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A GUIDEBOOK ON HONEY BEE HEALTH: Honey Bee Immunity — Pesticides — Pests and Diseases

Joey Caputo

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Honey Bee Immunity ■■■ Pesticides ■■■ Diseases and Pests

Photo by David Cappaert, Bugwood.org

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Honey Bee Immunity — Pesticides — Pests and Diseases

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Introduction

David Cappaert, Bugwood.org

Pedigo and Rice (2009) described a concept that some ecologists subscribe to called the "balance of nature" phenomenon. This idea holds that species in communities achieve certain status in their ecosystem and that this status becomes fixed and resistant to change. On average, individuals are only able to replace themselves. Fluctuations may occur, but ultimately the various species in the community will retain their position and relative population size in the ecosystem.

According to these ecologists, when humans alter and reduce the diversity of an ecosystem they are acting counter to this balance. In an attempt to return the altered system to its ordinary state, extraordinarily strong forces of nature will act in opposition to these activities. It could be argued that among these forces are biotic maladies which impair or destroy European honey bee (*Apis mellifera*) colonies. Oftentimes when honey bee diseases and pests explode and devastate apiaries, these activities are merely a reaction to the "overpopulation" of the single species which humans have selected. Thus many of the problems with honey bees should come as no surprise; they function just as they would in any other scenario where a single species becomes too numerous. The only distinction is these insects are of value to humans.

This is not to suggest that honey bees should be kept at "natural" rates. Honey bees provide approximately $15 billion dollars in annual pollination services in the United States (U.S.) (Morse and Calderone, 2000). If the environment is left on its own to determine how many honey bee colonies are to exist, it could have severe humanitarian and economic consequences. Such a proposal is just as absurd as keeping apples, melons or tomatoes at the rate which nature sees fit.

Honey bee diseases and pests are considered in ecology to be perfectly density-dependent, meaning that an increase in the density of the honey bees will result in more intense pressure from honey bee pests. To attribute all of the problems in beekeeping to this single notion is a gross oversimplification. Indeed many European honey bee pests came from other hosts such as the Asiatic honey bee (*Apis cerena*); therefore their deleterious effects are much more severe than would be if they had coevolved with their host. Furthermore, some of the problems with honey bee health have been attributed to abiotic factors such as inadequate nutrition and pesticide exposure. Yet the point regarding density-dependence is made because popular sentiment often suggests that the solution to problems with honey bees is simply that more honey bees are needed. The human population on Earth is expected to reach 10 billion in the 21st century (Bongaarts, 2009). As a result, there will likely need to be more honey bees added to our global agroecosystems in order to meet future food demands and keep food affordable. However, as new colonies are added it is imperative that disease and pest issues are kept under control, colonies are managed to maximize pollination capabilities and alternative pollinators are incorporated. Merely adding honey bee colonies without any consideration for the pest and disease "reaction" will only exacerbate problems in beekeeping .

This guidebook is meant to assist in the promotion of honey bee health and prepare for the likely inevitable need for an increased number of managed colonies. However it is not intended to be a diagnostic tool or a prescription for solutions. Rather it is a summary of scientific knowledge about honey bee immunity, disease etiology, pest problems and abiotic stressors. The goal of this guide is for the reader to: 1) develop a deeper familiarity with honey bee biology and the conditions that harm these insects; and 2) better understand the relative importance of the various problems that negatively affect colonies.

Honey Bee Immune System

Mechanical and Biochemical Immunity

The honey bee exoskeleton provides structure for the body and serves as an important barrier from diseases. In entomology the exoskeleton is also referred to as the integument. There are three main components to the integument: the basement membrane, the epidermis and the cuticle (Klowden, 2007). The insect cuticle portion of the integument is a critical first line of defense. The cuticle is subdivided into the epicuticle, exocuticle, mesocuticle and endocuticle (Elzinga, 2004). The innermost segment, the endocuticle, is comprised of chitin and proteins which cross link to form a rigid structure; this structure serves as an insurmountable obstacle to many pathogens (Kaltenpoth and Engl, 2014).

Honey bees also have internal adaptations which aid in mechanical defense. The proventriculus is a specialized apparatus that serves as a valve for the movement of food from the crop to the midgut in insects (Klowden, 2007). In honey bees the proventricular valve serves as a filter which reduces the ingestion of pathogenic spores (Sturtevant and Revell, 1953). Another example of internal mechanical defense is found in the anterior portion of the midgut. In this part of the honey bee there is a peritrophic membrane, which acts as a physical barrier to pathogens that have been digested (Cornman et al., 2013).

The biochemical composition of the honey bee midgut provides some degree of protection against certain diseases which are ingested (Aronstein and Murray, 2010). For instance regulation of gut pH is a means of preventing the growth of harmful microbes and potential infection (Fries and Camazine, 2001). Chalkbrood (*Ascosphaera apis)* is one such fungal disease that can be prevented by these biochemical protections (Aronstein and Murray, 2010). Yet it should be noted that in other instances, the environment of the midgut is conducive to pathogenesis of other fungal and bacterial diseases (Chen et al., 2009).

Innate and Cell-Mediated Immunity

Klowden (2007) summarized two of the cell-mediated immune responses in insects. The first described response is the deployment of hemocytes, which are cells that devour pathogens by a process known as phagocytosis. This progression begins when pathogens enter an insect's body and hemocytes recognize the foreign entities. Upon detection, the hemocytes move toward the invading microbes and fuse with the foreign bodies. The pathogens are destroyed by digestion. In the second described cell-mediated response, hemocytes bind together to sequester pathogens too large for phagocytosis. This phenomenon is known as encapsulation and it protects the insect by separating the pathogens from host cells, thereby depriving the invaders of oxygen and nourishment. The formation of nodules may also occur. Nodules are large accumulations of hemocytes, which create a bacteria-intercepting extracellular matrix. Bacteria are sometimes captured and encapsulated by these structures. The honey bee immune system employs these strategies with much success in certain instances. For example Chan et al. (2009) point out that the highly infectious *Paenibacillus larvae* bacteria which causes American foulbrood can sometimes be effectively phagocytized. This is an example of a cell-mediated response which suppresses an infection.

Humoral Immunity

Cell-mediated immunity is augmented by humoral immunity. Klowden (2007) describes humoral defense as the production of various antimicrobial peptides (AMPs), which are amino acid chains created by an insect's fat body organ in response to an infection. The author notes that this process is fast—peptides are employed 2 to 4 hours after the contagion is recognized and they have the capacity to replicate at a pace significantly faster than the reproductive rate of the pathogen. However, speed does not come at the cost of precision. Indeed a fungal invader will trigger an antifungal peptide without triggering the release of an

BOX A Enzymes are proteins which make chemical reactions occur faster by lowering the activation energy of a reaction (Soloman, et al. 2005). Honey bees, like most insects, use detoxification enzymes to rid the body of foreign chemicals known as xenobiotics (Johnson et al., 2012). Xenobiotics include naturally occurring substances and human-made chemicals, such as pesticides. Cytochrome P450 is a principal detoxifying enzyme in honey bees (Feyereisen, 2006). Diet appears to be a significant factor in the expression of genes that regulate cytochrome P450. Johnson et al. (2012) illustrated this point in an experiment where bees fed sucrose or high fructose corn syrup experienced reduced cytochrome P450 activity. This appeared to make honey bees more susceptible to the fungal toxin aflatoxin. Therefore the authors suggested that sugar diets do not result in detoxification capacities which are equivalent to diets composed of honey. This is important because beekeepers commonly provide honey substitutes to colonies during a nectar dearth.

antibacterial peptide. Antimicrobial peptides are known to protect honey bees against certain diseases. For instance, immature bees infected with *P. larvae* are found to have dramatically increased levels of antimicrobial peptides such as hymenoptaecin and apidaecin (Chan et al., 2009). Up-regulation of antimicrobial peptide expressions is thought to be an important component of honey bee larval defenses against diseases (Chan et al., 2009; Cornman et al., 2013).

Social Immunity

Darwin (1859) observed that worker honey bees behave altruistically toward nestmates. Altruism is behavior that reduces an actor's fitness but improves a recipient's fitness (Freeman and Herron, 2004). Hamilton (1964) proposed that altruism may be favored by evolution if the actor and beneficiary are closely related. He claimed that individuals can improve their fitness "indirectly" by taking actions which hinder their own fitness, but increase the reproductive capabilities of relatives far beyond what would have been achieved acting selfishly. It has been suggested that the high-relatedness among nestmates of ancestral bees (Hughes et al., 2008) explain the altruistic behavior in *A. mellifera*.

Unlike most female animals, worker bees do not usually reproduce but instead serve as helpers for their mother and siblings for the entirety of their lives. This form of extreme altruism is characteristic of a social system known as eusociality. It is a highly advanced arrangement of social behavior and is described by three main criteria: overlap in generations between progeny and parents, members of the group engaging in cooperative brood care and the group producing a self-sacrificing sterile caste (Freeman and Herron, 2004). Insects which exhibit eusocial behavior possess two distinct advantages over insects that live solitary lives: 1) Resources are swiftly exploited by social groups through communication and collective action; 2) A social group can maintain territory and quickly construct nests—both of which provide protection from competitors, natural enemies and harsh environments (Triplehorn and Johnson, 2005).

In the context of disease and pest transmission, social behavior is a double-edged sword. On the one hand, pathogens can spread quickly amongst social insects due to sharing of resources and numerous individuals living in high densities (Schmid-Hempel, 1998). Yet novel group-level tactics for disease resistance have also evolved to compensate.

Simone et al. (2009) found evidence that worker bee collection of plant-based resins are a means of altering the colony's environment in a way that contributes to "social" defenses. Resins are collected from various trees and shrubs and mixed with wax to form an adhesive substance called propolis, which is used to seal gaps in the nest's construction. However these resins also contain chemical compounds that are helpful to colonies in other ways. For instance propolis contains sesquiterpenes, which have antimicrobial, antifungal, anti-inflammatory and antioxidant properties. In the authors' experiment, hives treated with two different resins exhibited lower bacterial loads and bees with reduced expression of certain immune-related genes compared to controls. This suggested that resins are not only collected for the purpose of sealing the hive, but also have a role in reducing pathogens in the hive's environment and thereby reducing the need for immune-gene expression. Likewise, antimicrobial peptide fractions found in royal jelly have been found to inhibit *P. larvae* and these fractions may serve as a mechanism for larval host

defense (Bilikova et al., 2001). The chemical properties of honey and pollen also have antimicrobial qualities that bees can sometimes rely on to prevent infections (Fries and Camazine, 2001).

It has also been proposed that colonies can produce social fevers in response to contraction of disease. The fungal disease Chalkbrood caused by *Ascosphaera apis* favors temperatures that are lower than normal honey bee brood-rearing conditions between 33-36° C (Bailey, 1991). When infected larvae are exposed to temperatures around 30° C, the conditions are prime for mycelium growth of the pathogen (Bailey, 1991). Starks et al. (2000) found that colonies demonstrated an up-regulation in normal brood comb temperatures after being inoculated with *A. apis*. Since this pathogen is heat sensitive and there is no evidence to suggest that elevated brood comb temperatures confer any other benefit, it was concluded that the rise in temperature was a deliberate means of defending the colony by creating conditions unfavorable to the microbe.

