# *Parasites of the Honeybee*

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# Table of Contents

# Introduction

The greatest value of honeybees is in their service as pollinators and this far outweighs their value as producers of honey or honeybee products. In Ireland, the use of honeybees for the pollination of commercial crops is limited and though they facilitate in the pollination of many plants in the countryside; they are kept primarily for honey production. Apiary hygiene and early identification of pests and diseases is crucial in achieving this goal. This publication endeavors to assist beekeepers in detecting, monitoring and treating when possible these diseases in their colonies, thereby improving the general health of bee stocks and subsequently maximizing honey yields.

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# Bee Origin and Classification

## Bee Origin

Bees are a large and diverse group of **Hymenoptera** that includes several taxonomic families (Appendix 1). They are thought to have evolved from the **sphecid** wasp and thus were originally predators, but later abandoned predation in favor of provisioning their nests with **nectar** and **pollen**. In order to ingest nectar, bees developed specialized mouthparts and adaptations for pollen collection: all bees have at least a few plumose hairs and broadened hind legs, which are used to gather pollen and transport it back to their nests.

Although fossil records of bees are far from complete, the sphecid wasp was thought to occur during the middle of the Cretaceous period, about 100 million years ago[45], coincidental with the appearance of flowering plants (**angiosperms**) as the dominant vegetation. In fact evolutionary history suggests that bees and flowering plants have closely co-evolved throughout the last 100 million years. Angiosperm flowers developed odors, varied in shape and produced nectar, which are all characteristics that attract bees and thus facilitate in pollination. Some flowers have even evolved to mimic female bees to attract pollinating males.

Bees are classified into the super-family, Apoidea[18] because of their distinctive pollen collecting structures and habits. Within Apoidea there are currently 10 or 11 families[46], with approximately 700 genera[42] and 20,000 living species[44]. These are divided into two groups; the primitive short tongue bees and the advanced long tongued bees. Long-tongued bees are able to take advantage of the increasing complexity of advanced angiosperms, while the short tongued bees prefer to visit more primitive shallow angiosperms. In Ireland, there are 101 bee species, there is one native honeybee species (*Apis mellifera mellifera*), 19 species of bumblebee and 81 species of solitary bees[25]. Honeybees and bumblebees are highly social insects, living in colonies with a queen, some males and large numbers of female workers, while solitary bees prefer to live alone, although some species build their nest in little groups or aggregations. The mechanism for over wintering also varies between the bee species. Honeybees successfully maintain their colony throughout the year, while in bumblebee species only the young queen survives, emerging in the spring after hibernation. Solitary bees take one year to complete their life cycle and may only survive two weeks as an adult, thus these species over winter as pupae and in the following spring the young adults emerge, mate and the cycle begins again.

## Honeybees

Honeybees are classified in the family Apidae, subfamily Apinae, tribe Apini (Appendix 1). Close relatives of the honeybees are the orchid bees (Euglossini), bumblebees (Bombini), and stingless bees (Meliponinae). All members of the Apidae show some degree of **social behavior**, with Meliponinae and Apinae having the most elaborate social

behaviors of all bees. Social insects are best described as insects which live in a society with each society consisting of two parents (or at least a **fecundated** female) and their off-spring. The two generations live together in a common abode, exhibiting some degree of mutual co-operation. In such a society, there is a necessary lengthening of the life span of the colony mother, which is associated with a degree of specialization for a particular type of work. The queen in the honeybee colony may live for 3-4 years and is specialized for egg production. This specialization has not occurred by the queen adapting to undertake a new type of work, but rather by the suppression of any tendency to perform other types of work[10].

## Honeybee Species

Honeybees (Apidae: Apinae) are classified into the genus ***Apis*** which includes five main species: the common honeybee (*Apis mellifera*), the giant honeybee (*Apis dorsata*), the Indian honeybee (*Apis laboriosa*), the Asian honeybee (*Apis cerana*) and the little honeybee (*Apis florea*). Four of these species, *A. dorsata*, *A. laboriosa*, *A. cerana*, and *A. florea* are abundant in the wild in Southern India, Ceylon and other parts of Southern Asia, while *A. mellifera* is native to Europe and possibly North Africa (Figure 1).

