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PATHOGEN TRANSMISSION IN INSECT POLLINATORS

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***Image cover**: Honeybee and bumble bee sharing the same flower (Corydalis cava)*

ABSTRACT

The importance of pollination is undisputed. However, declines in wild and commercially reared pollinators have been reported. Pathogenicity has been indicated as one of the main causes for this decline in the number of pollinators. Bees perform the majority of pollination activity in natural and agro-ecosystems. Not much is known about pathogenicity in wild bees, whereas more information regarding commercially reared bees exists. The European Honeybee (*Apis mellifera*) is extensively used for pollinating monocultural crops worldwide. The use of *A. mellifera* in the agro-cultural industry has led to the global distribution of this pollinator. Similar to the range expansion of *A. mellifera*, other pollinator species such as bumble bees (*Bombus* spp.), have been globally distributed as well. Commercially reared bees come into contact with closely related native wild bees, and transmission of diseases can occur. Therefore, immunological information regarding honeybees can be of importance for wild bees as well.

Honeybee and bumble bee pathogens can be vertically and horizontally transmitted. Vertical pathogen transmission is the transmission of pathogens from parent to offspring. Horizontal pathogen transmission is the transmission of pathogens between individuals of the same generation. Honeybee pathogens have also been transmitted between bee species in a process called pathogen spill over; where bee pathogens have been transmitted from commercially reared bees to closely related wild bee species. Shared use of flowers and drifting is the most likely pathway for transmission.

Various bee pathogens are sensitive to transmission, especially prone to be transmitted between bee species, such as *Nosema* spp. *Crithidia* spp., . Therefore, *Nosema* spp. and *Crithidia* spp. are likely pathogen agents which would be transmitted from commercially reared bee species to wild bee species. The spread of *Varroa destructor* in synergy with viruses could also lead to the infection of wild bee species with viruses such as Deformed Wing Virus. Recent infection discoveries of *Nosema ceranae* and Deformed Wing Virus in wild bumble bee species (Genersch et al., 2006; Plischuk et al., 2009) further fuel the necessity to conduct more research, since these pathogens were thought to be host specific to honey bees.

Given the importance of commercially reared and wild pollinators for the pollination in our agro- and natural ecosystems, the Dutch and Belgian commercial rearing facilities are inspected to prevent pathogen spreading via the use of these bees for pollination in greenhouses and orchards

Recommendation for further study

Still little is known about the transmission of pathogens to wild pollinators. More study on should be done on

1. Transmission of microsporidia via shared use of flowers. the prevalence of *Nosema* spp. in flowers.
2. Prevalence of *Nosema ceranae* in wild and commercial reared bumble bee colonies
3. Prevalence of *Nosema apis* and *N. ceranae* in solitary (wild) bees.
4. Viruses in wild bees e.g. *Osmia* spp. and *Megachile* spp. Both species are commercially used for pollination.
5. Deformed Wing Virus in commercial reared and wild bumble bee colonies

INDEX

1. Pathogen transmission from *Apis mellifera* and *Bombus terrestris* to wild populations 5
1.1. Pathogen transmission 5
1.2. Transmission pathways 7
1.3. Inter species pathogen transmissions and host shifts 8
1.3.1. Nosema ceranae 8
1.3.2. Aethina tumida 8
1.3.3.Tropilaelaps spp. 9
1.3.4. Varroa destructor 9
1.3.5. Nosema bombi 9
1.3.6. Crithidia bombi 10
1.3.7. Locustacarus buchneri 0
1.3.7. Locustacarus buchneri 11
1.3.8. Ascosphaera apis, Paenibacillus apis and *Melissococcus pluton 11*
1.3.9. Nosema apis 11
1.4. Insect defence against pathogens 11

2. Intra-species (*Apis mellifera*) pathogen transmission 12
2.1. Microsporidia 12
2.2. Mites 12
2.3. Viruses 12
2.4. Bacteria 14

3. Intra-species (*Bombus* spp) pathogen transmission 15
3.1. Microsporidia 15
3.2. Viruses 15

4. Honeybee pathology 16
4.1. Mites 16
4.1.1. Varroa destructor 16
4.2. Viruses 16
4.2.1. Deformed Wing Virus 16
4.2.2. Black Queen Cell Virus 16
4.2.3. Acute Bee Paralysis Virus 17
4.3. Bacteria 17
4.3.1. American Foul Brood 17
4.3.2. European Foul Brood 18
4.4. Microspiridia - Protozoa 18
4.4.1. Nosema spp. 18
4.4.2. Crithidia mellificae 19

5. Bumble bee parasites and diseases 20
5.1. Microsporidia - Protozoa 20
5.1.1. Nosema bombi 20
5.1.2. Crithidia bombi 20
5.1.3. Apicystis bombi (Mattesia bombi) 21
5.2. Mites 21
5.2.1. Locustacarus buchneri 21
5.3. Nematods 21
5.3.1. Sphaerularia bombi 21
5.4. Wasps 21
5.4.1. Mellitobia acasta 21

6. Diseases of solitaire bees 22
6.1. Microsporidia 22

Annex 30

INTRODUCTION

The importance of pollination, provided as an ecosystem service, has been repeatedly stressed (i.e. Costanza et al., 1997; Kearns et al., 1998; Klein et al., 2007). Plants and pollinators provide mutual benefits, whereby pollinators transfer gametes between plant individuals, and plants subsequently can set fruit. In return, pollinators receive their nutrients: pollen and nectar.

Declines in wild pollinator populations have been recently reported (Biesmeijer et al., 2006; Brown and Paxton, 2009; Potts et al., in press). In an extensive review, Goulson (2003) discusses the potential effects of the introduction of commercial pollinators on native ecosystems. The transmission of pathogens from non-native to native pollinators could be a cause for the decrease in wild pollinators.

Bees are the major pollinator of wild plants and crops (Winfree, 2010). Within the superfamily of bees (*Apoidea*), the European Honeybee (*Apis mellifera*) is extensively used for pollinating monocultural crops worldwide. Their use in the agricultural industry is so widespread that it has led to a distribution of European Honeybees over all continents, except Antarctica (VanEngelsdorp and Meixner, 2010). Although *A. mellifera* is mainly used for pollination services, other pollinators are more effective for some crops. Therefore solitary bee species and bumble bees (*Bombus spp.*), are increasingly used for pollinating crops such as tomatoes or alfalfa (Peterson, 1992; Velthuis and Van Doorn, 2006). This process has led to global range expansions of various pollinators, similar to the range expansion of *Apis mellifera.* With these range expansions, commercially reared pollinators have come into close contact with wild native pollinators.

Infectious diseases in *A. mellifera* have been intensively studied, since disease was hypothesized as one of the main causes of population declines (Genersch, 2010c). Infestations by parasitic mites, such as *Varroa destructor,* in combination with the transmission of specific viruses can induce severe colony losses (Todd et al., 2007).
Much less is known of infectious diseases in wild pollinators. Commercially reared bees come into close contact with closely related wild species. Pathogens in commercially reared bees could potentially target closely related species of wild bees. Therefore, pathogen information regarding commercially reared bee species could be of importance for wild bee species as well.

In chapter 1 this report reviews evidenced transmissions of pathogens and the potential risk between commercially reared bees and wild populations. Chapter 2 and 3 are about intra-species pathogen transmission in honeybees and bumble bees, respectively. Chapter 4, 5 and 6 give a brief overview of the main honeybee, bumble bee and solitary bee parasites and diseases. In the Annex, the current disease check procedure for Dutch bumble bee rearing facilities is given.

1. Pathogen transmission from *Apis mellifera* and *Bombus terrestris* to wild populations

1.1. Pathogen transmission

In the evolution of a host-pathogen interaction, pathogen transmission plays an important role (Schmid-Hempel, 2001). In order to maximize a parasites reproductive output, a parasite is dependent on its ability to transmit. If transmission is restrained, the negative effect of parasitation should be minimal due to the dependence of the parasite on its host (Lipsitch et al., 1996). If transmission is not a restriction, the virulence of pathogens is often increased, since the pathogen is less dependent on its current host. Therefore, pathogen transmission is an important aspect in host-pathogen evolution. Pathogen spill over occurs when epidemics in a host population are not driven by pathogen transmission within that population, but by the transmission of pathogens from a closely related reservoir population (Goulson et al., 2008).