Other social immunities include hygienic behavior, which is the ability of bees to recognize and remove diseased or parasitized brood (Aronstein and Murray, 2010). For instance certain bees are able to sense when brood is infested with Varroa mites (*Varroa destructor*). These hygienic individuals proceed to uncap and remove these parasitized developing pupae from the colony (Navajas et al., 2008). Immature Varroa development requires the unique environment of the honey bee brood cell, so removal of the bee pupae from the colony essentially dooms the larval mites (Spivak, 1996) and reduces the colony mite load. However, it has been observed that most adult mites appear to abscond from brood cells throughout the opening process (Boecking and Spivak, 1999). This would suggest that much of the reduction in the mite load via hygienic behavior is actually due to a disruption in the mite reproductive cycle and a lengthening of mite's phorectic phase (a period when the mite is attached to the adult bee) and not the physical removal of mites (Rosenkranz et al., 2009).

Hygienic behavior is likewise useful for the control of diseases. American foulbrood has been found to be controlled when worker bees quickly detect, uncap and rid the colony of infected brood (Spivak and Reuter, 2001). Removal of larvae while the pathogen is still in its non-pathogenic rod stage is key to the success of this strategy (Woodrow and Holst, 1942). Chalkbrood can also be controlled by worker hygiene (Spivak and Reuter, 2001).

In addition to hygienic behavior, honey bees exhibit grooming behavior which can be useful in removing mites from the honey bee body. When honey bees groom themselves and dislodge mites this is known as auto-grooming and when bees groom other nestmates it is known as allo-grooming (Rosenkranz et al., 2009). This behavior likely reduces harm to the colony either by the physical removal of Varroa mites from the bee's body and/or by causing injury to the mite, which makes them less effective at parasitism (Spivak, 1996).

If honey bees are to be anthropomorphized, then surely the most sentimental of their behavioral defenses are those categorized under the umbrella of altruistic suicide. The most famous example of this behavior is sting autonomy, which is the thrusting of the poison apparatus into a perceived enemy that result in self-amputation and ultimately death of the bee (Hermann, 1971). A more obscure activity in this suite of behaviors is altruistic self-removal, or the self-imposed exile of individuals that have become compromised by disease or parasitism (Rueppel et al., 2010). Rueppell et al. (2010) demonstrated that most honey bees made artificially ill by exposure to CO_2 or cell growth inhibiting drugs absconded from the hive and failed to return. These authors purposely used artificial means for sickening bees because the previous anecdotal evidence which supported altruistic self-removal was perceived to have short-comings (i.e. parasitism may merely cause the affected bee to lose orientation abilities or the infected bees are ejected by healthy bees). Likewise, McDonnell et al. (2013) found that honey bees afflicted with Varroa and *Nosema ceranae* which absconded from the nest did not have significant differences in behavior and were not met with hostility amongst nestmates. This augmented previous data that described altruistic self-removal in honey bees.

Detoxification Complexes

Honey bee cells are capable of protecting the insect from dangerous natural and synthetic chemicals in the environment. This protection comes in the form of enzymatic complexes that can detoxify xenobiotics (foreign chemicals). In honey bees these include systems such as cytochrome P450 monooxgenase (P450s), glutathione S-transferases (GSTs), and carboxyl/cholinesterases (CCEs) (Claudianos et al., 2006; Feyereisen, 2006). According to Elliot and Elliot (2009), P450s are considered a phase I metabolic process, whereby a hydroxyl functional group is added to either aliphatic or aromatic groups. GSTs and CCEs are categorized as phase II metabolic systems; these involve adding highly polar groups to a hydroxyl group. Simply put, these systems make xenobiotics more water soluble and thus easier to excrete.

Much research has been conducted on P450s and their benefits to honey bee immunity. Johnson (2008) discovered that the honey bee genome includes approximately 46 genes that code for P450s. The author noted that this is far fewer than typically found in other insects. Yet the scheme serves the insect well in many instances. P450s are known to provide protection from a wide range of potential dangers in animals generally (Elliot and Elliot, 2009). In honey bees this is true of everything from pathogens to pesticides. For instance, data presented by Niu et al. (2010) suggest that P450s are instrumental in honey bee tolerance to mycotoxins which are produced by saprophytic fungi. These authors point out that these fungi are common in hives and were it not for P450s, the bees would likely suffer. P450s are also important in the context of in-hive treatments. For instance, Mao et al. (2011) discovered that honey bee tolerance to the pyrethroid taufluvalinate (used to control parasitic mites) is due to the detoxification abilities of P450s in the midgut. In the absence of these enzymatic complexes, useful acaricides would hurt the bees and therefore be useless as a mite control. Interestingly, the effectiveness of P450s are improved by consumption of honey and beebread, which serve as nutraceuticals (Berenbaum, 2015). **Box A** on page 3 discusses how the abilities of P450s to detoxify xenobiotics are hampered by poor nutrition.

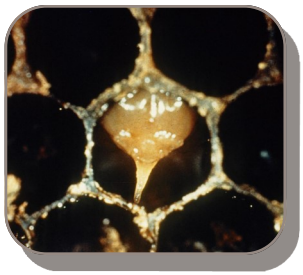
Problems in Beekeeping

In recent years both North American and European beekeepers have reported unusually high annual losses of honey bee colonies (Oldroyd, 2007). This is sometimes correctly or incorrectly referred to as Colony Collapse Disorder (CCD)—see **Box B**. In response to reports of CCD and high annual losses of colonies due to other problems, a workshop in Warrenton, Virginia was organized in 2012 which gathered 19 leading honey bee experts to evaluate the numerous threats to honey bees. The findings of this work group were published by Staveley et al. in 2014. This workgroup utilized a "causal analysis framework" which is means of organizing expert opinion on potential causes of specific problems. The process essentially ranked the importance of various threats to honey bees. Candidate causes were evaluated on their probability of reducing overwintering success of colonies and categorized into the following groups: probable, possible, contributing factor, unlikely alone and indeterminate. Throughout this guide, conclusions reached by the Causal Analysis Workgroup and other scientific authorities will be presented when specific maladies are reviewed. Honey bees face a plethora of problems—and there is no shortage of opinions as to where to assign blame. Therefore this guide will provide the reader with context regarding the importance and, at times, uncertainty regarding the various biotic and abiotic pressures that honey bees experience by referring to the conclusions of this workgroup.

BOX B The term Colony Collapse Disorder (CCD) often becomes an umbrella description for all problems in beekeeping, especially in the popular media. However, CCD is a specific honey bee condition with a number of observable signs. Underwood and vanEngelsdorp (2007) provided the following description of a hive with CCD: 1) A rapid loss of most adults in the colony 2) There are ample food stores and brood present 3) A queen remains with a small band of younger workers 4) Food stores will remain untouched by robber bees and secondary pests for an extended period. Reports of this malady were numerous between the winter of 2006 and spring of 2007 (Oldroyd, 2007). However, since that time confirmed cases with this specific set of symptoms have declined drastically and high annual losses are being framed in terms other than CCD according to the U.S. Environmental Protection Agency.

Bacterial, Fungal and Microsporidian Diseases

American foulbrood



Georgia Department of Agriculture

American foulbrood is a highly contagious and deadly honey bee brood disease caused by the persistent endospore-forming bacteria (*Paenibacillus larvae*) which has spread worldwide (Genersch, et al., 2006). Adult bees do not develop symptoms of the disease, but they can vector the pathogen. Infection reduces the immature bee to brown viscous remains (Sturtevant, 1932), which can as soon as one month later become a hardened, infectious, crust-like scale (Ritter and Akratanakul, 2014). A colony can fail within years or even months as a result of infection (Hansen and Brodsgaard, 1999). It is undoubtedly the most devastating of honey bee brood diseases. Interestingly, the disease's presence can even displace other existing bacterial infections due to the pathogen's production of a powerful antibiotic (Shinmanuki and Knox, 2000). American foulbrood has been known to spread and kill honey bee colonies that are unmanaged in some instances (Fries and Camazine, 2001). Yet the disease is likely to be of reduced importance in nature. Its virulence in apiculture appears to be due to beekeeper practices which intensify infective pressures (Fries et al., 2006). Practices which facilitate transmission include the movement of colonies, congregation of hives closely together and, perhaps most significantly, swapping frames from one hive to another hive.

The causative agent is a Gram-positive bacterium that in the vegetative state is slender, rod shaped and 2.5 to 5 micrometers (µm) in length and 0.5 µm wide; in the spore stage it is oval and is 0.6 x 1.3 µm (Shimanuki and Knox, 2000). The spores can remain viable for over 35 years and are able to withstand extreme heat, cold, drought and humidity (Hasemann, 1961). The spore's resilience is aided by seven defensive layers of lamella, which act as protective sheaths. Vegetative bacteria cannot cause infection, indeed only spores have the capacity to cause illness (Ritter and Akratanakul, 2014). Larvae are the only stage that is susceptible and they can become infected by ingesting 10 or fewer spores (Brodsgaardet al., 1998). The disease can affect the larval stage of any caste, yet it is quite rare for drone or queen immatures to develop infections (Ritter and Akratanakul, 2014). When adjusted for body size, both workers and drones appear to have a similar lethal thresholds to the disease, though drone death occurs one day later than worker expiration (Behrens et al., 2010). Immature bees are most vulnerable one day after hatching from their eggs (Crailsheim and Riessberger-Galle, 2001). However, larvae become immune to the disease after the third instar which is about 48 hours after eclosion (Chan et al., 2009).

Yue et al. (2008) was able to elucidate the pathway of pathogenesis using a technique known as fluorescence *in situ* hybridization (FISH). Once a spore has germinated in the larvae, the vegetative state of *P. larvae* begins to reproduce in the gut lumen. For two to six days this proliferation occurs at a rapid rate. The bacteria accumulate until they reach a threshold at which the peritrophic membrane is overcome and the epithelium is attacked. The epithelium is bypassed via paracellular space and this destroys cell to cell junctions. The pathogen proceeds to either degrade the basement membrane or undermine bonding of the cell matrix. This activity forms seepages in the tissue which separate the gut from other tissues and permits bacteria to invade the haemocoel. Ultimately the larvae die of septicemia and the corpse is devoured by vegetative bacteria (Cornman et al. 2013).

The production of new spores (sporulation) occurs throughout the infective process (Yue et al., 2008). However, spore production is higher as the infected larvae transition to a 5th instar prepupae—about 10 -11 days after egg hatching (Spivak and Reuter, 2001). A single larvae infected with the disease will produce approximately 2.5 billion spores

(Sturtevant, 1932). Throughout much of the 19th and 20th century this disease became epidemic and caused massive disruptions in honey production. This led to the formation of regulatory inspection programs (Humphreys, 1995). The importance of this disease has diminished in recent decades, due to the advent of antibiotics, inspection services and improved beekeeping practices. Yet the disease can still be problematic due to antibiotic-resistant strains of the disease and lack of knowledge among some beekeepers. The Causal Analysis Workgroup ranked American foulbrood as unlikely as a major contributor to high annual losses of colonies.

