Honeybee health (risks) in England and Wales

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Executive Summary

The health of honey bees in England and Wales is important for both the production of honey but perhaps more importantly for the pollination service they provide to the wider crop production industry. There are a significant number of threats facing honey bees both endemic and exotic and it is the role of the National Bee Unit(NBU), funded by Defra, to provide support to bee keepers through the continued research and development of these threats, surveillance of existing threats and the prevention of new threats entering or becoming established in the country.

Recent surveys have shown an increase in the level of hive mortality in the winter of 2006 and 2007 and this has raised concerns for the continued functionality of the industry. The abnormally high levels of mortality are not isolated to England and Wales but have been recorded in North America and mainland Europe. Since the NBU is in the forefront of the effort to ensure continued good bee health it is appropriate that the function and performance is examined.

An extensive review of the literature reveals that there is a great deal of activity in bee research at the moment with a strong focus on getting a better understanding of the impacts of the various pests and diseases on bee populations. The NBU has a healthy programme of research, often in conjunction with other organizations which help to ensure the sharing of new results and therefore the possible findings of research from other countries being applied to England and Wales. The research strategy of collaborating with institutes either within the UK or elsewhere appears to be a sensible approach given the constraints of funding.

The NBU has a surveillance programme that appears to be comparable with other countries in terms of the proportion of the hives inspected. However, the fact that not all beekeepers are registered means that it is not possible to have a fully stratified sample and not all high risk apiaries can be checked. A programme to encourage all beekeepers to register on a central database is therefore highly desirable and should be pursued further.

There is scope for greater coordination of the resources of the NBU and bee keepers to develop strategy for control of new threats whether they stem from new introductions (e.g. small hive beetle) or develop from existing threats (e.g. resistance to control methods).

The increased pressure of pests and diseases could result in increased use of antibiotics and pesticides for control which may then produce problems with contamination of honey with the associated human health issues. The development of integrated control methods that minimize the use of chemicals should be encouraged both for reasons of minimizing health risk but also reducing the likelihood of resistance developing in pests and diseases.

The current draft strategy on protecting and improving the health of honey bees in England and Wales should be exploited to develop stronger links between the NBU, beekeepers and other interested parties. Improvement in levels of education, awareness and a coordinated response should be at the core of the strategy.

Background

Honey bees provide a significant benefit to the environment, pollinating a wide variety of plants (Biesmeijer et~al., 2006).. It is likely that honey bees provide over 50% of pollination of naturally occurring plants on which wild birds and mammals depend in the UK. It has been estimated that there are 44,000 beekeepers in the UK, with a total of 274,000 hives. Of these some 300 are commercial beekeepers (members of the Bee Farmers Association) with 50,000 hives. The remainder are keen amateurs. 100 years ago there were around 1million bee hives; this had reduced to 400,000 in the 1950s and further reduced to the 274,000 today. It is estimated for the UK that the pollination services from honey bees are worth £120-200 million annually and honey production is worth an additional £10-30 million (Defra, 2008). The feral honey bee population is reported to have been largely wiped out by disease in the last 15 years.

The objective of Defra's bee health programme is to reduce the risk of introduction and spread in the UK of serious bee pests and diseases. Defra will be spending about £1.235m on bee health measures in 2006/07 (a small part of which is claimed from the EU under the England element of the UK National Apiculture Programme). These measures are delivered by the National Bee Unit (NBU), part of the Central Science Laboratory, an executive research agency of Defra. The NBU provides scientific advice and laboratory services and manages the bee health inspectorate. Disease controls are governed to a large extent by national bee health legislation and EU veterinary checks Directives. The bee health programme includes hive inspections and sample analysis, and where necessary, treatment or destruction of hives infected with notifiable diseases or pests. Training and education is also provided to make beekeepers more self-reliant through improved bee husbandry.

The Central Science Laboratory (CSL) National Bee Unit (NBU) has been responsible for maintaining the Integrated Bee Health Programme in England and Wales since the early 1990s. The role of the Bee Health Programme is to protect the honey bee, a major pollinator of agricultural and horticultural crops and wild flora, and to provide up-to-date technical support to beekeepers. The Bee Health Programme is funded in England by the Department for Environment, Food and Rural Affairs (Defra) and in Wales by the Welsh Assembly Government (WAG). The work includes inspection of honey bee colonies, disease and pest diagnosis, development of contingency plans for emerging threats, minimising the

risk of introduction of potentially serious exotic pests and diseases through importation by import risk analysis and related extension work and consultancy services to both government and industry. There is also an underpinning programme of research and development.

There are a number of bee pests and diseases that can have a detrimental effect on bee colonies and the services they provide both within agriculture and the wider environment. Some of the pests and diseases are already established in England and Wales e.g. American and European foulbrood, and require careful monitoring and management to ensure losses are kept to a minimum. However, there are a number of pests and diseases that are not yet in the UK (or Europe) and if introduced could have serious consequences for the bee populations within this country; examples of these are the small hive beetle and Tropilaelaps mites. It is therefore very important that the existing problems are well managed to maintain bee health and that the risks and consequences of introductions of alien pests and diseases are well understood and appropriate plans in place to deal with any such introduction. However, this needs to be done against a background of limited funding with the objective of implementing and maintaining a cost effective bee health programme. The objective of this report is to help in informing the National Audit Office about the current and past research activities on bee health both in England and Wales and in other parts of the world with the aim of identifying the current and potential threats to bee health, the measures already available to control these threats and the priority research areas to close any significant knowledge gaps.

Diseases of Bees Endemic to England and Wales - A review of the current status and research.

American foul brood (AFB; Paenibacillus larvae)

American foulbrood (AFB) is a bacterial disease of honeybee larvae (brood) caused by the spore-forming bacterium Paenibacillus larvae var. larvae. The spores are extremely resistant to heat and chemical agents and can survive for many years in scales (from diseased dead brood), hive products and equipment. Only the spores are capable of inducing the disease. It is a notifiable disease in England and Wales. All colonies found infected with AFB are compulsorily destroyed by burning, hives are sterilized by scorching with a blowtorch and affected apiaries are placed under the conditions of a 'Standstill Notice' prohibiting movement of bees or equipment until certified clear of infection.

The proportion of the colonies inspected by NBU inspectors in England and Wales found to be infected with AFB has fluctuated between about 0.1% and 1.4% over the last 10 years, though has remained at about 0.2% (about 0.7% of apiaries or beekeepers) since about 2003 (see Annex 1

Table 1, Table 2 and Table 3 in Annex 1) (
https://secure.csl.gov.uk/beebase/public/BeeDiseases/diseaseIncidenceM
aps.cfm)

Sources of infection are infected combs, honey and beekeeping equipment, while spread of the disease is by the beekeeper through management practices, the transfer of combs between colonies, and by worker bees robbing honey, drifting or swarming. There have been few studies on the horizontal transmission rates of AFB between honey bee colonies. A recent study in Sweden has shown that *P. larvae* spore loading on adult bees was a function of distance from an AFB killed colony and that most transmission between apiaries occurs within 1 km distance from clinically diseased colonies, but is significantly lower at 2 km distance or longer (Lindström *et al.*, 2008). However, colonies may

develop considerable spore densities on adult bees without exhibiting visible symptoms of disease. A model has been developed to determine how many adult bees to sample from a colony or apiary in order to be able to detect colonies with low levels of AFB infection. (Lindström, 2008).

No antibiotic treatments for AFB are approved in England and Wales. Elsewhere, for example in Argentina, the antibiotic oxytetracycline is widely used for prevention and control of AFB. This has however resulted in the development of tetracycline resistant strains of *Paenibacillus* larvae. Resistant strains were found to carry the tet(K) determinant on a plasmid (Alippi et al., 2007). Because of the problems with tetracycline resistance, the search is on for other treatments to control AFB. The antibiotic, tylosin A, was shown to be active at very low concentrations against all the many strains of P. larvae screened in Argentina (Alippi et al., 2005). The NBU/CSL showed that tylosin A depletes to desmycosin (tylosin B) in honey and can still be detected 238 days after dosing. Thus a more accurate residue definition is the sum of tylosin A and desmycosin (Adams et al., 2007). The macrolide antibiotic, tilmicosin, developed for exclusive use in veterinary medicine has also recently been shown to have good activity against AFB; a dosage of 1000 mg a.i. of tilmicosin applied in a 55 g candy resulted in a total suppression of AFB clinical signs in honeybee colonies 60 days after initial treatment in Argentina (Reynaldia et al., 2008). An essential oil extract from grapefruit (Citrus paradisi) was bactericidal to P. larvae at a concentration of 770.0 mg/l in broth culture, and if found to be effective in the bee hive it would be a welcome addition to the beekeepers control repertoire since it is a natural product without the risk of toxic residues in the honey (Fuselli et al., 2008).

Initially, two subspecies of *Paenibacillus larvae* were recognized on the basis of pathogenicity tests; *P. larvae* subsp larvae (*Pll*) the cause of AFB and *P. larvae* subsp *pulvifaciens* (*Plp*) which is know to cause powdery scale disease. However some *Plp* isolates have the ability to cause AFB. NBU/CSL studied the molecular phylogenetic relationships between these subspecies and found that they do not cluster separately – i.e. there is no molecular evidence for them being classed as different subspecies. These molecular fingerprints can be used to identify different genotypes of the pathogen and possibly could be used to trace new sources or routes of infection (Saville *et al.*, 2007). Genersch, *et al.* (2006) compared isolates from Sweden, Norway and Germany in molecular phylogenetic studies and also reclassified *Paenibacillus larvae* subsp. *pulvifaciens* and

Paenibacillus larvae subsp. larvae as Paenibacillus larvae without subspecies differentiation.

Studies in Germany with *in-vitro* reared drone larvae of several sister queens from an *Apis mellifera ligustica* and a Buckfast breeding line showed that there were differences within and between lineages in susceptibility to the strain of P. larvae used. Infection sensitivity was higher in the A. m. ligustica line compared to the Buckfast line. Different infection thresholds were found among sister queens of the *A. m. ligustica* line suggesting a considerable genetic variance for larval resistance against AFB (Behrens et al., 2007).

European foulbrood (EFB; Melissococcus plutonius)

European foulbrood is an economically significant disease of honey bee larvae present across most regions of the world and caused by the bacterium *Melissococcus plutonius* (Bailey 1983). It is a notifiable disease in England and Wales where it is prevalent in the south, southwest, and midlands. There are few outbreaks in the northern areas of England. Diseased apiaries are placed under the conditions of a Standstill Notice prohibiting movement of bees or equipment. Lightly infected colonies are treated with an antibiotic by an authorised bee inspector under the powers of the Bee Diseases and Pests Control (England) Order 2006, although the shook swarm technique without antibiotic is increasingly being applied. Colonies that are considered to be too weak or too heavily infected are destroyed as with AFB (Brown & Ball, 2007).

With the extensive use of tetracycline antibiotics to control EFB it was predicted that resistance to these antibiotics would eventually develop in *M. plutonius*. However, a NBU/CSL study failed to identify any such isolates and concluded that oxytetracycline (OTC) could continue to be used to treat European foulbrood and that resistance may not explain why some treatments fail (Waite *et al* 2003).

A recent NBU/CSL study on the efficacy of the shook swarm (SS) method found that although both oxytetracycline and SS reduced the amount of bacteria present, EFB reoccurred in 22% of colonies treated with OTC but only in 4% of colonies treated using SS, suggesting SS was more efficient at preventing EFB. In the same study, Mp was found not to be ubiquitous across all regions of England and Wales. Mp was detected in 54% of larvae and 43% of adult bee samples from asymptomatic contact

colonies and at greater concentration and frequency from EFB infected colonies (PH0502; Budge *et al.*, 2007b).

NBU/CSL are exploring alternative non-chemical approaches for the control of *M. plutonius* based on the identification and use of bacteriophage and bacterial antagonists (component of PH0505)

Although the main route of infection with EFB is through the activities of the beekeeper, it is also possibly for infection to be transmitted by the *Varroa destructor* mite (Kanbar et al., 2004). A recent study in Switzerland using a novel real-time PCR assay for *M. plutonius* has shown that worker bees from brood nests have about 20-times higher bacterial loads than those from flight entrances, suggesting that the former are more suitable for EFB-monitoring. Moreover, current sanitation measures in Switzerland appear to be insufficient because only three out of eight apiaries were free of *M. plutonius* one year after sanitation. While no clinical symptoms are observed below 50 000 CFU of *M. plutonius* per bee, workers can nevertheless be carriers and likely responsible for bacterial propagation (Belloya *et al.*, 2007; Roetschi *et al.*, 2008).

Some bee genotypes appear more resistant or tolerant of infection by EFB and DEFRA are funding work (component of PH0505) to try to identify microsatellite markers in honeybee linked to the apparent EFB resistance.

Chalkbrood (Ascosphaera apis).

Chalkbrood is an extremely common brood disease caused by the fungus *Ascosphaera apis*. Worker, drone, and queen larvae are susceptible. Spores of the fungus are ingested with the larval food. The spores germinate in the hind gut of the bee larva, but mycelial (vegetative) growth is arrested until the larva is sealed in its cell. When larvae are about 6 or 7 days old and sealed in their cells, the mycelia break through the gut wall and invade the larval tissues until the entire larva is overcome. This process generally takes from 2 to 3 days (Borum, & Ulgen, 2008).

Good husbandry is important; hives should be kept well ventilated and free from damp, with plenty of food. Where persistant chalkbrood infections occur re-queening is advisable. No commercial treatment for chalkbrood is currently available. However, Apiguard (Slow release Thymol) has been shown to have an effect on chalkbrood. Genetically

diverse *A. mellifera mellifera* colonies had a lower variance in chalkbrood disease prevalence than genetically similar colonies, which suggests that genetic diversity may benefit colonies by preventing severe infections (Tarpy, 2003).

Amoeba (Malpighamoeba mellificae)

Cysts of the protozoan *Malpighamoeba mellificae* are ingested with food and germinate in the rectum of the bee. They migrate to the malpighian tubules (the 'kidneys') to create more cysts that then accumulate in the rectum and are excreted. It has been suggested that infections of *M. mellificae* are associated with spring dwindling, dysentery and shortening the lifespan of infected bees. However, there is no evidence to support this and the effect of an infection is not clearly known. *M. mellificae* infections are very often found in association with nosemosis and it is likely that a dual infection will be more damaging to the health of the bee. The grainy circular cysts are larger than the rice shaped nosema spores and are often seen under a microscope when examining a sample for nosema. Hygiene and good management is the key to controlling spread of the organism, as with nosema. There are currently no approved proprietary products registered for the control of *M. mellificae* in the UK, although the spores are destroyed by acetic acid.

https://secure.csl.gov.uk/beebase/public/BeeDiseases/adultDiseases.cfm

Nosema (Nosema apis, Nosema ceranae)

Nosema belongs to a unique group of primitive spore-forming fungi known as Microspora, many of which are parasites of insects. They are transmitted via spore ingestion. Although *Nosema* can be spread by dysentery it is not the cause of dysentery. *Nosema apis,* which causes nosema disease, is found worldwide. *Nosema ceranae*, a similar parasite, was found in Asian honey bees (*Apis cerana*) in 1996. In 2005 it was found in *Apis mellifera* in Taiwan, and since in Europe, North America and Australia.

Nosema causes increased mortality in adult bees, decreased honey yield, poor overwintering capability, reduced spring build up and colony dwindling. *N ceranae* has been reported to cause greater mortality in honey bees compared to *N apis*.

Nosema is readily spread through the use of contaminated combs. The spores can remain viable for up to a year, it is therefore important not to transfer contaminated combs between colonies and as always to practice good husbandry and apiary management, maintaining vigorous, healthy stocks, which are better able to withstand infestations (Bailey, 1955, 1967.)

A multiplex PCR-based method, in which two small-subunit rRNA regions are simultaneously amplified in a single reaction, has proved efficient and reliable at detecting single and mixed infections of *N. apis* and *N. ceranae* in surveys across Europe (Martin-Hernandez et al., 2007).

There are indications that *N. ceranae* acts in concert with other pathogens or conditions resulting in Colony Collapse Disorder (CCD) in the USA. In Europe *N. ceranae* was the reported cause of 20,000 abnormal colony losses in the Salamanca region of Spain in November 2004 (Klee *et al.*, 2007).

Nosema infection can be eliminated from the hive with the specific antibiotic, fumagillin, in the last sugar feed before winter, though hives may become re-infected within six months of treatment (Higes et al., 2008; Williams et al., 2008). In an Italian search for alternative methods for treating nosema, bees fed with candies prepared with the natural products thymol or resveratrol had significantly lower nosema infection rates, and the bees supplied with the resveratrol prepared candy also lived significantly longer (Maistrello et al., 2008).