Commercialized pollinators, such as *A. mellifera* and *B. terrestris*, are globally distributed, and beekeepers keep them at much higher densities than would normally occur in the wild. The rate of pathogen transmission within domestic populations and between domestic and wild populations is increased with high host densities (Power and Mitchell, 2004).

Commercially reared bee species can function as parasite reservoirs, which can lead to the spill over of pathogens to wild bee species. They are able to interact with wild pollinators, since commercially reared bee species often escape their designated area of pollination. In a study by Whittington et al. (2004), 73% of the pollen collected by bumble bees in a tomato greenhouse originated from plants outside of the greenhouse. When outside the greenhouse, commercially reared bees can interact with wild bees. Most interaction between pollinators takes place upon flowers, which are the centres of pollination activity. At these pollination hotspots, horizontal transmission of pathogens between commercially reared bees and wild bees is possible. Durrer & Schmid-Hempel (1994) showed how the shared use of flowers could lead to the horizontal transmission of *Crithidia bombi* between bumble bees. *C. bombi* is also increasingly prevalent in wild bumble bee species in the vicinity of greenhouses with commercially reared bumble bees (Colla et al., 2006; Otterstatter & Thomson, 2008).

Pathogens can be transmitted vertically and horizontally. Vertical transmission of parasites occurs with the transfer of parasites from parents to their progeny (Fine, 1975). Vertical pathogen transmission is expected to select for less virulent pathogen strains since the pathogen is dependent on the ability of its host to reproduce (Chen et al., 2006a; Fries & Camazine, 2001). If the detrimental effects of a parasite on its host are lethal, the fate of the host is shared by the parasite. Therefore, vertical transmission has an important function in the long-term maintenance of viruses (Chen et al., 2006a). Horizontal pathogen transmission is the transmission of pathogens between individuals of the same generation (Chen et al. 2006a) both intra- and inter colonial. In contrast to vertical transmission, horizontal transmission tends to select for increased virulence, as long as the spread of the pathogen to a new host is sufficient (Fries, 2001; Chen et al., 2006a).

Pathogen transmission in eu-social bee systems can be divided into several components (Fries and Camazine, 2001): vertical pathogen transmission between individuals of different generations (parent – offspring) and horizontal pathogen between individuals of the same generation, both within a colony (intra-colonial) and between colonies (inter-colonial). Vertical transmission of pathogens in honeybees can be observed when the queen and/or drones are infected, and the pathogen is transmitted to the progeny of the colony. Reproductive swarming is the main cause of inter-colonial vertical pathogen transmission. During reproductive swarming,

propagules (swarms) bud off from the parent colony to reinstate new colonies (Fries and Camazine, 2001). Swarms are often headed by the old (infected) queen. She leaves the old colony behind with a new queen (or queen larva) and a part of the (infected) worker force. In this process of reproductive swarming, two infected colonies can arise from a single infected colony. Intra-colonial horizontal transmission is of importance in eu-social bee systems where populations are very densely packed within a hive and frequent physical contact between individuals is common. In addition, physical contact is often promoted, through grooming practices and intimate feeding activities, whereby food is regurgitated and fed to offspring (trophallaxis). Drifting, the entering of bees in colonies other than its own (Pfeiffer and Crailsheim, 1998; Birmingham et al., 2004), often occurs when multiple colonies are concentrated in a small area. Wild honeybee colonies are often scattered over large distances, but commercialized honeybee colonies are often concentrated in high densities. Occasionally, honeybees purposefully enter hives other than their own, in a process called robbing. When foraging resources are limited, bees enter weaker hives and rob the nutritional resources of that colony. Healthy honeybee colonies guard their hives to repel robbing bees, but colonies that are weakened by diseases are unable to fend off intruders. The robbing honeybees can thus become infected with the same pathogens that were responsible for weakening the invaded colony (Fries and Camazine, 2001). Transmission of pathogens via the robbery of contaminated waste may occur in case old, non-functioning commercial bumble bee hives, which are placed outside the greenhouses, or are left in the orchards.

Individuals can come into contact with individuals from other colonies while being outside of the hive, and pathogens can be transmitted. Previous research has indicated that shared use of flowers can lead to the horizontal transmission of a bumble bee pathogen, *Crithidia bombi* (Durrer and Schmid-Hempel, 1994). Flowers are the centres of pollination and are therefore visited multiple times by different pollinators. Other pathogens are also possible candidates to be transmitted through the shared use of flowers. Therefore, these centres of pollination activity could be potential hotspots for the horizontal transmission of bee pathogens.

Horizontal transmission can also occur between host species in a process called a host shift.

1.2. Transmission pathways

Intra-colonial horizontal transmission is of importance in eu-social bees where populations are very densely packed within a hive and frequent physical contact between individuals is common. Physical contact is often even promoted through grooming practices and intimate feeding activities in which food is regurgitated fed to offspring (trophallaxis).

Inter-colonial horizontal transmission can occur:

- via direct contact between individuals and feeding contact between nurse bees and larvae;
- via indirect contact via shared use of food sources;
- via robbery on contaminated waste products;
- via feeding of commercial reared bee species with products of other bee species.

Indirect contact via the shared use of flowers was described as a pathway for *Crithidia bombi,* and was transmitted between *Bombus terrestris* and *Bombus lucorum* (Durrer & Schmid-Hempel. 1994). This transmission pathway is suggested as one of the pathways *Nosema ceranae* and *Nosema apis* are transmitted between honeybee colonies, and how *Nosema bombi* is transmitted to other bumble bee colonies. Additional study is needed to investigate the importance of this pathway.

These transmission pathways exist for intra-species and inter-species. Since this report is about transmission of pathogens from commercially reared honeybees and bumble bees to wild bees, host shift is our man topic.

Fig 1. Shared use of flower: bumble bees bite little holes in the petals to rob nectar, and honeybees and wild bees will use these holes to gain easier access to the nectar.

1.3. Inter species pathogen transmissions and host shifts

Evidenced inter-species pathogen transmission and host shift pathways of the honeybee and bumble bee pathogens are described for: *Nosema bombi*, *Nosema ceranae*, *Crithidia bombi*, *Aethina tumida*, *Tropilaelaps* spp and *Varroa destructor* (table I and II).
Possible host shifts of *Nosema apis*, *Ascosphaera apis*, *Paenibacillus larvae* and *Melissococcus pluton* have been studied.

Table I. Pathogen host shift

pathogen	Host	Host shift to
Nosema ceranae	*Apis ceranae*	*Apis mellifera*
Nosema ceranae	Apis ceranae	*Bombus* spp. (South America)
Aethina tumida	Sub Sahara honeybee species	*Apis mellifera*
Aethina tumida	Sub Sahara honeybee species	*Bombus impatiens*
Tropilaelaps spp.	*Apis dorsata*	*Apis mellifera*
Varroa destructor	*Apis ceranae*	*Apis mellifera*

Table II Inter-species pathogen transmission

pathogen	Host	Host shift to
Nosema bombi	*Bombus* spp.	*Bombus* spp.
Crithidia bombi	*Bombus* spp.	*Bombus* spp.
Locustacarus buchneri	*Bombus* spp.	*Bombus* spp.

1.3.1. Nosema ceranae

At the end of the 20th century, *Nosema ceranae*, believed to be a specific parasite of *Apis ceranae*, the Asiatic honeybee, was found in *Apis mellifera* colonies (Fries et al., 1996). Cross-infection of *Nosema ceranae* to *Apis mellifera* was determined in wild specimens by Higes et al. (2006) and Huang (2007). Most honeybee colonies in the Netherlands are infected by *N. ceranae*. The impact of *N. ceranae* on honeybee colonies in the Netherlands is not yet clear. Until now, no evidence is provided that *N. ceranae* is a major factor in the recently reported colony losses, or that the parasitation harms the bees.

