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Project identification

1. Defra Project code: PH0509

2. Project title: Development of an Evidence Based Risk Assessment for Nosema Species that Infect Honey Bees

3. Contractor organisation(s): Risk & Policy Analysts Limited

   Professor Francis Ratnieks

4. Total Defra project costs (agreed fixed price): £ 25,060

5. Project: start date ..... 01 November 2009

   end date ..... 31 March 2010

6. It is Defra's intention to publish this form.
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Executive Summary

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Introduction

This study has identified and reviewed available data on *Nosema ceranae* and *N. apis* in order to estimate the geographical spread of these *Nosema* species, their virulence and to identify interactions/associations between the *Nosema* species and other bee pests and pathogens. In addition, an assessment has been made of current and possible future methods for the treatment of *Nosema* infections.

An initial impact assessment of *Nosema* species on the economy of England and Wales was carried out. Finally, this study makes suggestions for further research to fill identified data gaps.

Hazard

N. apis infections result in worker honey bees (*Apis mellifera*) being less able to feed their brood thereby slowing colony population build up in spring. Worker vigour and lifespan may also be reduced. Furthermore, the queen may be infected and cease egg laying. *N. ceranae* appears to have similar effects and may be more virulent.

N. apis infections have been a feature of beekeeping for many decades while *N. ceranae* is a relatively new pathogen of *A mellifera* in the UK and worldwide. There are contradictory reports about the virulence *of N. ceranae*. Evidence from Spain suggests that almost 100% of untreated colonies infected by *N. ceranae* may die within a year of infection while evidence from the USA shows no correlation between *N. ceranae* levels and colony mortality.

From the review of available literature it is not possible to state at this time whether or not *N. ceranae* is more virulent than *N. apis* that has infected Western honey bee (*A. mellifera*) colonies in the UK for many years.

Likelihood of Impacts

At least one strain of *N. ceranae* has been confirmed in areas widely dispersed across England and Wales. It is possible therefore that *N. ceranae* may be already widespread across the UK and so the likelihood of impacts to the UK must be considered to be high.

Consequences

A draft assessment of the potential impacts of *N. ceranae* on honey bee colonies England and Wales was conducted. However, due to the high level of uncertainty it was decided in consultation with the customer that this assessment could not form a robust basis for policy decisions at the present time and therefore a more detailed assessment was not conducted.

Response

Further research areas are suggested that would generate data on the level of virulence of *N. ceranae* likely to be experienced by honey bee colonies in the UK.

Important new data on the temporal and geographical spread of the two *Nosema* species across England and Wales and on the value of honey bee colonies to the UK economy are likely to be available in 2010 (beyond the timeframe for this study). It is therefore suggested that these data should be assessed and an estimation of the impact of *N. ceranae* should be made as soon as more definitive data becomes available on the virulence of this pathogen.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other

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- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

1. Introduction

1.1 Aims and Objectives of the Study

Nosema apis and *Nosema ceranae* are microsporidian infections that develop in the mid-gut of adult western or European honey bees (*Apis mellifera)*:

Nosema apis is a pathogen native to *Apis mellifera* and infects adult workers when they clean combs contaminated with bee faeces containing *Nosema* spores; spores can persist for one year or more in a hive or hive equipment; whereas
Nosema ceranae is a pathogen native to the eastern or Asian honey bee (*Apis ceranae*) but can also infect *Apis mellifera* (Paxton, 2007). *N. ceranae* has been found in *Apis mellifera* in many countries worldwide, including in Europe. *N. ceranae* was first identified as being present in the British Isles in 2008 but it is not as yet clear when infection may have first occurred.

This study has sought to:

(i) identify the information available on *N. ceranae* and *N. apis* from articles published in peer-reviewed journals, scientific reports, conference proceedings and other literature sources. With emphasis given to information relating to honey bees similar to the sub-species in the UK (European *Apis mellifera*) and to studies from areas where geographical and climatic conditions are similar to those in the UK;
(ii) review the information available in particular to consider the geographical spread of the two *Nosema* species and to identify interactions/associations between the *Nosema* species and other bee pests and pathogens;
(iii) assess the impact of *Nosema* species either alone or in association with other bee pests and pathogens to the UK beekeeping industry;
(iv) determine, if possible the extent to which the strains of *N. ceranae* that have been found in *A. mellifera* are significantly different to strains of *N. apis*; and
(v) review possible actions including potential treatment methods for managing *Nosema* species.

This study is primarily focused on the situations that may apply to England and Wales. However, there are no major distinctions between beekeeping in northern England and beekeeping in southern Scotland. In addition, the climate and beekeeping practices in Northern Ireland and the Republic of Ireland are very similar to those in the rest of the British Isles. Therefore, the findings of this study will be of relevance to the whole of the British Isles.

2. Hazard

2.1 Introduction

Nosema species, including *N. ceranae* and *N. apis*, are a genus of Microsporidia, a phylum of specialist unicellular fungi that are intracellular parasites of animals. Both species of *Nosema* affect a wide range of host organisms, especially insects, and produce spores that act as a vehicle for dispersal and infection.

Infection is mediated by a special organ (the polar filament) that pierces a host cell to inject the infectious contents of the spore (specifically the genetic material) into the host cell. *N. apis* and *N. ceranae* both infect gut (ventriculus) epithelial cells of the western honey bee species present in the UK (*Apis mellifera*).

Other *Nosema* species infect other bee species, including *N. bombi* which infects bumble bees, and other insects and *N. bombycis* which infects silk moth larvae and causes pebrine disease (Goulson, 2006).

2.2 Differences between *Nosema ceranae* and *Nosema apis*

General Differences

N. apis is a parasite of *Apis mellifera* that has been known for at least 100 years and has been extensively researched (Bailey, 1981; Furgala & Mussen 1990 and Fries, 1993). In contrast *N. ceranae* was only identified in 1996 as a parasite of the Asian honey bee (*Apis cerana*) (Fries et al, 1996) and only found to infect *A. mellifera* in 2007 (based on samples from 2005) (Huang et al, 2007).

Subsequent research shows that *N. ceranae* has a worldwide distribution in *A. mellifera* and has been present in Europe, North America and South America for approximately 10 years. Furthermore, *N. ceranae* has been identified as infecting *A. mellifera* in Africa and Australia. The symptoms associated with *N. ceranae* infections are similar to those from *N. apis* infections and are summarised in later sections of this report that consider the virulence of the two species.

N. ceranae is now more frequently detected than *N. apis* in most countries in Europe and the Americas and may even be replacing *N. apis*. However, data on *N. ceranae* infections are not yet sufficient to state whether or not these patterns are being repeated in the UK (Budge, 2008).

The *N. apis* infections exhibit a seasonal pattern with spore levels in worker bees rising from late autumn to spring, declining through the summer with, potentially, a second peak during the summer when extra boxes added to hives may be contaminated with spores (Bailey, 1981 and Furgala & Mussen, 1990). In contrast, infections of *N. ceranae* do not show the seasonal pattern described above for *N. apis* (Martín-Hernández et al, 2007).

Honey bees may be infected with *N. apis* and *N. ceranae* together. However, a 2007 study indicates that bees infected with *N. ceranae* (either alone or in combination with *N. apis*) contain five to eight times as many spores as bees infected with *N. apis* alone (Paxton et al, 2007).

N. ceranae infections produce similar symptoms to *N. apis* infections. However, there is some evidence to suggest that *N. ceranae* may be significantly more virulent than *N. apis* and this is considered further in sections below.

The spores of *N. apis* and *N. ceranae*, while not identical, are difficult to reliably differentiate by visual microscopy. However, the two *Nosema* species may be differentiated by genetic (DNA) analysis. This is mostly done with a single genetic marker, the 16s small sub-unit ribosomal RNA (i.e., a length of DNA in the nuclear genome). However, other markers may be developed now that much of the *N. ceranae* genome has been sequenced, as discussed below.

Identification of N. apis and N. ceranae - Introduction

N. ceranae can be differentiated from *N. apis* on the basis of DNA markers, spore morphology, and spore ultrastructure. The sample for analysis is generally an extract of the hind gut or gut contents from a sample of worker bees, but may also be from a single bee, or even from bee faeces.

There is a higher likelihood of *Nosema* species being identified and differentiated from an aggregated sample from a group of bees. This may allow earlier detection of *N. ceranae* in an area where it is colonising.

Transmission electron microscopy

Transmission electron microscopy of individual spores shows that *N. ceranae* spores have fewer polar filament coils than does *N. apis* (Fries, 2009). This is not thought to be a practical way of identifying species, but shows that the two species have different internal spore morphology as well as different spore size and shape.

Light microscopy: spore size & spore counting

Spore counts have long been used and have the advantage that they need only simple equipment (e.g. a regular compound microscope and a hemocytometer slide) and have an almost zero "consumables" cost. However, carrying out spore counts is time consuming[1].

Using light microscopy, the number of spores in the known volume of the slide can be counted and from this the average number of spores per bee, or the number per bee where the sample is from an individual bee, can be determined. *N. apis* spores have a characteristic barrel shape and size (c. 3 x 6 µm) and so can be easily distinguished from other materials in the gut such as pollen grains and gut cells.

There is a morphological difference between the spores of *N. apis* and *N. ceranae*. *N. ceranae* spores are slightly smaller and more rounded and less barrel-shaped than those of *N. apis* (Fries, 2009). However, the difference is quite slight and it is doubtful if this can be used diagnostically. In this respect Fries (2009) writes, "Spores of *N. ceranae* are distinctly smaller than spores of *N. apis*. Nevertheless, they can be hard to distinguish by light microscopy, in particular where mixed infections occur".

If a guide were available with the range of spore sizes and shapes for each *Nosema* species it may be possible to make a statistical comparison. That is, to count, say, 100 spores and from the distribution of lengths to plot a histogram and carry out a statistical analysis to ascertain whether the sample is most likely from *N. apis* only, *N. ceranae* only, or a mixed infection, and if mixed the relative proportions.