NBU/CSL are assessing different treatments for nosema and are trying to quantify the impact of *Nosema ceranae* on honey bee colonies in the UK. Colony loss data is being collected and the level of the pathogen quantified using real-time PCR. Apiaries testing positive for *Nosema apis* are sampled for a comparison of pathogenicity (component of PH0505)

Viruses of bees

To date, about eighteen viruses have been reported to infect honey bees, though for a number of these very little is known about etiology, geographical distribution or impact on the colony. At present there are no available treatments for viral diseases in honeybees. However, good husbandry and timely treatment of other pest and disease organisms can help to prevent any overt effects of viruses within the hive. Studies in

France by Tentcheva *et al.* (2004) indicate that bee virus infections occur persistently in bee populations despite the lack of clinical signs, suggesting that colony disease outbreaks might result from environmental factors that lead to activation of viral replication in bees. Shen *et al.* (2005) working with Kasmir Bee Virus (KBV) and Deformed Wing Virus (DWV) proposed that parasitization by varroa suppresses the immunity of honey bees, leading to activation of persistent, latent viral infections. In USA, Chen *et al.* (2006) have demonstrated that many of the viruses infecting honeybees (Black Queen Cell Virus [BQCV], DWV, Chronic Bee Paralysis Virus [CBPV], KBV, and Sacbrood Virus [SBV]) can be vertically transmitted from queens to their offspring, including eggs, larvae, and adult workers. Because of this finding, it is likely to be necessary to change the regulations and certification requirements for the importation of bee germplasm.

NBU/CSL and the University of Surrey have a project to study the molecular phylogeny of viruses infecting bees. They hope to be able to use the information to map the distribution of virus species and strains across Britain and possibly to associate symptom types to virus types or mixtures and presence of other pathogens or parasites (PH0410; Cordoni *et al.*, 2007).

NBU/CSL and the University of Sheffield have a NERC CASE PhD studentship, Investigating virus immune response in honey bees (with additional funding from Defra).

Deformed wings

Deformed wing virus (DWV) is a viral pathogen of the honeybee associated with clinical symptoms and colony collapse when transmitted by *Varroa destructor*. In the absence of *V. destructor*, DWV infection does not result in visible symptoms, suggesting that mite-independent transmission results in covert infections. In Germany Yue *et al.* (2007) demonstrated the occurrence of vertical transmission of DWV through both unfertilized eggs and semen. In a molecular phylogenetic study of DWV from bees from three different continents, Berenyi *et al.* (2007) found that in the sequenced regions, the genome turned out to be highly conserved (98 to 99% nucleotide sequence identity), independent of the geographic origins of the honeybee samples, indicating a recent global distribution of the virus.

Paralysis

Paralysis is a symptom of adult honey bees and usually is associated with viruses. Two different viruses, chronic bee paralysis virus (CBPV) and acute bee paralysis virus (ABPV), have been isolated from paralytic bees. Other suspected causes of paralysis include: pollen and nectar from plants such as buttercup, rhododendron, laurel, and some species of basswood; pollen deficiencies during brood rearing in the early spring; and consumption of fermented stored pollen.

Chronic bee paralysis virus (CBPV) is an infectious and contagious disease of adult honeybees causing chronic paralysis. Chronic paralysis is the only common viral disease of adult bees that has well-described symptoms, which include abnormal trembling of wings and body. Some individuals become almost hairless and dark in appearance and suffer nibbling attacks from healthy bees of their colony. Affected bees become flightless, often crawling on the ground and on the stems of grass. The bloated abdomen is caused by distension of the honey sac with fluid, leading to the so-called "dysentery" symptom. Sick individuals die within a few days of the onset of symptoms. CBPV infections have never been related to *Varroa destructor* infestations, and the virus has not been reported in this parasite. However, the virus is very contagious and in France Ribière *et al.* (2007) have shown that infectious CBPV particles excreted in the feces of infected bees can infect naive bees and provoke overt disease by mere confinement of naive bees in a soiled environment.

Acute bee paralysis virus (ABPV) is a virus that mainly affects the honeybee but it has also been found in bumblebees and is the only honeybee virus known to have a natural alternate host (Bailey & Gibbs, 1964). This virus spreads by way of salivary gland secretions of adult bees and in food stores to which these secretions are added (Ball, 1985). In Europe and North America, ABPV has been shown to kill adult bees and bee larvae in colonies infested with the mite *Varroa jacobsoni*. The mite damages bee tissues and, in so doing, may act as a vector, releasing viral particles into the haemolymph (Scott-Dupree & MacCarthy, 1995).

Sacbrood

Sacbrood is caused by *Sacbrood virus* (SBV) and alone does not result in severe losses. The virus can be transmitted by varroa, though it can also be transmitted by bee larvae ingesting virus contaminated brood and vertically from an infected queen; the virus has been present in the UK since long before varroa arrived (Bailey, 1969). It is most common during the first half of the brood-rearing season. Initially during an

infection, the virus particles replicate in the developing larva, which appear to develop normally until after being capped-over. The infected larvae turn a pale yellow colour; they remain stretched out on their backs, heads towards the top of the capped cell. Liquid accumulates between the body of the larva and its unshed skin, then larvae become fluid-filled sacs, hence the name. The larvae will eventually die and begin to dry out, turning a dark brown to black colour, giving rise to the characteristic 'Chinese slippers' or 'gondola-shaped' scales. As the larvae die, the workers will uncap the cells to expose them. It often goes unnoticed, since it affects usually only a small percentage of the brood. Adult bees typically detect and remove infected larvae quickly. Often, if sacbrood is widespread enough for the beekeeper to observe the symptoms, the disease may be so severe that the adult worker population is reduced. In cases where there are large areas of brood clearly affected then it would be best to re-queen the colony (https://secure.csl.gov.uk/beebase/public/BeeDiseases/minorDisorders.cf <u>m</u>)

Kashmir bee virus (KBV) is known to persist as a sub-lethal unapparent infection in *Apis mellifera* in several countries but has rarely been reported to cause disease outbreaks or mortality. Transpuparial (vertical) transmission of the virus does occur (Anderson & Gibbs, 1989). However, the virus has recently become more prevalent because of the spread of the parasitic mite Varroa destructor to new areas (Shen, 2005). KBV was reported in the UK for the first time in 2004.

Varroa Destructor Virus -1 (VDV-1) was first definitively identified in Europe in 2006. It is carried by both honeybees and the tiny varroa mites that affect them. VDV-1 is related to a family of paralytic viruses that causes a breakdown of some membranes. In silkworms the virus causes flaccid disease, which causes the worms to digest themselves internally (Ongus *et al.*, 2006). VDV-1 has recently been identified in USA where researchers are now assessing if there is any link with CCD (MarketWatch 2008).

Israeli acute paralysis virus (IAPV) has been correlated to Colony Collapse Disorder (CCD – see later) in the USA (Cox-Foster et al., 2007).

Abnormal Honeybee Colony Loss

Historically annual colony losses have fluctuated greatly in the UK, with severe weather increasing colony losses. Recently, significant bee losses across Europe and N. America (see CCD below) have given cause for concern and CSL's National Bee Unit (NBU) is working to investigate this. NBU inspectors have sampled over 500 colonies across England and Wales. Samples of bees and brood have been taken from apiaries for screening for a range of endemic and exotic pests and pathogens. The cause of much of the early 2007 colony mortality was failure to control a serious parasitic mite, *Varroa destructor*, often coupled with honeybee viruses as secondary pathogens transmitted by the mite. Later in 2007 Chronic bee paralysis virus (CBPV) and *Nosema* spp. were found associated with colony dwindling, correlating with poor weather conditions. Some commercial beekeepers lost 30% of their bees when the bees were confined for long periods with little opportunity to forage, allowing the pathogens to spread rapidly (CSL 2008).

Parasites of Bees endemic in England and Wales

Honey bee tracheal mite (Acarapis woodi)

This internal parasitic mite lives within the tracheae (breathing tubes) inside the thorax of adult honey bees. Tracheal mites also may be found in air sacs in the thorax, abdomen, and head. The mites pierce the breathing tube walls with their mouth parts and feed on the haemolymph of the bees. It was probably the cause of or a major contributory factor to 'Isle of Wight disease' first seen in the early 1900s on the Isle of Wight. It decimated the honey bee population, later spreading to mainland UK. In more recent times, the tracheal mite has had a serious economic impact on the beekeeping industry in North America after it's introduction in the 1980s from Mexico. However, in the UK acarine is not usually a serious disease, with relatively small numbers of colonies being affected.

There are currently no approved proprietary products registered for the control of acarine in the UK. As with most parasites it is important to practice good husbandry and to maintain vigorous, healthy stocks, which are better able to withstand or tolerate infestations. Some strains of bee are also more prone to infestations than others, for example strains that have not been exposed to tracheal mites before. It is therefore important to carefully select which strain of bee you maintain and not to breed from those stocks that appear predisposed to acarine.

https://secure.csl.gov.uk/beebase/public/BeeDiseases/adultDiseases.cfm

Varroa (Varroa destructor)

The ecto-parasitic mite Varroa destructor (Previously reported as V. jacobsoni) is a serious world-wide pest of the honeybee Apis mellifera and has been linked with the death of millions of colonies, although its role in colony death remains elusive. In temperate regions infested Apis mellifera colonies usually collapse within one to three years if Varroa is not controlled in some way (Bowen-Walker & Gunn, 2001). The first honey bee colony losses attributed to the Varroa mite were reported in the Far East during the 1960's; the mites have since spread to most areas of the world where Apis mellifera are kept. Varroa is endemic in England and Wales; present in south and central Scotland; and detected in Northern Ireland in April 2002. It is also widespread in Eire. Varroa is no longer a notifiable pest since the Bee Diseases and Pests Control (England) Order 2006 came into force 17 March 2006. Defra formally revoked, under the powers of the Bee Diseases Control Order 1982, the Statutory Infected Area for varroa in England, which was declared by the Notice of Declaration of Infected Area (No 9) that came into force on 7 March 1997.

Since Varroa cannot be eradicated, every beekeeper with infested colonies must practice effective mite control. Once varroa is found in an apiary the level of infestation must be estimated regularly throughout each season to monitor how the mite population is developing. The information gathered can then be used to decide what control methods will be appropriate and when. The NBU has developed a model that allows the prediction of when treatments are needed. A representative proportion of colonies in an apiary should be monitored since the level of infestation may vary greatly. Varroa monitoring and control is expensive in terms of labour and cost of treatment, and unnecessary treatments should be avoided because they involve loss of time or money and may increase mite resistance and residues in honey. However, this should be balanced against the risk that the colony might collapse if treatment is delayed. The Varroa Mite Model produces a forecast of the future level of infestation from the present scale of the problem and thus increases the effectiveness of the mite control saving both time and money (Wilkinson & Smith, 2002).

Soon after the introduction of pyrethroid treatments in the UK in 1992 to control varroa it was realised that in time varroa would develop resistance to them and when this happened these treatments would become ineffective. Pyrethroid resistance has now developed in many countries worldwide, including much of Europe (Trouiller, 1998) Where this has happened, generally the first sign beekeepers have had of the problem is when their colonies collapse despite having received

treatment for varroa. First detection of pyrethroid resistance in varroa in England and Wales was in 2001 (Thompson *et al.*, 2002). Varroa mites have also developed resistance in northern Italy to the organophosphorous acaricide coumaphos (Spreafico *et al.*, 2001).

Some honeybee genotypes exhibit varroa sensitive hygiene (VSH) behaviour which it may be possible to exploit in the future (Boecking et al., 2000; Ward et al., 2008). Because of the problems with insecticide resistance, alternative methods of varroa control are being researched. Treatment of hives with strips coated with conidia of the entomopathogenic fungus Metarhizium anisopliae resulted in significantly reduced varroa populations in those hives at the end of the experimental period in Florida and Texas (Kanga et al., 2006). Similarly, a formulation of conidia of an isolate of Beauveria bassiana collected from Varroa mites is showing some potential as a biological control for varroa in experiments in the south of France (Meikle et al., 2008a,b)

In Minnesota USA, bee colonies selectively bred for both hygienic behavior and Suppression of Mite Reproduction (HYG/SMR) had significantly fewer mites on adult bees and in worker brood compared to the unselected control colonies, but there were no differences among the lines in mite reproductive success (Ibrahim et al., 2007). In France, Varroa infestation did not induce mortality in naturally occurring "varroa-surviving bee" (VSB) stock colonies. However, honey production was significantly higher (1.7 times) in the control treated than in VSB colonies (Le Conte et al., 2007).

Greater wax moth (Galleria mellonella) & Lesser wax moth (Achroia grisella)

The **Greater Wax Moth** (*Galleria mellonella*) larva hatches among the brood and chews its way through brood cappings in a straight line. The bees remove the silky tunnels and leave the bee larvae bare which are not recapped. The brood emerges normally but is sometimes crippled with deformed wings and legs due to faecal pellets from the wax moth larvae. Stored comb is vulnerable to damage since the larvae feed on wax, larval skins and pollen. Protect stored comb by stacking boxes, placing newspaper between each box. *Certan* is a solution of *Bacillus thuringiensis* and is sprayed on the combs – the larvae die after ingesting the insecticide. Deep freezing kills all stages of wax moth. Acetic acid kills all stages. Greater Wax Moth has become more evident in recent years, maybe resulting from the loss of feral colonies and the use of varroa

screens under which they pupate. The larva spins its cocoon and pupates in a boat-shaped depression scooped out of a wooden part of the hive. Combs can be attacked in weak colonies (Garedew *et al*, 2004).

The **Lesser Wax Moth** (*Achroia grisella*) can cause similar problems. The Greater Wax Moth has a wingspan of up to 3.6 cm. and the Lesser Wax Moth a wingspan of 1.8 cm.

Bee louse (Braula coeca)

Braula coeca, a wingless fly, is commonly known as the bee louse. The adults are small and reddish brown in colour and may initially be mistaken for immature Varroa mites. Although several adult flies may live on a queen, usually only one will be found on a worker. These pests apparently do little harm. They breed under cell cappings and adults feed on honey taken as queen or workers are feeding. Tunnels can spoil appearance of comb honey (Dobson, 1998).

Bee parasitic mite syndrome (BPMS)

This is generally associated with varroa mites, viruses, or a combination of both. Affected larvae die in the late larval or prepupal stage, stretched out in their cells often with their heads slightly raised. In the early stage of infection, they are white but dull rather than glistening, and they look deflated.

Exotic parasites of potential threat to Bees in England and Wales

The Asian honey bee mites, (Tropilaelaps species)

Tropilaelaps are serious parasitic mites affecting both developing brood and adult honey bees. Parasitisation by these mites can cause abnormal brood development, death of both brood and bees, leading to colony decline and collapse, and can cause the bees to abscond from the hive. Recent molecular and morphological studies have shown that the genus Tropilaelaps contains at least four species. Tropilaelaps clareae, previously assumed to be ubiquitous in Asia, was found to be two species, and was redefined as encompassing haplotypes (mites with

distinct mtDNA gene sequences) that parasitize native *A. dorsata breviligula* and introduced *A. mellifera* in the Philippines and also native *A. d. binghami* on Sulawesi Island in Indonesia. *Tropilaelaps mercedesae* n. sp., which until now has been mistaken for *T. clareae*, encompasses haplotypes that, together with haplotypes of *T. koenigerum*, parasitise native *A. d. dorsata* in mainland Asia and Indonesia (except Sulawesi Island). It also parasitizes introduced *A. mellifera* in these and surrounding regions and, with another new species, *T. thaii* n. sp., also parasitizes *A. laboriosa* in mountainous Himalayan regions (Anderson & Morgan, 2007). Tropilaelaps species are thought to be restricted to tropical or sub-tropical regions of Asia but their exact geographical range remains unclear. They are exotic to the European Community but are notifiable throughout.

The National Bee Unit (NBU) inspectorate carries out surveillance for *Tropilaelaps* targeting "At risk" apiaries, e.g. around ports and container freight terminals. Beekeepers are strongly encouraged to monitor their hives for Tropilaelaps as part of routine colony management. Debris samples submitted to the NBU laboratory are routinely screened for exotic pests. These are either in the form of debris samples or hive inserts collected and sent to the Bee Unit by Appointed Bee Inspectors (ABIs) (statutory samples) or beekeepers (voluntary samples) throughout England and Wales (Wilkins, , & Brown, 2005).