European foulbrood

Georgia Department of Agriculture, Bugwood.org

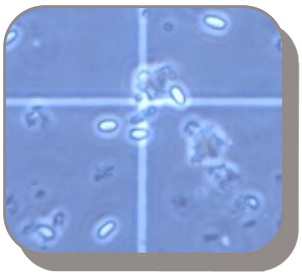
This disease is induced by the Gram-positive bacterium *Melissocccus plutonius* (Forsgren, 2010). European foulbrood cells are lancet shaped and shorter than American foulbrood—measuring just 0.5-0.7 µm x 1.0 µm (Shimanuki and Knox, 2000). This disease does not form spores (Shimanuki and Knox, 2000). Larvae of any age are susceptible to this pathogen, however it tends to kill the immature bees when they are 4-5 days old (Forsgren, 2010). Symptoms of the disease begin when the larvae lose their characteristic "C" shape and become twisted around the walls of the cell or are found lying lengthways (Forsgren, 2010). Occasionally larvae will die after their cell is sealed and this may result in two symptoms similar to American foulbrood: sunken caps and a foul smell (Forsgren, 2010).

While the disease is not ubiquitous in the environment, *M. plutonius* can be present in hives without colonies manifesting symptoms (Forsgren et al., 2005). One scientific estimate suggested that more than one-third of hives include adult bees that harbor the bacterium, but larvae do not exhibit symptoms (Forsgren, 2005). McKee et al. (2004) described factors involved in developing or resisting an infection. Infection begins when bacterium is ingested by the larvae and replication commences in the midgut. The authors note that in a clinical environment, an effective threshold for larval infection appears to by 200 organisms per mL. Yet, even larvae fed high concentrations of inoculum can demonstrate variable resistance. This is potentially due to genetic advantages or enhanced immune systems of individual larvae. Interestingly it is the larvae which survive infection that typically spread the disease to other susceptible bees. This is because surviving carriers of the disease pupate and defecate bacterial-laden feces into comb, whereas infected larvae that die before pupation are removed by housekeeping bees along with the bacteria (Forsgren, 2010).

The cause of larval expiration is ambiguous. According to Bailey (1983) the bacteria competes with larval cells for food resources and this essentially starves the immature bee to death. However McKee, et al. (2004) seemed to deflate this hypothesis by artificially rearing honey bee larvae and infecting them with European foulbrood in the presence of excess food. These authors found evidence for an alternative explanation for larval demise: the bacteria causes dissolution of the peritrophic membrane in the gut, leading to permanent physiological damage and possibly inhibition of proper digestion.

Although the disease is potentially lethal, the degree of fatality is variable around the world. Certain regions are impacted much worse than others (Forsgren, 2010). Since the bacterium is unable to persist both inside the hive and within the environment, it is certainly of diminished risk in both danger and transmission compared to American foulbrood (Mutinelli, 2011). The Causal Analysis Workgroup thought the disease was not a major contributor to honey bee losses.

Nosemosis



Katie Lee,
Bee Informed Partnership

Bee diseases in the genus *Nosema* are obligate, intracellular microsporidians (Gisder et al., 2011) meaning growth and division does not occur outside of the host cell. *Nosema apis* and *N. ceranae* are the two species which cause the condition known as Nosemosis (Chen et al., 2009). In laboratory settings all castes can become infected with this disease (Chen et al., 2009), though in the field workers are most commonly infected. It is possible that queen bees are frequently spared infection due to changes in behavior of infected workers that make them less likely to feed the queen (Wang and Mofller, 1970). The disease is more prevalent and infection intensity is higher in older, foraging worker bees compared to younger, house worker bees (Smart and Sheppard, 2012). According to annual surveys of honey bee health in the U.S., *N. ceranae* has largely displaced *N. apis* in recent years (Runckel et al., 2011). *N. ceranae* is a pathogen that recently arrived in the United States and the earliest known infection was detected in bees collected in 1995 (Chen et al., 2008). **Box C** describes some of the notable distinctions between the two species of Nosema and a possible explanation as to why one strain is becoming more prevalent than the other.

Both species of Nosema have been detected in the hypopharyngeal glands, thoracic salivary glands and mandibular glands which would suggest that food production and comb building may contribute to sinks and sources of the disease and promote horizontal transmission (Copley and Jabaji, 2011). An explanation of difference between horizontal versus vertical transmission of pathogens is provided in **Box D** on page 9. Risk of transmission may also be increased when bees are smashed by routine hive management (Mutinelli, 2011). Nosema spores cause infection in the digestive system of honey bees when food containing spores pass the proventricular valve of the foregut and enter the midgut

BOX C

Nosema apis

Spores of this species are large and oval measuring 4-6 µm long X 2-4 µm wide (Shimanuki and Knox, 2000). *N. apis* spores are heat sensitive and perish if exposed to temperatures of 60° C for 15 minutes (Fenoy et al., 2009). Nosemosis due to infection by this species can cause dysentery in bees, which is thought to enhance the fecal-oral route of transmission (Fries et al., 2009). In fact, comb soiled with feces is thought to be the primary source of transmission of this pathogen (Bailey, 1955).

Nosema ceranae

While difficult to distinguish, even with light microscopy, *N. ceranae* spores are on average 1 µm smaller than those produced by *N. apis* (Fries et al., 2006). Spores of this species demonstrate tolerance to heat. Fenoy et al. (2009) found that 90% of spores were still viable after a six-hour heat treatment at 60° C. *N. ceranae* exhibits far higher spore intensity than compared to *N. apis* (Williams et al. 2014).

Bees infected with this species notably lack the dysentery symptoms which are characteristic of the disease Nosemosis caused by *N. apis* (Fries et al. 2006). It is not clear why this is the case, however Chen et al. (2009) suggested that *N. ceranae* may lack specific PCR signals that affect muscles and fat bodies, which induce such symptoms. Infections by this species appears to result in higher worker mortality than compared to *N. apis* (Williams et al. 2014).

Williams et al. (2008) demonstrated that the antibiotic Fumagilin-B (fumagillin dicyclohexylammonium) was effective in controlling both species of Nosema. However, Huang et al. (2012) presented data which suggested that the two species respond differently to treatment with this antibiotic. The medicine is effective at reducing spore loads of both diseases when initially applied. However, there is a rebound of spore production as the chemical degrades. These authors monitored responses of both microsporidia to diminishing concentrations of the drug and found that *N. ceranae* spore production recovered significantly faster than *N. apis*. It was also discovered that Fumagilin-B may result in hyperproliferation of *N. ceranae* and exacerbate the infection. This information led the authors to conclude that fumagillin may be a factor in the replacement of *N. apis* by *N. ceranae* because widespread use of the drug is controlling the former while invigorated the latter.

physical environment of the midgut that induces germination of the spores (Chen et al., 2009). Infection of a host cell unfolds in this manner: 1) spore germination begins with an extension of the polar tube; 2) the tube penetrates the host cell membrane of a midgut cell; 3) sporoplasm is forced into the host cell (Gisder et al., 2011). The microsporidian devours the nutrients of the cell and grows until it eventually splits; this continues until the cell is exhausted (Mussen, 2011). These spores can infect other cells in the midgut or they are excreted from the host and act as infectious agents for other honey bees (Chen et al., 2009). Spore formation (sporulation) occurs sometime between 4 and 9 days post infection (Mussen, 2011).

Visual symptoms are not sufficient to determine if there is an infection, as positive diagnosis of the pathogen can only be done by microscopic examination (Shimanuki and Knox, 2000). Infection results in bees becoming energetically stressed and hungry (Mayack and Naug, 2008). This prompts the infected to be more likely to solicit food from nestmates and less likely to share food with others (Naug and Gibbs, 2009). Queen bees infected with *N. apis* early in their life are generally superceded (replaced by worker bees) within a month (Mussen, 2011). The queen is more likely to become infected in winter months in temperate regions, since the bees are confined and there are more opportunities for her to come into contact with infected workers (Higes et al., 2009). Nosema infections can significantly increase worker bee mortality. An infection of *N. ceranae* can reduce an average worker's lifespan by 9 days (Goblirsh et al. 2013). Kralj and Fuchs (2010) found that workers artificially infected with *Nosema spp.* were 2.5 times less likely to return to a colony than disease-free bees. The authors could not explain why this occurred, but suggested that inoculated bees may have experienced fatigue as a consequence of infection. Infection can also circumvent age polyethism of adult workers, causing them to abandon brood rearing altogether and prematurely become foragers (Mussen 2011). McDonnell (2013) supported the notion that these behavioral changes were a means of preventing transmission of the disease.

BOX D Chen et al. (2006) explained that transmission of honey bee pathogens occurs by one of two routes. The first is horizontal transmission, where individual bees are infected by other individuals of the same generation. The second is vertical transmission. This is where adults transmit maladies to their offspring. Fries and Camazine (2001) explained that the degree of virulence of honey bee pathogens is complex, however it often corresponds with the mode of transmission. Vertical transmission tends to select for reduced virulence and horizontal transmission favors increased virulence. In the case of vertical transmission the objective of the parasite and the host are one and the same: effective reproduction. Extreme virulence in this case will result in pathogens with no progeny in which to reproduce. However in horizontal transmission of disease, there is reduced advantage in lower virulence because the pathogen does not need host offspring to reproduce, it merely needs a new host of the same generation.

Effects of the disease at the colony level has also been studied. For example the pathogen has been found to act in a synergistic manner with certain pesticides and increase colony mortality rates (Alaux et al., 2012).

Despite all of the documented deleterious effects of this condition, it is not clear how problematic Nosemosis is to beekeeping by and large. For instance, *Nosema spp*. tends to be seasonal in prevalence and intensity because colonies typically exhibit infections more often in spring than in fall (Gisder, et al. 2010). Data presented by Dainat et al. (2012) downplayed the role of *Nosema* in widespread colony losses by demonstrating that overwintering deaths were generally the same between infected and uninfected colonies. Cornman et al. (2012) discovered that colonies infected with *N. apis* tended to be associated with extensive losses, but the same could not be said of *N. ceranae*. Yet the authors note that *N. ceranae* is often found alongside various honey bee viruses, which would suggest that the disease makes bees more vulnerable to other pathogens and abiotic stresses. Further complicating matters are data revealed by Zheng et al. (2014), which showed a direct correlation with

sterile pollen feeding and increases in *Nosema ceranae* spore load, which is commonly measured to determine the level of infection. Based on what is known about Nosemosis, increases in spore loads would hypothetically reduce worker longevity. Yet when pollen feeding was halted in test bees this resulted in higher mortality levels compared to bees that continued to receive pollen and consequently had higher spore loads. Simply put counting spore loads by itself is not likely useful in determining the severity of *Nosema ceranae* infections. This finding seriously hampers the development of proper treatment thresholds, since spore loads may not correspond with hive health. It also may partially explain why healthy colonies can sometimes have elevated Nosema spore loads. The Causal Analysis Workshop participants determined that both species of Nosema were unlikely alone responsible for widespread losses in honey bee colonies. However they may be contributing factors.