Nosema ceranae is also detected in the South American bumble bees *Bombus altratus*, *B. morio*, *B. bellicossus* (Plischuk et al., 2009).

1.3.2. Aethina tumida

Aethina tumida Murray originated from sub-Saharan regions. In its natural environment, the beetle is not considered to be a parasite; only weakened honeybee colonies die from the infestation. Since 1996, *Aethina tumida* is prevalent in honeybee colonies in other parts of the word. Besides the USA, *Aethina tumida* is found in honeybee colonies in Egypt (since 2000), Canada (since 2002), and in Australia (since 2001). In honeybee colonies of the European bee *Apis mellifera*, *Aethina tumida* is a severe plague. In the USA, a mere 2 years after the first recording, the beetles contributed to la loss of at least 20 000 colonies. The impact on Egyptian beekeeping is still unknown. In Australia, at first only weak colonies died, but at the moment also strong colonies are also becoming damaged. In North America, the beetle is now sympatric with bumble bees not occurring in its native region. *Aethina tumida* can reproduce in bumble bee colonies (*Bombus impatiens*) within the laboratory. They are attracted to bumble bee workers and the pollen from bumble bee nests. Reproduction of the beetle is not destructive to field colonies, but lowers the number of worker bees and destroys nests (Spiewok & Neumann, 2006).

Aethina tumida in not yet recorded in the EU.

1.3.3.Tropilaelaps spp.

The genus *Tropilaelaps* has four species: *T. clareae, T. koenigerum, T mercedesae* and *T. thaii*. The natural host is *Apis dorsata*, but *T. clareae* and *T. mercedesae* have been recorded and can reproduce in the European honeybee *Apis mellifera*. Up till now *Tropilaelaps* spp. has not been found in the Netherlands.

1.3.4. Varroa destructor

V. destructor is not native to the European honeybee, but to its sister species, the Asian honeybee (*Apis cerana;* Oldroyd and Wongsiri, 2006). *Varroa destructor* is one of the most harmful pathogens to European honeybees. Their interaction with other pathogens, such as viruses, can lead to the collapse of whole colonies. Prior to the research of Anderson and Trueman (2000), *V. destructor* was assumed to be *Varroa jacobsoni*. Although *V. destructor and V. jacobsoni* mites are physically alike, their virulence toward *A. mellifera* is not uniform (Rosenkranz et al., 2010). *V. jacobsoni* is a parasitic mite of the Asian honeybee, *Apis cerana,* and is unable to colonize *A. mellifera* colonies. *V. destructor* is capable of colonizing both bee species. The effect of *Varroa* infestation on *A. cerana* is minimal, whereas *Varroa* infestations on *A. mellifera* can have severe consequences. The long existing parasite-host relationship observed in *Apis cerana* has presumably coevolved to this stable equilibrium (Royce and Rossignol, 1990; Oldroyd, 1999). Two strains of *V. destructor* on *A. mellifera* have been discovered (Oldroyd, 1999; Rosenkranz et al., 2010), indicating that the host shift from *A. cerana* to *A. mellifera* occurred twice, independently. Much is unclear about There is not much clear, when and how this shift took place. General assumption is that with the transport of *A. mellifera* to the Far East, somewhere in the first half of the past century, *V. destructor* was given the possibility to move to a new host (Oldroyd, 1999). Since the host shift occurred, infested *A. mellifera* colonies have been moved around the world, spreading *V. destructor* as well.

1.3.5. Nosema bombi

Nosema bombi is a microsporidian parasite of multiple *Bombus spp.* species (Larsson, 2007). *N. bombi* was detected in *B. hypnorum, B. jonellus, B. lapidarius, B. lucorum, B. pascuorum, B. pratorum, B. subterraneus* and *B. terrestris* from across Northern and Central Europe (Tay et al., 2005). The impact of *N. bombi* on different *Bombus* spp differs. Where a *N. bombi* infection does not result in a significant reduction of colony size in *B. terrestris*, it does in *B. lucorum* colonies. In *B lucorum* colonies the production of gynes was reduced by infection with *N. bombi,* whereas this is not the case in *B. terrestris.* On the other hand, infected gynes of *B. lucorum* had no decrease in mating success, whereas infected gynes of *B. terrestris* were unable to mate (Rutrecht & Brown, 2009). Cross-infectivity experiments with *N. bombi* originating from different *Bombus* spp. demonstrate that cross-infectivity is also possible. Niwa et al. (2003) demonstrated in laboratory tests that isolated microsporidia (presumably *N. bombi*) from *B. terrestris*, imported from the Netherlands, were able to infect *B. hypocrita* and *B. diversus*, two native Japanese bumble bee species. Schmid-Hempel & Loosli (1998) showed that *N. bombi*, originating from *B. terrestris*, was infective for workers of *B. lapidarius* and *B. hypnorum*; although infections in *B. lapidarius* and *B. hypnorum* were less severe than in workers of *B. terrestris*, but mortality was significantly higher in the foreign hosts than in *B. terrestris*.
From 1992 to 1994, native North American bumble bees were shipped to European rearing facilities. They were reared alongside the European bumble bee *B. terrestris* and then transported back to North America. In the European rearing facilities they could have been infected with parasites and diseases of *B. terrestris*. Since that time, a severe decline has been recorded for three widespread North American species, B*. occidentalis, B. affinis, B. terricola* and *B. ashtoniam* a specialized parasite of *B. affinis* and *B. terricola.* The cause of this decline is not clearly understood, but may

have been the result of infestations of *N bombi*, imported from Europe (Winter et al., 2006).

1.3.6. Crithidia bombi

Crithidia bombi (Trypanosomatidae) is a widespread parasite of bumble bees (Schmid-Hempel, 1998). Transmission of this organism between bumble bee species via shared use of flowers was demonstrated by Durrer & Schmid-Hempel (1994). Colla et al., (2006) compared the prevalence of pathogens in wild bumble bees caught in the vicinity of commercial greenhouses, with the prevalence of pathogens in wild bumble bees caught in areas distant from commercial greenhouses. In these greenhouses commercially reared bumble bees were used for the pollination of crops. The results have indicated that wild bumble bees caught in the vicinity of greenhouses have a higher pathogen load than wild bumble bees caught in areas distant from greenhouses. A similar result was obtained by Otterstatter and Thomson (2008), which showed a decrease in *C. bombi* and *N. bombi* prevalence in wild bumble bee species, with decreasing geographical distance from greenhouses where commercially reared bumble bees were used for pollination activities.

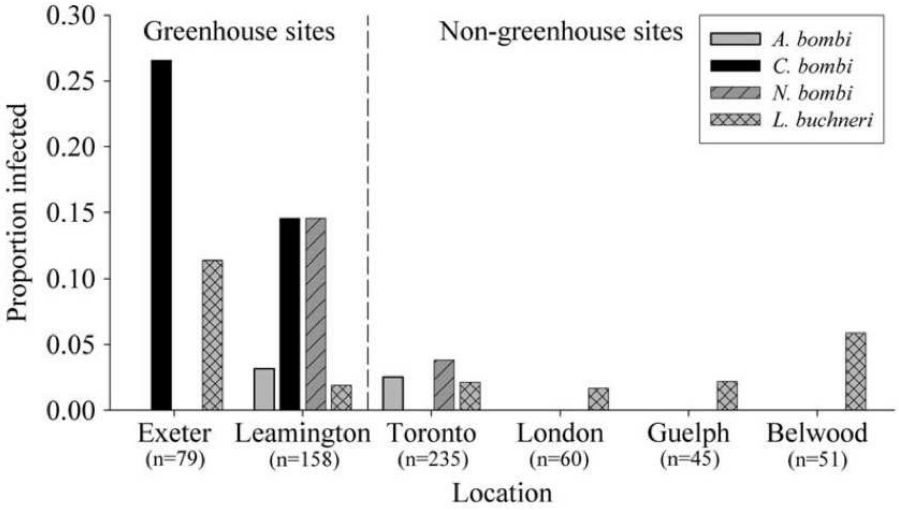

Fig. 4. *The infection rate of 4 diseases in wild bumble bees. Wild bumble bees collected in the vicinity of commercial greenhouses have a higher pathogen load than wild bumble bees collected at a site distant from commercial greenhouses. Greenhouses use commercial bumble bees for the pollination of their crops (Colla et al., 2006)*

1.3.7. Locustacarus buchneri

Locustacarus buchneri reproduces in the air sac in the abdomen of bumble bees. Intra- and inter-species transmission goes via direct contact. They can migrate via, and hibernate, in young queens. Also, pupae of *Bombus* spp can be infected (Eijnde, & Ruijter 1998).
L. buchneri occurs both in native Japanese bumble bee e.g. *Bombus ignitus* and in the European bumble bees e.g. *B. terrestris*. However, the haplotypes of *L. buchneri* in natural *B. ignitus* colonies differs from the haplotypes of *L. buchneri* in *B. terrestris*. *B. ignitus* is reared in the Netherlands and transported back to Japan. It was found that the *L. buchneri* in the commercial reared and retransported *B. ignitus* colonies had the same haplotype as *B. terrestris,* indicating an overseas migration of the parasitic mite of different origins Goka et al., 2001).