(South African) Small Hive Beetle (SHB; Aethina tumida)

The small hive beetle (SHB) is a parasite and scavenger of honey bee colonies. The beetles multiply to huge numbers, their larvae tunnel through comb to eat brood, ruin stored honey, and ultimately destroy infested colonies or cause them to abscond. Adult beetles can fly long distances to infect new hives. Larvae crawl out of the hive to pupate, usually in soil outside the hive. Warm sandy soils are preferred for pupation, which is a vulnerable time for the SHB and there is probably high natural mortality. This is a point in their lifecycle where they could be eliminated by the beekeeper - for instance using pesticides or a biological control method.

SHB is indigenous to Africa, where it is considered a minor pest of honey bees. It is not yet in England or Wales, but was first observed in Florida USA in 1998; at least 20,000 colonies were destroyed in USA within 2 years of first observation. It is now widespread in USA and as far north as Manitoba, Canada, and was observed in Australia in October 2002

where it is now widespread and causing considerable damage. The beetle is exotic to the European Community, but is a serious threat to the sustainability of European apiculture. It was made notifiable throughout the European Community in 2003 (Commission Decision 2003/881/EC).

To date there has only been one recorded incident of SHB entering Europe. This was in September 2005 when two SHB larvae were identified on the queen boxes and attendant workers from a consignment of *A. m. ligustica*-like mated queens imported to Portugal from USA for research purposes. The importation was under the conditions of EU Decision 2003/881/CE so once the queens had been transferred into new queen cages and introduced into receiving nuclei in Portugal, all original (US) queen cages and escorting workers were sent to the National Veterinary Services (NVS) for further analysis. Once the identity of the larvae found in the original queen boxes was confirmed, an order was issued by NVS to kill and burn all honeybee colonies in both apiaries where the queens had been introduced. The prompt action of the Portuguese authorities and perhaps because the two apiaries concerned were somewhat isolated from other apiaries, means that SHB have not established in Portugal (Murilhas, 2005).

Until 2004, SHB was the only Nitidulid species reported associated with honeybee colonies, but then Neumann and Ritter (2004) identified *Cychramus luteus* Fabricius (Coleoptera: Nitidulidae) associated with honeybee colonies in an apiary close to Freiburg (im Breisgau), Germany. The *C luteus* were observed to feed on pollen but although they caused no apparent harm to the brood, it is important to have reliable diagnostics that can distinguish between this species and the SHB for surveillance purposes.

African honeybee colonies (*Apis mellifera scutellata*) show some resistance to SHB; workers quickly remove both unprotected eggs and larvae of SHB from the hive, often carrying and dropping the larvae several meters from the hive (Neumann & Härtel, 2004).

NBU/CSL is collaborating with USA and South Africa to identify volatile chemical attractants from bee colonies that can be used in lures for trapping and monitoring SHB populations (PH0503; Wakefield *et al.*, 2007). Similarly, a study in USA is looking at volatile attractants of SHB from a pollen-based diet conditioned by the feeding of adult virgin female or male SHBs and compared to that of the same diet fermented with the yeast *Kodamaea ohmeri* isolated from the beetle (Torto *et al.*, 2007).

In a study across South Africa, Australia, Florida and Maryland, apiaries next to large scale honey extraction facilities (honey houses) showed higher SHB infestation levels but colony phenotypes (number of bees, amount of brood or stores) did not influence colony infestation levels. Inside colonies, SHB distribution was influenced by the presence of bees with more SHB in the brood nest in the absence of bees. The conclusion was that methods of reducing SHB populations, such as the removal of dead colonies and the prevention of SHB reproduction in honey houses are important means of controlling SHB (Spiewok et al., 2007).

NBU/CSL have been operating targeted surveillance for incursions of SHB to England and Wales since 2003 by inspecting colonies at apiaries at high risk locations such as near airports, sea ports and distribution depots for imported horticultural produce in the warmer southern areas of the country (Brown & Morton, 2003). To date, no SHB have been detected in any of these inspections (See Table 4 in Annex 1)

As well as the targeted apiary inspections, 49 samples have been submitted to NBU/CSL by beekeepers for exotic disease inspection over the last 3 years; no SHB or *Tropilaelaps* were observed in any of these.

To improve the sensitivity of the colony inspections, NBU/CSL have developed a method to screen hive debris for the presence of SHB using real-time PCR in conjunction with an automated DNA extraction protocol. This will be a valuable support tool enabling species identification and rapid screening of hive debris in delimiting surveys if the SHB were to establish at new locations (Ward et al., 2007).

Because measures for SHB control are limited and their use has given variable results, NBU/CSL are exploring the use of novel fusion proteins for SHB control (component of project PH0505).

Varroa jacobsoni

In 1999 it was discovered that a second *Varroa* species occurs in Asia that can parasitise honeybees (hence the change of name of the more widespread species to *V. destructor*). This species is now known as *V. jacobsoni* and so far it has not been demonstrated to reproduce in *A. mellifera*. It has not yet been comprehensively studied, but it is clearly desirable to avoid its introduction to EU and UK honey production areas

until more is known about its biology. NBU vigilance will be necessary to monitor the situation.

Additional risks for Apiculture; pests not currently notifiable within EU or UK or listed in the World Organisation for Animal Health (OIE) Terrestrial code.

Colony Collapse Disorder (CCD; formerly Fall Dwindle Disease)

In 2007, there were numerous press reports about the abnormally high incidence of colony loss in the USA. In the condition described as Colony Collapse Disorder (CCD) colonies suffer a rapid decline of adult bees (Glinski and Kostro, 2007), the colony is abandoned and the bees are never found. It has caused 50-90% loss of honeybee colonies in USA beekeeping operations (Cox-Foster *et al.*, 2007). The cause(s) of CCD is the subject of much research in the USA and elsewhere where severe colony losses are happening. The box below is an adaptation of a note on the causes of CCD by Jamie Ellis 16 April 2007 at http://pestalert.ifas.ufl.edu/Colony Collapse Disorder.htm

What causes CCD?

The cause of CCD is under investigation. At this point, almost every conceivable and realistic cause remains a possibility. The leading candidates and a brief explanation of their potential role are listed below. This list is not a comprehensive list and the candidates occur in no particular order. It is important to note that this list may change as new information on CCD becomes available. Such changes could result in the addition or exclusion of any of the following potential causes. The author makes no attempt to promote or undermine any one of the following theories.

Traditional bee pests and diseases (including American foulbrood, European foulbrood, chalkbrood, nosema, small hive beetles, and tracheal mites): "traditional" bee maladies (those nearly-cosmopolitan throughout the US and globally). Although traditional bee diseases and pests (fungi, bacteria, virus, parasitic insects and mites) are considered potential causes of CCD by some, likely they are not the primary cause. This is because they do not have a history of promoting CCD-like symptoms. Rather, they may exacerbate the disorder; for example *Israeli acute paralysis virus* (IAPV) has been correlated with CCD in USA (Cox-

Foster et al., 2007);

Style of feeding bees and type of bee food: The style of feeding bees and types of bee food used to feed bees vary considerably among beekeepers reporting CCD losses. As such, no correlation has been found between what colonies were fed and their likelihood of survival. Despite this, many beekeepers have abandoned the practice of feeding high fructose corn syrup to bees due to indications that it can form byproducts that are harmful to bees

How the bees were managed: Management style is a broad category but it can include the type of income pursued with bees (honey production, pollination services, etc.) or what routine colony management beekeepers perform (splitting hives, swarm control, chemical use, etc.). Both of these vary considerably among beekeepers so this possible cause of CCD is given less attention. That said, poor management can make any colony malady worse. In Spain, Pajuelo et al. (2008) concluded that environmental and husbandry factors play an important role in colony collapse

Queen source: Scientists are investigating the lack of genetic diversity and lineage of bees, both related to queen quality, as possible causes of CCD. Regarding the former, relatively few (in the hundreds) breeder queens are used in the U.S. to produce the millions of queen bees (and therefore all bees) used throughout the U.S. Geneticists refer to this as a genetic bottle neck. This lack of genetic biodiversity can make bees increasingly susceptible to any pest/disease that invades the system.

Chemical use in bee colonies: Like farmers in other agricultural sectors, beekeepers often attempt to chemically-control the various maladies affecting their honey bees in an effort to keep their bees healthy and productive. Investigators recently have found a number of sub-lethal effects of these chemicals on honey bees (workers, queens, and drones) even when the chemicals were used according to label and in accordance with best management practices suggested by specialists. These sub-lethal effects have led some to consider the role of in-hive chemical use in the CCD paradigm.

Chemical toxins in the environment: Another chemically-oriented theory is that toxins in the environment are responsible for CCD. Because pesticides are used widely in cropping systems in an effort to kill herbivorous insects, one is left to consider the potential for non-target chemical effects on foraging bees (Barnet *et al*, 2007). In addition to being exposed to toxins while foraging, honey bees also may encounter toxins by drinking water contaminated with chemical runoff, encountering various chemicals (household, commercial, etc.) through contact outside of the hive, or via direct inhalation. There is also the possibility of contamination of pollen and nectar by insecticides such as imidacloprid

and fipronil (Bonmatin et al., 2007); Environmental and consumer advocates in Germany and the U.S. blame pesticides, particularly Bayer CropScience's clothianidin (successor to imidacloprid); the concerns gained urgency in May 2008 when more than 11,000 German honeybee hives were poisoned by clothianidin. German regulators banned some uses of the pesticide (Vollmer, 2008).

Genetically modified crops: Some people have proposed that genetically modified crops may be responsible for the widespread bee deaths. Interestingly, many seeds from which genetically modified crops are grown are dipped first in systemic insecticides that later may appear in the plants' nectar and pollen. This makes genetically modified plants suspect because of their chemical treatment history, not just because they are genetically modified. Scientists have begun initial investigations into both theories but no conclusive data have been collected.

Electronic smog: Dr Warnke – who has been researching the effects of man-made electrical fields on wildlife for more than 30 years – suggests that "an unprecedented dense mesh of artificial magnetic, electrical and electromagnetic fields" has been generated, overwhelming the "natural system of information" on which the species rely and he believes this could be responsible for the disappearance of bees in Europe and the US in what is known as colony collapse disorder (Warnke, 2007; Lean, 2008). Similarly, Kievits, (2007) also suggests that electromagnetic radiation may be the cause.

Varroa mites and associated pathogens: Even with the concerns surrounding CCD, varroa mites remain the world's most destructive honey bee killer. As such, varroa and the viruses they transmit have been considered as possible causes of CCD. Varroa mites may cause the serious demise of honey bees by suppressing bee immunity and by boosting the amplification of *Deformed wing virus* (DWV) in bees exposed to microbes (Yang & Cox-Foster, 2007). Further, varroa often are controlled chemically by beekeepers. So varroa (perhaps not directly) has been considered a potential cause of CCD because the mite itself is damaging, it transmits viruses to bees, and it can elicit chemical responses from beekeepers. Despite this, there have been instances of colonies showing symptoms of CCD when their varroa populations were under control.

Nutritional fitness: Scientists have proposed nutritional fitness of adult bees as a potential cause of CCD. This topic is being investigated although little information exists currently to support/refute the role of nutrition. Malnutrition is a stress to bees, possibly weakening the bees' immune system. A weak immune system can affect a bee's ability to fight pests and diseases and immunosuppression may be caused by pathogen

or parasite attack (Glinski & Kostro, 2007).

Undiscovered/new pests and diseases: Finally, undiscovered or unidentified pests/pathogens are considered possible causes of CCD. Some believe that a new pest/disease may have been introduced into the U.S. and is causing CCD. To give one example, Nosema apis (a microsporidian that lives in the digestive tract of honey bees) has been present in the U.S. for many years. In 2006, scientists discovered and identified a new nosema species, Nosema ceranae, present in some colonies displaying symptoms of CCD (it also has been found in bee samples dating back to 1995). When this disease is present in bees in elevated levels, the bees leave their colonies, never to return. Although the role of N. ceranae in the CCD complex is not understood, it and other new pathogens may play an important role in elevated bee deaths. In Spain, Higes et al. (2008) have proved Koch's postulates between N. ceranae infection and a syndrome with a long incubation period involving continuous death of adult bees, non-stop brood rearing by the bees and colony loss in winter or early spring despite the presence of sufficient remaining pollen and honey.

Many scientists believe that CCD is caused by a combination of the factors above. To illustrate this point, some dead bees showing symptoms of CCD have had elevated levels of normally-benign pathogens in their bodies, possibly indicating a compromised immune system. In theory, any stress or combination of stresses (chemicals, genetic bottlenecks, varroa, etc.) can suppress a bee's immune system. Considering synergistic effects as a potential cause of CCD makes the disorder increasingly harder to study.

Adapted from Jamie Ellis 16 April 2007 at http://pestalert.ifas.ufl.edu/Colony Collapse Disorder.htm

It would appear that no single factor is responsible for CCD or honeybee decline in general (Frazier *et al.*, 2008).

The Cape bee (Apis mellifera capensis)

A single clonal lineage of socially parasitic Cape honeybee workers, *Apis mellifera capensis*, has caused dramatic losses in managed populations of *A. m. scutellata* in South Africa (Härtel *et al.*, 2006). There is the risk that if *A.m. capensis* were deliberately (for improvement purposes) or accidentally introduced into England and Wales they could affect the western honeybee (*A.m. mellifera*) in a similar way.

The Africanized honey bee and its hybrids (*Apis mellifera scutellatta*)

Africanised bees (*Apis mellifera scutellatta*) are a subspecies of the honey bee (*Apis mellifera*). They were introduced into South America (Brazil) from Africa in 1956 (and the "swarms escaped" in 1957) in an attempt to breed a strain of bees that would be more suitable to tropical conditions. Africanised bees have a number of behavioural traits that make them difficult to manage. Their introduction would consequently have a negative impact on beekeeping in England and Wales. The most important trait is that they show an exceptionally high level of defensive behaviour (Goodwin, 2002).

Other mites species such as *Euvarroa* spp.

Euvarroa wongsirii and Euvarroa sinhai have been identified in colonies of Apis dorsata and A. m. mellifera across South Asia and the Middle East, though there is little indication of how much damage they do. The US government prohibits the import of bees or bee products from areas where hives have been identified as having been infested with E. sinhai (Koeniger et al., 2002) while the NBU screen the queen boxes and accompanying worker bees from imported queens for Euvarroa species (BeeBase, 2008).

Asian/Japanese Hornets

The oriental hornets *Vespa orientalis* and *Vespa mandarinia* are social wasps that display an annual nesting cycle. The new colony is founded between spring and early summer by a single overwintered queen that copulated in the proceeding autumn. *V. orientalis* is distributed from the Mediterranean to Japan and is an important predator of honey bees in the Mediterranean area. It has been known to destroy whole apiaries. The rate of *V. orientalis* predation has been estimated at 33 bees per hornet per day. *V. mandarinia* is found in Japan, China and India and can also have catastrophic effects on honey bee colonies₃. It has been classed at the most serious enemy of Japanese apiculture. Ten workers of *V. mandarinia* can kill 40 bees per minute with their mandibles and a colony of 30,000 bees can be killed in 3 hours by a group of 20-30 wasps (Goodwin, 2002)

Exotic honey bee viruses

Israeli acute paralysis virus (IAPV) and Apis iridescent virus (AIV) appear not to have been detected in England and Wales so far. IAPV has been associated (correlated) with Colony Collapse Disorder in USA (Cox-Foster et al., 2007), but not in Brazil(?)

Thai sacbrood virus has been reported causing severe brood mortality in Apis cerana (Verma et al., 1990). Although it has been found to multiply in A. mellifera in the laboratory, it has not been reported to cause disease signs in localities where both A. m. mellifera and A. cerana coexist (Allen, 1995).

Egypt virus has been isolated from dead bees from Egypt (Bailey et al., 1979) and France. Its epidemiology is unknown. Young pupae injected with the virus die in about 7 or 8 days₂.

Resistant organisms imported either with hive products or bees

It is worth noting that the importation of a chemical or drug resistant form of any currently endemic pest or disease that does not currently exhibit a level of resistance in the UK would also result in reduced control and increased impact. Therefore the fact that a disease or pest is endemic does not imply that it should not be monitored for during the importation of bees or bee related products or equipment.

NBU recent and current activities on bee health and protection

CSL overall has performance indicators, which apply to NBU. Actions they take are specified in a MoU with the Plant Health Division. Responsibility is shared with beekeepers, but rather informally – not like in NZ which has for example an American Foulbrood (AFB) Disease control plan (National Pest Management Strategy) that sets specific outcomes and responsibilities between Ministry and beekeepers and retailers. See http://afb.org.nz/biosecurity-national-american-foulbrood-pest-management-strategy-order-1998

Disease and parasite identification and diagnosis

Accurate and robust methods for identifying and diagnosing parasites and diseases of bees are essential for surveillance both for the better management of endemic problems and for the detection of exotic new arrivals. CSL/NBU have a very strong team working on developing new and improved diagnostic methods for plant and bee pathogens and pests (Boonham *et al.*, 2008).