Production of such a guide would however require a comprehensive analysis including measurements of the sizes and shapes of large numbers of spore for the two *Nosema* species and to date such an analysis has not been carried out.

Spore microscopy is, however, a good way of quantifying levels of infection. One advantage of spore counts is that they can be undertaken by well-organised beekeepers rather than by trained researchers only. Indeed, some beekeeper associations have the equipment needed for undertaking spore counts such as suitable microscopes and club buildings in which to undertake the work.

It should however be noted that where such assessments are carried out good hygiene practices should be adopted to ensure that further spread of the pathogens is prevented.

DNA Markers

The use of DNA markers is now common in the identification of samples to a particular species and is based on the fact that although two species may share a large amount of their DNA (especially closely related species) there will also be consistent differences.

When *N. ceranae* was first described by Fries et al (1996) part of the *N. ceranae* genome, corresponding to the 16s small sub-unit ribosomal RNA (i.e. a length of DNA in the nuclear genome) was sequenced and this sequence was deposited in GenBank.

Subsequent research on the identification of *N. ceranae* and *N. apis* has involved the sequencing of this part of the genome. For example, Williams et al (2008a) note that 5/8 of their samples

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[1] A hemocytometer is a slide marked with a grid and which will hold a known volume of fluid (e.g., diluted suspensions of macerated bee abdomen(s), macerated gut(s), gut contents or faeces).

corresponded to the GenBank entry for *N. ceranae* and 3/8 to the entry for *N. apis*, showing the presence of *N. ceranae* in Canada.

Small DNA sequence differences were also noted (two sample differences each of one base pair) from their *N. ceranae* to that from GenBank. This shows differences within *N. ceranae* but these are not sufficient to prevent the sample being assigned to *N. ceranae*. Sequence differences were also noted by Chen et al (2009) in samples from China and the USA indicating that different strains of *N. ceranae* may be grouped phylogenetically.

Paxton (2010) notes that the "rRNA gene sequence seems to be an excellent DNA barcode (sensu Valentini et al, 2009) to differentiate among these and other *microsporidian* species (Klee et al, 2007), but not for intraspecific characterisation of variants (O'Mahony et al, 2007)" .

The reason why rRNA is not good for "intraspecific characterisation" is that "in the closely related *Nosema bombi*, each spore contains a range of rRNA sequence variants, making invalid the use of rRNA as a haplotype marker because homologues cannot be reliably compared between host isolates" (O'Mahony et al, 2007). Thus, a single chromosome may have multiple copies of the rRNA gene and these copies may not all be the same.

This complicates the issue of differentiating between different strains of a particular *Nosema* species using this gene. It is possible that there are multiple copies of the rRNA gene of *N. ceranae* which may result in complications similar to those noted for *N. bombi*. This is why both Fries (2009) and Paxton (2010) note that there is a need for single locus markers.

Single locus markers are DNA markers that are found at only one location in the entire genome (= 2 locations in diploid organisms like Nosema with 2 sets of chromosomes). However, at present there are no DNA markers which can be used to distinguish between different strains of *N. ceranae*. The identification of different strains of *N. ceranae* may be helpful in understanding the pathogen better, for example by allowing different strains to be assessed for variation in virulence.

2.3 Virulence of *Nosema ceranae*

For the purposes of this study virulence is defined as the harm done to a host (e.g. *A. mellifera*) by a pathogen (e.g. *Nosema* species).

N. apis makes infected workers less able to feed their brood thereby slowing colony population build up in spring. The queen may also be infected and cease egg laying, resulting in the queen being superseded. Consequently, the worker population may be reduced for several months, leading to a lower honey yield and may even result in the colony dying if the new queen is unsuccessful in mating.

As stated above, *N. ceranae* infections produce similar symptoms to *N. apis* infections in both worker and queen bees (Paxton et al, 2007). However, there is evidence to suggest that the symptoms are more severe with *N. ceranae*. For example, a 2007 study of caged bees suggests that *N. ceranae* is more virulent than *N. apis* as measured by bee mortality (Paxton et al, 2007). However, the researchers concerned urge caution in interpreting their preliminary results because of the small sample size.

A separate 2007 study of caged worker bees found a very high rate of mortality for caged workers fed *N. ceranae* spores and took this as evidence for high virulence and higher virulence than *N. apis* (Higes et al, 2007). Doses of spores used were much higher than is usual in cage studies. The median infective dose is c. 20 to 90 *N. apis* spores per bee therefore these doses are probably at a level that exceeds natural infection (Fries, 1993).

In experiments carried out in Spain involving colonies with naturally occurring infections of *N. ceranae* it was found that without treatment such colonies suffered almost 100% mortality (Higes et al, 2008). In addition, the corpses of worker bees collected from a nectar foraging site had very high levels of *N. ceranae*, which may suggest a possible role of *N. ceranae* on individual worker mortality.

N. ceranae was also implicated in the loss of colonies owned by professional beekeepers in Spain (Higes et al, 2009b). Furthermore, in samples provided by beekeepers from colonies in Spain, the presence of *N. ceranae* (alone or in combination with *N. apis*) increased the risk of a colony dying by a factor of almost six; whereas, the presence of *N. apis* alone was not found to increase the risk of colony mortality (Martín-Hernández et al, 2007).
However, Fries (2009) notes that, "Several studies from Spain suggest that *N. ceranae* is a colony level virulent parasite and that infections eventually lead to colony collapse unless the infections are controlled. However, most published data on colony losses linked to *N. ceranae* infections are correlations and fail to provide evidence of cause and effect".

Current concerns about honey bee colony mortality, including Colony Collapse Disorder (CCD), mean that there is considerable research interest in honey bee pathogens and diseases. As a novel parasite of *A. mellifera*, there is understandable concern that *N. ceranae* may be harmful and may even be the cause of CCD (Oldroyd, 2007).

On the one hand it should be noted that there is a risk that a novel parasite such as *N. ceranae* may simply be blamed for CCD because its appearance correlates with the advent of CCD. Alternatively, there is the possibility that a new parasite such as *N. ceranae* may be harmful itself, and may also interact with other pathogens, especially viruses, causing them to become more harmful.

Evidence to date from the USA indicates that *N. ceranae* and *N. apis* are poorly correlated with severe CCD symptoms in infected hives versus controls (Cox-Foster et al, 2007 and van Eaglesdorp et al, 2009). This evidence therefore does not suggest that *N. ceranae* is the cause of CCD/colony loss, contrary to the evidence from Spain, as documented above.

To add to the uncertainty regarding the virulence of *N. ceranae*, in Uruguay the replacement of *N. apis* by *N. ceranae* has not been accompanied by an overall increase in colony mortality (Invernizzi et al, 2009). Furthermore, a recent study undertaken for Defra found that infections of both Nosema species may die away over time, without treatment (PH0505, 2009).

In an attempt to make sense of the contradictory evidence one recent study notes that, "Spanish *A. mellifera* may be more susceptible to *N. ceranae* than other honey bee races, or the variant of *N. ceranae* within Spain may be more virulent than that found elsewhere, suggestions which deserve attention" (Paxton, 2010).

In support of the above statement, there is evidence that not all strains of *N. ceranae* infecting *Apis mellifera* are identical. In particular, detailed genetic studies involving DNA sequencing show that there are different strains of *N. ceranae* from North America and Spain (Williams et al, 2008a).

Finally, it should be noted that no experimental evidence has been identified which either proves or disproves that Spanish *A. mellifera* are more susceptible to *A. ceranae* than other *A. mellifera*. Nor is there experimental evidence on the relative virulence of *N. ceranae* strains from different parts of the world.

Summary

Some studies suggest that almost 100% of untreated colonies infected by *N. ceranae* may die within a year of infection while other evidence shows no correlation between *N. ceranae* spore levels and colony mortality.

From the review of available literature it is not possible to categorically state whether or not *N. ceranae* is more virulent than the *N. apis* that has infected Western honey bee (*A. mellifera*) colonies in the UK for many years.

2.4 Nosema and Other Pests and Pathogens

Current Understanding

If *N. ceranae* causes harm to *A. mellifera* colonies it may do so on its own or via interactions with other pathogens and other factors that can influence honey bee colonies. From studies on *N. apis*,

interactions would seem to be inevitable with virulence being affected by factors such as the seasonal cycle of the honey bee colony and the year to year variation in weather.

For example, the levels of spores may be greater in years with poor summers that prevent colony build up or harsh winters that prevent cleansing flights for defecation outside the hive (Bailey, 1981, Furgala & Mussen, 1990).

However, what has happened with *N. apis* is not necessarily a good guide to what will happen with *N. ceranae*. It has been noted, for example, that infection by *N. ceranae* leads to higher spore levels in workers (Paxton et al, 2007) but such spore loads do not show the seasonal cycle found with *N. apis* (Martín-Hernández et al, 2007).

Interactions are known to occur between the mite *Varroa destructor* and various viruses (Ratnieks & Carreck, 2010). For example, there are data to suggest that in some cases it is the presence of mites plus virus that is needed to kill a colony (Martin, 2002) and that virus levels rise once the mite is established (Carreck et al, 2002). However, interactions may be complex and no data exist on the specific interaction(s) between *V. destructor* and *Nosema* (*N. apis* and/or *N. ceranae*).

N. ceranae affects the gut of worker honey bees (*A. mellifera*) and so could increase the likelihood that pathogens are able to enter the body following oral ingestion. Interactions of *N. ceranae* with other pathogens are therefore considered by the authors to be likely.