Chalkbrood

Jeff Pettis, Bugwood.org

Ascosphaera apis is the causative fungal agent of Chalkbrood infections (Shinmanuki and Knox, 2000). The disease exclusively affects bee brood (any caste) (Aronstein and Murray, 2010). Adults are not susceptible, however they can act as disease vectors (Aronstein and Murray, 2010).

Chalkbrood is a heterothallic organism (Shinmanuki and Knox, 2000), which means that spore formation only results when fungal hypha mate with different mating types (Solomon et al. 2005). Different mating types are designated as (+) and (-) and not male and female, since there are no physical distinctions between the different hypha (Solomon et al., 2005). When a (+) and (-) strain combine, a spore cyst is formed; these cysts contain spore balls, which hold individual spores (Shinmanuki and Knox, 2000). The cysts measure 47-140 µm in diameter, the spore balls are 9-19 µm in diameter and the spores are 3.0-4.0 X 1.4-20 µm (Shinmanuki and Knox, 2000). Spores can remain viable in hives for up to 15 years (Toumanoff 1951, reviewed in Aronstein and Murray, 2010).

Infection occurs when spores are ingested by honey bee larvae and germination begins in the gut. Fungal mycelia penetrate mechanical defenses in the gut and the pathogen proceeds to infiltrate internal organs and devour nutrients (Cornman et al., 2012). Mycelia eventually emerge from the host cadaver and transform it into a cotton-like mummy (Shinmanuki and Knox, 2000). The mummies range in color from white to brown to black. Lighter colors typically indicate that the mummy is young and few ascospores are present (Aronstein and Murray, 2010). This transformation of the larval host into mummies makes diagnosis of the disease simple.

The pathogen is cosmopolitan (Aronstein and Murray, 2010). Chalkbrood spores are likely to be ubiquitous within individual honey bee colonies, yet many colonies never demonstrate symptoms due to hygienic behavior (Spivak and Reuter, 2001). However if climate conditions are conducive to fungal growth or larvae are exposed to high doses of spores, the disease can lead to severe colony losses (Cornman et al., 2012). The Causal Analysis Workgroup suggested that this disease is unlikely alone to be responsible for major losses of colonies, however it could be a contributing factor.

Crithidia

Crithidia mellificae is a trypanomatid parasite first described by Langridge and McGhee (1967). These authors reported decades ago that the disease was not known to be deleterious to honey bees. The relative importance of this little studied disease is unknown even today. Recent data suggests that this pathogen may be linked to high overwintering mortality in Belgium, especially when found in combination with other stressors like *Nosema ceranae* (Ravoet et al., 2013). This is potentially troubling considering that a survey of large scale migratory beekeepers in the U.S. found that Crithidia was present in roughly one-third of colonies (Runckel et al., 2011). It was also discovered that in contrast to

many other honey bee maladies which reach their zenith in the summer, this disease peaks in the winter (a time of year when colony mortality is common). The disease has a worldwide distribution (Runckel et al., 2014). The Causal Analysis Workgroup deemed that the importance of Crithidia in beekeeping losses was indeterminate.

Stonebrood

Aspergillis spp. are fungi that tend to be beneficial decomposers, however some are pathogenic to honey bee larvae such as *A. flavus*, *A. fumigatus* and *A. niger* (Foley et al., 2012). Symptoms of the disease begin with a yellowish collar-like ring appearing around the larval head; afterwards the immature bee develops a hardened exterior and various colored powdery fungal spores are discharged (Shinmanuki and Knox, 2000). The colors of the spores are loosely diagnostic: *A. flavus* spores are yellow-green, *A. fumigatus* are gray-green and *A. niger* are black (Shinmanuki and Knox, 2000). Foley et al. (2014) discovered that individual larvae easily succumb to infection in lab experiments. Yet this disease is quite rare in colonies. The authors suggested that colony-level defenses are enormously helpful in suppressing the disease. However nutrition is likely a factor in keeping infections under control. For instance, it was discovered that larvae in colonies subjected to insufficient nutrition were significantly more likely to contract the disease than hives sufficiently nourished (Foley et al., 2012). Members of the Causal Analysis Workgroup discounted the importance of this disease.

Varroa Mite and Viruses

Varroa Overview

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It has been frequently proclaimed that the ectoparastic Varroa mite (*Varroa destructor*) is the single greatest global threat to the health of the managed European honey bee (Francis et al., 2012; Rosenkranz et al., 2009). It is without doubt the most destructive honey bee pest (Spivak 1996). The Causal Analysis Workgroup participants ranked Varroa mite and the viruses it transmits as probable in causing high annual colony losses. Interestingly, the mite is of little detriment to the fitness of its natural host *A. ceranae* (Asiatic honey bee) as detailed in **BOX E** on page 12 (Sumpter and Martin, 2004). The genus Varroa contains four species: *V. underwoodi, V. rindereri, V. jacobsoni,* and *V. destructor* (Rosenkranz , et al. 2009). Initially, the penultimate species was incorrectly identified as the mite that had spread to Europe and the Americas. The only species in this genus that is of economic importance is *V. destructor* (Rosenkranz et al., 2009). The term "Varroa mite" in this guidebook will be in reference to this specific species.

V. destructor is found on every continent that produces honey with the exception of Australia (AQIS, Australian Government, 2016). In regions of the world where European honey bees are unmanaged and population densities are low there are few opportunities for horizontal transmission of this pest (Fries and Camazine, 2001). However, apiculture practices promote opportunities for horizontal transmission of Varroa (Fries and Camazine, 2001) and if mites are not controlled in managed systems by external human intervention, colonies with high

infestations typically perish (Tentcheva et al., 2004).

Varroa Biology and Life Cycle

Varroa mites are found on adult bees, on immature bees, inside brood cells and throughout other parts of the hive (Shinmanuki and Knox, 2000). These mites are so closely linked with their host that there is no free living stage in their life cycle (Rosenkranz et al., 2009). When mites are attached and feeding on adult bees they are considered to be in a phoretic phase (Sumpter and Martin, 2004). When these parasites are inside sealed brood they are in a reproductive phase (Rosenkranz et al., 2009). There is sexual dimorphism amongst this species. Adult females are pale to reddish brown, ovoid and measure 1.1 mm long X 1.55 mm wide (Shinmanuki and Knox, 2000); males are markedly smaller, round and pale to light tan in color (Delfinado-Baker, 1984). Male mites only live during the reproductive stage and do not become phoretic mites (Boecking and Genersch, 2008).

A pregnant female mite usually lays five eggs in a single worker cell, but in some instances can produce six (Martin, 1994). The mother mite begins by moving down into the cell, past the prepupal bee into the larval food where it becomes stuck; it will stay in this area until the brood is capped and the larvae consumes the food (Boecking and Genersch, 2008). It is possible that staying at the bottom of the cell is a means of avoiding early detection by hygienic worker bees (Rosenkranz et al. 2009). The first mite larva is a haploid male and is deposited about 60 hours after the host brood cell is capped (Martin, 1994). The remaining mite larvae are females and are laid in 26-32 hours segments (Martin, 1994). The mite larvae feed on the honey bee host's hemolymph, undergo several nymphal stages and ultimately mate (Boecking and Genersch, 2008). Many of the mother mite's progeny naturally perish before reaching maturity, resulting in an average of just 1.45 female adult offspring which emerge from the host bee (Martin, 1994). However, in drone brood this reproduction rate is almost doubled due to a more conducive reproductive environment for the parasite (Martin, 1994). **BOX F** on page 13 provides an explanation of Varroa mite's penchant for drone brood and aversion to queen brood. 4-14 days after emergence mated female daughters crawl into new brood cells and lay eggs of their own (Boecking and Genersch, 2008). This parasite can be passed to other colonies in many ways. However, transmission principally occurs when mites are attached to bees and the infested hosts invade other hives (Shen et al., 2005).

Varroa Mite Damage and Parasitic Mite Syndrome

There are many negative consequences of Varroa parasitism. First, the mite can cause physiological damage to the host. These injuries include:

BOX E The Asiatic honey bee (*Apis cerana)* is a host of Varroa mite (*Varroa destructor*). However, the health effects of infestation on the Asiatic honey bee are marginal compared to the negative responses exhibited by the European honey bee (*A. mellifera)*. The difference in seriousness on host health can be attributed to a number of factors.

First, Varroa mites are only able to reproduce in drone brood of *A. cerana* (Boecking and Genersch, 2008), whereas they can breed in both worker and drone brood of *A. mellifera*. The Asiatic honey bee also exhibits three behavioral adaptations that aid in tolerance: 1) advanced grooming behavior which dislodges the mites from themselves and nestmates (Spivak, 1996); 2) enhanced hygienic behavior, allowing the bees to remove mites from the colony (Spivak, 1996); and 3) the ability to close the central pore of a cell's capping of infested drones; this process is known as "entombing" and it kills both the host and the mites (Boecking and Spivak, 1999).

A thorough reader may have remembered in the "Honey Bee Immune System" section that European honey bees also exhibit some of the behavioral adaptions to Varroa mite infestations mentioned above and wonder why these do not provide effective control. While European bees do exhibit grooming and hygienic behavior, they are expressed to a much lesser extent than in their Asian cousins. Indeed an astonishing study conducted by Peng et al. (1987) found that 98% of mites artificially implanted into an Asiatic honey bee colony were groomed from the bodies of bees and removed from the hive within minutes. It is also worth noting that European honey bees do not perform entombing, a practice of sealing infested brood which prevents adult emergence from the cocoon (Rosenkranz et al., 2009).

interference in production of molting hormone (Amdam et al., 2004), decreases in the protein content of the honey bee body (Yang and Cox-Foster, 2007) and reduction of the adult bee's eventual body weight (Rosenkranz et al., 2009). These physiological impairments result in bees that are shorter-lived and less adapted for overwintering survival (Amdam et al., 2004). A second problem with Varroa is that they induce immunosuppression in afflicted bees (Yang and Cox-Foster, 2005), making them more susceptible to diseases and stressors. Yang and Cox-Foster (2007) found that mite infestations reduced the expression of genes involved in antimicrobial peptides and immune-system related enzymes. This impaired both cellular and humoral immunity functions. Di Prisco et al. (2011) discovered that increased levels of Varroa correlated with a decrease in the level of antimicrobial peptides (apidaecin) in colonies. The effects of parasitism on host physiology and immune function are harsh. Colonies that are excessively parasitized usually die within months if left untreated (Shen et al., 2005).

The set of common visual indications of severe mite-related stress on a colony is collectively called honey bee Parasitic Mite Syndrome (PMS). Shinmanuki and Knox (2000) described honey bee PMS as a colony which exhibits a spotty brood pattern, queen supersedure (replacement) and the presence of easily removed scale (dead and dried remains of brood). Individual larvae may also become twisted in the cell, liquefied and change color to light brown, gray or black.

Viruses

As Soloman et al. (2005) explain, viruses are small, acellular, infectious particles. They do not exhibit characteristics commonly found in living organisms. For instance, viruses contain either deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) but not both. This differs from living organisms, which have both DNA and RNA. Viruses cannot conduct metabolic processes or reproduce on their own. They must hijack the machinery of living cells to perform these functions.