- NBU & University of York PhD: Investigating the genetic differences between *Paenibacillus larvae* subspecies (Defra seedcorn studentship)
- NBU & Defra Biosecurity Developing micro-array (Bio-Chip) screening methods for the detection of animal, fish and plant viruses.
- NBU & University of Surrey PhD: Investigating the taxonomy of UK honey bee viruses: A molecular approach (Defra funded project number PH0410)
- NBU Investigating abnormal colony losses in England and Wales (Defra funded)
- NBU/CSL -Evaluation of Metagenomic Sequencing (Pyrosequencing) as a Diagnostic Tool for the Characterisation of Disease of Unknown Aetiology (Defra seedcorn)
- NBU/CSL Streamlining honey bee diagnostic services (Defra funded)

Monitoring and control of diseases and parasites already present

AFB and EFB are the two notifiable diseases of honeybees currently present in England and Wales and thus they are the primary focus of the NBU inspections. All the NBU inspections are recorded on a custom-made database and live summaries of the results can be accessed on the www (https://secure.csl.gov.uk/beebase/public/BeeDiseases/diseaseIncidenceMaps.cfm).

Over the last 10 years, on average, the NBU inspectors have inspected about 25,000 colonies from about 4200 apiaries representing about 3000 bee keepers each year. The proportion of these colonies found to be infected with AFB has fluctuated between about 0.1% and 1.4% over the last 10 years, though has remained at about 0.2% (about 0.7% of apiaries or beekeepers) since about 2003 (see Annex 1

Table 1, Table 2 and Table 3 in Annex 1). The proportion of colonies found to be infected with EFB has been much higher than for AFB, but has been declining from over 4% in 2000 to about 2.7% in 2007. These figures suggest that the level of inspection and destruction of colonies with AFB is not managing to decrease the incidence of AFB across England and Wales, while the treatment or destruction of colonies with EFB does appear to be reducing the incidence of this disease. The NBU started

trialling the shook-swarm procedure instead of oxytetracyclin treatment for EFB infected hives in 2006, but it is too early to tell what effect this will have on the overall trend in incidence of EFB. From the data available it would appear that in order to reduce the incidence of AFB or EFB to less than 0.1%, the number of colonies inspected each year would have to be significantly increased from current levels. This would necessitate increasing the number of inspectors and the number of beekeepers registered with NBU (on BeeBase).

- Varroa was first identified in England in 1992 when it was classified as a notifiable pest and NBU inspectors were responsible for inspecting for it and managing its control. However, since it has rapidly spread and is now endemic across most of England and Wales, it has ceased to be a notifiable pest since the Bee Diseases and Pests Control (England) Order 2006 came into force 17 March 2006. Since pyrethroid treatments were first used to control varroa in the UK in 1992 it was realised that in time varroa would develop resistance to them and when this happened these treatments would become ineffective. NBU inspectors have been carrying out varroa pyrethroid resistance surveillance since 2000 and first detected resistance in August 2001 in north Devon. The incidence of resistance is steadily creeping up, and though the greatest density of detections is still in the southwest (Devon, Cornwall, South Wales), resistance has been detected in apiaries near the Scottish border. Although testing for pyrethroid resistant varroa is now the responsibility of beekeepers rather than the NBU, the NBU plans to continue resistance surveillance as part of their routine apiary inspection work in the short term.
- NBU & Vita evaluating a new biological control agent (*Bacillus* spp.?) for AFB and EFB.
- NBU Assessing the effectiveness of the shook swarm method for controlling European Foul Brood (Defra funded project number PH0502)
- NBU & EU partners Working group "Co-ordination in Europe of integrated control of varroa mites in honey bee colonies" (originates out of the EU Project FAIR CT97-3686)
- Investigating novel control methods for honey bee pests and diseases -PH0505

Alternative non-chemical approaches for the control of *Mellisococcus* plutonius.

Monitoring the impact of *Nosema ceranae*

Risk assessment, surveillance and eradication of exotic incursions

With globalisation, trade and movement of bees around the world have increased the risks to bee health. Potential exists for major pest threats of the honey bee to reach Europe and the UK. Recent concern has focused on Aethina tumida (the small hive beetle (SHB)) and the Tropilaelaps mites. In 2003, the European Commission stepped up measures to protect EU apiculture against these pests by making both notifiable throughout the Community and establishing additional import controls to reduce the risk of their introduction from third countries (Commission Decision 2003/881/EC). Since 2003, NBU Appointed Bee Inspectors (ABIs) have been increasing the statutory surveillance programmes to specifically monitor for SHB and *Tropilaelaps* mites. The NBU has been using its beekeeper and apiary database ('Beebase') and Geographical Information Systems (GIS) to help prioritise this programme and target 'At Risk Apiaries' (ARAs). For example, apiaries within close proximity to high-risk areas such as ports, freight terminals or fruit and vegetable importers are targeted and regularly inspected. Using the GIS systems, ARAs are given mathematical risk scores and inspections can be prioritised readily. Since both are notifiable in England and Wales, beekeepers are also required by law to notify NBU and send samples to NBU/CSL for inspection if they suspect the presence of either SHB or *Tropilaelaps* in their hives (Brown et al., 2002). In the case of confirmation of the SHB or *Tropilaelaps*, Defra's Contingency Plan for Exotic Pests and Diseases of Honey Bees will be invoked and emergency searches and control measures will be commence immediately (https://secure.csl.gov.uk/beebase/public/BeeHealth/indexInspection.cf m).

NBU ABIs inspected about 2590 colonies at high risk apiaries in 2007. To date, neither SHB nor *Tropilaelaps* have been detected in any of the apiary inspections since they began in 2003 (See Table 4 in Annex 1). Similarly, none of the 50 suspect samples sent by beekeepers to NBU/CSL since 2006 for examination have proved to contain SHB or *Tropilaelaps*.

NBU are also required to undertake a laboratory examination of queen boxes and attendant worker bees which accompanied Queen Bees imported into England/Wales under license for the presence of pests and diseases. These samples are examined and tested for the presence of Acarine, Nosema, Amoeba, Varroa, Mellitiphis (pollen mite), SHB and *Tropilaelaps*. Neither SHB nor Tropilaelaps has been found in the queen boxes or on the attendant workers of queens imported to England/Wales. So far in 2008, queens have been imported from Argentina, Hawaii and

New Zealand. Nosema has been detected in some samples from each country (Budge, 2008), Varroa in one sample from Hawaii and Mellitiphis in three samples from New Zealand.

- NBU & Chiang Mai University (Thailand) PhD: Assessing risks posed by both exotic *Tropilaelaps* mites and the viruses they may carry (Jubilee Fellowship)
- NBU Development of a method to screen hive debris for the presence of SHB using real-time PCR in conjunction with an automated DNA extraction protocol.
- NBU & South Africa Development of a monitoring system for the small hive beetle, Aethina tumida (Murray) (Defra funded project number PH0503) http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=14474&FromSearch=Y&Publisher=1&SearchText=bee%20health&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description
- Use of fusion proteins for control of the small hive beetle (component of PH0505)

Breeding and selection of bees for disease and parasite resistance or tolerance

Some species or genotypes of honeybee appear to have some innate physiological resistance, tolerance or immunity to certain of the diseases or parasites; whiles others exhibit behavioural traits, such as increased hygienic behaviour, which results in lower rates of establishment of parasites or diseases in the hive. The aim here is to identify genotypes with such useful traits and then combine these with genotypes with other commercially desirable agronomic traits to produce commercially useful bees with greater parasite or disease resistance or avoidance.

NBU & University of Sheffield - PhD: Investigating virus immune response in honey bees (NERC CASE studentship with additional funding from Defra)

Microsatellite markers and European foulbrood resistance (component of PH0505)

Current bee health surveillance and research activities in other countries

This section aims to review the current research being undertaken in a range of countries around the world, examine the structures, regulations and institutions in place to monitor and regulate the beekeeping industry.

Europe

A recent survey by the European Food Standards Agency on Bee mortality and Bee Surveillance in Europe shows the following results for colony mortality in the different member states submitting statistics Table 1. (EFSA, 2008). Countries were also asked to supply information on the general state of bee colonies in the country in terms of whether they were "weakening", close to "collapse" or suffering "mortality" defined as follows

weakening lack of strength (or vigour) of a beehive. It is linked to

a decrease in the hive population density over a period of time combined, mostly, with a decrease in the hive activity. Bee disorders can be observed, for example, growth or behaviour disorders. Weakening is combined

with a loss of honey production

collapse rapid loss of bees in the hive, leading to its destruction

mortality death of bee colonies

The results of the health of colony assessment are shown in table 2.

Table 1. Questionnaire data, honey production, beehives, beekeepers and mortality rate for the year 2006-2007 by country (EFSA, 2008).

		2006					2007					
Country	Source	Prod	Beehives	Yld	Bee- keepers	Mortality rate (%)	Prod	Beehives	Yld	Bee- keepers	Mortality rate (%)	
Austria	ÖIB - Österreichischer IMKERBUND	4.5-7										
Belgium	CARI	2.513	110000	23	8600							
Cyprus	Beekeeping_Data - Department of Agriculture, Ministry of Agriculture, Natural Resources and Environment	0.63 (0.389)	41478 (21633)	15	707 (120)		0.72 (0.383)	40533 (22500)	18	712 (129)		
Czech Republic	Statistic of Beehives and Beekeepers - Czech Beekeepers Union	9.081 (0.570)	525560 (19155)	17	46647 (83)	10	8.466 (0.6)	520084 (20521)	16	48919 (90)	20	
Denmark	Spørgeskemaundersøgelsen 2008/2007 - Danmarks Biavlerforening	2.5 (0.5)	80000 (15000)	31	4100 (150)	15	2 (0.5)	- (15000)		4100 (150)	7	
Estonia	The Estonian National Program of Development of Honey Production and Marketing - The Estonian Beekeepers Association	1.4 (0.4)	48000 (12000)	29	7000 (60)	8-10	1.1 (0.385)	48000 (12000)	22	7000 (60)	8-10	
Finland	membership survey - Finnish Beekeepers Associoation	2.3 (1.26)	53000 (28900)	43	3300 (77)	9.3	3.04 (1.78)	54000 (29306)	56	3200 (78)	10.2	
France	Ministère de l'agriculture et de la pêche	20	1324565	15	66924	808*	18	1243046	14	65050	142'	
Germany	Deutscher Imkerbund	25	700000	36	82000	13	20	710000	28	82000	9	
Greece	Min. Rural Dev. & Food, Dir Animal Production	14 (11.2)	1380000	10	23000 (5000)		14 (11.2)	1380000	10	23000 (5000)		
Hungary	Ministry of Agriculture and Rural Development	20-22	923103	24	15764		24-26	897670	29	15320		
ireland	Estimated figures – Department of Agriculture, Fisheries and Food and the Federation of Irish Beekeepers Associations	0.350 (0.125)	20000 (7000)	6	2200 (70)		0.140 (0.049)	20000 (7000)	2	2200 (70)		
Italy	MIPAAF - Ministero Politiche Agricole, Alimentari e Forestali	15	1083266 (350000)	14	75000 (1.5%)	30-40	12	1100000 (400000)	11	55000 (1.5%)	40-50	
Latvia	Latvian Beekeepers Association	2.232	62000	35	3300 (53)		2.1	70000	30	3400 (53)		

Table 1 (contd.). Questionnaire data, honey production, beehives, beekeepers and mortality rate for the year 2006-2007 by country (EFSA, 2008).

				2006			2007					
Country	Source	Prod	Beehives	Yld	Bee- keepers	Mortality rate (%)	Prod	Beehives	Yld	Bee- keepers	Mortality rate (%)	
		(0.420)	(11687)				(0.35)	(11700)				
Lithuania	Statistical data of several institutions - Department of Apiculture of the Lithuanian Institute of Agriculture	4	100000 – 120000	33- 40	11000 (20-40)		3	100000- 120000	25-30	11000 (20-40)		
Luxembourg	Administration des Services Veterinaires	0.092	5637	16	369 (3)	16	0.088	5300	17	358 (3)	20	
Netherlands	bijen@wur- Plant Research International, Wageningen University & Research		80000 (5000)		7500 (20)	26		80000 (5000)		7500 (20)	15	
Norway	Norges Birøkterlag	1.8	70000 (10000)	26	3500 (35)	10.6	2	70000 (10000)	29	3500 (35)		
Portugal	Direcção Geral de Veterinária							555049		15267 (4.1%)		
Romania	Program of strategic actions in sanitary veterinary field - National Sanitary Veterinary and Food Safety Authority	19	1100000 (165000)	17	3200 (480)	10	16.5	996000 (199200)	17	2942 (588)	>20	
Slovakia	Statistic data of the annual report of the Slovak beekeeping - Ministry of Agriculture of the Slovak Republic	3.862	217338 (7852)	18	12797 (49)		4.628	247678 (57)	19	14854 (57)		
Sweden	Swedish Beekeepers Association	4.100	105000	39	13000	18	3.6	110000	33	12000	12	
United Kingdom	ADAS Economic Evaluation (last survey 2001) – ADAS	6.5	274000 (40000)	24	43900 (300)	11.1	4.4	274000 (40000)	16	43900 (300)	11.7	

Production (Prod) x 1000 tonnes, Yield (Yld) Kg/beehive

Figures in brackets are for professional beekeepers

^{*} Mortality data expressed as number of statements

Table 2. Bee surveillance programmes relevant for assessment of colony weakening, colony collapse or mortality

Country	Name of Surveillance Programme	Responsible Institution	Weakening / Collapse / Mortality
Austria		Institut für Zoologie, Universität Graz	
		Beekeeping Association of the Austrian Federal Province of Upper Austria	
Czech Republic	Statistical data	Czech Beekeepers Union	Mortality
Denmark	Questionnaire to local associations	Danmarks Biavlerforening	Collapse & Mortality
Estonia	The Estonian National Program of Development of Honey Production and Marketing	The Estonian Beekeepers Association	Weakening & Collapse & Mortality
Finland	member questionnaire, 2008 started bee yard monitoring	Finnish Beekeepers Association	Weakening & Collapse & Mortality
France	"Réseau de surveillance des troubles des abeilles " (NS 2002-8110 du 2/08/02 modifiée) (Network for surveillance of bee disorders)	Ministère de l'agriculture et de la péche - AFSSA Sophia-Antipolis (National reference laboratory for pour bee diseases) - GIRPA (Reference laboratory for plant health)	Weakening & Collapse & Mortality
Germany	Project *Deutsches Bienenmonitoring*	Beekeeping Organizations, Farmer Organization, Agricultural Institutes, Ministry of Agriculture, Chemical Companies	Weakening & Collapse & Mortality
Country	Name of Surveillance Programme	Responsible Institution	Weakening / Collapse / Mortality
Italy	Ligustica.IT & COLOSS project Liebefled Institute	FAI–Federazione Apicoltori Italiani and other associations	Weakening & Collapse & Mortality
Luxembourg	Surveillance sanitaire des abeilles	FUAL (Federation des Unions d'Apiculteurs du GD. de Luxembourg)	Weakening & Collapse & Mortality
Netherlands	Monitor Bijensterfte	ICR Beemonitoring	Mortality
Norway		Norges Birøkterlag	Weakening & Collapse
Portugal	Programa Apícola Nacional	Faculdade de Medicina Veterinária & Federação Nacional dos Apicultores de Portugal	Mortality

Romania		Institute for Beekeeping Research & Development	Weakening & Collapse & Mortality
Sweden		Swedish Beekeepers Association (SBR)	Mortality
Switzerland	COLOSS Network for Prevention of honeybee colony losses	Swiss Bee Research Centre	
United Kingdom	Bee health programme	Central Science Laboratory National Bee Unit	Weakening & Collapse & Mortality

Information on the activities of a number of individual countries is given in more detail by way of example and contrast to the situation in England and Wales.