Studies into the cause/s of Colony Collapse Disorder (CCD) by van Engelsdorp et al (2009) and van der Steen et al (2009) implicated a number of pathogens in combination. Three viruses have been associated with *N. apis* (Bailey 1981) but these associations were not thought to be harmful. However, studies that focus directly on potential interactions between *Nosema* species and other pathogens have not been identified.

Furthermore, factors affecting immunity may also influence the pathogenicity of *N. ceranae* and indeed all pathogens. However, when Johnson et al (2009) investigated this indirectly by examining gene expression levels in the gut of worker honey bees (*A. mellifera*) from colonies affected or not affected by CCD, their results did not show increased expression levels of immune function genes.

Summary

There is evidence to suggest that *N. apis* maybe associated with three viruses. Viruses are also known to infect/kill honey bee colonies and can have a significant impact in combination with *Varroa destructor*. However, it should be noted that no direct information on the interactions of *N. ceranae* with other pathogens has been identified.

2.5 Treatment Methods

The Veterinary Medicines Directorate have identified three products authorised for the treatment of *Nosema apis* in at least one of the 27 EU member states, as summarised in Table 2.1 (VMD, 2009).

Table 2.1: Products Authorised for the Treatment of Nosema Apis in EU Member States

Trade Name	Active Ingredient	Member State/s	Notes
Nonosz	Ortho-hydroxybenzoic acid (salicylic acid) sodium salt	Hungary and Slovakia	External application product for the treatment and prevention of *Nosema apis*
Formidol Dosticky	Formic acid	Slovakia	External application product to decrease the summer and after summer population of *Varroa destructor*, *Nosema apis* and *Ascosphaera apis*
Fumidil B	Fumagillin biclohexylamine salt	United Kingdom	Internal treatment for the control of *Nosema* species in honey bees

The effectiveness of salicylic acid and formic acid for the treatment of either *N. apis* or *N. ceranae* could not be established as part of this study. However, Nonosz and Formidol Dosticky appear to be external treatments to remove spores from hives rather than internal treatments of *Nosema* infections in adult *A. mellifera*.

N. apis can be controlled by the antibiotic fumagillin (trade name Fumidil B). This is normally fed to honey bee colonies in the autumn and/or spring, dissolved in sugar syrup. The fumagillin provides an ongoing source of antibiotic as it is quite stable in the syrup fed to the bees, stored syrup and honey.

The fumagillin syrup also acts as additional winter food for the colony and treatment may therefore be linked with general hive preparation for winter. In addition, recent research in Canada (Williams et al, 2008) and Spain (Higes et al, 2008) both indicate that fumagillin is also effective against *N. ceranae*.

Fumagillin is proven to be effective in controlling infections of both *N. apis* and *N. ceranae* but recent data suggest that it may have carcinogenic/mutagenic properties (COM, 2009). It is understood that further testing is being conducted to confirm or refute this suggestion. However, in the likely event of the future withdrawal of this product from UK shelves suitable alternative replacements need to be identified.

Defra have commissioned a research project to be carried out by the Food and Environment Research Agency (Fera) to "evaluate the field efficacy of the fungicide enilconazole (trade name Imazalil) as a potential alternative chemical treatment for the control of *Nosema spp*" (Project VM0139). The project will be completed in 2010 but results were not available for evaluation as part of this study (Defra 2009).

Interest by amateur beekeepers has been expressed in the potential of acidic syrup feeds as treatments of *Nosema* infections in adult *A. mellifera*. However, a study of such feeds for the treatment of *N. apis* found them to be ineffective (Forsgren & Fries, 2003).

Beekeeping methods that reduce the number of infective spores in stored wax combs may reduce the impact of infections on bee colonies (Fries, 1993; Bailey, 1981 and Furgala & Mussen, 1990).

Such methods include replacing old wax combs with new foundation (wax sheets) and keeping colonies populous and healthy, and thus more able to maintain clean combs. However, whilst this practice is thought to help there is no evidence that demonstrates its effectiveness. In particular, it is not known to what degree spore-contaminated equipment is significant in the build up of infections of *N. ceranae*.

Excessive management of hives is also implicated as a factor increasing *N. apis* levels due to the stress that this can cause to the bees and because worker bees may be crushed during hive manipulation releasing gut contents (including *N. apis* spores) into the hive (Fries, 1993 and Bailey, 1981).

N. apis spores contaminating stored hive equipment (e.g. boxes of wax combs) can be killed by various treatments. Fumigation with ethylene oxide gas is effective but is not practical (Fries, 1993 and Michael, 1964). Gamma irradiation can be used to kill the spores of any microorganism but is not generally practicable, as hive equipment must be taken to a radiation facility (Hornitzky, 1986; Ratnieks, 1989 and Ratnieks et al, 1996).

More practical to beekeepers are storage of combs at moderate temperatures (49°C) (Fries, 1993 and Cantwell & Shimanuki, 1969) and fumigation with acetic acid vapours (Fries, 1993; Bailey, 1981 and Furgala & Mussen, 1990). It should be noted that heat treatment is not generally a practical method for killing spores on stored combs because the wax combs melt at a relatively low temperatures.

There are also recent data to show that *N. ceranae* spores can be killed by freezing (Fries & Forsgren, 2009, results presented in Fries, 2009). After 1 week at -18C a 1000 spore dose of *N. ceranae* was not effective at infecting worker bees and at a dose of 10,000 spores the infection rate rose to only 10%. It should be noted that such freezing was not effective on *N. apis* spores (100% infected at both doses).

3. Likelihood of Impacts (Geographical Spread)

Note on Sample Sizes
In this section studies are considered which have used different sample sizes and methods for determining presence or absence of a pathogen. It should be noted that in many cases a small number of samples have been assayed and therefore the results are less statistically robust than if a large number of samples had been considered. This section briefly highlights the issues.

If a pathogen has recently been introduced to an area it would not be expected to be common and hence many colonies would have to be sampled to definitively detect or reject the presence of the pathogen in that area. If the aim is to monitor the spread of a pathogen then the sampling should be repeated at intervals.

Positive results from a small number of samples can flag up the presence of a pathogen in an area. However further samples will need to be assessed in order to confirm the presence of the pathogen and to determine whether the pathogen is limited to one colony or spread across many or all colonies in a particular area. Furthermore, a negative result from a small sample may not be sufficient to state that a pathogen was not present.

These points should be borne in mind when considering the conclusions drawn below.

3.1 Evidence from Around the World

Nosema ceranae
As stated in the introduction, it was in 1996 that *N. ceranae* was first described as infecting Asian honey bee workers (*Apis cerana*) from colonies in Beijing, China from samples taken a few years earlier (Fries et al, 1996). The first infections of *A. mellifera* were determined from samples of worker bees collected in 2005 in Taiwan (Huang et al, 2007) and Spain (Higes et al, 2006).

N. ceranae is now found to infect *A. mellifera* in all continents and in most countries from which samples have been examined using DNA markers to differentiate *N. ceranae* from *N. apis*. Klee et al (2007) analysed samples of *A. mellifera* from 13 countries worldwide including 9 in Europe, 2 in the Americas (Brazil and USA), 1 in Asia (Vietnam), and 1 in Australasia (New Zealand).

The 9 European countries studied were Denmark, Finland, Germany, Greece, the Republic of Ireland, UK (Northern Ireland), Italy, Serbia, Spain and Sweden. Data relating to Northern Ireland was recorded (correctly) as relating to the UK but geographically may best be associated with the Republic of Ireland.

Country samples for 2004 to 2006 (most were 2006) were negative only for Ireland and New Zealand, with *N. ceranae* present in the other 11 countries. In the same samples, *N. apis* was detected in 5 countries, 4 of them in Europe including the UK.

Samples from before 1990 were available for 4 European countries, none of these contained *N. ceranae* but all contained *N. apis*. Subsequently, *N. ceranae* has also been found in samples of *A. mellifera* from North Africa (Higes et al, 2009a) and Australasia (Giersch et al, 2009).

N. ceranae was found in Uruguay from a single sample taken in 1990 (and also in further samples from 2007-8) (Invernizzi et al, 2009), in the USA from samples taken in 1995 (Chen et al, 2009) and in Finland from a sample taken in 1998 (Paxton et al, 2007). These findings indicate that *N. ceranae* has been present in the continents of South America, North America and Europe for over a decade.

The latest evidence for Europe shows that *N. ceranae* has also been found in *A. mellifera* samples from the UK (Budge 2008), the Republic of Ireland (McCabe, 2009), Hungary (Tapaszti et al, 2009) and the Netherlands (van der Steen et al, 2009). In other continents *N. ceranae* has been identified in *A. mellifera* samples from Argentina (Sarlo et al. 2008), Canada (Williams et al, 2008a and Pernal, 2009) and Uruguay (Invernizzi et al, 2009).

N. ceranae has also been detected in bumble bees (*Bombus* species) in Argentina (Plischuk et al, 2009).

Pattern of Spread

Some recent studies suggest that wherever *N. ceranae* occurs it predominates over, and may even replace, *N. apis*. Stored samples have allowed the determination of temporal trends in the prevalence of *N. apis* and *N. ceranae* in southern Finland (Paxton et al, 2007). Paxton et al studied 40 colony samples and identified three distinct periods of time:

(1) no *N. ceranae* present. This period occurred prior to 1995 (Samples were analysed from six colonies, all six of which were infected with *N. apis* with no colonies infected with *N. ceranae*);
(2) *N. ceranae* found as a mixed infection in only some colonies. This period extended from 1998 to 2002 (Samples were analysed from twelve colonies, ten of the twelve were infected with *N. apis* alone, and the remaining two colonies were infected with both *N. apis* and *N. ceranae*. No colonies were found to be infected with *N. ceranae* alone); and
(3) *N. ceranae* found in all colonies, either alone or as a mixed infection with *N. apis*. This period extends from 2006 to the present. (Samples were analysed from ten colonies, none of the colonies were infected with *N. apis* alone, five colonies were infected with both *N. apis* and *N. ceranae* and five colonies were infected with *N. ceranae* alone).