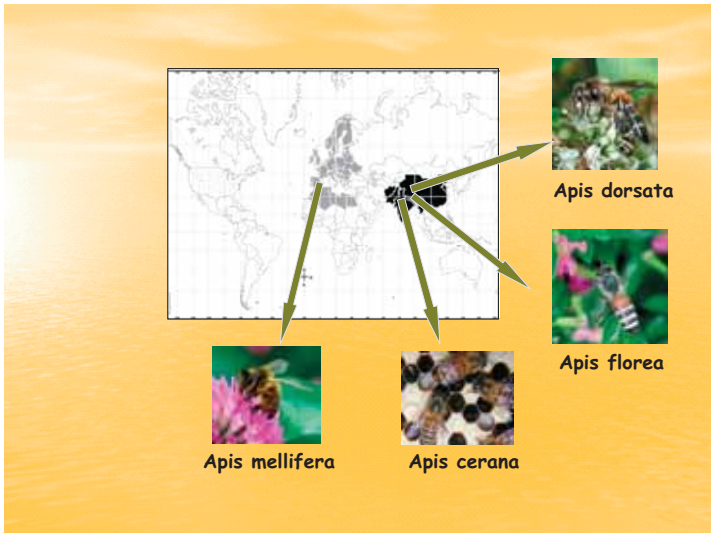



Figure 1*: Honeybee species and their native location*

*Apis cerana* and *A. mellifera* are medium sized bees (10-11mm). In the wild, they build multiple comb nests in cavities and thus have adapted well to life in a hive. The worker population in *A. cerana* is relatively small, 6000-7000 workers, while *A. mellifera* colony members often exceed 80 000 individuals. In contrast *A. florea, A. dorsata and A. laboriosa* build single comb nests in the open and have not been induced to life in a hive. *A. florea* workers are small, approximately 7mm in length. Colonies also tend to be small <5000 individuals. They build their nest suspended from branches, surrounded by dense vegetation. In contrast, *A. dorsata and A. laboriosa* are large bees, 17-19mm in length, with colony members reaching 20 000 workers. Their nests, which consists of a single comb is constructed high in trees or suspended from open cliff faces. The workers are

very aggressive and their nests do not need to be concealed. Nests are frequently aggregated and colonies migrate up and down mountains to take advantage of seasonal nectar sources.

## *Apis Mellifera* Subspecies

Different subspecies of *Apis mellifera* have originated in Europe (A. *mellifera ligustica, A. mellifera carnica, A. mellifera* caucasica and *A. mellifera mellifera*), Africa (*A. mellifera scutellata* and *A. mellifera capensis*) and the Middle East and Asia (*A. mellifera macedonica*) (Appendix 1). In Ireland there is only one subspecies, *A. mellifera mellifera*, commonly known as the dark bee of northern Europe. It has been domesticated successfully and during colonial times introduced into America. The introduction of the African honeybee (*A. mellifera scutellata*) to South America constitutes part of the ancestry of the Africianized bee, colloquially known as 'killer' bee. The latter is a hybrid of the African honeybee (*A. m. scutellata*) and a European honeybee subspecies such as *A. m. ligustica*[17]. Today, in Central America and in tropical areas of South America, the Africanized hybrids are the preferred type of bee for beekeeping, largely due to their relatively high productivity. However, in South Africa, the integration of the two traditional subspecies *A. m. scutellata* (north) and *A. m. capensis* (south) has created the Cape bee problem. The latter has a trait scientist's call thelytoky. This is the reproduction of female queens or workers by a laying worker. *Scutellata* colonies readily accept laying workers from *capensis* colonies, which eventually destroy their true queen in favor of their infertile Cape honeybee queen. This inevitably results in population decline and colony demise. This phenomenon is called "social parasitism," and has been responsible for tens of thousands of colony losses in *scutellata* country. Research is being carried out, but there is no immediate answer to the problem.