Belgium

(Information obtained from http://www.afsca.be/sp/pa/prod-api-2 fr.asp#21 16/09/08)

There are currently about 7,850 beekeepers in Belgium managing about 110,000 hives and of these there are only 4 professional beekeepers responsible for 1000 hives. All beekeepers are required to register with the authorities. The following pests and diseases are all notifiable; *Acarapis woodi*, small hive beetle, European foul brood, American foul brood, Varroa and Tropilaelaps. If colonies are ordered to be destroyed there is compensation of €125 for each hive

Results of testing for American foul brood are shown in Table 3

Table 3. Cases of American foulbrood detected in hives teste in Belgium during 2006/7

Date	Hives tested	Cases detected	Location
20/09/2006	3510	1	Kermt
04/10/2006	9990	1	Maldegem
25/10/2006	8340	1	Sijsele
08/03/2007	3650	1	Dilsen-Stokkem
15/05/2007	2340	1	Beerse
18/06/2007	2340	2	Beerse
19/10/2007	9800	1	Deinze

Beekeepers currently pay a "tax" related to the controls imposed by l'Agence Fédérale pour la Sécurité de la Chaîne Alimentaire (l'AFSCA). In

2008, it was €193.5 for bee-keepers who have more than 24 hives. In 2009 the tax will be reduced to €108. Beekeepers with fewer than 24 hives do not have to pay a contribution but still have to register. There is no indication whether this money is used to fund research and development, the bee health programme or funds the compensation scheme.

France

(Information obtained from presentation by Dr Françoise Liébert, Directrice DDSV du Nord, Académie Vétérinaire de France 20th March 2008).

In 2006 there were 66,924 beekeepers in France with 101,947 apiaries containing 1,368,809 hives. All beekeepers have to register and obtain a "licence" to keep bees. Nosema and American foul brood are recognised as endemic but contagious and along with small hive beetle (not found in France) are notifiable and action will be taken to control these should they be found. Varroa is also a notifiable disease but no action will be taken by the state. *Acarapis woodi* and European foul brood are not notifiable.

Table 4. Details of inspections done at sites suspected of having bee health problems for 2005 and 2006 in France.

	2005	2006	Num	Number of sites with bee health problem						
Reason for visit	Numb visits	er of		rican rood	Nos	ema	Acarapis	Varrroa	European foulbrood	Other illnesses
Suspicion of problem	618	434	153	107	18	36	2	4	15	53
Health certificate for movement	714	677	8	0	0	0	0	0	0	4
Structured visits	602	452	125	69	72	26	7	5	43	2
Random visits	2108	2044	103	51	7	5	3	65	37	24
Swarm collection	119	116								
Other	658	645	49	6	1	2	0	9	6	10

reasons										
Total	4910	4368	438	233	98	69	12	88	101	93
visits										

The above table shows the level of monitoring done by the French and the number of cases of each disease detected in 2005 and 2006. There is currently a programme in France to improve the bee industry with money being provided by the government using 50% EU funds. This aims to spend epsilon1,000,000 a year for 2008-2010 on combating the effects of varroa in the industry and a further epsilon1,000,000 a year over the same period on bee research and the application thereof. Additional funds are also being made available for investment in the industry (MAP, 2007).

Inspection effort in France appears to be very high with 1717 apicultural specialists registered in 2006 with a total of 3353 visits being conducted by 755 inspectors. These visits were associated with suspected incidents requiring control measures, 385 of which resulted in some action being taken.

Switzerland

The exact number of hives and beekeepers is not known for sure in Switzerland as there is no requirement to register hives but it is estimated to be 19,500 beekeepers with 200,000 hives. The average annual production of honey is 337 tonnes (1997-2006). The pollination value of the bees is estimated at 256 million Swiss Francs (£128 million). There is Federal support of The Swiss Bee Research Centre of Agroscope Liebefeld-Posieux Research Station ALP Liebefeld-Bern amounting to 920,000 Swiss Francs per annum (£460,000) with an additional 150,000 Swiss Francs (£75,000) for the promotion of apiculture and another 40-50,000 Swiss Francs for research into potential epizootic diseases. Canton veterinary officers are also involved in the control and monitoring of American and European foulbrood. Currently there is no centralized registration but a recent report (OFAG, 2008) recommends the establishment of a central register.

There are a number of research projects currently underway in Switzerland.

• Development of a new PCR diagnostic method for early detection of Paenibacillus larvae larvae (American Foul Brood) and characterisation of the strains found in Switzerland (PhD thesis M. Gillard at the University of Lausanne with Prof. Ph. Heeb). Up to date epidolmological studies connected with prevention and control of the pest.

- Development of a new PCR diagnostic method for early detection of Melissococcus pluton (European Foul Brood) (collaboration with Prof. I. Fries, University of Uppsala, Sweden). The method will be then applied for epidemiological studies and for prevention and control of the pest.
- Development of new PCR diagnostic methods for detection of dangerous bee viruses (H. Berthoud). The methods will be then used in epidemiological studies.
- Laboratory testing of new essential oils for possible application against Varroa. Simple methods for application in practical beekeeping will be then developed.
- As a preparation for the feared emergence of Small hive beetle in Switzerland, the necessary know-how concerning the simple integrated control of the small hive beetle will be acquired, in collaboration with scientific teams outside Europe (P. Neumann and others).
- Effects of special honey plants on the health of bee colonies Specific plant species (sunflower, rape, maize), suspected to harm bees, will be examined in order to elucidate the possible toxic agents.
- The honey bee populations in Switzerland will be characterised by molecular genetic methods in order to elucidate their genetic diversity (PhD of D. Reckeweg at the university of Berne with Prof. L. Excoffier).
- The mineral balance of bee colonies will be determined, based on data on pollen supply, mineral content and colony development, in order to provide answers to current questions regarding bee nutrition. http://www.alp.admin.ch/themen/00502/index.html?lang=en

Australia

Because Australia is so large and covers such a range of ecologies the problems of beekeeping are different in different areas. Thus, each of the states of Australia has its own apiaries act legislating beekeeper registration, hive inspection and disease control, and the import of bees, beehives and hive products.

Under the Queensland *Apiaries Act 1982*, it is a requirement to become a registered beekeeper in Queensland if owning one or more beehives. Registration with the Department of Primary Industries and Fisheries is required for the orderly conduct of the industry, and to enable control of important honeybee diseases. A fee of \$11.70 is charged annually. Under the Act it is a requirement to notify DPI&F within 48 hours when a

beekeeper is aware of, or suspects, the existence of certain diseases in beehives or bee products. These diseases include AFB, parasitic mites, Small Hive Beetle and the bee louse.

Since Western Australia is free of European foulbrood, mites and SHB, the legislation under the Western Australian *Beekeepers Act (1963)* and the quarantine measures in place prevent the import of hive products, including foods containing them, into the State. Products that can not be imported into Western Australia include honey, honeycomb, unprocessed beeswax, pollen, bees, used hive equipment, used beekeeping appliances, queen bees, queen cells, packages (live bees in a wooden box), or any other hive product not mentioned above. Some low-risk products are permitted entry into Western Australia under certain conditions or having undergone a risk assessment. The Beekeepers Act (1963) requires the reporting of any honeybee pest, notifiable disease or exotic honeybees to the Department of Agriculture and Food. Beekeepers are also required to register in Western Australia, New South Wales, Tasmania, Northern territories and South Australia.

The Rural Industries Research and Development Corporation (RIRDC) have recently undertaken a review of Honeybee research and Development in Australia (RIRDC, 2007). This document provides a framework for the development and protection of the industry over the 5 year period 2007-2012. The planned spending on research and development over that time is A\$ 247,500 (£107,000) for the first 2 years rising to A\$270,000 (£117,000) in years 3-5. This expenditure is purely on R&D and does not include the activities of federal or state bodies relating to quarantine and inspections.

Some relevant research currently being undertaken and funded by RIRDC is given below.

- Development of Two Markers for Hygienic Behaviour of Honeybees;
 Project No US-123A
- Sustainable Control of Small Hive Beetle Through Targeting In-ground Stages: Project No. UWS-22A

Canada

Each of the Canadian states has its own Apiary Act. For example, the Alberta Bee Act regulates beekeeping in Alberta. Anyone offering used beekeeping equipment for sale (especially supers and frames) must have a permit to do so from Alberta Agriculture and Food. Anyone purchasing

such equipment must inform the Provincial Apiculturist of this purchase within 15 days. Anyone owning bees or used beekeeping equipment in Alberta, or operating bees in Alberta is required to register annually with the Provincial Apiculturist. State apiculture inspectors are employed to inspect apiaries and enforce the regulations. Indicative figures from Ontario show that in 2006 there were 2400 beekeepers with 76,700 colonies. Inspections revealed the following levels of disease (Table 5) (OMAFRA, 2006).

Disease/Pest	Number of Colonies Inspected	Disease Colony Incidence (%)	
AFB*	8,694	3.25%	
EFB	8,694	0.07%	
Chalkbrood	8,694	2.38%	
Sacbrood	8,694	0.15%	

Table 5. Levels of disease detected in hive inspections in Ontario in 2006

Increased levels of colony losses over the last two winters in Edmonton province (up from 15-20% to about 30% each year, with a further 14 per cent were severely weakened) have been attributed to the development of resistance to the approved acaricides in varroa and not as some had previously suggested to CCD. Incidence of Nosema in Alberta is also on the rise (Finlayson, 2008).

Two outbreaks of SHB (*Aethina tumida*) were reported in Alberta and Manitoba provinces in June 2006. In these outbreaks, only adult specimens of small hive beetle were found in a total of three colonies out of a total of approximately 1,700. Unlike the 2002 outbreak, which was contained and eradicated, the 2006 outbreak appears not to be the result of beetles from Texas, and more likely to have got in with queens imported from elsewhere – probably Australia.

Substantial honey bee losses have occurred in Canada in the past winter reflecting the situation in the USA although some regions, notably New Brunswick, having better than average figures although the majority of provinces showed substantially worse overwintering and spring survival (Table 6).

Table 6. Table showing the overwintering and early spring mortality of colonies in Canadian provinces.

Province	Number of colonies wintered	Number of colonies dead	Wintering losses (%)
British Columbia	45,648	17,346	38
Alberta	250,000	110,000	44
Sakatchewan	95,000	25,080	26
Manitoba	81,000	22,860	28
Ontario	75,000	24,563	33
Quebec	30,000	5,676	19
Nova Scotia	18,600	3,422	18
New Brunswick	9,434	2,765	29
Prince Edward Island	3,641	1,328	36
Canada	608,323	213,040	35

New Zealand

Varroa was first discovered in New Zealand in 2000 in South Auckland. By the time the mites were detected, they had spread too far to be eliminated. Instead, the government put in place a programme to slow their spread in the North Island and to try to keep the South Island free of the pest. This failed and in 2006 varroa was detected in the north of South Island; it was detected in the vicinity of Christchurch on 10th September 2008.

Small Hive Beetle, *Aethina tumida*, is not present in NZ and is a notifiable pest. There are strict restrictions on the import of queen bees to avoid the introduction of SHB and surveillance so that if SHB is detected measures for containment and eradication can quickly be set in motion.

European foulbrood (*Melissococcus pluton*) is notifiable under the New Zealand Biosecurity Act 1993 and the honey bee population is free from the disease.

American foulbrood disease management in New Zealand is controlled by law - the Biosecurity (National Foulbrood Pest Management Strategy) Order 1998. The aim of the strategy is to (eliminate) reduce the incidence of AFB in New Zealand to 0.1% calculated on the number of colonies identified each year with AFB divided by the total number of hives registered). This is to be done by a concerted campaign of surveillance and destruction of infected colonies. All beekeepers are required to register with the National Beekeepers' Association (as the Management Agency responsible for implementing the strategy), to pay a levy on each hive, to report and destroy any colonies confirmed to have AFB and to submit an annual disease report (ADR). In April 2008 the Management Agency sent to all registered beekeepers (2626 in total) an ADR for completion and return by 1 June 2008. Although only about 70% of ADRs had been returned by mid June, the strategy appears to be being successful since the incidence of infection of colonies is going down as is the proportion of honey samples found to contain AFB spores. The Management Agency for the American Foulbrood Pest Management Strategy has recently launched its own web site - www.afb.org.nz - to better publicize its aims and improve communication with beekeepers.

(The following information was obtained from M. Goodwin, (pers comm.) one of the two bee scientists in New Zealand).

All beekeepers have to register and an apiary levy is charged. There are no inspectors paid for by the government but a number of inspection programmes are conducted.

- 1) A surveillance programme for exotic bee disease and pests. This is paid for by the government and the cost thought to be something less than NZ\$200,000 (£74,400)
- 2)
 American Foulbrood eradication programme paid for by the National Beekeepers association. The money comes from a levy on all beekeepers Current cost something less than NZ\$180,000 (£66,900) per annum
- 3) Up to a year ago a surveillance programme in the south island of New Zealand designed for early detection of varroa which was stopped last year because varroa got there. Cost about NZ\$800,000 (£297,000) and was paid for by a levy on beekeepers and some producer organisations and some local councils.

Research is undertaken at The Horticulture and Food Research Institute of New Zealand and has a team of two scientists and 3 research associates and is worth about NZ\$1 million (£372,000) per year with no direct government funding. Typically funding for research consists of 25% from beekeepers with matching funding from government (25%) and 50% from a range of industries. Not all this research is targeted at New Zealand bee keeping but a proportion is consultancy for other countries. Currently half of the research is focused on bees and 50% on pollination.

Key threats to bee industry were identified as being Endemic pests - Varroa is most important then American Foul brood Exotic pests - Small hive beetle and then Tropolaelaps and tracheal mites

United States of America

Beekeeper registration and apiary inspections are generally legislated and administered at the state level in USA. For example, all beekeepers in Pennsylvania (PA) are required to register with the PA Dept. of Agriculture at a fee of \$10 per beekeeper regardless of how many apiaries are owned. Bees must be kept in modern type hives with removable frames so combs may be inspected for disease. Bee inspectors are employed throughout PA from May to September to inspect all

apiaries for brood diseases and parasitic mites. When a diseased colony is found, the bee inspector will recommend a treatment procedure. If the disease is too advanced to be treated, the inspector will require that the colony be destroyed. It is a violation of the bee law for any beekeeper to knowingly keep, without proper treatment, any colony of diseased bees or to expose any diseased equipment to flying bees. It is also a violation of the law to sell, receive, or transport any diseased bees. Also, bees transported into Pennsylvania must be accompanied by a certificate of inspection from the state of origin stating that the bees were inspected within 30 days of shipment date and that the bees are disease free. However, in some states the apiary legislations are less stringent than in PA, while many States do not have an apiary program at all.

American foulbrood, European foulbrood, chalk brood, Nosema, varroa, small hive beetle and Africanized honey bees are all present in USA. Each requires control measures to a greater or lesser extent depending on region.

Importation to USA of honey bee queens and packages (no brood) is permitted from Australia, Canada, and New Zealand. Bees are visually inspected at the country of origin 10 days or less prior to export and any diseases and pathogens are recorded on the export document sheet. Honey bees need to be certified as being from the country of origin and free of Thai sacbrood virus, *Tropilaelaps*, *Euvarroa sinhai*, Cape honey bee, *Apis cerana*, and Africanized bees. Once the bees enter the United States mainland they are free to move without restriction. A quarantine was tried on the United States mainland in the 1980s when Varroa was detected but was quickly abandoned as unworkable.

Import of honey bee germplasm from Australia, Bermuda, Canada, France, Great Britain, New Zealand, and Sweden is allowed without permit. This list was developed in the 1980s and carried through when the regulation was revised in November 2004. Based on concerns about the vertical transmission of bee viruses (Chen *et al.*, 2006; Yue *et al.*, 2007, this may be reconsidered. Any germplasm imported from non-approved regions has to enter under permit into a containment apiary on either an island or an isolated inland location. The inseminated queens or their progeny cannot leave without APHIS approval.

Dead bees as preserved specimens can be carried in without permit but there are notification requirements. The importation of used honey bee beekeeping equipment is not allowed. The importation of all pollen and royal jelly for bee feed is prohibited due to disease concerns. (At issue though is that large quantities of both are entering for human consumption and the cosmetic industry and USDA only have the authority to regulate what is being used for the bee trade. The USDA are looking at the use of radiation as a possible remediation measure but the issue remains problematic.)

The importation of 2 species of bumble bees (*Bombus impatiens, B. occidentalis*), alfalfa leafcutter bees (*Megachile rotundata*), blue orchard bees (*Osmia lignaria*), horn-faced bees (*Osmia cornifrons*) is permitted from Canada only.