The three periods described above are displayed in Figure 3.1.

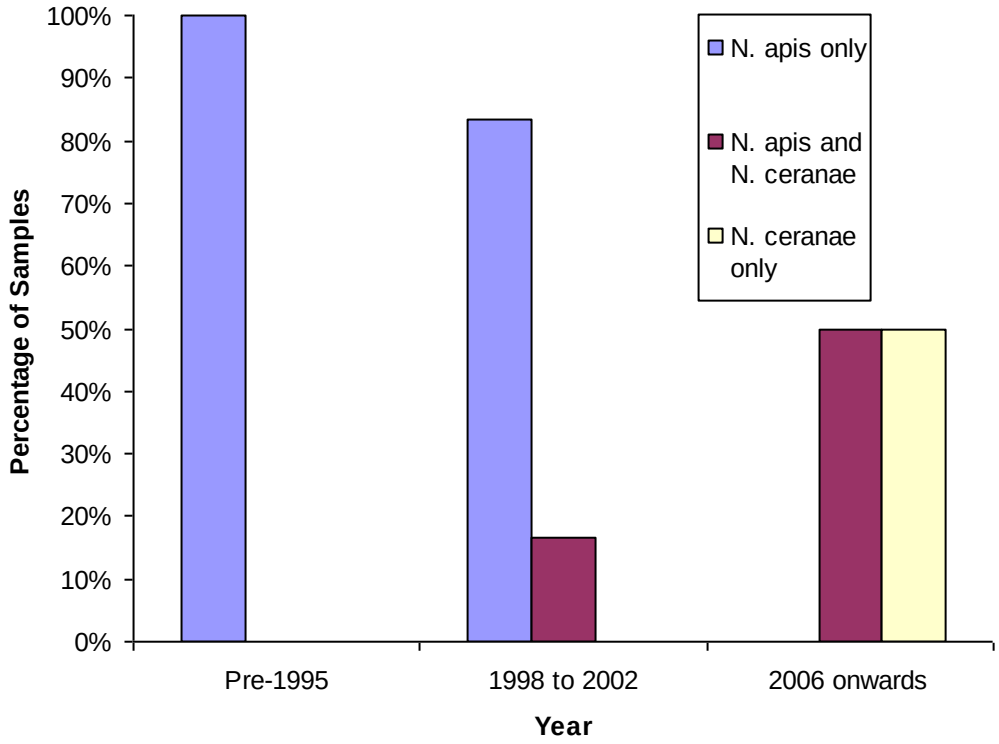

Figure 3.1: *N.* species Infections in Finland

The evidence that *N. ceranae* is spreading and even predominating over *N. apis* in some countries is further supported by data from studies undertaken on samples of *A. mellifera* taken from colonies in the Netherlands, Uruguay and the USA, as detailed below.

Samples of worker *A. mellifera* were taken from 170 apiaries across the Netherlands (5 hives per apiary) across the whole country in June 2008 (van der Steen et al, 2009).

10% of the samples were positive for *N. apis* and were mainly from the north. 87% were positive for *N. ceranae*, which is found throughout the country. Samples negative or positive for both species were infrequent. Of 13 samples that had *N. apis*, 11 (85%) of these also had *N. ceranae* but of the 153 samples that had *N. ceranae*, only 7% also had *N. apis*.

From the Netherlands study it is clear that *N. ceranae* was widespread. Although the data came from a single sampling period, the extensive geographic coverage suggests that *N. ceranae* may be replacing *N. apis* from south to north.

In Uruguay, 29 samples of honey bees infected with Nosema were analysed and only *N. ceranae* was detected. In the USA, Chen et al (2008) found only *N. ceranae* in their samples.

Several large studies have been carried out on Colony Collapse Disorder (CCD) in the USA. In these studies, samples of honey bee workers from hives and apiaries with and without CCD have been analysed for a wide range of pathogens and provide information on levels of *N. apis* and *N. ceranae*.

Van Engelsdorp et al (2009) collected samples in January and February 2007 from 13 apiaries in Florida and California. In terms of prevalence, *N. ceranae* was found in approximately half the colonies (55% in CCD colonies, 50% in normal [non-CCD] colonies) and *N. apis* in approximately one quarter (28% in CCD colonies, 18% in normal colonies).

Chen et al (2008) detected *N. ceranae* but not *N. apis* in samples from Oregon, California, Hawaii, Idaho, North Dakota, Minnesota, Texas, Ohio, Tennessee, Connecticut, Maryland and Florida. This would support the statement that *N. ceranae* is more prevalent than *N. apis.*

Cox-Foster et al (2007) analyzed samples of 4 to 15 worker bees collected per colony between 2004 and 2007 from 30 CCD colonies and 21 non-CCD colonies from Arizona, California, Florida, Georgia, Louisiana and Pennsylvania. Overall, *N. ceranae* was found in almost all the colonies (100% in CCD colonies, 81% in non-CCD, 92% overall) and *N. apis* in three quarters (90% in CCD colonies, 48% in non-CCD, 73% overall).

Johnson et al (2009) analysed samples taken from various states in 2006 and 2007 and found that 66% of the colonies were infected with *N. ceranae* and 11% with *N. apis*.

In a Swedish study, 319 samples (identified as being infected with *Nosema* species by light microscopy) were analysed to determine the *Nosema* species responsible (Fries and Forsgren, 2008 cited in Fries, 2009). It was found that 17% of samples were mixed infections of *N. ceranae* and *N. apis*, 83% were infected with *N. apis* alone and none with *N. ceranae* alone.

The low prevalence of *N. ceranae* in Sweden, as compared to Finland, despite the similar climate, may be because Finland imports queen bees from Southern Europe whereas Sweden does not (Fries 2009). However, only time will tell whether the spread of *N. ceranae* in Sweden will follow the pattern identified in Finland.

Martín-Hernández et al analysed 290 samples from apiaries in France, Germany, Spain and Switzerland which show a significant predominance of *N. ceranae* over *N. apis* (Martín-Hernández et al, 2007). The samples were sent in by beekeepers mostly in 2006 but some were supplied in 2005 and 2003. Between 10 and 20 *A. mellifera* workers per sample were pooled, the abdomens were removed and macerated in water.

The results of this study are displayed in Table 3.1.

Table 3.1: *Nosema* Infections Identified by Martín-Hernández et al (2007)

Result	Samples Analysed									
	France		Germany		Spain		Switzerland		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Absence of *N.* species	3	8.3	8	11.6	65	43.6	12	33.3	88	30.3
***N. ceranae* alone**	27	75	54	78.3	52	34.9	23	63.9	156	53.8
***N. apis* alone**	0	0	5	7.2	21	14.1	1	2.8	27	9.3
N. apis* and *N. ceranae	6	16.7	2	2.9	11	7.4	0	0	19	6.6
Total	36	100	69	100	149	100	36	100	290	100

The samples used by Martín-Hernández et al (2007) were not random and there is no information to indicate the region of origin within of the countries concerned. In addition, although most samples were from 2006, not all samples were collected in that year and so annual variability may have biased the results to some extent. However, these findings mirror those from Finland and the Netherlands and add to the weight of evidence towards the conclusions that *N. ceranae* is spreading and predominating over *N. apis* in these and other countries.

The findings of Martín-Hernández et al (2007) are therefore of interest to this study and hence are displayed graphically in Figure 3.2.

Summary of the Situation Around the World

Evidence from Europe, North and South America suggest that *N. ceranae* may be steadily replacing *N. apis* in colonies of Western honey bees (*A. mellifera*). However, the data are as yet far from conclusive on this matter (Fries, 2009).

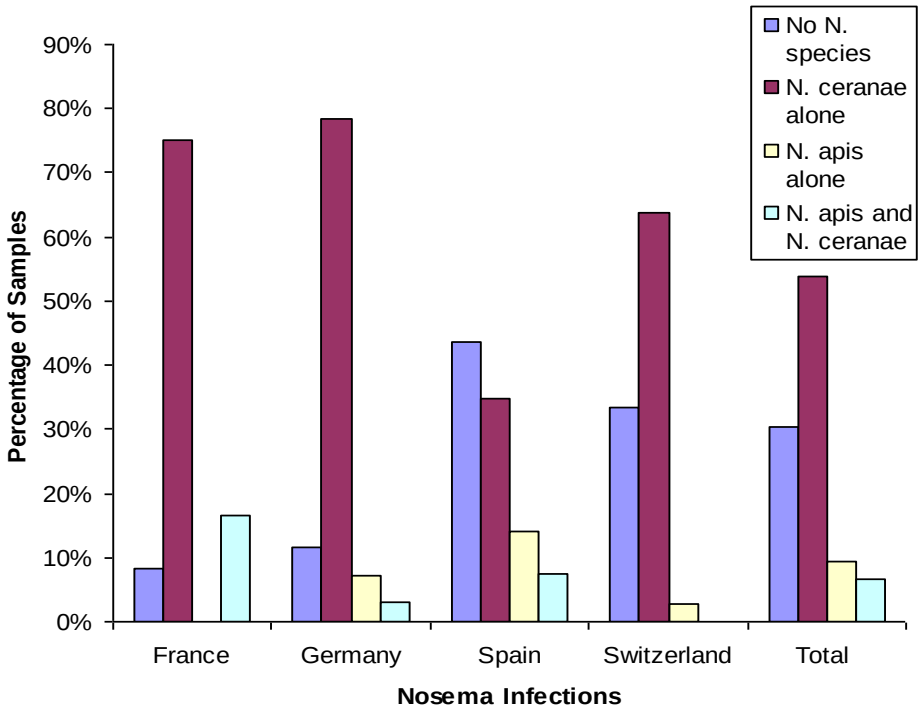

Figure 3.2: % *Nosema* Infections Identified by Martín-Hernández et al (2007)

3.2 The Situation in the UK

The Presence of Nosema ceranae

In the British Isles, to date *N. ceranae* infections have been confirmed in six English counties (Cornwall, Essex, Lincolnshire, Hertfordshire, Greater London and North Yorkshire) and 3 Welsh counties (Glamorgan, Powys and Dyfed) from samples taken in 2007 (Budge, 2008).