# The Honeybee Colony

Honeybees cannot survive as individuals, but require the social setting of a colony. Within the colony there are three **castes**, the queen, the worker and the drone (Plate 1).

(a) (b) (c)

Plate 1*: Honeybee castes: (a) worker; (b) queen; (c) drone*

The queen and the workers are females that develop from a fertilized egg, have 32 chromosomes and are referred to as **diploid**. The drone is the male and develops from an unfertilized egg, thus has only half the number of chromosomes and is described as **haploid**. The caste determination between queen and worker cannot be attributed to any genetic differences and is primarily due to the methods of feeding during larval development.

A typical honeybee colony in early spring consists of one queen and approximately 10 000 adult workers, which increases to approximately 50 000 in summer. The colony will also support 200-1000 drones between May-August, but by late autumn they will be removed by the workers. In addition to the adult bee population, the colony will have variable amounts of **brood** at different developmental stages throughout most of the year. Within the life cycle of the bee, there are four distinct stages; egg, larva, **pupa** and adult (Plate 2).

(a) (b) (c) (d)

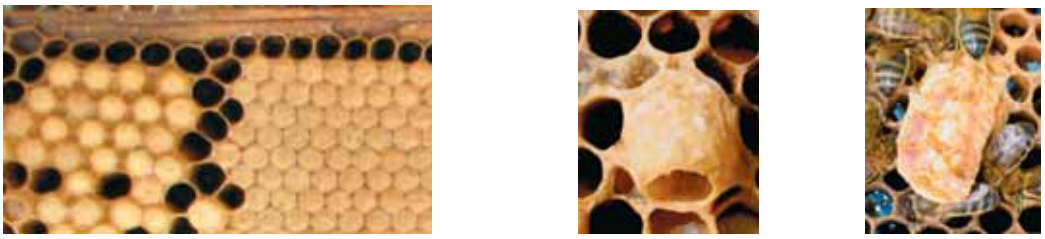
Plate 2: *The different stages of metamorphosis: (a) egg; (b) larva; (c) pupa; (d) adult*

This series of development is referred to as **complete metamorphosis**. During the first three days, the embryo inside the egg develops rapidly. Prior to hatching, irrespective of caste, the egg is provided with a minute drop of bee milk, hereafter referred to as royal jelly. The newly born worker larvae are liberally fed royal jelly for two to three days followed by a honey and pollen diet. This change of food determines the worker caste. When the diet is not changed and remains royal jelly throughout the larval period, a queen will develop. During the larval stage five **molts** take place. After eight days, for both the queen and the worker, cells are sealed with a porous capping. This is called the capped brood stage. Inside the sealed cell, the larvae spin a cocoon and undergo the process of pupation. The duration of the developmental stages depends on the caste (Table 1).

Table 1*: The duration of the different developmental stages (days) of the three honeybee castes*

| | | Days | |
|---|---|---|---|
| **Open cell** | **Worker** | **Queen** | **Drone** |
| Egg | 3 | 3 | 3 |
| Larvae | 5 | 5 | 7 |
| **Sealed cell** | | | |
| Pro-pupae | 3 | 2 | 4 |
| Pupae | 10 | 6 | 10 |
| **Egg-emergence** | 21 | 16 | 24 |

Workers and drones are reared in the hexagonal shaped cells on the comb, while a queen is reared in an acorn shaped cell, normally protruding vertically from the comb surface, with the opening at the bottom (Plate 3(a-c)). Drone cells are larger than worker cells as indicated by the following width and depth approximations; drone=6.5mm; worker=5.5mm (width); drone=14mm: worker 11/12mm (depth).