Colony Collapse Disorder, also known as Fall-Dwindle Disease, is of increasing concern to beekeepers across the USA (and worldwide). Beekeepers are reporting the sudden disappearance of adult bees from their colonies – few, if any, adult bees are found in or near the dead colonies. Queen and baby (brood) bees remain in the colonies, but the adults are not returning to provide food, so the colonies collapse or die. CCD was first reported in 2006 by a Pennsylvania beekeeper overwintering his hives in Florida. Subsequently, over 22 US states reported significant colony losses in the autumn of 2006. Now some commercial migratory and nonmigratory beekeepers are reporting losses of 50% to 90% of their colonies. There is extensive effort across the USA, both at state and federal level to identify the cause(s) of and how to control CCD. The USDA published a Colony Collapse Disorder Action Plan (CCD-Steering-Committee, 2007) in June 2007.

One of the higher profile bee health coalitions is the Mid-Atlantic Apiculture Research and Extension Consortium (MAAREC; http://maarec.cas.psu.edu/). Established in 1997, this is a regional group focused on addressing the pest management crisis facing the beekeeping industry in the Mid-Atlantic Region. A task force has been established with representation from the departments of agriculture, state beekeeping organizations, and land-grant universities from each of the following states: New Jersey, Maryland, Delaware, Pennsylvania West Virginia, and Virginia. Also participating in the task force is a representative of the USDA/ARS (Beltsville Bee Lab, MD). MAAREC has recently published "Pest Management Strategic Plan For Honey Bees In The Mid-Atlantic States (DE, MD, NC, NJ, PA, SC, VA, WV)" (7/8/2008) which provides a comprehensive account of the bee health problems and the identified priority activities to understand and manage the problems in the region.

Israeli acute paralysis virus has been associated with Colony Collapse Disorder (CCD) in USA, but was not present in any of the dead or failing colonies tested in England in 2007.

Varroa mites were first detected on Honolulu Hawaii in April 2008 and more were detected on 29th August 2008. This is a great concern to the beekeepers of Hawaii since if the outbreak cannot be contained and eradicated it could potentially destroy the states \$4M/year queen bee export industry (Hao, 2008)

Florida

The Apiary inspection programme in Florida can be used to illustrate the effort devoted to this activity in one state. However the effort varies from state to state. During 2007-2008, of the 186,345 honey bee colonies maintained by registered Florida Beekeepers, there were 49,757 colonies inspected from 2,414 apiaries. Compensation in the amount of \$3,480.00 was paid to beekeepers for 196 honey bee colonies destroyed because of infestation of American Foulbrood Disease

Research Activities in Florida

- Determining Optimum Varroa Mite (*Varroa destructor*) Economic Treatment Thresholds and Powdered Sugar Efficacy Trials.
- Investigate the Use of small cell Foundation (5.1 mm to 4.9 mm) as a tool for Varroa Mite (*Varroa destructor*) Control.
- Use of Certin, B401, *Bacillus thurigrensis* (Bt) for Control of Wax Moth (*Galleria mellonela*) in Florida's Apiary Industry.
- Identification of a Diet Supplement to Improve Honey bee Health.

Funding was also supplied by the state to the following projects

- Entombed Pollen: A New Phenomenon in Bee Hives. Dennis vanEngelsdorp, Pennsylvania State University
- African Honey Extension Education William H. Kern, Jr., University of Florida/Institute of Food and Agricultural Sciences
- Hygienic Removal by Honey Bee of Parasitic Varroa Mites: Identifying Genetic Loci Responsible for the Trait Dr. H. Glenn Hall, University of Florida
- Effect of Honey bee Queen Insemination Quantity on Supercedure Rates in Florida, Christina Grozinger, North Carolina State University

- Increasing African Honey Bee Awareness via the **A**frican Honey **B**ee **E**xtension and **E**ducation Program (AFBEE) Dr. Jamie Ellis, University of Florida/Institute of Food and Agricultural Sciences
- The Sub-lethal Effects of Imidaclopid and Amitraz on Honey Bee Susceptibly to Varroa Mite Mites Dr. Jamie Ellis, University of Florida/Institute of Food and Agricultural Sciences

Varroa Mites, continues to be the most significant honey bee health concern. Controlling the mite without damaging the honey bees or colony is still a challenge

In Florida colony and Apiary inspection was conducted with a staff of 12 inspectors (includes section Chief) which indicates each inspector examined on average about 200 apiaries each (Table 7).

Table 7. Inspection details and detection of American foulbrood in hives in Florida during 2008

•							
(Figures from FDAC 2008)							
Apiary Inspection Summary							
	Colonies		Apiaries				
	Inspected		Inspected				
	49,757		2,414				
American Foulbrood Disease Report							
Hives Destroyed		Amount Compensate					
196 \$3,480			0				
	se Report Hives De	Colonies Inspected 49,757 se Report Hives Destroyed	Colonies Inspected 49,757 se Report Hives Destroyed Amou				

Inspection in Maine

In 2006, 479 Maine beekeepers registered 7,476 hives. Four thousand, eight hundred and ninety eight colonies were surveyed at random with 2,361 opened and inspected for disease and parasites. American foulbrood (AFB) was found in 61 (2.6%) of inspected hives. The incidence of AFB was 0.75% higher in 2006 than 2005. European foulbrood (EFB) was found in 106 hives (4.5%). The incidence of EFB in 2006 was the second highest level in the last 23 years of apiary inspection (8% in 1987). Sacbrood virus was detected in 31 colonies (1.32%). Colony inspections identified additional commercial beekeeping operations and several apiaries managed by hobby beekeepers with American foulbrood (*Paenibacillus larvae*) strains resistant to Terramycin (oxytetracycline hydrochloride) (Maine, 2006).

Additional research is being conducted at a number of sites in the USA but the box below shows a number of current projects and level of funding

USA National Honey Board (NHB)-Funded Bee Health and CCD Research Projects

Cyclodextrins as Carriers of Essential Oils for Varroa Control in Honeybees. Blaise LeBlanc, Carl Hayden Bee Research Center NHB Funding: \$17,350

Contaminants in High Fructose Corn Syrup and Their Possible Effects on Bees. Blaise LeBlanc, Carl Hayden Bee Research Center NHB Funding: \$24,850

Identification of the Chemical(s) Associated with the CCD and the Observed Loss of Bee, Lack of Robbing, and Exclusion of Hive Beetle and Wax Moths. Jerry Bromenshenk, Bee Alert NHB Funding: \$24,000

Emergency Funding for Colony Collapse Disorder: PCR Quantification of Pathogens in Target CCD Colonies. Diana Cox-Foster, Penn State University NHB Funding: \$36,000

Nutritional Changes caused by pollen exposure to Miticides and colony health implications. Dennis vanEngelsdorp and Maryann Frazier, Penn State University NHB Funding: \$25,500

Quantifying Pesticides in Bees from Declining Colonies and Assessing Gamma irradiation as a Tool for Pesticide Reduction.

Jim Frazier, Maryann Frazier and Chris Mullin, Penn State University NHB Funding: \$11,875

Physiological changes in migratory and non-migratory honey bees: a comparative study. Zachary Huang, Michigan State University NHB Funding: \$12,000

Development of Reproductive Technologies to Facilitate the Safe International Exchange of Genetics in the Honey Bee. Dr. John Pollard, Dr. Claire Plante and Susan Cobey, UC Davis NHB Funding: \$21,700

Improving Honey Bee Health and Reducing Pesticide Use with Mite-Resistant Bees. Greg Hunt, Purdue University NHB Funding: \$24,715

Haagen-Dazs (owned by Vevey, Switzerland-based Nestle) has a campaign to raise \$250,000 to donate to researchers at Pennsylvania State University and the University of California at Davis for research into

what's ailing the honey bees (Colony Collapse Disorder).

Burt's Bees, which makes natural personal care products, unveiled produced a public service announcement in November on Colony Collapse Disorder and also donated money to researchers at The Honeybee Health Improvement Project.

The Almond Board of California has invested about \$200,000 a year, for a total of about \$1.4 million in bee research.

Analysis

The issue of bee health is currently high on the agenda of many countries due to an increase in the level of colony mortality.

Evidence for increased mortality

Figures reported from a number of countries show unusually high numbers of bee colonies dying, this is true of North America and for many European countries. The cause of the "colony collapse" does not appear to be a single pest or disease but causes appear to vary dependent on the geographical location. In North America a number of pressures appear to have combined to produce highly unusual levels of hive mortality and these include the overwintering conditions in Canada, the continued development of resistance to controls in the varroa mite, the increase in incidence of a number of viruses including Israeli acute paralysis virus which has shown a high degree of association with large scale colony mortality in the USA but not always in other countries. In England and Wales it appears that weather conditions, a high level of varroa mites and associated viruses have been the cause of the decline in bee numbers. It is worth noting that significant declines in honey bees have been recorded before with examples in the USA in the 1880s, the 1920s and the 1960s. In England there was a large decline in bee numbers on the Isle of Wight in 1906 (Silver, 1907). Descriptions of the symptoms are similar but there is no way of knowing if the causes are the same. One of the biggest problems in analysing the changes that have occurred is the lack of any long term data on hive mortality with the NBU only providing data on dead hives found on inspection in the last three years. This makes it very difficult to assess if levels are changing although this would be possible by collating all the information from a survey of beekeepers who will generally have records of hives dying out. This data may now be available from the survey of UK beekeepers done by the NAO.

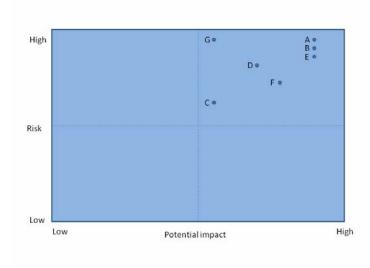
Proposed causes of mortality

There have been a number of proposed causes for the increased levels of mortality ranging from microwaves to genetically modified crops. The research reported above appears to indicate that it is a number of different factors acting upon the bees which varies from location to location. Certainly adverse weather conditions are known to increase mortality, whether this is during the winter, spring or summer. The reduction in numbers in Canada appear to be attributable to differing weather with New Brunswick bees doing rather better because of more clement weather than bees in other provinces. In England and Wales the combination of poor summer conditions with reduced foraging opportunities, the presence of varroa and associated viruses would increase the stress on bees unable to feed properly and suffering greater exposure to the mites and the viruses associated with it. When found together Deformed wing virus (DWV) and Chronic bee paralysis virus (CBPV) appeared to double the risk of bee death in a survey of 700 dead or failing colonies in the England and Wales in 2007 and DWV presence was consistently the best risk indicator of a weak/lost colony (Budge et al, 2007a). In the USA the presence of IAPV has been associated with colony collapse (Cox-Foster et al, 2007) although no such association has been found in the UK (Budge et al, 2007a). Most reports appear to agree that it is the accumulation of a number of factors that leads to the death of the colony i.e. an accumulation of stress and would appear to indicate that reducing stress as far as possible with good husbandry should reduce the likelihood of the colony succumbing to the stresses.

Current research on bees and bee health

It is clear from the amount of literature and the information reported here that there is a large amount of activity relating to research on the potential causes of the bee mortality being reported. The bee research community is relatively small and it appears that communication and collaboration is already happening and there appears to be little duplicated research. There is much research that has been done and is being done that will be of value to the NBU in developing a good strategy and programme for the continued protection of bees in England and Wales and there is no reason to suppose they are not already making good use of this. There will inevitably be research that is peculiar to the UK and the NBU should focus its attention on this work and contribute to other research programmes where appropriate and where feasible. Most of the work being done on the pests and diseases facing bees will be universally applicable and the development of integrated control methods

for varroa and the detection and control of small hive beetle should be especially useful. Most countries identify the same pests and diseases of being high importance with American and European foulbrood, *Varroa destructor*, small hive beetle and Tropalaelaps mite being top of the lists with the degree of importance shifting depending on whether the disease is endemic or not. Figure 1 shows the relative risks of different scenarios as identified for the Australian beekeeping industry but which is probably equally applicable to other bee industries.



- A Introduction of varroa mite (Australia)
- B Introduction of exotic pest/disease
- C Increase in domestic disease
- D Introduction of foreign bee species
- E Further decrease in access to resources
- F Contamination of honey
- G Contamination of other bee products

Figure 1. Risk assessment of pest and diseases threats facing bee industry (adapted from CIE, 2005).

Risk to human health of bee disease

There are reports of chemical residues being found in honey but they are generally extremely rare and at low levels. Within the EU, Directive 96/23/EC requires member states to monitor for certain substances in animal products to ensure compliance with MRLs for veterinary medicinal products and pesticide residues. No MRLs for pesticide residues are set in Directive 86/363/EEC for honey and there are no specific requirements concerning the sampling of active substances covered by this Directive. Therefore national residue monitoring plans for honey require monitoring of veterinary medicinal products and environmental contaminants, for the substances B1 (antibacterial substances, including sulphonomides, quinolones), B2(c) (carbamates and pyrethorides), 3a, 3b and 3c (other

substances and environmental contaminantsorganochlorine compounds including PcBs, organophosporous compounds and chemical elements). The results of the national residue monitoring plans for the years 2003-2005 are summarised in Table 1. Certain active substances such as certain antibacterials, carbamates, pyrethroids, organophosphorus and organochlorine compounds are used in plant protection products and therefore might cause residues of products to be detected in honey. The following substances streptomycin, fluvalinate, B-HCH, DDT, organophosphates and chlorfenvinphos have been detected at noncompliant levels in honey and are known to be substances used in plant protection (EFSA, 2008). Canadian testing of domestic and imported honey has revealed levels of substances such as Tetracycline, oxytetratcycline, Chloramphenicol, Sulfamethoxazole and Sufathiazole that exceed limits (CFIA, 2008). It would therefore appear reasonable to assume that an increase in the frequency and/or quantity of chemicals within the hive could result in increased health risks to consumers if quidelines were not developed and implemented carefully. A more extreme case of increased risk to human health in England and Wales would be through the introduction of the Africanized bee which has caused the death of individuals in countries where it is present.

Expenditure on bee health

The National Bee Unit (Part of the Central Science Laboratory and funded by DEFRA) implement beekeeping regulations and undertake risk-based apiary inspections and enforcement to control notifiable pests and diseases. They also undertake research and development, and contribute to policy development and contingency planning for emerging threats. Through training and education programmes the NBU also supports good bee husbandry. Current expenditure is approximately £1.3million per year which covers all the above activities. Funding models in other countries vary; some have relatively generous budgets funded by the federal or state governments whilst others have to raise their own funds from a variety of sources. Comparing the resources available to the NBU it appears that they are largely comparable to operations in New Zealand and Australia in terms of the amount of money that is being spent on R&D. The picture with inspection is rather more difficult to fathom out as data is rather more sporadic but it does appear that the level of surveillance that the NBU undertakes is also comparable to other countries as a percentage of the hives checked each year at around 10% e.g. Belgium, Alberta etc. France appears to have many more inspectors but only seems to inspect about 0.3% of the hives every year.

Conclusions

Through this examination of the available literature, it would appear that the NBU is well aware of the health threats to bees in the UK. The foulbrood diseases (AFB, EFB) are being kept reasonably under control, but to further decrease incidence there will need to be an increase in the proportion of apiaries inspected each year and beekeepers need to be further educated and encouraged to report suspected diseased colonies as soon as possible. Ideally, NBU should have the resources to provide a service to all the beekeepers in England and Wales who should, in turn, be registered on the NBU database – Beebase. A practical and sustainable means of persuading all beekeepers to register on Beebase should be sought and the current situation of only knowing the location of a proportion, albeit a significant one, of the hives in England and Wales is clearly an issue. The prohibition of prophylactic treatment of colonies with antibiotics to prevent EFB is justified since this can mask AFB infections and also increases the likelihood of antibiotic resistance developing in both AFB and EFB. NBU appear to have led the way in developing the shook swarm approach to dealing with EFB and are also involved in researching other cultural/biological methods, including the development of phage and other bacterial species as biological control agents for EFB. The research on the shook swarm approach is being trialled in other countries clearly indicating the value of the NBU in developing practical solutions.

Varroa is once again of increasing concern to beekeepers. This is primarily because of the development of resistance in varroa to the acaricides that were originally used to control the mites and the increased costs in terms of time and materials required to control the resistant types. NBU have been very active in monitoring and researching the resistance and in researching for different procedures and treatments for managing the mites. NBU should continue to be an active member of the EU working group "Co-ordination in Europe of integrated control of varroa mites in honey bee colonies". Varroa is also of concern because of the large number of viruses infecting bees it can vector. NBU have active projects investigating the identity, diversity and epidemiology of viruses infecting bees and aim to look at the interaction between the viruses and the varroa vector on symptom development in the bees. NBU should continue to liaise with and monitor the results of similar studies in USA, France and other countries.