In total, 471 DNA extracts from 421 colonies (268 colonies in 2007 and 153 colonies in 2008) were tested for the presence of both *N. ceranae* and *N. apis*. Overall, *N. apis* was found in twice as many (10%) of the samples as *N. ceranae* (4.5%), with 1% testing positive for both species (PH0505, 2009).

Two samples from Northern Ireland taken between 2005 and 2006 and one from the Republic of Ireland (Tipperary) in 2005 were negative for *N. ceranae* (Klee et al. 2007). However, as noted in Section 3.1, *N. ceranae* has subsequently been identified in the Republic of Ireland (McCabe, 2009).

Figure 3.3 displays the English and Welsh counties with confirmed infections of *N. ceranae*. From the wide spread of the counties infected it is possible that *N. ceranae* is present but as yet unconfirmed throughout England and Wales.

As part of the healthy bees plan a random apiary survey is being carried out to investigate the national incidence of the full range of bee pests and diseases. It is hoped that the results will provide an accurate picture of pest and disease incidence, including those that occur rarely, which should provide a clear picture of the spread of the two *Nosema* species within in England and Wales.

Figure 3.3: UK Counties with Confirmed Infections of *N. ceranae*[2].

Pattern of Spread

Data from the Netherlands (van der Steen et al, 2009), a country with a very similar climate and with similar honey bees to Britain, are probably the best available guide to the likely pattern of spread of *N. ceranae* infections across the British Isles. These data suggest that *N. ceranae* will become more common and may come to predominate over *N. apis*.

Data from Uruguay and Finland indicate that the total replacement of *N. apis* is a possibility. However, it should be noted that the climate in Uruguay is very different from the climate in England and Wales or that across the British Isles as a whole.

While, evidence from Finland suggested that all colonies of *A. mellifera* infected with *Nosema* species in England and Wales will include infection with *N. ceranae,* Martín-Hernández et al (2007), it should be noted that infection rates may not reach this level.

Data relating to spread of *N. ceranae* in the Netherlands and Finland are based on large numbers of samples which together with data from other countries such as Spain and France add to the weight of evidence towards the conclusions stated here. However, it should be noted that these conclusions are suggestions only and the British situation can only be determined from samples collected in Britain.

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[2] Map sourced and enhanced using SmartDraw 2008. Map was provided by Google Maps.

Summary of the Situation in the UK

At least one strain of *N. ceranae* has been confirmed in five single or multiple-county areas widely dispersed across England and Wales (see Figure 3.3). It is possible therefore that *N. ceranae* is already widespread across the UK and so there is a high potential for impact.

4. Consequences

A draft assessment of the potential impacts of *N. ceranae* on England and Wales was conducted at an early stage in the study. However, due to the high degree of uncertainty and in consultation with the customer, it was agreed that this initial assessment was unsuitable for making robust policy decisions at this time. Therefore, more detailed studies on the impacts were not carried out.

The key uncertainties identified are summarised below:

Geographical Spread: Data available to this study were sufficient for an estimation to be undertaken into the spread of *N. ceranae* across England and Wales. However, it is understood that results from the random apiary survey being carried out as part of the Healthy Bees Plan are due shortly (in 2010) and these will provide an accurate assessment of the spread of *N. ceranae* and other pathogens (see Section 5.1). It was therefore considered premature to proceed with detailed assessment at this time;

Value of a Honey Bee Colony: A range of studies were assessed in order to estimate the value of a colony of honey bees. However, it is understood that two studies due to be published in 2010 will provide important new data on the value of a honey bee colony to the UK economy (see Section 5.3). It was therefore considered premature to proceed with detailed assessment at this time; and

Virulence of *N. ceranae*: The data available for this study indicate that it is possible that *N. ceranae* may be universally fatal to all infected honey bee colonies; equally it is possible that *N. ceranae* may be no more virulent than the *N. apis* which is already widespread in the UK. The high level of uncertainty regarding the virulence of *N. ceranae* is the major reason why further detailed assessments were not carried out as part of this study.

5. Response

5.1 Geographical Spread

This study has identified gaps in current data on the identification of infections of *Nosema* species across England and Wales. As part of a ten year plan to protect and improve the health of honey bees (*A. mellifera*) in England and Wales, the National Bee Unit (NBU) is undertaking a survey to assess the health status of apiaries in England and Wales during 2009 and 2010.

This survey will include assessment of the prevalence of *N. apis* and *N. ceranae* and will determine the current spread of *Nosema* species. However, until the findings of NBU study are known it is not possible to say whether further research should be considered.

It is apparent however that further data detailing when *N. ceranae* first infected honey bees in England and Wales would improve the accuracy with which a future impact assessment could construct a baseline scenario. The determination of such data may be possible from an analysis of historic *A. mellifera* samples from British honey bee colonies.

It is understood that two researchers, Dr Paxton at Queens University, Belfast and Dr Hughes at Leeds University, are currently undertaking research on *Nosema ceranae* in the UK. Until the findings of all of these research efforts are published it is not possible to say whether further research to construct a robust baseline for future impact assessment is appropriate.

5.2 Virulence

Two recent reviews of current research into the virulence of *N. ceranae* draw attention to the need for studies that are not simply based on correlations (Fries, 2009 and Paxton, 2010). Paxton (2010) states, "Experimental infection of colonies is also necessary to show causation between *N. ceranae* and colony collapse; such experiments are currently lacking".

Further research is therefore suggested to determine whether or not there is a link between *N. ceranae* infection and colony mortality, and to determine the nature of any such link.

Evidence that *N. ceranae* is significantly more virulent than *N. apis* mostly relates to studies undertaken of Spanish *A. mellifera* infected by a strain of *N. ceranae* which may be different from those identified in the USA, Uruguay and elsewhere in Europe. It is therefore suggested that any future research into both the virulence of *N. ceranae* and its spread in the UK should differentiate between different strains of this pathogen.

It is suggested that further studies into the virulence of *N. ceranae* take into account both the possible variation in virulence of different strains of *N. ceranae* and the possibility that there may be increased susceptibility of Spanish *A. mellifera* compared to other strains of *A. mellifera*.

It is not known to what degree spores contaminating equipment are significant in the build up of infections of *N. ceranae*. It is therefore suggested that research be conducted into this area and into the effectiveness and practicality of hive management techniques in the control and management of *N. ceranae* infections including the freezing of hive equipment. Furthermore, the interactions between *N. ceranae* and other pathogens are unclear and therefore further research into such interactions is suggested.

5.3 Valuing a Colony of Honey Bees

It is understood that two studies due to be published in 2010 will provide important new data on the value of a honey bee colony to the UK economy. One study, for Fera by Marris et al, will apply the principle of replacement pollination costs to the valuation of honey bee pollination services in the UK (details from poster presentation of unpublished results Marris et al, 2009).

A further study being undertaken at Reading University is not yet published but is understood to have calculated the proportion of honey bee pollination services relative to other pollinators in the UK to be significantly different to those from previous studies (Postnote 348, 2010).

Detailed findings from the two studies mentioned above were not available for this study. It is therefore suggested that an estimation of the value of a colony of honey bees should await publication of these studies since it is possible that they may significantly change any future impact assessment.

5.4 Impact of *N. ceranae*

It is suggested that the impact of *N. ceranae* on the UK be estimated in terms of predicted number of colonies lost and cost to the UK economy. Given the potential for impact, it is suggested that such an estimation is made as soon as sufficient data are available on the virulence of *N. ceranae* to allow development of a sufficiently robust indicator for policy decision making.

Annex 1: Literature Review

A1.1 Introduction

A detailed literature review of peer reviewed published and other relevant material on the two *Nosema* species, *N. ceranae* and *N. apis*, that infect the Western honey bee *Apis mellifera* has been undertaken. Information has been gathered on the extent of the impact of both *Nosema* species on the UK and other countries, the measures taken to treat infections and control their spread, and the

success or failure of these measures in different environments (i.e. climate conditions, commercial hives, etc.).

The literature review has sought to gather background information on the two *Nosema* species and also to provide a basis for the subsequent tasks of this study. Where gaps have been identified these have been documented along with indications of potential future research needs.

Particular preference was given to sourcing information of greatest relevance to the UK. The publication of honey bee research is not limited to a number of specific journals so the literature review included published material from a wide range of journals.

A1.2 *Microsporidia*

Nosema are a genus of *Microsporidia*, a phylum of specialist unicellular fungi that are intracellular parasites of animals. They affect a wide range of host organisms, especially insects. They produce spores that act as a vehicle for dispersal and infection. Infection is mediated by a special organ, the polar filament, that pierces a host cell to inject the infectious contents of the spore, including genetic material, into the host cell.

Two species of *Nosema, N. apis* and *N. ceranae*, infect gut (ventriculus) epithelial cells of the Western honey bee (*Apis mellifera)*. Other *Nosema* species are pests of other bee species, including *N. bombi* which infects bumble bees, and other insects and *N. bombycis* which infects silk moth larvae and causes pebrine disease (Goulson, 2006).

A1.3 *Nosema apis*

Nosema apis has been known for 100 years and probably has a long history as a parasite of *Apis mellifera* and is the subject of much research that was reviewed by Bailey (1981), Furgala & Mussen (1990) and Fries (1993). *N*. *apis* is found worldwide including in Britain. There is no general agreement on the economic effects of *N. apis* on *A. mellifera* colonies. Some researchers consider it to be a serious disease (Furgala & Mussen, 1990 and Fries, 1993). However, Bailey (1981) notes that others consider it to be of lesser concern. Fries & Camazine (2001) refer to *N. apis* as “benign”.