When viruses are present in non-parasitized honey bees they tend to be persistent, yet latent, and are likely suppressed by the host's immune system (Shen et al., 2005). In fact, preceding the introduction of Varroa to European honey bee colonies, *A. mellifera* had an arguably commensal relationship with their RNA viruses. Sumpter and Martin (2004) explain that while individual bees may have exhibited reduced foraging ability or decreased life-span from viral infections, the consequences at the colony level was negligible. However once Varroa reached previously uninfested regions of the world, the viruses gained a new route of transmission via mite feeding. As a result, many of the previously innocuous viruses became severely injurious and epidemic within colonies.

This change in virulence is due to a number of possible causes. Viral genotypes may have evolved to become more deadly, however it seems more likely that Varroa has dramatically increased the frequency of lethal viral phenotypes as a result of their role in vectoring many of these pathogens (Sumpter and Martin, 2004; Bowen-Walker et al., 1999). Previous to Varroa, deadly phenotypes often perished along with their host, but now these strains can be transmitted before host death occurs. It may also be the case that mite feeding itself activates normally benign viruses which are already commonly present in the honey bees (Bowen-Walker et al., 1999).

BOX F While Varroa can parasitize the brood of all honey bee castes, the mite demonstrates a clear preference for drone larvae. It commonly afflicts worker brood, but almost never parasitizes larval queens. Male bees take three days longer to develop than female workers and it is presumed that this additional time is helpful for the Varroa mite's reproductive success (Boecking and Genersch, 2008). Calderone et al. (2002) attributed the low incidence of mites in queen brood to the repellant effects of royal jelly and variances in larval chemistry among different castes. In a repellant bioassay, these authors found that mites exposed to higher concentrations of royal jelly resulted in a higher repellant effect. Since larval queens are fed more royal jelly than larval workers, this may partly explain the mite's distaste for queens. They also discovered in binary-choice tests, that mites preferred the chemical environment of 5th instar worker brood to that of a queen larvae's environment of the same age.

Moreover, viruses may have made the mite itself more damaging—creating a sort of synergistic feedback loop of honey bee pestilence. For instance, Boecking and Genersch (2008) chronicled that when Varroa first arrived in Germany an established colony could tolerate up 10,000 mites before dying. Today's German bees are fortunate to survive an infestation less than 1/3 of that. These authors attributed the markedly reduced honey bee tolerance of Varroa infestation to the rise of galvanized viruses, which have possibly weakened the ability of bees to cope with parasitism itself. Even so, the ultimate failure of an excessively infested colony is more likely a consequence of viral infections than of the direct feeding of the mites (Rosenkranz et al., 2009).

Tentcheva et al. (2004) demonstrated through a survey of honey bees in France that certain viral infections are common in apiaries and often persist without inducing clinical symptoms. Nonetheless the high rate of certain viruses in mites led the authors to the conclusion that Varroa acts as both a vector and activator of many different viruses.

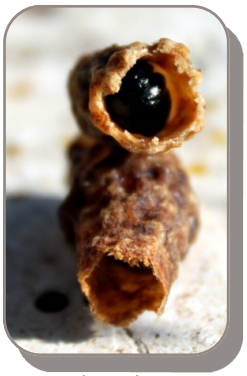
However not all honey bee viruses are transmitted by Varroa. For instance, evidence suggests that many viruses can be transmitted by the consumption of contaminated food products such as honey, pollen, bee bread and royal jelly (Shen et al., 2005). Transmission may also occur via the fecal-oral route (Chen et al., 2006). There are data to suggest that queen bees may contract viruses through trophallaxis with infected workers and occasionally from mating with drones (Francis et al., 2013). It is also possible that queen bees can transmit viruses to offspring via infected ovarian tissue (vertical transmission) (Chen et al., 2006). Furthermore, external environmental factors are thought to be important in facilitating the spread of viruses from their beginning replication sites to targets in the honey bee body (initial viral infections typically begin in the epithelial cells and pass to the nervous system). Regardless of the means of transmission, high viral loads are often correlated with significant colony losses (Cornman et al., 2012).

Acute Bee Paralysis Virus

This disease appears to be transmitted by both Varroa mite and through bee to bee contact (Tencheva et al., 2004). Larvae with Acute Bee Paralysis Virus (ABPV) may turn brownish black and experience impediments in weight gain (Azzami et al., 2012). Martin (2001) proposed that due to this pathogen's extreme virulence, nearly all pupae infected with this virus die before becoming adults as a consequence of infection. When honey bees die in the pupal stage, so too do the parasitic mites which may have vectored the disease. Since the virus is not favorable to Varroa reproduction, this author suggested that there must be a large population of mites present for this disease to be solely responsible for a colony's death.

Azzami et al. (2012) found that upon viral infection by injection, the honey bee immune system does not respond with either a cellular or humoral immune response. Indeed, inoculation failed to produce a nodulation response or AMPs from the honey bee when exposed to the virus alone. Yet the immune system responded when the virus was presented with a bacterial coinfection. The virus reproduces prolifically in the bees' hemolymph—which allows it to spread to the brain and other parts of the body—and the hypopharyngeal glands appear to be the major target of this virus (Bailey and Milne, 1969; Azzami et al., 2012). A survey of winter colony losses found that this virus along with deformed wing virus were both present at high levels in colonies that didn't survive, suggesting a link between this disease and seasonal survival (Berthoud et al., 2005).

Black Queen Cell Virus



Rob Synder,
Bee Informed Partnership

This virus affects immature queens bees and infections typically occur in the spring time (Locke et al. 2014). Larval queens that demonstrate clinical symptoms become darkened, hence the name Black Queen Cell Virus (BQCV) (Leat et al., 2000). The worker caste act as carriers but rarely

develop the disease in their larval stage; effects of the pathogen on adult workers are unknown (Locke et al., 2014). Tencheva et al. (2004) determined that transmission of this virus by Varroa was probably minimal. It appears that the virus is ubiquitous among honey bee colonies (Madella et al., 2015) and its importance in honey bee health is likely to be negligible.

Chronic Bee Paralysis Virus

Adult bees with symptoms of Chronic Bee Paralysis Virus (CBPV) are unable to fly and are found on the tops of hive frames or on the ground in a relentless shaking frenzy (Shinmanuki and Knox, 2000). Some bees afflicted with this disease will become shiny, hairless and black (Shinmanuki and Knox, 2000) which makes them sometimes mistaken for robber bees. This disease has been commonly called "hairless black syndrome" or "little blacks" because of symptomatic characteristics (Ribie're et al., 2007). Like Black Queen Cell Virus, this disease does not appear to be readily transmitted by Varroa (Tencheva et al., 2004). However symptomatic bees will sometimes exhibit dysentery and this is thought to be a route of continued infection amongst the nest mates in a soiled hive (Ribie're et al., 2007). This disease was rarely found in U.S. hives in the past, however in recent years the virus has become more prevalent (Madella et al., 2015).

Cloudy Wing Virus

As the name implies, Cloudy Wing Virus can sometimes cause the wings to become whitish and opaque (Bailey and Ball, 1991). Carreck et al. (2010) provided data on this little studied virus and proposed some conclusions from their research. First, there are no reliable overt symptoms to diagnose the disease. Second, the disease is not likely to be highly pathogenic, however it may be more problematic if it is present among other infections. Third, the disease is probably not transmitted by Varroa, but instead is passed by nurse bees to developing larvae via an oral route. Bailey and Ball (1991) reported that it is possibly transmitted by direct contact, when conditions in the hive become too crowded. It is not likely a disease of major significance.

Deformed Wing Virus

This is a virus of great importance in honey bee health. As the name suggests, this virus can cause a crippling of honey bee wings (Gisder et al., 2009). High levels of adult bees manifesting symptoms of Deformed Wing Virus (DWV) will exhibit reduced survivorship and imperil the colony (Francis et al., 2012). Physical symptoms of the disease in the absence of Varroa are possible, but rare (Bowen-Walker et al., 1999). It has been established that Varroa is not merely a potentiator of this disease, but indeed acts as a host vector (Bowen-Walker, et al. 1999). Mites can also transmit the disease to other mites by contaminating communal food sources (bees) (Bowen-Walker, Martin and Gunn, 1999).

Sumpter and Martin (2004) proposed that there were two requirements for a colony to express symptoms of DWV: 1) The disease must be transmitted by Varroa mite feeding; and 2) The mite population must be high. Gisder et al. (2009) agreed that transmission via Varroa parasitism was a prerequisite to the deformation of wings. However, they suggested that in order to induce clinical symptoms, the virus must first replicate within the mite—thus making the mite carry a higher viral load. Based on their data, which measured the viral titre of numerous phoretic mites, they found that bees with deformed wings were parasitized by mites with a DWV titre of $10^{\wedge 10}$ viral genomes per mite and mites with lower viral titres did not induce wing crippling in their host. The authors offered two explanations for this observation. First, there is a threshold at which the number of viral particles must reach to sufficiently circulate within the hemolymph and induce symptoms. Second, DWV is largely benign to bees. What induces detrimental symptoms are mutated virulent strains of the disease. The higher the viral load, the more likely it is to contain mutant, injurious strains. Yang and Cox-Foster (2005) concluded from their data that dramatic increases in the replication of DWV were associated with a bacterial coinfection. Consequently, the authors hypothesized that antibiotic treatments may reduce the replication of this virus.

If a colony of 30,000 to 60,000 bees is infested with roughly 6-9% mites carrying DWV, this will likely result in overwintering death in temperature regions (Martin, 2001). Colonies in temperate regions are much smaller during winter, therefore between 2,000-3,000 virus-vectoring mites need only infect two adult bees for roughly half of the colony to be dead by December (Martin, 2001). Members of the Causal Analysis Workgroup suggested that DWV in combination with other factors may be possibly responsible for high annual losses or a contributing factor.

Invertebrate Iridescent Virus

In 2010 Bromenshenk et al. described Invertebrate Iridescent Virus-6 (IIV-6) which was discovered using mass spectrometry-based proteomics (MSP). The virus is said to be a large DNA virus (in contrast to small RNA viruses which characterize most honey bee viruses). This pathogen in combination with Nosema disease was said to be tightly correlated with widespread losses in beekeeping. The basis of this claim resided in consistent coinfection of both pathogens in samples collected from colonies in the U.S. that experienced rapid declines. The methodology behind this study was disputed by Foster (2011) and the importance of this virus in honey bee health has been questioned by other researchers (Cornman et al., 2012). This pathogen's contributions to colony losses was classified as indeterminate by the Causal Analysis Workgroup.