Whereas in USA the increased frequency of colony losses over the last three seasons is being attributed to colony collapse disorder (for which the primary cause has not been identified) in UK the losses are being attributed to adverse weather conditions for bees for the last couple of seasons. Prolonged wet and cold inhibits the bees from leaving the hive to forage and this in turn promotes disease build up in the colony and colony weakening or collapse. Although the current losses in the UK are no greater than have occurred occasionally in other years, NBU are investigating whether the current losses are associated with increased incidence of any particular pest or disease or combination of these (e.g. Nosema and *Distorted wing virus*), potentially in relation to other stress factors such as apicultural or agricultural pesticides. Since this is also the approach being taken by researchers of CCD in USA and elsewhere, contact should be maintained between NBU and these other groups so that results can be shared; a standardized research protocol would aid in comparing results from different groups.

SHB and *Tropilaelaps* mites appear to be the priority exotic pest threats to UK apiculture at present. Either has the potential to cause major losses should it become established in the UK. NBU operate surveillance for both these pests at high-risk target apiaries (near ports, airports and horticultural distribution depots) and in queen boxes and on the accompanying workers of imported queens. NBU are conducting research in collaboration with USA and South Africa on lures for trapping and monitoring SHB and on the use of fusion proteins for the control of the beetle. Improved methods for the detection of both pests have also been developed and a pest risk analysis is in progress for *Tropilaelaps*.

NBU appear not to have much activitity concerned with bee breeding for increased pest/disease resistance. This is probably justified since it is a long-term and expensive pursuit, and there is some evidence that part of the cause of the increasingly poor health of honeybees in many areas is the rather narrow genetic diversity of commercial honeybees. The limited diversity of the large commercial stocks will in time have the effect of reducing the diversity within the less commercial (amateur) and feral populations. Little genetic diversity will mean greater selection pressure on the pathogens and parasites of the bees and when a pathogen or pest better adapts to the host bees, it is likely to be better adapted to the commercial bee genotypes as a whole. A better approach is to try to increase the genetic diversity of honeybees within England and Wales. This could be by importing A.m. mellifera genotypes and other *Apis* species from across Europe (centre of diversity of the western honeybee). Proper precautions would have to be taken to avoid the concomitant introduction of new races/pathotypes, or exotic type of pests or diseases of bees, or of the introduction of unsuitable bee genotypes such as the highly defensive Africanized bees (A, m, scutellata).

In developing a strategy for the continued protection and improvement of bee health in England and Wales the current consultation document is a step in the right direction. The key points should be the strengthening of the relationships between the NBU, the beekeepers and other stakeholders and a central part of this is to establish exactly what the key benefits arising from beekeeping are. Clearly honey production, whilst important, is a relatively minor activity in the overall scheme of agricultural and horticultural production. However, the estimates of the value of the pollination services indicate that there is significant benefit from the industry that is currently going unrewarded from the perspective of the bee keeper but is providing a free service for those producers reliant on pollinators for production of their crop. Since it would be impractical to collect money from a large number of people where the individual benefit was hard to calculate it seems sensible that the cost of bee health related activities should continue to fall to the Government. The NBU appears to fulfil its remit and provides the services that are required of it and to the extent that its budget permits, its activities are comparable to those of similar units in other countries. The current Defra consultation exercise and future strategy should aim to identify what exactly is required or expected from the bee industry and therefore provide a baseline against which performance and progress could be measured. This would allow the activities of the NBU and the bee keepers to be better targeted in the future.

There are a number of areas where information appears to be lacking and future research should focus on this. A key area is that there is very little hard evidence on the distribution, abundance and health of wild colonies of bees. There are anecdotal reports that numbers are very low but these need some substantiation. Another key area appears to be quarantine with the possibility of either new pests and diseases or pathotypes/strains being brought into the UK with imported queens and attendant workers or in nuclei. It is imperative that adequate checking and monitoring of imports is continued or improved and that well developed response plans have been developed in consultation with bee keepers so all parties are clear on what their role and response should be in the event of the importation of a new threat such as small hive beetle. Early action is likely to be more successful in eradicating or containing an introduced species than a poorly targeted response. Given that the majority of UK bee keepers are part time it would seem reasonable that the bulk of quarantine containment activities should fall to the NBU and associated personnel since this will enable a more rapid response to be

achieved and should ultimately prove to be a more reliable method of protecting the health of the wider bee population in the UK.

The Honeybee R&D plan developed by the RIRDC (RIRDC, 2007) may provide a useful source of additional ideas for inclusion in the development of the current strategy document.

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Other links

Apiary Inspectors of America Link http://www.apiaryinspectors.org/

Bee Alert Technology, Inc. <u>Link: www.beealert.info</u>

MAAREC - http://www.ento.psu.edu/MAAREC/index.html

Beebase https://secure.csl.gov.uk/beebase/

http://eur-

<u>lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32003D0881:EN:HT</u>ML

http://www.opsi.gov.uk/si/si2006/20060342.htm

http://www.defra.gov.uk/hort/Bees/index.htm

http://riley.nal.usda.gov/nal_display/index.php?info_center=8&tax_level =2&tax_subject=10&want_id=1322&topic_id=1006&placement_defa

http://www.cari.be/article/bibliographie-abeille--

pesticides?PHPSESSID=b844244aaf3476c0f9ef4bd81b34f496

Overseas information

NSW Department of Agriculture, Australia

http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/223349/AFB-tracing-the-source.pdf

Queensland Department of Primary Industries, Australia

Web: www.dpi.qld.gov.au/bees/

Department of Entomology, University of Georgia, USA

Small Hive Beetle Fact Sheet

Web: www.bugwood.org/factsheets/small_hive_beetle.html Florida Department of Agriculture and Consumer Services,

USA

Web: doacs.state.fl.us/~pi/enpp/ento/aethinanew.htm United States NAPIS Cooperative Agriculture Pest Survey Programme

Web: www.ceris.purdue.edu/napis/pests/shb/

USDA Bee Research Laboratory

Beltsville, Maryland, USA

Web: www.barc.usda.gov/psi/brl/

USDA Beneficial Insects Research Center

Weslaco, Texas, USA

Web: weslaco.ars.usda.gov/biru.html

ARC Plant Protection Research Institute Honey Bee Research Stellenbosch, South Africa

Web: http://www.arc.agric.za/institutes/ppri/main/divisions/beekeeping/honeybeeresearch.htm

Annex 1

Table 1. Results of honeybee colony inspections by NBU in England and Wales over the last $10\ \text{years}$

	Total	Dead	No. of colonies destroyed after diagnosis of AFB	No. of colonies diagnosed positive for EFB †	No. of colonies destroyed after diagnosis of EFB ‡	No. of colonies treated with antibiotic after diagnosis of EFB ‡	No. of colonies treated with shookswa rm after diagnosis of EFB ‡	No. of colonies sampled but laboratory diagnosis negative	Percent of inspected colonies which had AFB	Percent of inspected colonies which had EFB
England 1999	21916		122	853	418	435		269	0.6	3.9
England 2000	23297		89	1007	385	622		272	0.4	4.3
England 2001	19051		73	816	296	520		292	0.4	4.3
England 2002	20912		232	649	202	447		334	1.1	3.1
England 2003	20882		45	653	209	444		264	0.2	3.1
England 2004	21234		42	672	226	446		249	0.2	3.2
England 2005	19338		27	665	215	450		254	0.1	3.4
England 2006	20219	1666	56	548	169	227	172	28	0.28	2.71
England 2007	22264	2039	46	611	201	195	235	11	0.21	2.74
England 2008*	17968	1874	54	674	276	92	222	4	0.30	3.75
Wales 1999	3958		26	12	8	4		16	0.7	0.3
Wales 2000	3785		14	34	16	18		39	0.4	0.9
Wales 2001	3004		38	23	12	11		38	1.3	0.8
Wales 2002	3475		33	18	5	13		31	0.9	0.5
Wales 2003	4252		62	20	7	13		37	1.5	0.5
Wales 2004	4464		40	19	6	13		13	0.9	0.4
Wales 2005	4598		22	10	7	3		14	0.5	0.2
Wales 2006	4751	1098	12	8	4	0	1	0	0.25	0.17
Wales 2007	5117	1147	14	15	9	0	6	0	0.27	0.29
Wales 2008*	2860	818	4	32	7	0	6	2	0.14	1.12

^{† -} This figure may include colonies diagnosed but not yet treated.

2008 figures obtained 'live' from beebase reports

^{‡ -} This figure may include contact colonies which have a negative diagnosis but which are treated on request of the beekeeper.

Table 2. Results of honeybee apiary inspections by NBU in England and Wales over the last 10 years $\,$

	<u> </u>				
County	Apiaries Inspected	No. of apiaries diagnosed positive for AFB	No. of apiaries diagnosed positive for EFB	Percent of inspected apiaries diagnosed positive for AFB	Percent of inspected apiaries diagnosed positive for EFB
England 1999	3612	45	343	1.2	9.5
England 2000	3797	43	408	1.1	10.7
England 2001	3076	42	351	1.4	11.4
England 2002	3450	62	246	1.8	7.1
England 2003	3459	26	289	0.8	8.4
England 2004	3312	25	240	0.8	7.2
England 2005	3080	21	238	0.7	7.7
England 2006	3141	21	221	0.67	7.04
England 2007	3553	25	219	0.70	6.16
England 2008	2952	17	243	0.58	8.23
Wales 1999	812	15	8	1.8	1.0
Wales 2000	748	8	14	1.1	1.9
Wales 2001	630	16	14	2.5	2.2
Wales 2002	715	18	12	2.5	1.7
Wales 2003	810	29	12	3.6	1.5
Wales 2004	859	28	12	3.3	1.4
Wales 2005	955	16	5	1.7	0.5
Wales 2006	938	7	4	0.75	0.43
Wales 2007	1005	11	8	1.09	0.80
Wales 2008	634	4	11	0.63	1.74

Table 3. Results of beekeeper inspections by NBU in England and Wales over the last 10 years $\,$

County	Beekeepers Inspected	No. of beekeepers diagnosed positive for AFB	No. of beekeepers diagnosed positive for EFB	inspected beekeepers whose colonies were	Percent of inspected beekeepers whose colonies were diagnosed positive for EFB
England 1999	2554	28	256	1.1	10.0
England 2000	2774	19	323	0.7	11.6
England 2001	2109	28	259	1.3	12.3
England 2002	2510	32	176	1.3	7.0
England 2003	2459	19	218	0.8	8.9
England 2004	2449	22	174	0.9	7.1
England 2005	2351	16	173	0.7	7.4
England 2006	2260	12	148	0.53	6.55
England 2007	2542	19	147	0.75	5.78
England 2008	2217	14	164	0.63	7.40
Wales 1999	667	13	8	1.9	1.2
Wales 2000	620	6	11	1.0	1.8
Wales 2001	539	9	10	1.7	1.9
Wales 2002	565	10	10	1.8	1.8
Wales 2003	612	17	9	2.8	1.5
Wales 2004	668	12	9	1.8	1.3
Wales 2005	737	13	3	1.8	0.4
Wales 2006	723	6	4	0.83	0.55
Wales 2007	777	10	8	1.29	1.03
Wales 2008	505	3	9	0.59	1.78

Table 4. Inspections in England and Wales since 2003 for SHB and Tropilaelaps

County	No. of Apiaries Inspected	Total colonies	Dead colonies	No. of Colonies with Small hive beetle	No. of Colonies with Tropilaelaps
England 2003	131	745	73	0	0
England 2004	40	150	19	0	0
England 2005	104	434	79	0	0
England 2006	333	1607	190	0	0
England 2007	477	2002	236	0	0
England 2008*	270	982	185	0	0
Wales 2003	28	122	15	0	0
Wales 2004	41	205	45	0	0
Wales 2005	79	389	81	0	0
Wales 2006	151	741	171	0	0
Wales 2007	128	588	204	0	0
Wales 2008*	25	88	28	0	0

^{*} live figures from BeeBase on 22/08/08

Table 5. Distribution of bee pathogens and parasites

	1	p				1	1	1	
	England & Wales	Rest of Europe	North America	Central and South America	Australia	New Zealand	South Asia	North Asia - China	Africa
American foul brood (AFB) Paenibacillus larvae var. larvae	Y#	Y#	Y	Y	Υ#	Υ		Y	Υ
European foul brood (EFB) Melissococcus pluton	Y#	Y#	Y		Υ#	N#			
Chalkbrood (Ascosphaera apis)			Y			Y			
Malpighamoeba mellificae									
Varroa destructor	Y	Y	Υ	Y	N#	Υ	Υ	Υ	Υ
Varroa. jacobsoni					Υ#				
Asian honey bee mites <i>Tropilaelaps</i> spp.	N#	N			N#	N	Y	Y	
Nosema apis	Υ	Υ	Υ						
Nosema ceranae	Υ	Υ							
Honey bee tracheal mite Acarapis woodi	Y		Y		N#	N			
Bee louse (Braula coeca)			Y						
Small Hive Beetle, (Aethina tumida) (SHB)	N#		Y		Υ	N#			Y
Colony Collapse Disorder			Y						
Greater wax moth (Galleria mellonella)			Υ						Y
Lesser wax moth (Achroia grisella)									
Deformed wing virus	Y	Y	Υ	Y		Υ			
Black queen cell	Υ	Υ		Υ		Υ			Υ

virus BQCV								
Kashmir bee virus KBV	Υ	Y			Y	Y		
Sacbrood virus SBV	Y	Y	Y	Y	Y	Y		
Acute bee paralysis virus ABPV	Y	Y	Y	Y				Y
Chronic bee paralysis virus CBPV	Y	Y	Y	Y		Y		
Israeli acute paralysis virus	N		Y		Y			
Apis iridescent virus	N							
Cloudy wing virus CWV								
Africanised Honeybees (Apis mellifera scutellata)			Y	Y	N			Y

Defra Research

PhD: Investigating the genetic differences between Paenibacillus larvae subspecies (Defra seedcorn studentship)

This studentship is run with Dr Thorunn Helgason at the University of York. *Paenibacillus larvae* subspecies cause a serious disease of honey bee called American foulbrood. The classification of the disease causing bacteria has been the focus of much research in recent years. This project aims to collect a significant amount of genetic data which will allow recognition of the different subtypes. In addition, the strains of bacteria causing outbreaks of AFB in England and Wales will be characterised to identify the source of the bacteria. https://secure.csl.gov.uk/beebase/pdfs/paenibacillus.pdf

PhD: Investigating the taxonomy of UK honey bee viruses: A molecular approach (Defra funded project number PH0410)

This studentship is run with Professor Mike Carter at the University of Surrey. The main aim of this study is to investigate the viruses in UK honey bee populations by characterising the genetic material of each virus. The data will allow us to construct virus family trees (also known as phylogenetic trees) and detail the distribution each virus in England and Wales. (Cordoni1 *et al* 2007 - https://secure.csl.gov.uk/beebase/pdfs/taxonomyOfViruses.pdf)

Defra Research

Investigating abnormal colony losses in England and Wales (Defra funded)

Historically colony losses have fluctuated greatly in the UK, with severe weather increasing colony losses. However, the last 7 years have seen a trend of slowly rising colony losses. Beekeepers reported increased colony losses in Spring 2007 and the NBU responded by securing funding to investigate all reported abnormal colony losses. Samples of adult bees and brood will be collected from dead and healthy colonies. These samples will be analysed for a range of honey bee pests and pathogens. In addition, wax samples will be analysed pesticides and vetinary medicines.

https://secure.csl.gov.uk/beebase/pdfs/abnormalColonyLoss2007.pdf

PhD: Investigating virus immune response in honey bees (NERC CASE studentship with additional funding from Defra)

This studentship is run with Professor Mike Boots at University of Sheffield. Scientists at the University of Sheffield are experts in insect immune response. The way insects respond to bacterial pathogens is well characterised, however, little in known about virus immune response. This PhD will explore the way honey bees respond to honey bee virus infection and may help us to develop more resistant bees in the future.

