seen globally. This appears to have arisen from a combination of events and interactions:

- The arrival and spread of the mite, *V. destructor*, in the Western honey bee population;
- Insufficient control of the growth of the Varroa population by the new host, the Western honey bee, to prevent spread and damage to the infested colonies;
- The developing association between the mite, *V. destructor* and a range of honey bee viruses, such that apparently harmless, low level viral infections are able to replicate, be transmitted far more effectively and be more pathogenic to the bees;
- The dynamic interactions that occur between the bee, the mite, viral infections and environmental conditions that can favour high levels of viral replication, which ultimately affects colony survival.

The active role of *V. destructor* as a vector of bee viruses, and in destabilising the critical balance between viral pathogens and the bees' defences, seems to be the most significant factor in increased colony losses. Therefore more effective control of Varroa and understanding the potential for *V. destructor* to influence other viruses that infect bees is both urgent and important.

In addition, land management and environmental conditions affect the availability and quality of food sources and also affect conditions in the hive, and effective management of bee colonies under changing situations is dependent on bee keeping practices and bee selection/breeding. All of these factors can impact on bee vitality and the bees' ability to deal with pests and diseases. It is not surprising, therefore, that there may not be one single identifiable cause for the "colony loss" phenomenon, but rather that the gradual changes seen in bee infections, vectors of infection, disease control, weather, and the quality and quantity of food resources, singly or collectively lead to colony losses.

Appendix I: Global Honey Bee Viral Landscape Altered by a Parasitic Mite

Martin et al. (2012), Global Honey Bee Viral Landscape Altered by a Parasitic Mite, *Science* 336, 1304

The more recent arrival and spread of the Varroa mite across parts of the Hawaiian archipelago provided an opportunity to survey of bee colonies in the initial phases of the honey bee–Varroa–DWV association and its effect on colony survival. Hawaii was Varroa-free until August 2007, when the mite was discovered throughout Oahu Island. A subsequent survey during 2007–2008 recorded significant colony losses (274 of 419) from the untreated colonies. In reports covering up to 2010, the islands of Kauai and Maui remained mite-free, and no unusual colony losses or disease problems were reported there. As with other studies, the colony losses did not coincide with the arrival and establishment of the mite, but there was a lag time of 1-3 years.

This study of 293 honey bee colonies found that the mite had caused DWV to occur much more widely. Increases were not found in other bee viruses such as KBV, ABPV and IAPV. The introduction of Varroa caused DWV to be detected in more colonies, but also significantly raised levels were present and the strain diversity of DWV was affected. It was apparent from the results that arrival of Varroa in Hawaii had fundamentally altered the viral landscape in both managed and feral bee colonies.

The study found that the presence of Varroa over time appeared to be selecting for particular variants of DWV and this may be giving them a competitive advantage. Many factors are likely to influence the DWV variant population in different colonies, but the arrival of DWV variants that can replicate in the mite would certainly mean that these strains would rapidly increase. There was also some evidence that after 1 year of effective Varroa control the same DWV strain remained dominant, suggesting that Varroa-induced changes were capable of persisting despite the Varroa populations being under control.

The authors conclude that these findings provide strong evidence that the association that develops may be responsible for the death of millions of colonies worldwide wherever Varroa and DWV co-occur. It is expected that the current Varroa-adapted DWV variants will continue to evolve and other viruses, which showed an apparent lack of association in this study, may evolve differently under different conditions and/or require a longer period of time to develop.

Appendix II: Predictive Markers of Honey Bee Colony Collapse

Dainat et al. (2012), Predictive Markers of Honey Bee Colony Collapse, PLoS ONE 7(2): e32151. [oi:10.1371/journal.pone.0032151](https://doi.org/10.1371/journal.pone.0032151)

A survey of bee colonies in Switzerland looked at the presence and levels of eleven honey bee pathogens and three Western honey bee genes involved in bee immunity. 13 colonies died during winter and 16 survived. Those that survived winter had all received a treatment against *V. destructor*, whereas 11 out of 13 of the colonies that died, had received no treatment against *V. destructor*. Colonies that died during winter had significantly more mites than the surviving group either in the summer, autumn or winter. *V. destructor* levels were a good predictor of colony loss and, in fact, provided the only predictive marker in summer of subsequent winter colony losses.

The study found higher levels of DWV in collapsing colonies than in the surviving ones, plus there was a significant increase in the levels of DWV between the summer and the autumn. This is consistent with previous reports showing an increase in DWV levels in the autumn and it is believed to be a reflection of the close link between DWV and *V. destructor*, since mite numbers climb rapidly from summer to autumn. Furthermore, proper treatment of bee colonies against *V. destructor* drastically reduced levels of DWV in the colonies, supporting the argument that this virus is not efficiently transmitted in the absence of mites.

Another factor in this relationship is that the virus is shown to be able to replicate in various bee tissues, including the fat body. The fat body is the site for production of the egg yolk protein, vitellogenin which is involved in immunity and ageing. For this reason vitellogenin is used as a common marker for the overall health and lifespan of individual bees. As with a previous study, where the levels of vitellogenin were reduced in mite infested worker bees, this study found significantly lower production of vitellogenin in the collapsing colonies and it is suggested that DWV replication is in some way impairing its production.

The authors conclude that their study shows that the mite *V. destructor* is a key player in winter colony losses and that there is an urgent need for efficient treatments against this parasite. Their data suggests that it is not only the direct effect of the mite on the ability of honey bees to overwinter successfully, but the role it plays in promoting opportunistic viral infections, which eventually impair critical physiological functions in the bees.