N. apis can weaken hives by reducing their worker population. It does this by shortening the lifespan of workers and by making infected workers less able to produce the hypopharyngeal gland secretions necessary for feeding brood, thereby slowing colony population build up in spring. It can also harm a colony if the queen is infected. An infected queen is likely to cease egg laying and be superseded. Loss of the queen will often result in a colony having a lower worker population for several months, leading to a lower honey yield and less pollination effectiveness, and may even result in the colony dying if the new queen is unsuccessful in mating.

N. apis is spread from bee to bee via oral contact with spores in the faeces of infected workers leading to the ingestion of spores that germinate in the gut. Faecal transmission is exacerbated if workers defecate in the hive, as may occur under winter conditions when flight is not possible. This is known as dysentery and is recognisable by faecal marks on and inside the hive (worker *A. mellifera* normally defecate outside the nest on cleansing flights even in winter, when cleansing flights occur on warm days).

N. apis levels are generally quantified by macerating worker abdomens in water and counting the spores in a haemocytometer. The spores have a distinctive cylindrical shape and size (Bailey,1981; Furgala & Mussen,1990; Fries, 2009; Fries, 2010 and Paxton, 2010). Spore counts indicate that *N. apis* infections are generally at their peak in autumn and especially spring, and diminish during the summer. Average levels of infection can run to more than 10 million spores per bee. Conversely, a worker bee can be infected by relatively few spores (median infective dose 20-90 spores per bee [Fries, 1993]). As such, *N. apis* infections have the potential to increase rapidly in a colony if faecal contamination occurs. Spores can live for more than one year so that infection from stored hive equipment (e.g. wax combs) can occur.

When *N. apis* affects adult bees there are no macroscopic symptoms that can readily be seen by a beekeeper as is the case in brood diseases (e.g. sac brood, American foulbrood, European foulbrood and chalk brood) in which dead brood can be seen in their cells or *Varroa destructor* mites, which are large enough to be seen by the naked eye. In addition, shortening of the worker life span is not easily detectable. Infected workers forage at an earlier age and foragers normally die away from the hive. Lack of consensus about the seriousness of *N. apis* may be because it is not possible for a beekeeper to directly see the harm being done by infection to the colony. Even spore levels in worker bees are not a direct indication of harm although they are a good indication of the extent of infection. Furgala and Mussen (1990) note that the damage done by *N. apis* "must not be measured by colony mortality" but by reduced honey yields.

N. apis can be controlled by the antibiotic fumagillin, known commercially as Fumidil B. This is normally fed dissolved in sugar syrup in the autumn and in spring. Furgala and Mussen (1990) recommend 8 litres of syrup with 20 to 25 mg of active ingredient per litre. Fed in this way, the fumagillin provides an ongoing source of antibiotic as it is quite stable in the syrup being fed to the bees. The syrup also acts as additional winter food for the colony, so that autumn treatment with Fumidil B can be linked with general hive preparation for winter.

N. apis can also be controlled by beekeeping management methods that are aimed at reducing the number of infective spores in stored wax combs. These include replacing old combs with foundation (wax sheets that the bees draw into cells) and keeping strong colonies so that the large number of worker bees has time in the summer to clean the combs, including any additional used combs added by the beekeeper to give the colony more space for honey storage and population build up. Weather conditions are an important factor and severe winters, which prevent cleansing flights, and poor summers, which reduce foraging and colony population build up, are both considered to increase infection rates via increased faecal contamination and reduced cleaning of combs. Manipulation of hives is also implicated as a factor increasing *N. apis* levels due to the stress that this can cause to the bees and because worker bees can be crushed during hive inspection releasing gut contents, including *N. apis* spores, into the hive.

N. apis spores contaminating stored hive equipment (e.g. boxes of wax combs) can be killed by various treatments. Fumigation with ethylene oxide gas is effective but is not practical. Gamma irradiation can be used to kill the spores of any microorganism but is not generally practical as hive equipment must be taken to a radiation facility. More practical to beekeepers are storage of combs at moderate temperatures (49°C) or fumigation with acetic acid vapours. It should be noted that heat treatment is not generally a practical method for killing disease organisms or their spores that contaminate stored combs because the wax combs melt at a relatively low temperature.

A1.4 *Nosema ceranae*

Nosema ceranae was described in 1996 as a parasite of the Asian honey bee *Apis cerana*, based on samples from Beijing, China (Fries et al, 1996). DNA markers, particularly 16s the small sub-unit ribosomal RNA (i.e., a length of DNA in the nuclear genome), are used to differentiate *N. ceranae* from *N. apis* (Fries et al. 1996). Samples taken in 2005 showed infections in *Apis mellifera* in Taiwan (Huang et al, 2007) and Spain (Higes et al, 2006). Subsequent research shows that *N. ceranae* has a worldwide distribution in *A. mellifera* and has been present in Europe, North America and South America for at least 10 years, maybe even 20 years. *N. ceranae* is now more frequently detected than *N. apis* in most countries in Europe but there is not yet sufficient data to determine whether this is also the case for Britain (Budge, 2008).

Current concerns about honey bee colony mortality, including Colony Collapse Disorder (CCD), mean that there is considerable interest in honey bee pathogens and diseases. As a novel parasite of *A. mellifera*, there is understandable concern that *N. ceranae* may be harmful and may even be the cause of CCD. There is the risk that a novel parasite such as *N. ceranae* may simply be blamed for CCD because its appearance correlates well with the advent of CCD. In addition, there is also the possibility that a new parasite such as *N. ceranae* may interact with other pathogens, especially viruses, causing normally benign viruses to become harmful. Bailey (1981) notes that three types of virus, including Black Queen Cell Virus, are associated with *N. apis*. It is also known that the mite *Varroa destructor*,

which also originated on *Apis cerana* and which has been present in Britain for approximately 20 years can exacerbate virus diseases (Ratnieks & Carreck, 2010).

A1.4.1 Is *N. ceranae* the Cause of Colony Collapse Disorder (CCD)?

On balance, the current evidence does not suggest that *N. ceranae* is a universal cause of Colony Collapse Disorder (CCD). However, it should be noted that CCD is a syndrome involving the death of colonies, with dwindling/collapsing worker populations despite abundant food sources and without large numbers of dead bees being present inside or immediately outside the hive. There is no fundamental reason why this syndrome should have the same cause at all locations. However, it should be noted that CCD is not considered to occur in Britain (although colony death/loss does occur in Britain). It is therefore unhelpful to dub every unexplained colony death as CCD, especially given that CCD is not well understood. In principle, it could be caused by any agents or factors or combinations of these that kill adult worker honey bees away from the colony, including *Nosema*. A wide variety of possible factors have been suggested, including unlikely candidates such as mobile phones interfering with navigation, GM crops etc. Serious attention has been given to pathogens and pesticides (Ratnieks & Carreck, 2010).

In experiments carried out in Spain in which colonies with naturally occurring infections of *N. ceranae* were studied, colonies with *N. ceranae* suffered almost 100% mortality, and mortality could be reduced close to zero by treating colonies with fumagillin, which is known to be effective against both *N. apis* and *N. ceranae* (see also below) (Higes et al, 2008). In addition, the corpses of worker bees from a nectar foraging site had very high levels of *N. ceranae*, indicating a possible role of *N. ceranae* on individual mortality. *N. ceranae* was also implicated in the loss of colonies owned by professional beekeepers in Spain (Higes et al, 2009b). In samples provided by beekeepers from colonies in Spain, the presence of *N. ceranae* (alone or in combination with *N. apis*) increased the risk of a colony dying by a factor of almost six. In contrast, the presence of *N. apis* alone was not found to increase the risk of colony mortality (Martín-Hernández et al, 2007).

In the USA, the presence of *N. ceranae* and *N. apis* are poorly correlated with severe CCD symptoms in infected hives versus controls (Cox-Foster et al, 2007, van Engelsdorp et al, 2009). Cox-Foster et al (2007) suggested that Israeli Acute Paralysis Virus (IAPV) was a significant risk factor in CCD. However, van Engelsdorp et al (2009) found a much poorer link between CCD with specific risk factors including pathogens. Van Eaglesdorp et al (2009) investigated 61 quantifiable variables including ca. 18 pathogens and pests as well as pesticides, and colony and bee conditions. The P (probability) values for effects of either *N. apis* or *N. ceranae* prevalence or load on the presence or absence of CCD were all non-significant ($P > 0.05$). The only P value that came close to being significant, 0.06, was for an unexpected trend in which samples of worker bees from non-CCD apiaries had higher *N. ceranae* prevalence than samples from CCD apiaries. In other words, the opposite to what would be predicted if *N. ceranae* was linked to CCD.

In Uruguay, the replacement of *N. apis* by *N. ceranae* has not been accompanied by an overall increase in colony mortality (Invernizzi et al, 2009). However, researchers in Spain are convinced that *N. ceranae* is a major cause of colony mortality. The current data do not allow a definitive statement to be made to the effect that *N. ceranae* either is or is not a cause of significant losses and/or weakening of *A. mellifera* colonies in Britain.

Three reviews of *N. ceranae* have been written recently, and these all make comments on its role in CCD. The review of Fries (2010) “Nosema ceranae in European honey bees (*Apis mellifera*)” is not yet published. Fries is a Professor at the Swedish Agricultural University in Uppsala. He has 25 years experience studying honey bees and *Nosema apis*, and is also the discoverer of *N. ceranae*. His long experience with bees and *Nosema* means that his review and opinion carries weight. Fries writes “Several studies from Spain suggest that *N. ceranae* is a colony level virulent parasite and that infections eventually lead to colony collapse unless the infections are controlled. However, most published data on colony losses linked to *N. ceranae* infections are correlations and fail to provide evidence of cause and effect”. This is true, but the same could be said about most research being done on colony losses including the two large studies done in the USA (Cox-Foster et al, 2007, van Eaglesdorp et al, 2009).