Defra Biosecurity Chip:

Developing micro-array screening methods for the detection of animal, fish and plant viruses. Micro-array includes

- Acute bee paralysis virus
- Apis iridescent virus
- Arkansas bee virus
- Bee virus X

Bee virus Y

• Berkeley bee virus

Black queen cell virus

Chronic paralysis bee virus

Deformed wing virus

Egypt bee virus

Kashmir bee virus

- Sacbrood virus
- Sacbrood virus (Thai strain)

Slow paralysis virus (bee)

http://www.bio-chip.co.uk/viruses.cfm

Vita (Europe) Limited in conjunction with the National Bee Unit and Cardiff University have won UK government funding for a research project evaluating a new biological control agent for foulbrood. A harmless bacterium found as a commensal in beehives has been shown to control *Paenibacillus larvae* var. *larvae* (as well as *Melissococcus plutonius*) infections under laboratory conditions. Studies on the toxicity and palatability of the bacterium show no effect on the bees. Field trials against both European foulbrood and American foulbrood are underway and current progress is positive. A new, natural product may be available from Vita (Europe) Limited for the treatment of foulbrood within the next few years.

http://www.vitaeurope.com/Map enscript/frmbuilder.php?dateiname=%2Fen%2Fnews%2FNewsRelNov02200 71512.html

Defra Research

PhD: Assessing exotic threats (Jubilee Fellowship)

This studentship is run with Assistant Professor Panuwan Chantawannakul at Chiang Mai University in Thailand. The main aim of this study is to investigate the risk posed by *Tropilaelaps* mites. There are at least four known species of these mites. Although *Tropilaelaps* mites are smaller than *Varroa*, they have a much greater potential to reproduce on honey bee brood. These mites could also carry exotic viruses that may impact UK honey bees. This project will assess the risks posed by both mites and viruses

Defra Research

Assessing the effectiveness of the shook swarm method for controlling European Foul Brood (Defra funded project number PH0502)

European foul brood (EFB), caused by the bacterium *Melissococcus plutonius*, is the most prevalent brood disease in England and Wales with over 600 cases reported each year. Several treatment options exists in the UK including the application of the antibiotic oxytetracycline and a husbandry method of control known as Shook swarm. Shook swarm involves transferring all adult bees from an infected hive into a clean hive, thereby removing the infected brood from the colony

(https://secure.csl.gov.uk/beebase/pdfs/shookSwarmInstructions.pdf). This project aims to investigate the success of these two treatments and to investigate asymptomatic infection of the bacteria.

https://secure.csl.gov.uk/beebase/pdfs/shookSwarmYear1Results.pdf

European group for integrated Varroa control

This working group originates out of the EU Project FAIR CT97-3686 "Coordination in Europe of integrated control of varroa mites in honey bee colonies" The main objectives of the Working Group are to co-ordinate research efforts in Europe on integrated Varroa control and to disseminate information to the beekeeping community on how populations of the bee parasite, Varroa destructor, can be kept below the damage threshold by alternative strategies. With these methods it is possible to both avoid short term development of resistance and to produce high quality bee products.

The specific objectives are:

- To maintain an European network for efficient exchange of information on methods that minimise the use of pesticides for control of the parasitic mite Varroa destructor in honey bee colonies. This is supported by an annual meeting of members.
- To highlight future research needs in this field and to initiate new collaborative research.
- To compile information about integrated control of Varroa in a format suitable both for publication in national beekeeping journals (or as a separate publication), and to initiate seminars on this subject.
- To make available a bibliography on ecological control of Varroa mites.
- To harmonise efficacy evaluation methods.

NBU - Development of a monitoring system for the small hive beetle, *Aethina tumida* (Murray) (Defra funded project number PH0503)
The small hive beetle (SHB), *Aethina tumida*, is an invasive species originating from Africa which has proved to be a serious pest of honeybee hives in the USA and Australia. The SHB has been made notifiable within the European Community (Commission Decision 2003/881/EC). This project aims to develop attractant lures to use in traps that will assist with monitoring and surveillance for this pest.

NBU Research:

SHB has recently become an invasive species creating the need for an efficient and reliable detection method. BHU have led the development of a method to screen hive debris for the presence of SHB using real-time PCR in conjunction with an automated DNA extraction protocol. The method was able to detect DNA from SHB eggs, larvae and adult specimens collected from Africa, Australia and North America. The method was used to successfully detect SHB DNA extracted from spiked and naturally infested debris. An *Apis mellifera* 18S rRNA real-time PCR assay was used as an internal positive control (IPC). The IPC showed that the method was reliable for detection as extraction efficiency was consistent between hive debris samples. If the SHB were to establish at new locations, the availability of such a method would be a valuable support tool to enable species identification and rapid screening of hive debris for delimiting surveys (Ward *et al.*, 2007)

NBU -Evaluation of Metagenomic Sequencing (Pyrosequencing) as a Diagnostic Tool for the Characterisation of Disease of Unknown Aetiology (Defra seedcorn)

Pyrosequencing represents a recent advance in sequencing methodology. Such methods offer a non-targeted screen of all the organisms present in a sample. These methods are useful for identifying the causes of disease with

no prior knowledge of the cause. Samples will be taken from lost colonies to investigate whether any new or previously unknown organism is present in the samples.

NBU - Streamlining honey bee diagnostic services (Defra funded)

Scientists at CSL are experts in developing tests for a range of honey bee pests and diseases. Although we have advanced methods for determining the presence of many pests and pathogens, we are constantly looking to improve our methods. This project will investigate ways of streamlining our diagnostic services to allow higher sample throughput and shorter turnaround times to assist with future contingency situations. Using a combination of conventional and leading edge diagnostic techniques, CSL has developed a suite of assays based on a generic platform – real-time (TaqMan®) PCR. Currently CSL can detect and quantify 16 of the 19 major threats to bee colonies - and with the remaining three in development the suite will soon be complete.

USA

USDA National Research Initiative Coordinated Agricultural Project (No. GEO-2008-02374) *Sustainable Solutions To Problems Affecting Health Of Managed Bees*

START: 15 JUL 2008 **TERM:** 14 JUL 2009 **GRANT YR:** 2008

GRANT AMT: \$1,152,062

PI = Delaplane, K. S., University of Georgia, Athens

The long-term goal is to restore large and diverse populations of managed bee pollinators across the United States to sustain natural and agricultural plant communities. The specific goals of this project are to:

- 1. Determine and mitigate causes of Colony Collapse Disorder: study the interactive effects of disease agents (pathogens, parasites) and environmental factors (pesticides, nutrition) on honey bee health,
- 2. Incorporate traits that help honey bees resist pathogens and parasitic mites and increase genetic diversity of commercially available stocks,
- 3. Improve conservation and management of non-Apis pollinators by identifying new or emerging pathogens and parasites, abiotic stresses, and practices that optimize their pollinating efficacy,
- 4. Deliver research knowledge to client groups by developing a technology transfer program for queen breeders and a literature on Best Management Practices for queen breeders and managed pollinators as an eXtension Community of Practice.

Research Project: Honey Bee Microarray Slides

Location: Bee Research

Project Number: 1275-21000-174-08

Project Type:

Start Date: May 12, 2008 - End Date: Feb 01, 2009

Objective:

The University of Illinois and the Agricultural Research Service (ARS) desire to enter into this Agreement for the purpose of supporting research to be carried out at ARS and Cooperator facilities. ARS desires the Cooperator to provide goods and services necessary to carry out research of mutual interest.

Approach:

The Location is engaged in research including activity levels of honey bee immune genes. Under the authority of 7 USC 3319a, ARS desires to acquire goods and personnel services from the Cooperator to further agricultural research supporting the independent interests of both parties. This Agreement serves as an order for services to be funded on an annual basis.

Research Project: Improving Honey Bee Health, Survivorship and Pollination

Availability

Location: Bee Research

Project Number: 0500-00044-024-00

Project Type: Appropriated

Start Date: Oct 01, 2007 End Date: Sep 30, 2008

Objective:

The objective of this program is to improve overall colony survival and availability for pollination by bringing together recent ARS research findings on mite-resistant bee stocks, improved diets, mite and disease control alternatives and general colony management techniques into a comprehensive bee management system. The overarching goal of this Areawide program is to increase colony survival and availability for pollination and thus increase the profitability of beekeeping in the U.S.

Approach:

The Program will focus on bringing together recent ARS research including: 1) two ARS bee stock improvements, Russian bees and the Varroa Sensitive Hygiene (VSH) trait (Baton Rouge); 2) improvements in nutrition, Mega Bee® (Tucson), HFCS research results (Weslaco); 3) parasitic mite management techniques including new chemical controls 2-heptanone (Tucson), Hivastan® (Weslaco) and non-chemical controls plastic drone comb (Beltsville) and screen bottom boards (Beltsville); 4) management practices including the use of antibiotics, Tylosin® (Beltsville) and Nosema controls (Weslaco and

Beltsville). A year-round management scheme will be tested in large migratory and smaller non-migratory beekeeping operations with an emphasis on the larger migratory beekeepers that supply bees to almonds (almost half of all managed bees in the U.S.) The country will be divided into geographic regions as follows; East, Mid-West & West. It is imperative to tests in many geographic regions as bees and bee pests and diseases grow at different rates in different parts of the country.

Research Project: Managing Diseases and Pests of Honey Bees to Improve

Queen and Colony Health Location: Bee Research

Project Number: 1275-21000-174-00

Project Type: Appropriated

Start Date: Sep 10, 2003 - End Date: Sep 09, 2008

Objective:

Develop new, cost-effective strategies for controlling parasitic mites like *Varroa jacobsoni*, and bacterial disease like American foulbrood (AFB); Develop molecular markers and determine gene function in honey bees as they relate to disease resistance; Develop molecular techniques to diagnose and characterize pests and pathogens of honey bees.

Approach:

(1) An integrated pest management strategy for controlling parasitic mites of honey bees will be approached by examining the interaction of chemical, cultural and biological (genetic) control methods. (2) Alternative controls for American foulbrood disease examined by screening and evaluating antibiotics against terramycin-resistant bacteria. (3) Use molecular methods, like PCR, for virus diagnosis. (4) Use microsatellite DNA markers to characterize honey bee pests and gene expression arrays to isolate disease-related genes in honey bees.

Research Project: Preservation of Honey Bee Germplasm

Location: Bee Research

Project Number: 1275-21220-212-00

Project Type: Appropriated

Start Date: Sep 10, 2003 - End Date: Sep 09, 2008

Objective:

Improve our ability to maintain specific genetic types of honey bees by developing methods for the *in vitro* preservation of honey bee semen. Determine the biochemical and physiological environment that enables honey bee sperm to remain viable in queen spermathecae. Develop methods for the

in vitro preservation of honey bee embryos. Design and implement a germplasm collection protocol.

Approach:

The focus of this project is to develop practical methods of germplasm preservation for the honey bee, using cryopreservation and non-frozen systems. This technology is needed to preserve the genetic diversity of this species in the United States, especially because of severe colony losses to parasitic mites and diseases, and to assist in the selection of superior stocks of bees. Once preservation methodology is available, a collection scheme to maximize diversity in preserved germplasm will be needed.

Research Project: Applied Genomics Add Complete-Genome Microarray for

Honey Bees

Location: Bee Research

Project Number: 1275-21000-174-03

Project Type: Reimbursable

Start Date: May 01, 2004 - End Date: Apr 30, 2008

Objective:

Honey bees are key beneficial insects as pollinators and food providers. To better understand honey bee disease, reproduction and behavior, we will develop a state-of-the-art genomic resource and deploy it to address fundamental issues in the bee biology that are of critical importance to apiculture. The new microarray will help genomic studies in honey bees advance rapidly, leading to improvements in breeding stock and management, and helping to realize the promise of the bee genome project. Data collected will provide insights into how honey bees respond to challenges and how non-desirable honey bee traits, including defensive behavior arise. We will use interdisciplinary approaches, from field tests to whole-genome expression analyses.

Approach:

Taking advantage of the sequencing of the bee genome, we propose to create a microarray to study gene expression that contains all the genes identified in the genome. We will use a newly established method of designing one diagnostic 70mer oligonucleotide for every gene identified in the honey bee genome. This approach, already successfully used with Drosophila, will be further tested against an already functional bee cDNA miroarray. We will use this new microarray to begin to identify genes and pathways that are involved in: 1)the response of larvae to the pathogen that causes American Foulbrood, the most deadly bee disease; 2)metamorphosis and caste determination, to provide a basic understanding of developmental processes that occur in the life stages that are both most vulnerable to pathogens and parasites and critical to the formation of a vigorous queen; and 3)differences in behavior

between European and (more defensive) African bees. We also will provide training to students in genomic and entomological techniques, and make the microarray available to the scientific community at minimal cost.

Research Project: Evaluation of Pathogens and Pesticides Affecting Honey

Bee Health

Location: Bee Research

Project Number: 1275-21000-174-06

Project Type: Specific C/A

Start Date: Sep 03, 2007 - End Date: Sep 03, 2012

Objective:

The objectives of this agreement are to determine the effects of pathogens and pesticides on honey bee health. We propose to conduct pathogenicity experiments with specific pathogens recently identified and linked to colony collapse disorder (CCD). Additionally, sequencing of the genomes of two microsporidain species (*Nosema apis* and *N. ceranae*) is proposed under this agreement.

Approach:

Honey bees will be exposed to viruses, bacteria, fungi or pesticides and monitored for effects including longevity. Interactions between pathogens and pesticides will be explored by exposing bees to pesticides and pathogens in specific pairs. Fluvalinate and coumaphos will be tested along with newly identified bee pathogens to determine if either act alone or in combination to impact bee health. The sequencing of the *Nosema* genomes will be accomplished through collaboration with the 4-5-4 sequencing center.

Research Project: Evaluation of Pesticides on Honey Bee Health

Location: Bee Research

Project Number: 1275-21000-174-05

Project Type: Specific C/A

Start Date: Sep 25, 2007 - End Date: Sep 03, 2012

Objective:

The objectives of this cooperative agreement are to determine the lethal and sub-lethal effects of crop pesticides on honey bee health. We propose to examine the exposure rates and effects of pesticides used on crops pollinated by bees in the mid-Atlantic region. Pesticide exposure could be one stress factor involved in colony collapse disorder (CCD) and bees used for pollination appear to be particularly at risk for CCD. Thus, the research will document to level of pesticide exposure from specific crops and determine the effects, if any, of this exposure on bee health.

Approach:

Crops that rent bees for pollination including watermelons, cucumbers, and other cucurbits will be the target crops for these studies. Honey bee colonies will be placed in test plots were specific pesticide regimes are administered and the nectar and pollen from these crops collected and analyzed for pesticide residues. Following documentation of the levels of exposure in the nectar and pollen, cage studies will be conducted that expose bees to these concentrations of pesticides in protein and or carbohydrates diets and longevity used to determine effects. Companion studies may be carried out in larger fields where whole colonies can be exposed under real-world situations and colony health monitored.

EU

Current Call: FP7-KBBE-2009-3

KBBE-2009-1-3-03: Bee health: identification of emerging honey bee pest and diseases and re-emergence of pathogens and explaining the intimate mechanisms and the reasons for increased honey bee mortality

The project will fill knowledge gaps in honey bee pest and diseases, including the 'colony collapse disorder' and help explain the intimate mechanisms and the reasons for increased honey bee mortality, relating to the emergence of new- or re- emergence of well known pathogens, as well as interactions with endemic infections and parasitism, in different European areas. Genomics knowledge of the bee genome and of some pathogens (such as viruses) will be used to differentiate and explore host-pathogen interaction. As latent infection is commonly observed in bee colonies the qualitative assessment should be complemented with a quantitative approach. Special focus should be made on Nosema disease (N. apis and ceranae), viruses and the impact of Varroa destructor infestation. Environmental factors, including chronic exposure to pesticides, as well as husbandry and management practices should be carefully considered. The final aim would be the development of diagnostic screening methods and sustainable disease prevention and control strategies. Practical transferability of results for use by beekeepers should be ensured. Funding scheme: Collaborative Project (small or medium- scale focused research project) Expected impact: The European added value lies in the pooling of interdisciplinary research expertise, thus creating economies of scale to address a cross-border issue and provide support to agricultural policies. Indeed, the massive loss of honey bee colonies may impact on

agriculture via the pollination network. The role of pollinators is important both for our food supply and for the preservation of natural ecosystems.

New Zealand - HortResearch

- The first programme is to develop technologies for the control of varroa to ensure that enough insect pollinators remain in New Zealand's environment for New Zealand to be able to meet its economic and social goals. The key outcomes include:
- Honey bee stock that is at least partially resistant to varroa.
- Recommendations for the use of the major synthetic pyrethroid control products that will reduce rates and consequently costs, residues, and resistance.
- Improved methods for using organic products without the current variation in effectiveness.
- Hive management practices to increase the effectiveness of varroa control.
- Economic thresholds for treatment to reduce the current overuse of chemicals.
- Knowledge of the factors causing Parasitic Mite Syndrome and possible leads removing the symptoms.
- Improved understanding of how varroa invades colonies. http://www.hortresearch.co.nz/index/page/414

Australia - Rural Industries Research and Development Corporation

- Sustainable Control of Small Hive Beetle Through Targeting In-ground Stages: Project No. UWS-22A
- Development of Two Markers for Hygienic Behaviour of Honeybees; Project No US-123A