Appendix III: Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive the Collapse of Honey Bee Colonies

Nazzi et al. (2012), Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive the Collapse of Honey Bee Colonies, PLoS Pathog 8(6): e1002735. doi:10.1371/journal.ppat.1002735

This paper looks further into the dynamic interactions that occur between the bee and the pests and diseases that infect the colony. In this study, mite levels were actively controlled in 6 bee colonies, while the other 6 were left untreated. In the untreated colonies there was a steady increase in the mite population, peaking at the end of the season.

A survey of the colonies for the most common pathogenic bee viruses revealed the widespread presence of Blackqueen cell virus (BQCV), Deformed wing virus (DWV) and Sacbrood virus (SBV) only. The occurrence of both BQCV and SBV fluctuated and generally declined over the season, whereas DWV increased over time. Consistent with reports in other studies, DWV occurred at higher levels in bees with high levels of mites.

There is a critical balance between the viral pathogens and the bees' defences that can effectively maintain DWV at low levels. In this study bees from the highly infected colonies were found to have a suppressed immune system, when the viral replication rate was high. This alteration in the bees' immune system leads to uncontrolled viral replication and appears to be a key strategy adopted by DWV to overcome one of the central components of the antiviral immunity in insects.

There was evidence of reduced expression of certain genes involved in insect immunity, one of which was from a family of genes that are central in insect immunity. This gene is involved in the complex network of responses to infection and environmental stress and optimises energy allocation in the bee. Therefore, any additional stress factor triggering a response mediated by this gene will deplete the immune system and will allow a gradual increase in the viral replication until uncontrolled viral replication ensues. For example, cold temperatures would favour channelling the bees' energy into metabolism rather than into an immune response. This hypothesis is supported by the reported induction of DWV replication in bees exposed to cold stress. Indeed, the wounds inflicted on the bee by the feeding Varroa mite would also make demands on the same immune defence system.

The authors conclude that their findings start to explain the multi-factorial nature of bee colony losses. A combination of situations can result in intense viral replication that affects colony survival, where bees were previously harbouring harmless, low level viral infections. This de-stabilisation of the DWV infection results from a widespread suppression of the bees' immunity.

Appendix IV: Source References

AFSSA (2009), Mortalités, effondrements et affaiblissements des colonies d'abeilles (Weakening, collapse and mortality of bee colonies). <http://www.afssa.fr/Documents/SANT-Ra-MortaliteAbeilles.pdf>.

Cresswell, J (2012), Written evidence submitted by Dr James Cresswell, University of Exeter to the Commons Select Committee, Environmental Audit. <http://www.parliament.uk>

Dainat B, Evans J D, Chen Y P, Gauthier L and Neumann P. (2012), Predictive Markers of Honey Bee Colony Collapse. PLoS ONE 7(2): e32151. oi:10.1371/ journal.pone.0032151

DEFRA (2012), Neonicotinoid insecticides and bees. The state of the science and the regulatory response. 13 September 2012. <http://webarchive.nationalarchives.gov.uk/20130123162956/http://www.defra.gov.uk/publications/2012/09/18/pb13818-pesticides-bees/>

EFSA (2012), Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe. <http://www.efsa.europa.eu/fr/efsajournal/doc/2752.pdf>

FERA (2013), Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions. <http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportPS2371Mar13.pdf>

FAO (2009), ProdSTAT Database. Food and Agriculture Organization of the United Nations. Available at <http://faostat.fao.org/default.aspx>

Genersch, E (2010), Honey bee pathology: current threats to honey bees and beekeeping. *Appl Microbiol Biotechnol* 87:87–97.

Genersch, E and Aubert, M (2010), Emerging and re-emerging viruses of the honey bee (*Apis mellifera*), *Vet Res* 41(6):54. Published online 2010 April 29 doi: [10.1051/vetres/2010027](https://doi.org/10.1051/vetres/2010027) PMID: PMC2883145

Guzma'n-Nova E, Eccles L, Calvete Y, Mcgowan J, Kelly PG, and Adriana Correa-Ben'itez (2010), *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41: 443–450.

Martin S J, Highfield A C, Brettell L, Villalobos E M, Budge G E, Powell M, Nikaido S and Schroeder D C (2012), Global Honey Bee Viral Landscape Altered by a Parasitic Mite. *Science* 336, 1304.

Nazzi F, Brown S P, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E, and Pennacchio F (2012), Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive the Collapse of Honey Bee Colonies. *PLoS Pathog* 8(6): e1002735. doi:10.1371/journal.ppat.1002735

Neuman P and Carreck, N L (2010), Honey bee colony losses. *Journal of Apicultural Research* 49(1): 1-6.

Oliver R (2012), Neonicotinoids: Trying To Make Sense of the Science. First published in *Bee Journal*, August, 2012.

OPERA Research Centre (2013), Report on "Bee Health in Europe - Facts & Figures – 2013". www.operaresearch.eu

Potts S G, Roberts S P M, Dean R, Marris G, Brown M A, Jones R, Neumann P and Settele J (2010), Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research* 49: 15-22.

Rosenkranz P, Aumeier P and Ziegelmann (2010), Biology and control of *Varroa destructor* (2010), *Journal of Invertebrate Pathology* 103 S96–S119.

Van Engelsdorp D, Hayes J Jr, Underwood R M and Pettis J S.(2010), A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. *Journal of Apicultural Research* 49(1): 7-14.