The recent review by Paxton (2010) entitled "Does infection by *Nosema ceranae* cause Colony Collapse Disorder in honey bees (*Apis mellifera*)?" was published on 5 January 2010 in a volume of the Journal of Apicultural Research that is dedicated to papers on honey bee colony losses. Paxton is a Lecturer in Genetics at Queens University, Belfast. He has 25 years experience studying bees, but is not exclusively a honey bee biologist. He worked on *Nosema bombi*, a pathogen of bumble bees, before beginning work on *N. ceranae*. Like Fries, with whom he collaborates, his long experience with bees and *Nosema* means that his review and opinion carries weight. He writes "Though it has been dismissed as a cause of CCD in the USA based on correlational analyses of snapshot sampling of diseased hives, observations of naturally infected colonies suggest that it leads to colony collapse in Spain". Paxton also echoes the concern of Fries (2009) by drawing attention to the need for studies that are not simply based on correlations thus, "Experimental infection of colonies is also necessary to show causation between *N. cerana*e and colony collapse; such experiments are currently lacking".

Paxton also notes that, "Spanish *A. mellifera* may be more susceptible to *N. ceranae* than other honey bee races, or the variant of *N. ceranae* within Spain may be more virulent than that found elsewhere, suggestions which deserve attention". There is evidence that not all strains of *N. ceranae* infecting *Apis mellifera* are the same. In particular, detailed genetic studies involving DNA sequencing show that *N. ceranae* from North America and Spain are not the same strain (Williams et al, 2008a) and also that the strains of *N*. *ceranae* found in China and the USA are not the same (Chen et al. 2009).

The third review is by Parnell; he writes a blog, is based in British Colombia, Canada, and says of himself, "I'm a professional biologist and environmental writer. My writing explores the broad topic of sustainable resource management - what it is, and the science and policy that support and guide it". On 26 September 2009 Parnell uploaded a lengthy essay entitled "Honey Bee Colony Collapse Disorder (CCD) VII: IAPV, *Nosema ceranae*, and CCD I". He has been following the honey bee/CCD study for several years. What he writes seems quite well balanced and is a useful perspective, but does not benefit from being personally involved with the research in the way that Fries and Paxton are. His writing pulls in some journalist-written articles and background information that would not normally find its way into a scientific paper. He comes down in favour of *N. ceranae* being causal writing, "The weight of scientific evidence favours *Nosema ceranae* over IAPV as a cause of CCD". This conclusion seems to be based, in part, simply on their being more papers on *N. ceranae*.

A1.4.2 Cage Studies

Studies of *Nosema* are often made using worker *A. mellifera* confined in small cages with food. The bees are generally newly emerged from their pupae and are dosed orally with a spore suspension in syrup. Several studies have been carried out with *N. ceranae* and show that it easily infects workers. However, cage studies need to be interpreted with caution as a cage is a very unnatural environment for honey bees.

Paxton et al, (2007) suggest higher virulence caused by *N. ceranae* versus *N. apis* on caged bees, but urge caution in interpreting their preliminary results based on a small sample size. Higes et al (2007) found very high mortality of caged workers fed *N. ceranae* spores and took this as evidence for high virulence. However, they treated their caged workers with extremely high doses of spores (100,000), much higher than is usual in cage studies and probably at a level that exceeds natural infection via the faecal-oral route, and certainly more than the c. 20-90 spores needed to infect a worker bee (Fries, 1993).

A1.4.3 Control of *N. ceranae*

In the event that *N. ceranae* proves to be harmful to *A. mellifera* colonies, there are several possible remedies. Recent research in Canada (Williams et al, 2008b) and Spain (Higes et al, 2008) both indicate that fumagillin is also effective against *N. ceranae*.

The study of Higes et al, (2008) is of particular interest as there was good evidence that *N. ceranae* was killing colonies in Spain. In Experiment 2, 50 colonies were set up and tested for *N. ceranae* in October 2006. 18 colonies positive for *N. ceranae* were treated with fumagillin at the recommended dose (120 mg fumagillin active ingredient per colony in 1 litre of syrup, ¼ litre per week for 4 weeks) in

November 2006. The other colonies (13 *N. ceranae* positive, 2 *N. ceranae* positive and *N. apis* negative, and 17 *Nosema* species negative) were treated only with syrup. The treated *N. ceranae* positive colonies were all *N. ceranae* negative on 15 December, with 5/18 and 18/18 becoming *N. ceranae* positive on 26 April and 26 September 2007 respectively. This shows that the effect of fumagillin wears off and that it must be reapplied. None of these colonies died. The untreated negative colonies all were positive for *N. ceranae* on 26 April 2007, although 3 of these were negative on 25 September 2007. None of these colonies died either. Conversely, all but 2 of the untreated positive colonies were dead by 26 September 2007.

The experiments described above were done using normal-sized bee hives. Experiments were also done using smaller "nucleus" hives, and the results mirror those found for the normal sized hives. Colonies (n = 5) that were not treated with fumagillin died, while those that were treated (n = 5) survived. Untreated colonies suffered a gradual loss of worker bees from November to April and tried to compensate by rearing brood during winter, and had more brood than the treated colonies. Williams et al (2008b) studied *A. mellifera* colonies kept by 8 beekeepers in Nova Scotia, Canada. Colonies treated with fumagillin according to the label instructions in September 2006 (n = 94) had significantly lower *Nosema* intensity than did colonies that did not receive treatment (n = 51) in spring 2007 but by late summer 2007 there was no difference. Using molecular analysis it was shown that *N. ceranae* was the predominant species of *Nosema* (93%).

Table A1.1 sets out the median intensity (number of spores/bee) and prevalence (percent of colonies) of *Nosema* in spring (20 April–4 May) and late summer (20–26 August) 2007 in western honey bee (*Apis mellifera*) colonies from 8 beekeeping operations in Nova Scotia, Canada that had been treated or untreated with fumagillin in September 2006 (Williams et al. 2008b).

Table A1.1: Intensity and Prevalence of *Nosema* Species in *Apis mellifera* Colonies

Beekeeper	Number of Colonies	Spring 2007		Late Summer 2007	
		Number of Spores (Median)	Prevalence (%)	Number of Spores (Median)	Prevalence (%)
Untreated with Fumagillin					
1	15	10,725,000	100	1,425,000	80
2	19	2,725,000	74	1,625,000	89
3	17	1,475,000	82	1,875,000	71
Treated with Fumagillin					
4	16	0	31	0	38
5	21	0	29	0	33
6	17	0	6	2,625,000	88
7	20	700,000	70	2,925,000	90
8	20	2,375,000	90	2,687,500	95
Source: Williams et al (2008b).					

The above studies show that fumagillin is highly effective against *N. ceranae*. However, fumagillin has an uncertain future in the EU. Although it is still sold in the UK its regulatory status is problematic.

Research on *N. apis* has shown that spores in stored combs can be killed via fumigation with acetic acid vapour or heat.

There are recent data (Fries & Forsgren, 2009, results presented in Fries, 2010) to show that *N. ceranae* spores can also be rendered less infective (probably killed) by freezing. Caged worker *Apis mellifera* were fed with 10 microlitres of syrup containing 1000 or 10,000 spores of either *N. ceranae* or *N. apis*. A spore suspension was made up using fresh infections, that were used fresh or after one week at +8°C or -18°C. The proportions of bees that became infected (n = 25 per treatment) are set out in Table A1.2.

Table A1.2: Proportions of A. Mellifera that Became Infected with *Nosema* Species.

Nosema Species	Number of Spores used for Infection	Treatment to Nosema Spore Suspension		
		Fresh	1 week +8°C	1 week -18°C

Table A1.2: Proportions of A. Mellifera that Became Infected with *Nosema* Species.				
Nosema Species	**Number of Spores used for Infection**	**Treatment to Nosema Spore Suspension**		
		Fresh	**1 week +8°C**	**1 week -18°C**
N. ceranae	1000	76%	48%	0%
N. ceranae	10000	100%	100%	12%
N. apis	1000	84%	88%	100%
N. apis	10000	100%	72%	100%
Note: Number of bees infected in each case = 12.				

If further research supports this initial study, it would be practical for a beekeeper to place unused boxes of frames into a chest freezer and several boxes could be placed there at one time (beekeepers generally add additional boxes to colonies in the spring and summer). Thus, over the course of 6 months about 100 boxes could be treated for one week using a single freezer.

However, caution is needed in interpreting these results in practical terms. In particular, it is not known to what degree spores contaminating equipment are significant in the build up of infections of *N. ceranae* in colonies of *A. mellifera*. One of the differences between infections of *N. apis* and *N. ceranae* is that *N. ceranae* is less seasonal, with consistently high spore levels seen in samples of workers (Martín-Hernández et al, 2007).

A1.4.4 Other Uncertainties: Interactions, Immunity etc.

If *N. ceranae* causes harm to *A. mellifera* colonies, it may do so on its own or via interactions with other pathogens and a multitude of factors affecting the honey bee colony. In fact, interactions would seem to be inevitable, even if "only" with factors such as the seasonal cycle of the honey bee colony or year to year variation in weather. *N. apis*, for example exhibits a characteristic pattern of infection, with spore levels in worker bees rising from late autumn to spring, and declining through the summer with, potentially, a second peak in summer when extra boxes of combs with contaminating spores are placed on hives. In addition, the levels of spores may be greater in years with poor summers that prevent colony build up or harsh winters that prevent cleansing flights for defecation outside the nest (Bailey, 1980 and Furgala & Mussen, 1991). However, what has happened with *N. apis* is not necessarily a good guide to what will happen with *N. ceranae*. It has been noted, for example, that *N. ceranae* leads to higher spores levels in workers (Paxton et al, 2007) and spore loads that do not show the seasonal cycle found with *N. apis* (Martín-Hernández et al, 2007).