1.3.8. Ascosphaera apis, Paenibacillus apis and *Melissococcus pluton*

Ascosphaera apis causes a fungal infection in honeybee brood. It is prevalent in the majority of honeybee colonies. An outbreak of the infection is related to colony conditions; in bad conditions when the brood is chilled the fungus will develop. *Paenibacillus larvae* is a much feared bacterial infection of honeybee brood, causing American Foulbrood. The bacterium is prevalent in about 1% of the Dutch honeybee colonies but is much more prevalent in surrounding countries. *Melissococcus pluton* infects honeybee larvae. The infection is called European Foulbrood. The bacterium is present in most Dutch honeybee colonies. The success of the infection depends on the infection pressure, which is high in the case of severe varroa infections. The micro-organisms can be transferred to commercial bumble bee rearing facilities along with the honeybee collected pollen, used as bumble bee feed. Since the start of the commercial rearing of bumble bees in the Netherlands, no infection of bumble bee brood by *Ascosphaera apis, Paenibacillus larvae* and *Melissococcus pluton* has been detected. Pridal (1997, 2001, 2002) studied microorganisms in laboratory reared bumble bee colonies. Bacteria detected in the pollen and honey feed of the bumble bees included: *Bacillus circulans*, *B. licheniformis, Paenibacillus pabuli*, *A. apis* and *B. circulans, B. licheniformis, B. pumilus*. It was demonstrated that none of the contagious honey bee diseases e.g. *Ascosphaera* caused bumblebee larval mortality. Larval mortality is caused by *Bacillus pumilus* and *Paenibacillus glucanolyticus*.

1.3.9. Nosema apis

Nosema apis is a microsporidian parasite of *Apis mellifera*. Bumblebee species and other pollinators may be contaminated via shared flower use. Another likely pathway is feeding bumble bee colonies with pollen collected by honeybees. This is a common feeding practice in commercial bumble bee rearing. Study of Eijnde & Vette (1993) demonstrated that *N. apis* could not infect *B. terrestris*.

1.4. Insect defense against pathogens

Durable specific immune priming for microorganisms results in an immune system in insects, which shows specific response to previous encountered pathogens. This was demonstrated in infection studies of adult *B. terrestris* individuals infected with *Pseudomonas fluorescens, Paenibacillus alvei* and *Paenibacillus larvae* (Sass & Schmid-Hempel, 2006).

2. Intra-species (*Apis mellifera*) pathogen transmission

2.1. Microsporidia

Nosema ceranae is a microsporidian parasite that was first described as a parasite of the Asian honeybee *Apis cerana* (Fries et al., 1996). However, *N. ceranae* is cross-infective with the European honeybee *Apis mellifera* (Higes et al., 2006). When and where the host shift from *A. cerana* to *A. mellifera* has taken place is unclear, but the oldest *A. mellifera* sample known to be infected with *N. ceranae* is a 20 year-old sample from Uruguay (Invernizzia et al., 2009). *N. ceranae* seems to be replacing *N. apis* in *Apis mellifera* over the last couple of years. This replacement of *N. apis by N. ceranae,* remains an enigma since the spores of *N. ceranae* are less durable than the spores of *N. apis* (Fries, 2010). Plischuk et al. (2009) also reported the infection of native South-American bumble bees with *Nosema ceranae*. Prior to this discovery, *N. ceranae* was thought to be host-specific to honeybees

In contrary to other insect species (Van Frankenhuyzen et al., 2007; Goertz and Hoch, 2008), no evidence for the vertical transmission of the microsporidia *Nosema apis.* has been found in honeybees. Webster et al. (2008) infected honeybee queens with *N. apis* and checked infected queens for *N. apis* development. Six out of seven queens developed a *N. apis* infection in their ventriculus, but none had infected ovaries. Additionally, their progeny was not infected by *N. apis*. However, further research might be necessary.

2.2. Mites

The ecto-parasitic mite *Varroa destructor* is closely linked to its honeybee host and lacks a free-living stage (Figure 3; Rosenkranz et al., 2010). The males and nymphal stages of the mite do not leave the brood cells. Adult females are able to infest adult worker bees and they can be horizontally transmitted within and between colonies. Mites can be dispersed between colonies by swarming or on foraging trips, when adult honeybees encounter bees from other colonies (Kuenen and Calderone, 1997). Due to the prevalence and proliferation of viruses in *V. destructor*, the spread of *V. destructor* has been linked with the collapse of whole colonies (Rosenkranz et al., 2010).

Fig. 3. Opened brood cells with Varroa destructor infested A. mellifera larvae (Rosenkranz et al., 2010)

2.3. Viruses

Around 18 viruses have been detected in honeybees (Chen and Siede, 2007). Viruses are horizontally transmitted between individuals via different pathways. Nutritionally related activities, such as foraging, are among the main activities of honeybees. Bees gather nectar, pollen and water from their environment and return

it to the colony. Upon return in the colony, worker bees regurgitate food and pass it on to nursery bees that add saliva and store this product in the colony. In times of need, nutritional resources that would have been stored are used. Worker honeybees feed on stored resources and feed other colony individuals by regurgitating the food. Viruses use these foraging and processing pathways to spread viruses between individuals of the colony. This food borne transmission pathway is an excellent carrier for pathogens to be transmitted. Shen et al. (2005a) and Chen et al. (2006a) detected viruses in colony food, including honey, pollen and royal jelly. The prevalence of viruses in colony food indicates that in the spread of virus infections, colony food stores could be involved (Chen and Siede, 2007). The elevated prevalence of virus particles in gut tissue of honeybee queens, in comparison with other body tissues, is another indication for a food-borne transmission pathway (Chen et al., 2006b). However, it is not clear what amount of ingested virus particles are sufficient to create an infection.

Chen et al. (2005) checked honeybee queens for the prevalence of six viruses. Five viruses (incl. DWV, BQCV and ABPV) were present in the queen bee samples. Virus prevalence in honeybee queens can be an indication of vertical transmission of viruses from mother to offspring. In a subsequent study, Chen et al. (2006b) observed a correlation between viruses present in queens and viruses present in their offspring (eggs, larvae and adult workers). Honeybee queens that did not harbour viruses produced non-infected progeny. A similar result was obtained by De Miranda and Fries (2008), who infected virgin honeybee queens with DWV particles and observed progeny with an almost 100% infection rate. Viruses possibly infect the ovary tissue of queen bees. However, viruses are likely in a latent stage, since a deleterious effects on embryos were not observed (Chen et al., 2006a). A study by Yue et al. (2006a) on the semen of honeybee drones has shown that bee sperm can contain viruses, indicating a venereal transmission pathway. Venereal transmission of pathogens occurs when pathogens are transmitted through mating. Yue et al. (2006b) fertilized DWV-negative unfertilized eggs with DWV-positive sperm and they observed that all of their fertilized honeybee eggs were DWV infected. Therefore, venereal transmission is a likely pathway to cause vertical transmission of pathogens. Interestingly, the relative frequency of viral infections found in the semen did not correspond with the relative frequency of the virus in the bee population (Yue et al., 2006a). For example, DWV is present, though often in latent state, in almost 100% of the population, whereas only 50% of the semen was infected by DWV. Taking these results together, a vertical transmission pathway of honeybee viruses is very likely, however, the venereal transmission pathway still has some unclear aspects which need to be examined.