Israeli Acute Paralysis Virus

Members of the Causal Analysis Workgroup suggested that Israeli Acute Paralysis Virus (IAPV) may be possibly responsible for extensive hive losses or a contributing factor. This disease infects all stages and sexes of honey bees (Chen et al., 2014). The virus has the ability to make replica in all bee tissues, yet it tends to collect in gut tissues, nerve tissues and in the hypopharyngeal gland (Chen et al., 2014). Presence in the hypopharyngeal glands may elucidate why the virus is found in royal jelly, as this is the gland which produces the substance (Chen et al., 2014). High concentrations in the gut would suggest that food acts as a source of transmission within the colony (Chen et al., 2014). Varroa have likewise been implicated in transmission. Di Prisco et al. (2011) established that Varroa was capable of acting as a vector and that there was a significant link between the occurrence of this disease and the parasite population. The authors found evidence that mites may transmit the disease amongst themselves if multiple parasites feed on the same bee. Replication of the virus may also occur within the mite.

Symptoms of the disease are similar to that of ABPV (Maori et al., 2007) and include shivering wings, paralysis and death (Li et al., 2013). High concentrations of the virus in nervous tissues may stimulate nerves that trigger behavioral characteristics of the disease (Chen et al., 2014). High levels of IAPV have also been found to adversely affect the homing abilities of infected honey bees and in some cases the bees are unable to return to the hive (Li et al., 2013). This virus is most closely related to Kashmir Bee Virus (KBV) and ABPV (Maori et al., 2011).

Kashmir Bee Virus

This virus is closely related to ABPV (de Miranda et al., 2004). It potentially causes premature death among adult and immature bees (Shinmanuki and Knox, 2000). Chen et al. (2004) presented evidence that this virus was transmitted by Varroa. It is also possible that the disease can be transmitted from the queen to eggs and from workers to larvae by food (this includes honey, pollen and royal jelly) (Shen et al., 2005). Hung (2000) found the disease in fecal material of both workers and queens, which inferred another route of transmission. Annual surveys of honey bee viruses in the U.S. have found the prevalence of this disease to be declining in recent years (Madella et al., 2015).

Lake Sinai Virus Group

It has been established that Lake Sinai Virus (LSV) is actually a complex of viruses (Ravoet et al., 2013) and there are at least seven strains (Daughenbaugh, 2015). This group is believed to be closely related to CBPV (Granberg et al., 2013). The strains LSV-1 and LSV-2 have been detected in Varroa mite and bees infected with LSV-1 tend to have high levels of the pathogen in their gut (Daughenbaugh, 2015). This suggests that transmission of the disease may

occur by mite vector, contaminated food or a fecal-oral pathway. In the U.S. LSV-2 demonstrates seasonal fluctuations; infected colonies seem to experience the highest viral loads in spring (Madella et al., 2015). The significance of the virus complex is still unknown (Granberg et al., 2013).

Sacbrood Virus

This virus causes clinical symptoms exclusively in bee brood (Shinmanuki and Knox, 2000), but adults may act as carriers (Shen et al., 2005). When infected the larvae changes color from pearly white to gray and the head region will become black (Shinmanuki and Knox, 2000). Affected larvae form a watery sac which can be removed from cells (Shinmanuki and Knox, 2000). The sac formation is due to buildup of fluid that amasses under the larval cuticle (Shen et al., 2005). Infected larvae are often found in capped cells, because death occurs just prior to pupation (Shinmanuki and Knox, 2000). This pathogen may be transmitted from queen to progeny via egg laying, workers to nestmates by glandular secretions mixed with food and through Varroa mite parasitism (Shen et al., 2005).

Slow Bee Paralysis Virus

Bailey and Woods described Slow Bee Paralysis Virus (SBPV) in 1974. The name is meant to differentiate it from the much faster acting ABPV. In a bioassay performed by these researchers, it was discovered that inoculation of the virus induced death of workers within 12 days. Anterior legs became paralyzed shortly before expiration. SBPV is not thought to be present in the U.S. based on annual state and federal surveys (Madella et al., 2015).

In conclusion, viruses are often present in honey bee colonies, but they are usually kept latent by properly functioning immune systems. These pathogens become problematic when bees are excessively parasitized, nutritionally deprived, exposed to xenobiotics or otherwise stressed. There are currently no treatments for honey bee viruses, however good management practices such as removing old brood comb, regular replacement of queens, minimizing nutritional stress and breeding resistant stock will reduce problems associated with these maladies (Somerville, 2010).

Other Honey Bee Pests

Small Hive Beetle

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Lundie (1940) provided the first record of the small hive beetle (*Aethina tumida*) in a beehive in South Africa over half a century ago. It was eventually transported into the U.S. in the 1990s and has proven to be a destructive pest of comb, honey and brood, especially in the southeast (Shinmanuki and Knox, 2000). Climate change is expected to expand the range in which this pest thrives and creates problems for beekeepers (Le Conte and Navajas, 2017). However in its native range, it is considered a secondary pest (Lundie, 1940). It has been suggested that this geographical-based distinction in pest status is due to the numerous defenses possessed by African honey bee (*Apis mellifera scutellata*) colonies but absent in European honey bees (*A. mellifera*) (Neumann and Hartel, 2004).

The larvae are white and can be as large as ½ inch, while adults are reddish-brown and half the length of an immature beetle; both life stages can be found in the hive, but pupation occurs outside the hive in nearby soil (Shinmanuki and Knox, 2000). Eggs are laid in cluttered groups often in crevices of the hive (Lundie, 1940). Yet gravid females will sometimes oviposit under capped brood cells where about 10 eggs are laid in each cell (Ellis et al., 2003). This pest voraciously devours pollen and honey however it appears that it has a preference for bee brood (Elzen et al., 2000).

Eyer et al. (2009) reported data suggesting that the beetles may transmit DWV. These authors report that beetles can develop an infection by feeding on adult bees with deformed wings, eating brood that are DWV-positive and engaging in trophallaxis with infected adult bees. Infestations of small hive beetle have also been implicated as a potential cause of colonies absconding (Ellis et al., 2003). Yet, the Causal Analysis Workgroup did not think this pest was responsible for high annual losses.

Tracheal mite

The tracheal mite (*Acarapsis woodi*) is a difficult to detect pest that lives in the honey bee's prothoracic trachea (air tubes) (Sanford, 1987). The female is 143-174 µm long; the male size ranges from 125-136 µm (Shinmanuki and Knox, 2000). Although positive diagnosis can only be made by dissection, visible symptoms of an infestation include bees with wings that are unhinged (k-wing) and bees that crawl on the ground (Shinmanuki and Knox, 2000).

Eischen et al. (1989) demonstrated a negative correlation between honey production and infestation. These results were especially dramatic in moderate to highly infested colonies. Colonies co-infested with both tracheal mite and Varroa mite have been documented to exhibit far higher mortality than colonies with Varroa mite alone (Downey and Winston, 2001). It has also been suggested that tracheal mites are more problematic in colder climates. For instance, a study demonstrated that honey bees infested with tracheal mite are less likely to return to colonies when day time temperatures are below 12° C (Harrison et al., 2001). This may be a consequence of reduced tracheal gas exchange due to parasitism.

Rennie et al. (1921) first reported on tracheal mite infestations and it was linked to what was known as "Isle of Wight disease," a mysterious malady that was reported to have decimated many colonies in Great Britain. Bailey (1964) later debunked this assertion. Today tracheal mites are a peripheral honey bee health concern. In 2011, the USDA-APHIS Honey Bee Pest and Disease Survey removed tracheal mite from their monitoring program, because subsequent years yielded no detections of this pest (Madella et al., 2015). Tracheal mites were considered by the Causal Analysis Workgroup to be unlikely contributors to major problems in beekeeping.

Tropilaelaps mites

Tropilaelaps mites (*Tropilaelaps clareae; T. mercedesae*) are ectoparasites that feed solely on immature bees (Sammataro et al., 2000). Their natural host is the giant honey bee *Apis dorsata* (Woyke, 1987), which is native to Asia. *T. clareae* females measure 1 mm long and 0.6 mm wide; the male mites are somewhat smaller (Shinmanuki and Knox, 2000). Mother mites will lay three to four eggs on larvae just before capping; one male and several females hatch and reach maturity within a week (Sammataro et al., 2000). *T. clareae* only spend 1-3 days outside of sealed brood cells, whereas Varroa mites will remain outside for nearly 10 times as long (Woyke, 1986). This reduced ability to survive outside of brood temporally may limit the spread of this mite into areas that have cold winters with extended brood-less periods (Forsgren et al., 2009).

Like Varroa mite, it has been demonstrated that *T. mercedesae* can vector viruses such as DWV in European honey bees (Dainat et al., 2009). It is also well established that *T. clareae* reproduce faster than Varroa mites (Sammataro et al., 2000). The capacity for substantially swifter reproduction compared to Varroa mite potentially make Tropilaelaps a more severe pest of European honey bees (Woyke, 1987). The annual USDA-APHIS National Honey Bee Survey has actively been monitoring for these mites but has not detected them in the U.S. (Madella et al., 2015). Preventing the entrance of this pest remains a high biosecurity priority.

Wax moths

The greater wax moth (*Galleria mellonella*) and the lesser wax moth (*Achroia grisella*) are secondary pests known to damage honey comb in weak or dead colonies (Shinmanuki and Knox, 2000). These moths can be especially problematic when beekeepers are storing equipment (Sanford, 1987).

Female moths lay their eggs in cracks and crevices of the hive (Shinmanuki and Knox, 2000). Once the eggs hatch, larvae emerge and create damage by burrowing into combs and leave tunnels filled with webbing (Sanford, 1987). Fecund female moths will be obstructed from entering the hive by guard bees in the day, however at night they manage to penetrate the hives and oviposit (Nielsen and Brister, 1976). Most hives likely have a wax moth infestation, however when colonies are strong, bees effectively remove the moth larvae once they hatch (Sanford, 1987). The Causal Analysis Workgroup determined that wax moths were not likely contributing to high overwintering losses.

Pesticides

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Pesticides are defined as chemical substances that are used to control unwanted pests (Yu, 2008). Pesticide risk is determined not merely by a chemical's toxicity, but also potential of exposure to toxic compounds (Krupke et al., 2012). In other words, if a compound is highly toxic to an organism, but the probability of exposure is low then the risk is correspondingly low. Depending on the toxicity of the chemical used and the degree of exposure, honey bee poisonings may manifest in different ways. Devillers (2002) described two scenarios for agrochemical exposure: 1) foraging bees can be exposed to lethal chemicals in the field and die there or 2) bees can become exposed to lethal or sublethal doses and then fly back into the hive. The former scenario may be the least devastating of circumstances because the toxin is not brought back to the colony. If contaminated foragers do manage to find their way back to the hive, the xenobiotic may poison younger adults performing nest duties or be fed to immature bees via pollen or nectar. This often results in neglect of the larvae due to fewer nurse bees or outright death of larvae on account of being fed toxic food. Pesticide exposure to the queen bee may result in diminished egg laying abilities; this often encourages the workers to attempt supersedure. If a toxic xenobiotic is brought back in large enough quantities, the colony may perish.