Interactions are known to occur between the mite *Varroa jacobsoni* (also known as *Varroa destructor*) and various viruses (Ratnieks & Carreck, 2010). For example, there are data to suggest that in some cases it is the presence of mites plus virus that leads to colony death (Martin, 2001) and that virus levels rise once the mite is established (Carreck et al, 2002). The importance of *V. jacobsoni* in the spread of pathogens is further supported by Neumann & Carreck (2010) and Carreck et al (2010). The evidence presented by these two studies would suggest that interaction between *V. jacobsoni* and viruses is causing colony death in England.

However, interactions may be complex and no research has been identified which specifically considers interactions between *V. destructor* and *Nosema* species. Possible interactions of *Varroa* and *N. ceranae* must not be entirely ruled out but their interactions with bees suggest that there may be no specific biological interaction. *Nosema* infect only adult bees and enter the cells of the gut via an oral route through contact with bee faeces containing *Nosema* spores. Conversely, *Varroa* uses its mouthparts to feed on the haemolymph (blood) or pupal and adult bees, and is unlikely to come into contact with honey bee faeces.

A1.5 Other Factors on the Impact of *N. ceranae*

Paxton et al (2007) show that the number of spores per worker bee is greater in bees infected with *N. ceranae*, either alone (mean c. 80 million) or in a joint infection with *N. apis* (c. 50 million) than in an infection on *N. apis* alone (c. 10 million). This may indicate greater virulence of *N. ceranae* to individual workers.

Higes et al (2009a) note that strong colonies with high levels of *N. ceranae* do not swarm in the spring and have greater brood rearing in the winter. Brood rearing in mid-winter (December-January) may provide a macroscopic diagnostic character for *N. ceranae*. However, this must be weighed against the possible harm that opening hives in winter may do. It can be done, but only with care and on a warm day, and there may be differences in the winter response of bee colonies in Britain versus Spain.

A1.6 Summary

There is insufficient data available to categorically establish whether *N. ceranae* will be a significant problem to *A. mellifera* colonies and to beekeeping and pollination in the British Isles. On the one hand Higes and his group believe that *N. ceranae* is the cause of CCD/colony losses in Spain. The data he has are persuasive and are arguably more convincing, in terms of identifying cause than the data so far available from studies in the USA, which do not present a clear picture as to particular pathogens or pests being the causative agent(s) of CCD/colony losses. Although Israeli Acute Paralysis Virus (IAPV) was found to be a significant risk factor in one study, this was not corroborated in a second study.

On the other hand, in Uruguay there appears to be no correlation between *N. ceranae* levels and colony mortality. In the USA, three extensive surveys of colonies showing symptoms of CCD have been carried out comparing levels of a wide variety of pathogens (normal/non-CCD colonies) and gene expression patterns in honey bees. These surveys have found both *N. apis* and *N. ceranae* to be common but statistical analysis have not detected either species playing a role. Data on the control of *N. ceranae* show two promising methods. Fumagillin, which has been used for decades to treat for *N. apis* is effective against *N. ceranae* and can be used to treat live colonies. However, there are potential regulatory issues with Fumidil B and therefore its availability as a long term treatment is unclear. Freezing for one week (-18°C) appears to kill *N. ceranae* spores in stored equipment.

Annex 2:
Models for Measuring the Economic Value of Pollination by Honey Bees (*Apis mellifera*)

Table A2.1: Models for Measuring of the Economic Value of Pollination by Honey Bees (*Apis Mellifera*)

Reference and Country	Model	Comments	Value (Units Specified)
Marris et al (2009): UK	Replacement Cost Method based on the cost of providing man-made substitutes for lost pollination. Hand pollination considered as substitute based on figures from China.	Focuses on costs of pollinating apple crops. No estimation provided of whether or not the UK apple market could accommodate the replacement costs calculated. No estimation of the value of pollination services to other crops	Apple Pollination Value: £520 per tonne (replacement costs) Current Annual Production Costs: £433 per tonne Replacement Costs: 120% of Current Annual Production Costs

Allsopp MH et al (2008): South Africa (Western Cape only)	Replacement Cost Method: Insect dependence factors are calculated taking crop type and cultivar into account, where possible. Insect dependence factors are adjusted to take account of replacement pollination. The value of insect pollination is calculated by multiplying the value of pollination by the insect dependence factor	Based on previous models but reduces the value of insect pollination by taking into account replacement pollination (hand or mechanical pollination)	Crop Production Value: $501.0 million US. Value of Insect Pollination without Consideration of Replacement Pollination: $312.2 million US. Value of Insect Pollination with Consideration of Replacement Pollination: $119.8 million US
Temple et al (2001): UK	1. Estimation of the value of pollination services from a literature review to quantify the effect of pollination. 2. Opinions of growers as to the value of honey bees as pollinators of their crops. 3. Estimation of the income to keepers of bees from pollination fees	Estimates exclude protected cropping and Carreck and William's weighting has been refined to more accurately reflect differences between individual crops grown in England. Estimates do not include a monetisation of the value of pollination services of honey bees to wild plants or garden crops	1. £117.20 pollination services. 2. £54.00 as valued by growers. 3. £11.30
Southwick E &. Southwick L (1992): USA	Evaluation of the economic gains due to the honeybee, focusing on the gains to consumers through lower prices for crops benefited by honeybees. Estimation of the economic demand functions for major agricultural crops and the amount of increase in yield due to pollination calculated from a variety of sources, also the amounts by which various crops would be reduced if honey bees were absent. Determination of the surplus realised by consumers of these crops that would be lost if honey bees were depleted	Impossible to assess how much of the pollination gap would be filled by other insects and bee species should honeybees become extinct	Annual Societal Gains are estimated to range from $1.6 billion (honey bees replacing other pollinators) and $5.7 billion, (honey bees replacing no other pollinators). This should be contrasted with Robinson's et al (1989) estimate of £8.3 billion where there are no replacement pollinators. More recent estimations give a figure of $10 billion for honey bees, who have in the past been credited with pollination carried out by other bee species

Carreck N & Williams I (1998): UK	Economic evaluation of the role of bees as pollinators of selected crops (arable, tree, soft fruit and seed crops) and as producers of honey and wax. Weighting of the crops dependency on insects which has then been used to multiply the crop value to estimate the proportion attributable to insects. 80% was attributed to honeybees in orchard and field crops commonly pollinated by honey bees	The market value is used for the calculation although acreage payments (subsidies) would bring this value down. Market prices are used as indicators of social benefit. The estimation of 80% pollination services for the selected crops listed in the previous column being due to honey bees contrasts starkly with the estimate by Ingram et al of 80% of pollination across all crops being carried out by wild and other bee species (although for total crops, not a select few)	Estimated total value of insect pollination (mainly bees) is £172.2 million (outdoor crops), and £29.8 million (glasshouse crops.) If the proportion of insect pollination due to honey bees is given as 80%, the estimated value of this service to the selected crops is £137.80 million. Estimate value of honey production = £15.7 million Estimate value of wax production = £120,000 Total value of honey bees =£153.6 million (roughly £770 per colony)
Borneck R & Merle B (1989): France	Crop pollination by honeybees alone is estimated to be worth €4.25 billion/year in Europe	N/A	Bumblebees together with other non-honeybee pollinators are estimated to provide services worth more than €750 million per year
Benedek P (1985): Hungary	Separate assessment of the percent contribution of honeybees to pollination of 24 different crops in Hungary	N/A	N/A
Levin MD (1983): USA	Annual value of bee-pollinated crops in the US	No estimate of the contribution to pollination made exclusively by honeybees, or dissagregration of bee species as pollinators	$18 billion US
Blawat P & Fingler B (1994): Canada	Calculation of the value per year of pollination services to the production of pedigree alfalfa seed in Saskatchewan, Manitoba, and Alberta	The value of pollination to alfalfa seed growers in the Canadian prairies is estimated to be 35% of annual crop production, based on a calculation of area (30,000 ha) x yield (300 kg/ha) x value (Can. $0.60 kg) x 35%	$2 million Can.
Provincial Ministries of Agriculture, Quebec (2000): Canada	Calculation of the production value and honey bee contribution by crop, using data to estimate the dependence on pollination of each crop, the proportion of pollination services given by honey bees expressed as a percentage of pollination due to honey bees	See Annex for Table of estimations	Production Value ($'000 CAN) = 365,692.5 Honey Bee Contribution ($'000 CAN) = 781,542.64

Kevan PG & Phillips TP (2001):	An economic model that can be used to measure some of the economic impacts of pollinator deficits on traded commodities	This economic analysis indicates that consumers of a commodity affected by a pollinator deficit may suffer because the commodity costs more and becomes less available. Producers of the affected commodity may experience crop declines and/or economic gains in the form of higher prices. The amount the producer gains or loses depends on the shape of the supply and demand functions, and the magnitude of these losses or gains	N/A
Kevan PG (1997): Canada	The provision of one hive of honey bees per hectare resulted in about one extra seed per apple, producing larger and more symmetrical apples. The improved apples were estimated to provide marginal returns of about 5–6%, or about Can. $250/ha, compared to an orchard without honey bees	The cost of pollination services in 1997 was about 1% of production costs, and the greater yield represented a return to the grower of 700% of the cost of pollination services.	The model gives an approach to evaluating pollinators as an agricultural production cost with huge potential benefits
Cane JH (1996): USA	Assessment of the value of individual wild bees (*Habropoda laboriosa*) as pollinators of rabbit eye blueberry (*Vaccinium ashei*)	N/A	US $20.00 per bee

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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