Vector-borne transmission is an indirect route of horizontal transmission and involves an intermediate biological host, a vector, which acquires and transmits viruses from one host to another (Chen and Siede, 2007). The most prominent vector of honeybee viruses is the ectoparasitic mite *Varroa destructor*. *V. destructor* is an obligate parasite of honeybees that penetrates the body wall of bees with piercing mouthparts to suck on their hemolymph. They are capable of transmitting viruses between hosts and act as an important vector for horizontal transmission. Bowen-walker et al. (1999) first suggested the spread of DWV by *Varroa jacobsoni* (probably *V. destructor*) and subsequent studies have confirmed the spread of DWV by *V. destructor* (Tentcheva et al., 2004; Shen et al., 2005). Since the discovery of the spread of DWV by *V. destructor*, multiple other viruses (incl. BQCV) have been found able to be transmitted between honeybees by using *V. destructor* as a vector (Chen et al., 2004; Shen et al., 2005; Chen and Siede, 2007; Todd et al., 2007). Besides transferring viruses between hosts, *V. destructor* also functions as an activator of latent viruses that are already present in the host, prior to *V. destructor* infestation (Shen et al., 2005). Additionally, Ongus et al. (2004) reported that viruses are not only capable of using *V. destructor* to be transferred between hosts, but also to replicate themselves and being transmitted between *V. destructor* individuals. *Varroa destructor* is therefore a very potent and harmful vector to transmit viruses between

A. mellifera individuals.

2.4. Bacteria

American Foulbrood, an infection of the larvae by *Paenibacillus larvae*, is a frequently occurring and a potentially deadly disease of honeybees (Genersch, 2010b), but not much is known regarding its horizontal transmission. However, a study by Lindstrom et al. (2008) did indicate that horizontal transmission of AFB in honeybees is of importance when healthy colonies invade and rob diseased colonies. Lindstrom et al. (2008) stress the importance of geographical distance between colonies, since less distant colonies have a higher likelihood to be robbed by neighbouring colonies. Closer colonies contained adults with high spore loads without causing clinical disease symptoms. It is unclear why these high spore loads do not lead to AFB infection with clinical symptoms. The functional mechanism behind transmission and the actual establishment of the disease in its host should be further explored.

American Foul Brood (AFB) spores can be carried by swarming individuals of honeybees when they move to new colonies. A study by Fries et al. (2006) indicated that swarms carry spores of *P. larvae*, and that these spores can cause infections in daughter colonies. Interestingly, besides the colonies that were latently infected, they also collected three colonies that swarmed, even though these colonies exhibited clinical symptoms of AFB. The swarming of diseased colonies can have stronger implications for vertical transmission than the swarming of colonies with a latent AFB infection, since pathogen load is higher in diseased colonies. Infected colonies that swarmed reduced the number of *P. larvae* spores present in infected individuals. High spore load levels, able to create AFB diseased colonies, are therefore not easily transmitted (Fries et al, 2006). Perhaps swarming can be opted as a possible method to reduce pathogen load. Dutch beekeepers create swarms to prevent natural swarming, and by doing this, they probably reduce the pathogen load of their honeybee colonies.

Melissococcus pluton, the bacterium that causes European Foulbrood, was tested on horizontal disease transmission (Roetschi et al., 2008). Adult worker honeybees carried high spore loads of *M. pluton* but the high spore load did not correlate with the prevalence of disease development in larvae.

3. **Intra-species (*Bombus* spp) pathogen transmission**

3.1. Microsporidia

Horizontal transmission between colonies can occur through drifting, shared use of flowers, and robbing. Interestingly, Rutrecht et al. (2007) observed low horizontal transmission levels of *N. bombi* in bumble bee species, indicating a low level of virulence.
However, further study revealed that the impact of *Nosema bombi* on different *Bombus* spp differs; significant differences were recorded between the impact of a *N. bombi* infection on *B. terrestris* and *N. lucorum*, related to the production of gynes and mating success (Rutrecht & Brown, 2009). Horizontal transmission ought to select for high levels of virulence, since the parasite is less dependent on the direct reproductive success of its current host, in contrary to vertical transmission of parasites (Fries, 2001; Chen et al., 2006a). Presumably, this is an indication that the parasite-host relationship is in equilibrium. Co-evolution has led to stability as a result of a long-term parasite-host relationship (Royce and Rossignol, 1990). The success of horizontal transmission also depends on the amount of social interaction. Otterstatter and Thomson (2007) observed the social interactions of bumble bees in *Bombus impatiens* colonies when infected with *C. bombi*. Individuals that interacted more frequently with other individuals of the colony had a higher chance of being infected with *C. bombi*. Additionally, differences in rates of infection among colonies resulted largely from differences in network density among hives. Durrer and Schmid-Hempel (1994) showed how the shared use of flowers could lead to the horizontal transmission of *Crithidia bombi* between bumble bees.

3.2. Viruses

Not much is known regarding virus host shifts between bee species. European honeybee viruses such as BQCV and DWV are known to also infect Asian honeybees (Oldroyd and Wongsiri, 2006).
Genersch et al. (2006) did report on two natural cases in which a virus of honeybees, DWV, was observed in wild bumble bee species (figure 4). This might indicate a host shift, and a broader host range for this previously assumed honeybee host-specific pathogen.

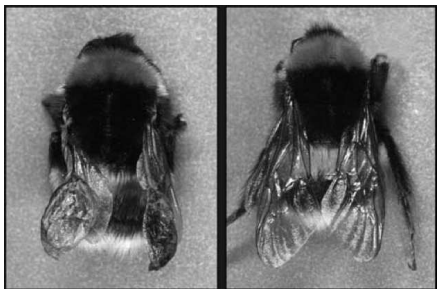

Fig. 4. Bombus terrestris individual with DWV disease (left) and a healthy Bombus terrestris individual (right; Genersch et al., 2006)

4. Honeybee pathology

4.1. Mites

Parasitic mites play a key-role in collapsing *A. mellifera* colonies. Therefore, parasitic mites are considered to be the single most detrimental bee parasite in the world today (Rosenkranz et al., 2010; VanEngelsdorp and Meixner, 2010). The direct effect of the parasite itself, however, is apparently not the main problem. Instead, synergisms between parasitation and virus infection are the most harmful, since mites are capable of transmitting specific viruses.

4.1.1. Varroa destructor

Varroa destructor (varroidea) is an obligate ectoparasitic mite that parasitizes pupae and adult honeybees. The mite depends on bee brood for reproduction. The life cycle of *V. destructor* is intimately linked to its host (Genersch, 2010b). Female mites deposit their eggs with bee larvae just before the brood cell is encapsulated. After hatching, the mites suck hemolymph through an opening in the larvae's cuticle. This process impairs the growth of the bee larvae (De Jong et al., 1982). The exact impact of *V. destructor* is difficult to quantify. Often, colony losses cannot be attributed directly to the effects of *V. destructor*. Beekeeping without *Varroa* control, however, is nearly impossible nowadays. Without control, colonies in *Varroa* infested areas with a temperate climate often collapse within two to three years after contamination (Rosenkranz et al., 2010).

4.2. Viruses

At least 18 viruses have been reported to infect honeybees worldwide (Chen and Siede, 2007; Genersch, 2010b). Most of these 18 viruses may exist and even co-exist in honeybee individuals or colonies, without causing any symptoms (Genersch, 2010b). However, in interplay with other pathogens such as *Varroa destructor*, these viruses can have an additive and lethal effect.