It is well understood that particular pesticides can result in honey bee mortality or have negative effects on development, longevity, immune function, and behavior by interfering with the activity of physiological processes (Chauzat et al., 2009; van der Sluijs et al., 2014). However not all pesticides are toxic to bees. There is a great variability in risk depending on the type of chemical, formulation, dose, etc. Insecticides—by definition—kill insects, thus many are toxic to honey bees (Johnson, 2014). Yet some insecticides, like tau-fluvalinate are relatively non-toxic to bees and in fact are used in the hive for Varroa mite control (Johnson et al., 2010). Herbicides and fungicides are not acutely toxic to bees, however certain chemicals have been found to cause sub-lethal effects or problems in brood rearing (Johnson, 2014). Surfactants are not pesticides, but are commonly mixed with pest control products to enhance penetration into plant surfaces or the insect cuticle. They are considered inert and therefore assumed to be non-toxic to bees, yet certain compounds have nonetheless demonstrated oral and topical toxicity (Goodwin and McBrydie, 2000).

Unfortunately pesticides are often detected in beehives; these include agrochemicals which are picked up by bees in agroecosystems and beekeeper-applied miticides deliberately introduced into the hive for Varroa mite control. For instance, a two-year multistate pesticide survey of commercial beehives in the United States by Mullin et al. (2010) found significant residues of 98 different miticides, insecticides, fungicides and herbicides in sampled hives. Other field studies have demonstrated similar findings (Chauzat et al., 2009; Wu et al., 2011; Rennich et al., 2014). Making matters worse, honey bees are generally known to be more sensitive to pesticides when compared to certain other insects. For instance Claudianos et al. (2006) demonstrated that honey bees possessed far fewer genes that encode for enzymes which detoxify xenobiotic chemicals, when compared to certain dipertans. These authors report that honey bees had about half the P450s, GSTs and CCEs of *Drosophila melanogaster* and *Anopheles gambiae*.

Yet it is unclear what severity of harm these various residues have alone or in combinations on bee health (Chauzat et al., 2009; Mullin et al., 2010; Staveley et al., 2014). This uncertainty is especially

augmented when pesticides are found in hives at low and chronic levels (Chauzat et al., 2009). The Causal Analysis Workshop participants deemed that the relationship between external (non-beekeeper applied) insecticides and high annual colony loss was indeterminate (Staveley et al., 2014). However a 2005 economic impact assessment suggested that pesticide exposure to both honey and native bees resulted in approximately $286 million in annual losses due to colony deaths, lowered pollination rates, reduced honey yields and other problems (Pimentel, 2005).

A comprehensive overview of pesticide effects on bee health is not reviewed here. Instead three different groups of chemicals will be explored. This will include the highly controversial class of insecticides known as the neonicotinoids. The less often discussed umbrella groups of herbicides and beekeeper-applied pesticides will also be examined.

Neonicotinoids

Perhaps no other class of insecticides has received as much attention and scrutiny in the context of honey bee health as have the neonicotinoids. Indeed some beekeepers have blamed certain neonicotinoids insecticides for high annual losses (Rortais et al., 2005). Neonicotinoids are plant protection chemicals that act as agonists of the nicotinic acetylcholine receptors (nAChR) (Fisher et al., 2014) and are often preferred to many other classes of chemicals by applicators because of their low toxicity to mammals (Yu, 2008). Neonicotinoids are known as systemic insecticides because they are absorbed upon application and transported throughout the plant, effectively making them toxic to target insects via feeding (van der Sluijs et al., 2015). These chemicals can be applied like other insecticides as a foliar application, however a substantial portion of their usage occurs in the form of root drenches and seed treatment (Pisa et al., 2015). There is special concern regarding seeds treated with neonicotinoids. Seeds treated with these chemicals are often mixed with talc in mechanical equipment to ensure that they do not become stuck together. During the planting process the talc becomes a pesticide-laden waste product, which in a dust form can be exhausted into the environment. This waste dust may be transported by wind away from the planting site and come into contact with honey bees (Krupke et al., 2012).

There are numerous routes in which honey bees may become exposed to neonicotinoids. Samson-Robert et al. (2014) presented data which demonstrated that puddles of water near corn fields became contaminated with neonicotinoid compounds shortly after treated seeds were planted. These puddles are attractive to bees, as they need to collect water for colony needs. Therefore, it is possible for bees to become exposed to these chemicals in this way. Krupke et al. (2012) outlined two other routes which honey bees can come into contact with neonicotinoids. First, honey bees may forage for floral resources during a treatment window and bring contaminates back to the hive. This is possible because the insecticide is transported to all plant parts, including nectar and pollen. Second, when treated seeds are being planted, the neonicotinoid-contaminated dust byproduct can be transported onto flowers which honey bees visit or the dust can land on the bees. These authors suggest that the latter route likely creates the greatest opportunity for exposure.

There is little question that neonicotinoids are acutely toxic to bees. For example, imidacloprid, the first chemical registered in the class, has a very low oral LD_{50} of 13 ng/bee and is therefore categorized as “highly toxic” (Sanchez-Bayo et al., 2016). Other chemicals in the class are also considered toxic to honey bees, especially the nitro-containing neonicotinoids (Pisa et al., 2015). Yet the problem with these chemicals isn’t merely that they are toxic to bees. There is an unfortunate overlap in the window of time in which treated-seeds are usually planted in fields and the period in which honey bee colonies are most vulnerable. Data suggests that small colonies are at highest risk from these chemicals, since fewer workers are able to provide a buffer between chemical exposure and the queen (Wu-Smart and Spivak, 2016). This is concerning since treated seeds are typically planted in early spring, when honey bee colonies are small.

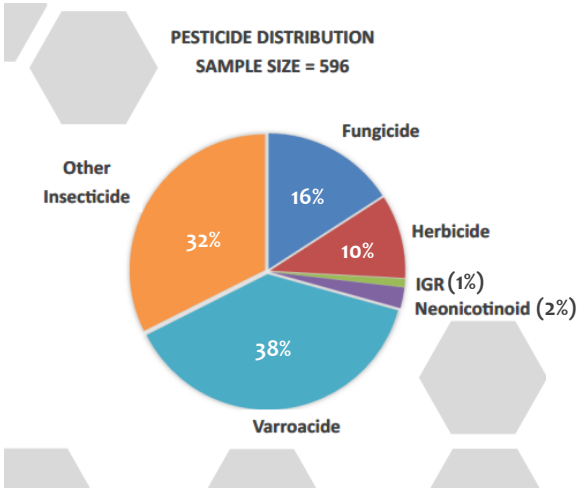
Due to the various concerns about this class of chemicals, the European Union restricted the use of

the neonicotinoids imidacloprid, clothianidin and thiamethoxam (metabolized into clothianidin) in 2012. These chemicals are part of the nitro-containing neonicotinoids, which are thought to be more toxic to bees than the cyano-containing neonicotinoids such as acetamiprid and thiacloprid (Pisa et al., 2015)—for this reason this section will focus on the nitro-containing chemicals.

However attributing widespread colony losses to a single or even a handful of chemicals has proved elusive. Firstly, it has been acknowledged that neonicotinoids are just one group among many chemicals found in hives and that many other classes of chemicals are likely to have negative effects on honey bees as well (Chauzat et al., 2009; Mullin et al., 2010). Indeed a multi-year survey of pesticide residues in pollen sampled from nearly 600 apiaries throughout the U.S. found numerous agricultural and beekeeper-applied chemicals, yet neonicotinoids comprised only about 2% of chemical residues that were identified (Rennich et al., 2014)—see **Box G**. Secondly, there has been much research which has documented various problems with neonicotinoid exposure to bees in lab settings, however these same issues at times do not manifest in field conditions under field-relevant doses (Blacquiere et al., 2012). Finally, studies on the survival of colonies exposed to specific neonicotinoids have not provided a "smoking gun." Dively et al. (2015) provides a prime example of this in a field study where full-sized honey bee colonies were chronically exposed to various concentrations of imidacloprid: 5µg/kg, 20 µg/kg and 100 µg/kg over a 12-week period. The lowest concentration was meant to simulate "normal" dietary exposure (where bees come into contact with the pesticide properly applied), whereas the highest concentration was intended to represent a "worst case scenario" of exposure (the pesticide applied during bloom). Colonies exposed to the higher concentrations exhibited significantly increased overwintering loss, however the bees subjected to the lower field-relevant dose was inconsequential on overwintering success. This study along with others, have led many researchers to suggest that neonicotinoids contribute to high annual losses of colonies, (Krupke et al., 2012; Di Prisco et al., 2013) however placing blame exclusively on this class of chemicals has yet to be demonstrated by indisputable research. The Causal Analysis Workshop participants buttressed this notion by noting that neonicotinoids were not likely alone responsible for reduced survival of colonies, however they were thought to be a possibly contributing factor. Furthermore the scientists involved in the E.U. ban noted that it was not clear to what extent neonicotinoids were responsible for widespread problems in beekeeping (O'Neal and Hodgson, 2013).

Yet high annual colony losses due to neonicotinioid exposure is not the only concern; there is also interest in the effects that sub-lethal doses of these chemicals cause and the possibility that these problems will topple hives in the presence of other biotic or abiotic pressures. It has been established that sub-lethal neonicotinoid exposure has been linked to impaired learning, memory loss, modifications of navigation abilities and immune-suppression (Desneux et al., 2007; Di Prisco et al., 2013).

BOX G USDA-APHIS coordinates the National Honey Bee Pest and Disease Survey, which monitors for exotic pests, overall honey bee health and pesticide residue in beehives. Perhaps surprisingly, the percentage of neonicotinoid residues found in honey bee pollen has consistently been found to be low. Below is a graphical breakdown of pesticide residues by category found in pollen samples that were collected from nearly 600 apiaries over multiple years.



Source: Rennich et al., 2014

Henry et al. (2012) performed a study to evaluate whether sub-lethal doses of thiamethoxam increased the rate of homing failure in exposed forager bees. Foragers were gathered and given 1.34 ng of the insecticide in a 20-µl sugar solution, which is considered a field-relevant dose. Exposed bees and control bees were monitored with radio-frequency identification technology to determine whether they returned to their colony after release. The bees given thiamethoxam were roughly twice as likely to fail to return as control bees. When these data were entered into a honey bee population dynamics model, it was discovered that colonies significantly suffered even in cases where only 50% of foragers were exposed. In instances where 90% of foragers were exposed a colony of 15,000 bees could dwindle to 5,000 bees in less than 40 days of foraging on treated crops. The authors noted that the negative effects of exposure were more pronounced if the bees were foraging in territory that had not been visited recently.

The sub-lethal effects of imidacloprid on honey bee health has also been reviewed. It is suspected that this insecticide can make honey bees prone to infection at concentrations not thought to be acutely harmful to bees. For example, Pettis et al. (2012) fed colonies protein patties spiked with sub-lethal concentrations of imidacloprid and demonstrated a clear correlation with increased susceptibility to Nosema disease. At concentrations of 5 and 20 ppb bees were found to have as much as a four-fold increase in the number of Nosema spores compared to bees from colonies fed patties without imidacloprid. Di Prisco et al. (2013) found a similar impairment of the immune system when bees were exposed to clothianidin and imidacloprid. In this study bees were exposed to various sub-lethal doses of both pesticides and it was discovered that as a consequence: 1) the transcription of the antimicrobial apidaecin genes were significantly reduced; 2) the rate of DWV measurably increased. It was determined that there was a dose-dependent relationship for the latter result: the more active ingredient bees were exposed to, the higher the DWV replication.