(a) (b) (c)

Plate 3: *(a) Drone and worker brood; (b) queen cup; (c) sealed queen cell*

## The Queen

Plate 4: *The queen*

The total development time of a queen from egg to emergence is approximately16 days (Plate 4). The first day of her adult life is spent seeking out and killing potential rivals. The virgin queen will then fly out on a number orientation flights to establish the location

of the hive, before she begins a series of mating flights. Such flights may continue for three weeks and during this period a queen may mate with 6-18 drones and store 5-12 million quiescent sperm in a special sac in her abdomen called spermatheca. Once this initial period of copulation is passed, the queen no longer attempts to mate. Instead, her abdomen enlarges to accommodate her fertile ovaries and egg laying is initiated. During peak season a queen may lay approximately 1000-2000 eggs per day, but does not assist in the nursing of the brood. The queen has the ability to fertilize eggs before laying and will measure the size of the cell with her front legs to determine whether an egg needs to be fertilized or not[41], drones will be unfertilized while worker eggs will be fertilized (Plate 5).

Plate 5: *Laying queen measuring cells with front legs*

Occasionally, the queen lays fertilized eggs in worker cells, which can potentially develop into males called **diploid drones**. Such drones result from the **match-mating** of queens and drones with identical sex alleles, due for example to inbreeding, which is exemplified in colonies by gaps in the brood area. In *A. mellifera* 'false diploid' male larvae are eliminated by worker cannibalism immediately after hatching.
Mating has a profound effect on queen behaviour and physiology. Not alone does a queen start laying, but she also produces mandibular gland **pheromone** or 'queen substance' which alters worker behavior. This pheromone is licked from the queen by a retinue of attendants and distributed around the hive by means of normal food transfer (Plate 6), a behavior known as **trophallaxis**.

Plate 6: *Retinue of workers licking pheromone from the queen*

Workers who receive more than a small amount of this pheromone in their food, are inhibited from building queen cells. In a normal colony this is the situation for most of the year. However, if workers receive insufficient pheromone, because of a reduction in the amount produced by the queen, due to age or physical disability, worker bees will construct queen cells in preparation to replace her, a process referred to as **supersedure**. If however, the inhibition occurs because of rapid colony growth and thus a breakdown in the food transfer, the **swarming** instinct will be initiated. Thus, to minimize the risk of a slowdown in brood rearing or queen failure in the middle of the production season, it is recommended to replace the queen every 1-2 years and alleviate congestion by putting on **supers**, approximately two weeks before they are required.

## The Drone

Plate 7: The *drone honeybee on the comb*

The drone is the male bee of the colony (Plate 7), and their rearing and feeding requires considerable resources from the colony. It develops from an unfertilized egg and is haploid, thus all its genetic characteristics originate from the colony queen. In summer, a colony may maintain 200-1000 drones, but in winter they are eliminated from the hive. Drones do not perform any hive duties, forage or sting and their sole purpose is to mate with a virgin queen from a different colony and thus dissipate the genes of the colony. Drones have specific areas where they collect to mate called drone congregation areas. In these areas, the drones release a pheromone from their mandibular glands which not only attracts queens, but also attracts other drones in the area, thus ensuring a good mix of genetic material which minimizes inbreeding. Mating occurs on the wing and the strongest flying drone in the '**comet**' usually fertilizes the queen. Immediately after mating the drone dies.

## The Worker

Plate 8: *The worker honeybee*

The worker (Plate 8), though originating from a fertilized egg, usually develops into an infertile female. This is caused by a reduction in feeding at the larval stage, which results in the dwarfing of the ovary. The winter worker bees have a life span of approximately six months, while summer worker bees have a much shorter life, usually about six weeks. The life of the worker is devoted to carrying out the many tasks necessary for colony development. Many duties carried out by the worker are the result of physiological changes that take place in her life, most notably glandular secretion of royal jelly and wax. Its life may be divided into three distinct phases: (1) the nurse phase, (2) the domestic phase and (3) the foraging phase (Table 2). However, due to variation in colony needs and other environmental factors, these phases are not always rigidly adhered to in nature.