4.2.1. Deformed Wing Virus

Deformed Wing Virus (DWV, Iflaviridae) is one of the few bee viruses that cause well-defined disease symptoms. Establishing causal relationships between observed symptoms and specific viruses can be a challenge in bees, since many viruses are often latently present (De Miranda and Genersch, 2010). Typical symptoms of DWV infection in adult bees include shrunken crumpled wings, decreased body size and discolouration (Chen and Siede, 2007). DWV has a very high prevalence among *Apis mellifera*. Recent findings in three studies indicate an infection rate between 90 and 100% (Tentcheva et al., 2004; Berenyi et al., 2006; Chen and Siede, 2007). However, the disease is often latent and symptoms are only observed after infestation by the parasitic mite *Varroa destructor.* In combination with *V. destructor,* DWV can have lethal consequences.

4.2.2. Black Queen Cell Virus

Black Queen Cell Virus (BQCV, Dicistroviridae) is the second most prevalent honeybee virus (Chen and Siede, 2007). Research on worker bees in Austrian and French apiaries detected infection rates of 30% and 86% (Tentcheva et al., 2004; Berenyi et al., 2006). As the name suggests, queen pupae are particularly susceptible. Infected workers often do not exhibit symptoms, but these workers may transmit the virus to queen pupae with secreted brood food (Tapaszti et al., 2009). Infected queen pupae have a pale yellow appearance and a tough, sac-like skin. When infection progresses, diseased pupae turn dark and die rapidly. The wall of the queen cell eventually becomes darkly coloured (Chen and Siede, 2007). Research

indicates that BQCV has a high co-incidence with the microsporidia *Nosema apis* (Tentcheva et al., 2004; Berenyi et al., 2006). Infection of the midgut by *N. apis* may increase susceptibility for BQCV (Chen and Siede, 2007).

4.2.3. Acute Bee Paralysis Virus

Acute Bee Paralysis Virus (ABPV, Dicistroviridae) has been closely linked to the numerous *V. destructor* induced colony losses in Europe and the United States (Chen and Siede, 2007; De Miranda et al., 2010). ABPV is closely related to the Kashmir Bee Virus (KBV, Dicistroviridae) and Israeli Acute Paralysis Virus (IAPV, Dicistroviridae; reviewed by De Miranda et al., 2010. ABPV distribution is widespread and found on all continents except Antarctica. ABPV normally persists as a covert infection within a colony but the virus is extremely virulent when injected into the hemolymph (De Miranda et al., 2010). Overtly infected individuals exhibit specific symptoms: Trembling and paralysis, followed by death. Since virulence is high when in contact with host haemolymphe, relationships between ABPV and *V. destructor* should be expected*. V. destructor* feeds on the hemolymph of hosts and is a likely vector for ABPV (De Miranda et al., 2010). Until now, this virus has not been detected in Dutch honey bee colonies.

4.3. Bacteria

Two bacterial diseases affecting honeybee brood are among the most economically important diseases globally: European Foul Brood (EFB) and American Foul Brood (AFB). Both diseases can be lethal at the individual level, and with high infection levels, at the colony level. Thereby, these diseases pose a serious threat to bee health. Genersch (2010a) and Forsgren (2010) provide detailed reviews. AFB shows low prevalence in the Netherlands. EFB is widespread.

4.3.1. American Foul Brood

AFB (figure 5) is globally a major threat to honeybee health, since it occurs frequently (5 – 10 % of colonies were infected over a 10 year observation period in Germany; Genersch, 2010b). However, in the Netherlands, prevalence is very low; 0 – 2 incidents per year (own data).

AFB is caused by the spore forming bacterium, *Paenibacillus larvae* (Genersch, 2006). Larvae are infected by consuming food with *P. larvae* spores. Vegetative bacteria colonize and massively proliferate in the midgut (Yue et al., 2008), living on food provided by the bee larvae. Eventually, the midgut is stocked with bacteria and the epithelial barrier is breached. Proteases secreted by the bacteria may disrupt cell structure and break down cell walls (Genersch, 2010a). After degradation, the larvae remains as a brownish fluid, which desiccates and adheres to the hive cell wall as a hard scale. This scale contains numerous *P. larvae* spores that can be infectious for more than 35 years, and can withstand significant changes in cold, heat, draught and humidity (Genersch, 2010a).

The presence of *P. larvae* can go unnoticed for some time as colonies show few, if any, symptoms (Schmid-Hempel, 1998). Individuals can contain low numbers of bacteria and develop into adults that can spread the disease in their faeces. A disease can build up latently over a period of time until symptoms reveal and the disease can subsequently lead to the collapse of a whole colony.

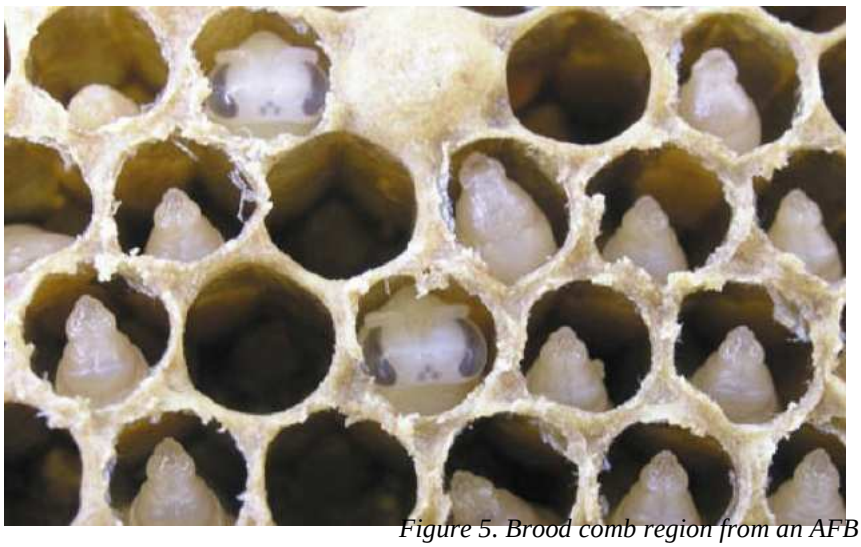

Figure 5. Brood comb region from an AFB infected colony. Uninfected larvae developed into dark-eyed pupae, infected individuals visible as engorged larvae (Genersch, 2010a).

4.3.2. European Foul Brood

The etiological agent causing EFB is a bacterium, *Melissococcus pluton.* This bacterium infects 4-5 day old honeybee larvae in open brood, which causes a 'foul' smell (Schmid-Hempel, 1998). *M. plutonius* colonizes the midgut of bee larvae after being consumed in contaminated food. Bacteria multiply within the host, and severe infections can have lethal consequences. A nutrient deficiency, due to nutrient competition, was thought to cause starvation of larvae. However, *in-vitro* experiments by McKee et al. (2004) still demonstrated lethal infections, even when excessive nutritional resources were provided. The exact cause of death due to EFB infection remains enigmatic (Forsgren, 2010).
Brood that has died before the capsulation of brood-cells is often removed. Adult worker bees clean out these cells and remove much of the bacteria; however, new brood can still get infected.

4.4. Microspiridia - Protozoa

4.4.1. Nosema spp.

Microsporidia are obligatory intracellular pathogens that damage many economically important insects (Schmid-Hempel, 1998). Members of this group produce spores, which are their only means of survival outside of the hosts. These spores germinate by extruding a polar filament, which is able to penetrate host cells. *Nosema spp.* generally infects the ventricular cells of the midgut. Dysentery has been associated with *Nosema apis,* but interestingly, this agent is not the primary cause of this condition. Nevertheless, dysentery certainly aids the transmission of *N. apis* (Fries, 2010). *Nosema ceranae* is less connected with dysentery, which might indicate a different main route of transmission (Fries, 2010).
Reports on the virulence and impact of *Nosema,* are contradictory (Genersch, 2010b). Much is still unclear about the importance of *Nosema* on honeybee colonies at a regional scale in comparison with the impact of *Nosema* on honeybee colonies on a global scale

N. apis infects the epithelium cells of the ventriculus. As infected cells don't function optimally, less protein, carbohydrates, fat, and minerals are absorbed, resulting in reduced longevity, reduced food glands and a small fat body. On a colony

scale, this means that subsequent generations are malnourished, resulting in an impeded colony development. Adequate pollen flow reduces the impact of a *Nosema* infection (PRI bees@wur 2010).