Data have demonstrated that neonicotinoid exposure can have negative impacts on honey bee colonies and mechanical planting of seeds treated with these chemicals presents special concern. However, it is also true that chemical residues in this class are not commonly detected by pesticide surveys of hives in the U.S. Determining what level of restriction should be applied to these chemicals is a matter of difficult deliberation. What makes this conundrum especially challenging is that extreme restrictions will potentially result in other classes of pesticides filling the need for plant-protection products that are likely also toxic to bees, but possibly more dangerous to mammals and other non-targets.

Herbicides

Herbicides are generally considered to be safe to use around honey bees. Many have high LD_{50}s for both oral and contact exposure to honey bees. Some of these chemicals work on plant-specific pathways, which likely reduces toxicity to non-targets (Herbert et al., 2014). However numerous sub-lethal effects have been documented when bees are exposed to certain herbicides.

Glyphosate has a high LD_{50} (>0.1mg/bee) with exposure having little effect on the survival of adult bees or bee brood (Thompson et al., 2014). Herbert et al. (2014) found that field-relevant acute and chronic exposure did not result in increased mortality of adult bees. Though, these authors found significant sub-lethal impairments of cognitive abilities such as learning and sensory sensitivity when bees were subjected to 0.125 to 0.25 µg of the pesticide. It has also been demonstrated that sub-lethal exposure can impair navigation. Balbuena et al. (2015) documented this by spiking sugar water with various concentrations of the chemical and feeding it to bees. Bees fed 0.5 µg of glyphosate took longer to return to hives after being released from novel locations and performed more indirect flights (flights with loops) than control bees. This is problematic because it suggests that forager efficiency is potentially impaired by field-relevant glyphosate exposure.

Like glyphosate, 2,4-D has a relatively high LD_{50} for honey bees at 11.5 µg/bee and is therefore

considered relatively non-toxic (Mayeret al. 1999). However, Papaefthimious et al. (2002) found that the honey bee heart has unusual sensitivity to 2,4-D. Indeed a mere 1 µM exposure to this chemical permanently reduced heart function of adult workers by 70%. This concentration is well below the recommended field application rate of 90-180 µM. Negative effects of 2,4-D have also been found on brood rearing. A study found that when bees are fed a sublethal concentration of 500 ppb, brood rearing stops altogether (Moffett and Morton, 1975). The same study found that at a fifth of the concentration, eggs do not hatch at normal rates and nurse bees have difficultly rearing larvae. In both cases the effects were found to be temporary and once 2,4-D was not fed to the bees, the colony recovered.

Another herbicide that is considered relatively non-toxic to bees (LD_{50} = 36 µg/bee) is paraquat (Mayer, et al., 1999). However at concentrations above what is recommended for field application, it can be deadly to bees. When workers are injected directly with 15 µg of paraquat they experience a tenfold reduction in lifespan (Corona et al., 2007). Likewise when bees are exposed to 4.5 kg/ha of paraquat in the field, they die within about three days (Moffett and Morten, 1972). This concentration is roughly four times the maximum recommended field application rate. These data underline the importance of not exceeding the concentrations prescribed by the herbicide label.

One may ask how this information is able to be reconciled with the notion that herbicides are typically safe for use around honey bees. First, toxicity data collected in the registration of most herbicides merely determine acute toxicity and not chronic effects or sub-lethal effects; second, it is also important to consider that honey bees have sophisticated detoxifying capabilities which may prevent active ingredients from reaching the organism's site of action (Papaefthimious et al., 2002).

However the most deleterious effects of herbicides on honey bees may in fact be indirect, since their use has the potential to significantly reduce the abundance and diversity of honey bee forage (Devillers, 2002). USDA has identified nutritional deficiencies as a major contributor to the problems in honey bee health. Yet it should be noted that in certain instances herbicides can be used for the long-term benefit of diverse floral resources. For instance, herbicides are sometimes used by weed-abatement programs to protect native plants and habitats (Goldner, 1984). Without the use of herbicides as part of an integrated weed management strategy, certain noxious plants may turn thriving, diverse habitats into monocultures. A noxious plant may provide nectar and pollen to honey bees for a short period of time. However if a single plant dominates the flora of an environment, the benefit of that forage source may be quite small. Indeed, honey bee health is improved when bees are provided with a diverse set of flora that bloom throughout the season, instead of small number of plants that bloom periodically (Decourtye et al., 2010).

Beekeeper-Applied Miticides and Medicines

Florida Department of Agriculture and Consumer Services

Perhaps unsurprisingly, residues from beekeeper-applied miticides are frequently found in honey bee colonies and often in very high concentrations (Rennich et al., 2014; Mullin et al., 2010). These pesticides are intended to control the honey bee parasite Varroa mite, which as previously mentioned is the most serious honey bee pest (Tarpy et al., 2007). One might question if the presence of these chemicals is problematic since miticides are thought to be selective (ideally killing mites, without harming bees). Despite the fact that miticides approved in the U.S. for use in hives exhibit high LD_{50}s for individual honey bees, they nonetheless can have an array of negative effects on

colonies (Berry et al., 2013). Beekeepers also use antibiotics for the treatment of honey bee pathogens; these medicines can have deleterious interactions with other chemicals found in hives (Hawthorne and Dively, 2011). These problems are often amplified when beekeepers use products not registered by regulatory institutions for Varroa mite control or fail to follow instructions on the label of pesticides legally permitted for use in hives. These actions have the potential to harm bees just as severely as misuse of chemicals by growers or pesticide applicators (Johnson et al., 2010).

Miticides used in the hive can be damaging to developing immature honey bees. Zhu et al. (2014) demonstrated this by exposing larvae to pesticides commonly found in honey bee hives, specifically: coumaphos (organophosphate), tau-fluvalinate (pyrethroid), chlorothalonil (organochlorine) and chlorpyrifos (organophosphate). The former two pesticides are beekeeper-applied miticides, and the latter two are plant-protection chemicals (a fungicide and an insecticide, respectively). Compared to controls, larvae exposed to this cocktail of chemicals (at concentrations frequently found in hives) exhibited more than a two-fold increase in mortality. The authors noted that the interactions between the chemicals were mostly additive (combined effect of chemicals equal to the sum), however the chlorothalonil fungicide was found to synergize both miticides as binary mixtures. In addition to this research, Berry et al. (2013) found that tau-fluvalinate and coumaphos exposure to immature bees reduced the 3-day brood survivorship rate.

Since queen bees are critical to the success of a colony, various studies have evaluated the effects of miticides on these reproductive individuals. Queens tend to be more tolerant of miticides than workers or drones (Dahlgren et al., 2012). However, in a queen rearing experiment, Haarmann, et al. (2002) found they were vulnerable to certain beekeeper-applied chemicals. When tau-fluvalinate was used per manufacturer recommendations (two strips per hive), there was no significant effect on queen bee health. However, at levels exceeding the label queen body weight suffered—emphasizing the importance of following the pesticide label. Yet coumaphos demonstrated harmful effects on queens even at low doses (1 strip). Exposure of this miticide during queen development caused high mortality rates and sub-lethal effects in survivors, such as physical deformation and behavioral abnormalities. Berry et al. (2013) also found problems with both coumaphos and tau-fluvalinate residues in hives and suggested that they catalyzed increased frequency of queen supercedure cells . Queen supercedure cells can be an indicator of queen health, since they are built by worker bees in response to a sick, injured or poorly laying queen.

Interactions of honey bee medications can also increase the susceptibility of bees to other pesticides. For instance, Ellis et al. (1997) discovered in a caged -bee bioassay that Apistan (tau-fluvalinate) made colonies more susceptible to harm from the agrochemical bifenthrin (pyrethroid). The authors didn't claim that this evidence could be extrapolated to field conditions, but they did suggest that beekeepers avoid using tau-fluvalinate at times when bees would forage on crops treated with bifenthrin. Likewise, hives that were previously treated with coumaphos or tau-fluvalinate exacerbated the toxicity of the essential oil thymol, which is the active ingredient in the miticides Apiguard and ApiLife Var (Johnson et al., 2010). A multiple drug interaction analysis of miticides and fungicides found that about half of miticide-miticide and miticide-fungicide combinations had a synergistic effect, and consequently made the miticide more toxic (Johnson et al. 2013). Zhu et al. (2014) found that larvae exposed to a cocktail of chemicals that were found in hives time and again suffered. Indeed the commonly detected combination of coumaphos, tau-fluvalinate, chlorothalonil and chlorpyrifos at field-relevant rates caused a two-fold increase in mortality of immature bees. The former two pesticides are beekeeper-applied miticides, and the latter two are plant-protection chemicals. Research has also suggested that the beekeeper-applied antibiotic oxytetracycline can increase the sensitivity of bees to the toxic effects of both coumaphos and tau -fluvalinate (Hawthorne and Dively, 2011). It should be noted that Varroa mite has developed widespread resistance to coumaphos and tau-fluvalinate

and therefore many beekeepers have ceased using these products. However, even after discontinuation these chemicals continue to be found in the hive for years because they persist as residues in wax (Johnson et al., 2010).

In an effort to find new, effective, "softer" mite treatments, some beekeepers have turned to natural chemicals and plant secondary products with miticidal properties. A few of these chemicals have been formulated into commercially available acaricides for beekeepers; this includes Apiguard (thymol), ApiLife Var (thymol, eucalyptol, menthol and camphor), HopGuard (salts of hops beta acids), Mite Away Quick Strips (formic acid) and Mite-A-Thol (menthol). Yet as Paracelsus (1493-1541 AD) famously remarked "All substances are poisons*:* there is none which is not a poison. The right dose differentiates a poison and a remedy." This notion applies to naturally occurring chemicals. A laboratory analysis evaluating the toxicity of various essential oils and organic acids by Ebert et al. (2007) revealed that compounds such as wintergreen, menthol, sage oil and cineole were found to be fairly benign. However, Carayon et al. (2013) found that there were negative effects resulting from honey bee exposure to thymol at approved concentrations. This study exposed honey bees to ApiLife Var (74% thymol) under laboratory conditions, and found that they exhibited significant impairment in phototaxis just one day after application. Thymol can also be problematic in combination with other chemicals as discussed previously.

■ ■ ■ ■ ■ Despite the negative effects on bee health associated with these inputs, data has consistently demonstrated that beekeepers which keep mites under control improve survival rates compared to apiaries which do not receive treatment (Traynor et al., 2016). The elimination of miticides would likely make modern, commercial beekeeping uneconomical. Therefore, it is often stressed that beekeepers should not aim to eliminate inputs but rather minimize them. Keeping an apiary clean, strictly following the label instructions on miticides and practicing Integrated Pest Management (IPM) techniques in Varroa mite control may reduce the degree of complications associated with these inputs. IPM efforts include consistent inspection for maladies, utilizing non-chemical methods of Varroa control (drone trapping), breeding pest resistant stock and making treatment decisions based on economic thresholds (MAAREC, 2000).

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