Table 2: *The duration of activities (days) of the worker honeybee at different stages of development*

| Worker Honeybee Life Phases | Time | Main Activities |
|---|---|---|
| **Nurse phase** | 1-9 days | Cell cleaning<br>Capping brood<br>Tending brood |
| **Domestic phase** | 10-21 days | Queen tending<br>Receiving nectar<br>Handling pollen<br>Comb building<br>Cleaning debris<br>Ventilation<br>Patrolling<br>Resting<br>Guard duties |
| **Foraging phase** | 3-6 weeks | Orientation flight<br>Foraging |

## Nurse Phase (Days 1-9)

Initially, young bees clean cells and incubate brood. After 3-4 days, the brood food gland is activated in the head of the young bee and it concentrates on feeding older larvae a mixture of pollen, honey and royal jelly. By day 7, the young bee has progressed to feeding young larvae with royal jelly only.

## Domestic Phase (Days 10-21)

During this phase the **hypopharyngeal glands** and the **wax glands** are activated in the young bee. The former produce the **enzyme**, invertase, which is required for the ripening of honey, while the wax glands, located on the ventral side of the abdomen enables the bee to secrete wax. Other bee activities during this phase include cooling of the nest, pollen packing and the evaporation of excess water from the nectar. Towards the end of this phase (days 19-21), the bees become guards and make their first orientation flight from the nest in preparation for foraging.

## Foraging Phase (Weeks 3-6)

Honeybees forage for nectar, pollen, water and **propolis** and their average flight speed is approximately 24km/h[27]. Nectar is the main source of carbohydrates for the colony. The fresh nectar is converted into honey by a series of physical and chemical changes and is then stored in the comb as a winter food supply. Pollen is the main source of protein and is fed in large amounts to developing brood. Water is used for cooling the hive and diluting winter stores. Propolis or bee glue is used to plug unwanted holes within the hive. Other activities of the worker bees include nest **homeostasis**, the most important aspect being **thermoregulation**.

Honeybees can survive periods of cold weather using energy derived from the consumption of stored honey to generate body heat and keep the nest at an adequate temperature for adult survival. Clustering helps to maintain colony temperature and as the temperature drops the cluster contracts. This conserves heat by diminishing the surface area over which heat can be lost and reducing internal convection currents. The minimum temperature required for workers to cling to the cluster is a centre temperature of 13°C, which maintains the outer cluster at 8°C[68]. In the presence of brood, thermoregulation is much more critical. Nest temperatures in brood areas must be maintained at 30-35°C. As ambient temperatures rise, nest cooling becomes increasingly important, especially in the presence of brood. Temperatures above 36°C for any appreciable time are harmful to brood and excesses of 1-2°C can cause developmental abnormalities and death. Ventilation is also employed by the bees to reduce nest temperature. Cooling air currents and suction which draws the warm air out of the nest are created, when workers line up at the entrance and in the brood nest and fan. If fanning is not sufficient, workers can further cool the nest by water evaporation.

## Development Phases and Colony Requirements

Although the above describes the transitional phases in the life of the worker honeybee, the high level of co-ordination apparent in honeybee colonies suggests a more profound level of organization than simply a gradual transition between highly overlapping task groupings.

The quantity and quality of nectar available affects brood rearing, food handling and comb building and the ages at which these tasks are performed[56]. Surplus nectar and in particular, pollen increases brood rearing, while surplus honey and fresh nectar stimulates wax secretion and comb building. Colony population is also a critical factor. If a colony is decreased by natural causes such as predation, swarming, nest damage or disease, workers forage at a younger age and have a shorter life span[69]. Another aspect of internal organization which influences cast ontogeny is the location of jobs within the nest or the spatial organization of the colony. A study by Seeley has shown that the youngest bees clean cells because they can be easily located and are close to the area from which they have just emerged, while older house-bees are more mobile and move to the periphery of the nest and undertake activities such as grooming, feeding and food handling chores. The final sub-caste involves foraging and other activities outside the hive[58]. The genetic component to caste determination was demonstrated by Winston and Katz who reported that there are genetically based differences in the **ontogeny** of at least one task, foraging, between temperate- and tropical-evolved bees[70]. Results indicate that **Africanized bees** (tropical) forage significantly earlier than the European (temperate) bees.