4.4.2. Crithidia mellificae

Crithidia mellificae belongs to the family of Trypanosomatidae (Langgridge and Barclay McGhee, 1967). *Crithidia mellificae* infests the pylorum of adult honeybees and does not affect honeybees younger than 6 days. Relatively little information regarding this parasite is present. Perhaps it cannot even be regarded as a parasite, but more as a commensal, since it is thought that infections cause little to no harm (Schmid-Hempel, 1998).

5. Bumble bee parasites and diseases

5.1. Microsporidia - Protozoa

5.1.1. Nosema bombi

Nosema bombi is a microsporidia that affects multiple bumble bee species (Tay et al., 2005; (Larsson, 2007). *N. bombi* infects the Malpighian tubules, the ventriculus, fat tissue and nerve tissues (Fries et al., 2001). An infection by *N. bombi* can significantly reduce the lifespan of infected *Bombus* spp individuals (Otti and Schmid-Hempel, 2007). Once a *N. bombi* infection is introduced into a *Bombus spp*. colony, via workers that were infected in the larval stage, the infection is transmitted to future generations and adults that were not infected prior to the introduction (Steen, 2007). Infection occurs through the ingestion of food that contains *N. bombi* spores. *Nosema* spores are released in the faeces of bees and new hosts become infected through the ingestion of spores in their food (Rutrecht et al., 2007, Steen, 2007). When a colony is infected in the early stage of its development, all individuals are infected, with spores found in a number of tissues e.g. Malpighian tubules, fat body, ovaries and testicular organs. The infection reduces the functional fitness of males and young queens to zero (Otti & Schmid-Hempel 2007). Studies of the impact of *N. bombi* in colonies of *B. lucorum* demonstrated that the parasite success was the highest where larval exposure was high. Once the adults emerged, the parasite load in the adults did not increase. This suggests a switch in the parasite's growth strategy after emergence of the adults, to the production of transmission stages, or the set of a balance between the internal infection and the production of transmission stages (Rutrecht & Brown 2008). *Nosema bombi* can also infect the ovaries of *Bombus lucorum* (Rutrecht and Brown, 2008).

5.1.2. Crithidia bombi

Crithidia bombi (Trypanosomatidae) is a widespread parasite of bumble bees that can decrease colony growth and infect up to 10% - 30% of different *Bombus* species (Schmid-Hempel, 1998). *C. bombi* targets the gut and results in large numbers of parasite cells lining the wall of the mid-gut and rectum from where cells are shed for further transmission (Schmid-Hempel, 1998). Bumble bees are annual social insects with queens that hibernate during the winter. In spring, these queens are the foundresses of new colonies. *C. bombi* can overwinter in these hibernating queens and be transmitted in spring. The virulence of a parasite affects both the temporal dynamics of host-parasite relationships and the degree to which parasites regulate the host populations. If the host can compensate for the parasitism, the parasite may exhibit condition-dependent virulence, where high virulence is seen when the host is under a condition of stress e.g. starvation. This was demonstrated in the host *B. terrestris*. Under favorable conditions, after infection, no mortality was recorded, but in the case of starvation, mortality exceeded 50%. Also, parasite related changes in host resources were recorded; infected bumble bees invested relatively more resources in the fat body and less in the reproductive system. It is unknown whether this is a parasite drive or a host response (Brown et al., 2000).

Crithidia bombi, a parasite of bumble bees (*Bombus spp.*), can be transmitted from mother to offspring. Bumble bee queens hibernate during winter and are foundresses of new colonies in spring. *C. bombi* is able to hibernate during winter in these queens and subsequently infect the spring progeny (Yourth et al., 2008.

5.1.3. Apicystis bombi (Mattesia bombi)

This protozoon is prevalent in *Bombus* spp. It infects the intestines and fat cells. Infected fat cells are reduced. Also, oöcysts are found in the spermateca of queens indicating congenital transmission of *A. bombi*. Little is known about the impact of *A. bombi* on bumble bees (Lui et al 1974).

5.2. Mites

5.2.1. Locustacarus buchneri

Locustacarus buchneri develops in the air sac of larvae, pupae and adults. In adults, the mite develops and reproduces. *L. bombi* hibernates in over-wintering queens. Infested bumble bees are weakened and colony development is impeded (Eijnde & Ruijter 1998).

5.3. Nematods

5.3.1. Sphaerularia bombi

This nematode only infects bumble bee queens during hibernation. The infection impedes the development of the corpora allata, resulting in reduced ovary development. Infected queens survive winter but are unable to found a new colony (Prys-Jones & Corbet 1991).

5.4. Wasps

5.4.1. Mellitobia acasta

The parasitic wasp *Mellitobia acasta* parasitizes the brood of bumble bees. It was found in bumble bee rearing facilities, but no observations of the parasitation in wild bumble bee colonies were recorded. (Wael et al. 1993).

6. Diseases of solitaire bees

Very little is known about diseases and parasites in solitary bees. Mites and parasitic wasps can parasitize solitary bees. In the frame of this report only a microsporidium is presented.

6.1. Microsporidia

An undescribed microsporidium was detected in the adipose tissues (fat body) of *Andrena scotica*. A high spore load reduces the female's reproductivty activity. The spores were only found in *A. scotica*, but not in other *Andrena* species, nor in *Nomada marshamella*, a common cleptoparasite that consumes eggs, young larvae, and food stores of A. scotica (Paxton et al., 1997).

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Annex

Raamwerk en verantwoording hommelziekte en parasietenprotocol hommelteeltbedrijven

Doel

Een wetenschappelijke onderbouwing van de Standard Operator Procedures (SOP) teneinde gezondheid van de hommelpopulatie en de algemene hygiëne in de teeltunit van een hommelteeltbedrijf X.

Methode

Verantwoording monstergrootte tbv controle op *Nosema bombi, Apicystis bombi, Crithidia bombi* en *Locustacarus buchneri*

Voor de controle op *Nosema bombi, Apicystis bombi, Crithidia bombi* en *Locustacarus buchneri* worden jonge koninginnen gebruikt.

Het aantal koninginnen dat wekelijks gemonsterd wordt door XX tbv ziektecontrole en werksters die bij de audit verzameld worden voor onafhankelijk controle op *Nosema bombi, Apicystis bombi, Crithidia bombi* en *Locustacarus bombi* door PRI bijen is gebaseerd de volgende gegevens:

1. Een hommelvolk is ongeveer 10 tot 12 weken in de kweekruimte van XX. Dit is de periode waarin de koningin uit de hibernatie komt, begint met de ovipositie en de volken uitgroeien tot commerciële bestuivingvolken. Dit betekent dat elke drie maanden de hommelpopulatie gewisseld is in de kweekruimte. Dit is uiteraard relatief omdat het een continu teelt is en nieuwe koninginnen voortkomen uit dezelfde SBB populatie. Om een verantwoorde steekproef te nemen worden elke drie maanden 150 hommels (zie statistische verantwoording) onderzocht op ziekten / parasieten. In de praktijk worden daarom wekelijks 18 koninginnen verzameld. De maximale monstergrootte komt hiermee op 216. Hierin is de minimale monstergrootte van 150 redelijkerwijs gegarandeerd omdat er koninginnen gedurende de opkweek van vijf weken uit kunnen vallen.
2. Waarom worden alleen koninginnen gecontroleerd? Koninginnen worden opgekweekt uit volken van de XX hommelpopulatie. In geval er een ziekte / parasiet in de populatie aanwezig is, zullen ook de koninginnen besmet zijn. Hommelparasieten hiberneren in / op de koningin omdat deze het enige individu is dat overwintert. Door wekelijks een steekproef van 18 jonge koninginnen uit de totale jonge populatie koninginnen te nemen en deze na paring en een CO_2 behandeling 5 weken op te kweken geeft een verantwoord beeld van de aanwezigheid van ziekten / parasieten in de SBB hommelpopulatie. Door de koninginnen minimaal vijf weken op te kweken wordt de fysiologie van de koningin en ook die van de parasieten gestimuleerd wat de kans op het vinden van parasieten verhoogt.