# Irish Floral Sources of Pollen and Nectar

Nectar and pollen come from a very diverse flora. The foraging season may be divided into four main periods: early spring period, main spring period, mid summer period and the autumn period[16]. Early spring period is dominated by a variety of trees and herbs. Important species include *Prunus/Pyrus* type, *Ranunculus ficaria*, *Salix* spp. and *Ulex* type. The latter, though nectarless is foraged solely for its large quantities of pollen, which are required by honeybee colonies for expansion during the spring period. *Acer* spp. and *Crataegus monogyna* are important pollen and nectar sources during the main spring period. *Ulex* type continues to supply pollen in addition to *Salix* spp, which is also a good source of nectar. Early June is a transitional period between the spring flow and the main summer flow and is often referred to as the June-gap. Flowers foraged during this period are predominantly pollen sources such as *Sambucus* spp., a nectarless species, *Ranunculus* spp. and *Rosa* spp., both producing very little nectar and *Trifolium pratense*, though producing nectar is not easily accessible to honeybees due to the corolla length. By late June, *Trifolium repens* s.l. and *Rubus* spp. are the main sources of pollen and nectar. *Filipendula ulmaria*, though nectarless is foraged extensively by bees for pollen. During the autumn period, bees continue to forage on *Rubus* spp. due to its long flowering season. *Impatiens glandulifera* and *Calluna vulgaris* can be important local sources of nectar in addition to *Epilobium angustifolium*. The final pollen and nectar source is *Hedera helix*, which under favorable weather conditions can be utilized until October. It is an important source of pollen and also a rich source of winter honey stores for bees. Common names for the above mentioned plant species are given in Appendix 2

# Pests and Diseases of the Honeybee

Honeybees are affected by a number of pests and diseases that may be subdivided into three main categories: (1) pests, (2) diseases and (3) viral infections. Good bee husbandry should include, monitoring colonies for diseases, becoming familiar with the symptoms of the various adult and brood diseases and sending samples regularly for disease diagnosis. Sampling methods for the diagnosis are given in Appendix 3. In the following chapters, these diseases will be briefly described, symptoms listed and guidelines for treatment and management outlined. Although not all the described pests and diseases occur in Ireland, for example the *Tropilaelaps* mite and the Small hive beetle, they are listed as quarantine species and thus are notifiable according to the European Commission. This means that all beekeepers who suspect their colonies to be infested must inform the appropriate authorities in their member states. Foulbroods which occur in Ireland are still notifiable diseases.

# Pests

## Varroa Mite

*Varroa destructor*, originally classified as *Varroa jacobsoni*, and commonly referred to as 'varroa' is a highly destructive pest that can severely reduce honey production. Varroa is a small mite that causes precocious reduction in foraging, increased drifting and high mortality during the winter. The mite originated on the Asian bee, *Apis cerana*, where a natural host-parasite relationship exists. However, by movement of *Apis mellifera* into areas where *Apis cerana* is endemic, varroa was transferred to a far less resistant host. Untreated colonies of *Apis mellifera* will collapse within two years of infection, thus indicating an **incomplete host-parasite relationship**.

### Biology

The adult female is a reddish brown, relative large mite, 1.1mm long x 1.5-1.6mm wide (Plate 9). It is flattened and oval in shape and its dorsal shell covers the entire **idosoma**. It has an indistinct head and four pairs of legs, which protrude from one side of this ellipsoid shell. It is difficult for the bee to remove the mite by grooming because of its flattened shape, the suckers at the base of each tarsus and the stiff hairs on the ventral side of the body. Mites are generally found between the first abdominal segments, but may also be apparent between the head and the thorax and between the thorax and the abdomen.

(a) (b) (c)

Plate 9: *Physical features of the varroa mite: (a) dorsal; (b) ventral; (c) on adult worker*

In these regions the inter-segmental membrane is more easily penetrated and the mite can access the **haemolymph** of the infested bee. The male is smaller (0.7mm x 0.7mm) and yellow-white in color. The **chelicerae** of the male are transformed for sperm transfer, thus males cannot feed and are never found outside the brood cells.