Verantwoording controle op *Aethina tumida* en *Tropilaelaps clareae*

Aethina tumida en *Tropilaelaps clareae*, respectievelijk een kever en een mijt komen niet in de EU voor. *Aethina tumida* kan zich ophouden / vermenigvuldigen in hommelvolken, *Tropilaelaps clareae* parasitering komt voor bij bijen en is nog niet bij hommels gesignaleerd. Oorspronkelijk komt deze mijt voor bij de reuzebij *Apis dorsata* maar ook parasiteringen van *Apis mellifera*, de westerse honingbij zijn beschreven. Volgens Europese richtlijnen dient hier op gecontroleerd te worden. Daarom zijn de medewerkers geïnstrueerd afwijkingen aan de hommels en aan het broed en vreemde organismen te melden. Deze volken worden vervolgens geïnspecteerd op *Aethina tumida* en *Tropilaelaps clareae*. De bevindingen worden geregistreerd.

Verantwoording controle op insecten in de kweekruimten van XX

Een hommelkweekruimte kan insecten van buiten aanlokken. Om dit te voorkomen zijn alle buiteningangen van het XX gebouw voorzien van plastic flappen en wordt erop toegezien dat de deuren zo kort mogelijk open zijn. Om te registeren of er insecten binnenkomen zijn 11 lichtvallen geplaatst. Deze worden wekelijks gecheckt op het voorkomen van tenminste *Aethina tumida*, *Tropilaelaps spp* en *Mellitobia acasta.*

Statistische onderbouwing

De steekproefgrootte voor controle van *Nosema bombi, Apicystis bombi, Crithidia bombi* en *Locustacarus buchneri* is gebaseerd op de binomiale verdeling en de OC curve (operation characteristics), approval criterion = 0, disapproval criterion is 1). Bij een monstergrootte van 150 hoort een kans van 22% op een onterecht (vals) goedkeuren van een partij indien 1% van de populatie is geparasiteerd en een kans van 5% in het geval 2% van de populatie is geparasiteerd. Een steekproef van 100 hommels uit een populatie geeft een kans op een onterechte goedkeuring van respectievelijk 37% en 13%.

Omdat parasieten en ziekten zich via teelthandelingen binnen de kweekruimte zullen verspreiden zal een onverhoopte parasitering snel meer zijn dan 2%. Hieruit kan geconcludeerd worden dat 150 een betrouwbare steekproefgrootte is.

XX ziekteprotocol

Het XX ziekteprotocol is opgebouwd uit SOP's

0. het verzamelen van koninginnen tbv ziekte controle

1. Het onderzoek van hommels op ziekte

2. Diagnose van *Locustacarus buchneri* in hommels

3. Diagnose van *Nosema bombi* in hommels

4. Diagnose van *Apicystis bombi* in hommels

5. Diagnose van *Crithidia bombi* in hommels

6. controle lichtvallen

Registratie van gemelde afwijkingen in hommelvolken.

Hygiëne

De vloeren, apparatuur en stellingen in de kweekruimtes en facilitaire ruimtes worden regelmatig schoongemaakt. Het kweekmateriaal bestaat uit wegwerp materiaal.

Audits

Het ziekteprotocol van XX wordt 2 x per jaar geaudit door PRI bijen@wur. Een audit bestaat uit het controleren van de uitvoering van de SOP's dmv documentcontrole, een visuele controle van hommelvolken op afwijkingen (minimaal 20 volken) en vreemde organismen en een algemene indruk van de hygiëne.

Om de betrouwbaarheid van de SBB interne ziekteprotocol te testen worden bij elke audit, ad random 150 werksters uit volken genomen (1 per volk met minimaal 20 werksters en een koningin) en door PRI bijen@wur gecontroleerd. Volgens de PRI bijen@wur SOP's.

142: Diagnose van *Locustacarus buchneri*

143: Diagnose van *Nosema bombi*

144: Diagnose van *Apicystis bombi*

145: Diagnose van *Crithidia bombi*

English summaries

Sampling for parasites check at PRI bijen@wur.

To check the robustness of the XX bumble bee health system, Worker bees were taken from colonies that were queen right and had more than 20 workers. Per colony one worker was taken for parasite diagnosis. About 150 colonies were sampled. The worker bees were checked for parasites in the laboratory of PRI bijen@wur.

Parasite diagnosis of adult bumble bees at PRI bijen@wur.

The bumble bees were diagnosed according to current PRI Biointeracties Bijen SOPs

142: Diagnose van *Locustacarus buchneri* / Diagnosis of *Locustacarus buchneri*

143: Diagnose van *Nosema bombi* / Diagnosis of *Nosema bombi*

144: Diagnose van *Apicystis bombi* / Diagnosis of *Apicystis bombi*

145: Diagnose van *Crithidia bombi* / Diagnosis of *Crithidia bombi*

Sample size

1. A bumble bee colony is approximately 10 to 12 weeks in the rearing unit. It takes this period from end of hibernation to commercial bumble bee colony suitable for pollination. So roughly every three months the population in the rearing unit has changed. Therefore within this 3 months period 150 individuals must be samples (see statistics). The weekly checks of 18 queens meet this requirement.
2. Queens are reared from colonies in the rearing unit. In case a parasite is prevalent in the rearing unit, also young queens will be infested. By checking a sample of 18 young queens from batches that are hibernated directly after mating the health status of the batch is checked and it is prevented that possible infected hibernated queens enter the rearing unit to start a new colony. To increase the chance to find parasites, the young queens are not hibernated but treated with CO2 and placed in starting boxes. This stimulated the physiology of the queens, and with that also the parasites.

In the rearing units 4 and in the central room 7 blue light traps are placed.

From week 21 (2009) the traps will be checked weekly (but not restricted) to for *Aethina tumida*, *Tropilaelaps spp*, *Mellitobia acasta.* All entry doors of the XX plant have plastic flaps to prevent the income of insects form outside.

Statistics

The sample size is based on the OC curve (operation characteristics), approval criterion = 0, disapproval criterion is 1. A sample size of 150 individuals results in a chance of 22% to a false approval of a population in which 1% is parasitized and of 5% in case 2% of the population is parasitized. A sample size of 100 bumble bees results in false approval of 37% and 13% respectively.

As parasites tend to spread within a bumble bee rearing unit because of handling and a closed room, parasitism will soon exceed 2%. It can be concluded that 150 is a reliable sample size to check the parasite load of the population.

XX bumble bee health system

0. het verzamelen van koninginnen tbv ziekte controle

1. Het onderzoek van hommels op ziekte

2. Diagnose van *Locustacarus buchneri* in hommels

3. Diagnose van *Nosema bombi* in hommels

4. Diagnose van *Apicystis bombi* in hommels

5. Diagnose van *Crithidia bombi* in hommels

6. controle lichtvallen

Registratie van gemelde afwijkingen in hommelvolken.

Hygiene check

The hygiene is checked visually in situ.

Sampling for parasites check at PRI bijen@wur.

To check the robustness of the SPP bumble bee health system, Worker bees were taken from colonies that were queen right and had more than 20 workers. Per colony one worker was taken for parasite diagnosis. About 150 colonies were sampled. The worker bees were checked for parasites in the laboratory of PRI bijen@wur.

Parasite diagnosis of adult bumble bees at PRI bijen@wur.
The bumble bees were diagnosed according to current PRI Biointeracties Bijen SOPs

142: Diagnose van *Locustacarus buchneri* / Diagnosis of *Locustacarus buchneri*

143: Diagnose van *Nosema bombi* / Diagnosis of *Nosema bombi*

144: Diagnose van *Apicystis bombi* / Diagnosis of *Apicystis bombi*

145: Diagnose van *Crithidia bombi* / Diagnosis of *Crithidia bombi*

Parasite diagnosis of brood parasites

The brood of 20 ad random selected colonies will be visually checked for the prevalence of *Aethina tumida* and *Tropilaelaps spp*.