## Life Cycle

The varroa mite life-cycle can be divided into two stages, the phoretic stage and larval stage (Figure 2).

### The Phoretic Phase

The adult bee is only an intermediate host and a means of transport for the mite. The female mite ingests small quantities of adult haemolymph but, to stimulate egg laying, she must consume larval haemolymph. Mites can live for several months on adult bees. The rate of infestation depends on brood ratio and the age of the mite. However, most mites enter brood cells for reproduction a few days after they have been released into the colony.

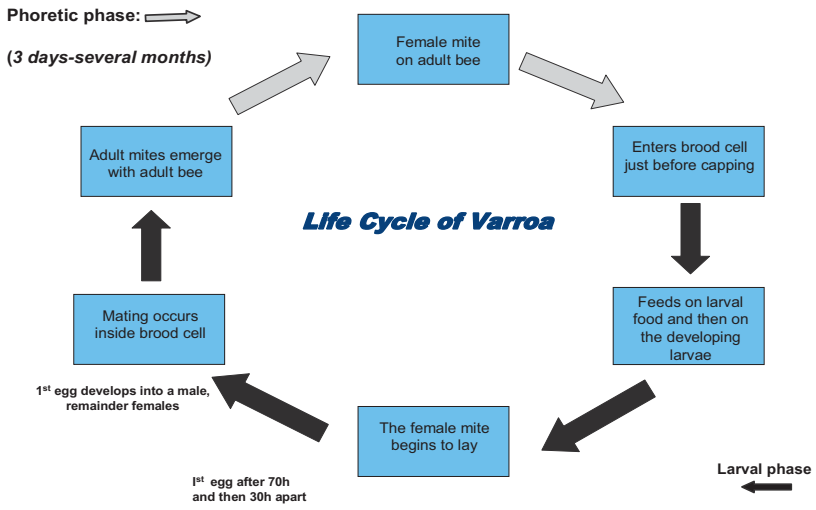



Figure 2: *Life cycle of the varroa mite (Varroa destructor)*

(a) (b)

Plate 10: *The varroa mite: (a) the male; (b) the different developmental stages of the mite*

### The Larval Phase

The adult female enters the brood cell 5-6hrs before capping. Drone larvae are preferred to worker larvae and queen cells are only entered in cases of high infestation. The mite immerses herself in the liquid brood food at the bottom of the cell until the cell is capped and the larva has finished spinning the cocoon. The mite then feeds on the developing larva and after three days starts to lay its eggs. At varying intervals, the female will lay 2-5 eggs. Inside the egg, within 24hrs, a six legged larva develops followed by the eight-legged protonymph. Once hatched, the protonymph feeds on the haemolymph of the pupa for 1-2 days, before molting into a deutonymph. Feeding continues for a further 3-4 days before molting to the adult stage. The first egg develops into a male the remainder into females. Maturation for female mites is 6.0-6.2 days, while for males it is 6.8-6.9 days (Plate 10). Mating takes place between males and females in the cell. The varroa mite exhibits **haplo-diploid breeding**, thus permitting very low levels of **deleterious recessive alleles**, which may be a pre-adaptation to inbreeding.

## Varroa and the Adult Bee

Individual bees infested with one or more offspring varroa mites during development can survive without any visible damage and are normal in appearance. However, in bees which have been more severely infested, deformed wings, bloated abdomens and discoloration of legs are typical symptoms of infection (Plate 11) (*see deformed wing virus*).

(a) (b) (c)

Plate 11: *The effect of varroa on the adult honeybee: (a) deformed wings; (b) bloated abdomen; (c) discoloration of legs*

Infested bees also show a marked reduction in body weight and their haemolymph proteins are reduced, with the low molecular components often being depleted. The longevity of the bee is also reduced and, in general, the bees become increasingly prone to viral and bacterial infections.

## Varroa and the Brood

The nutritional demands of the mites are very high owing to their inefficient metabolic activity. The mites will use up to 25% of the nutritional reserves of the pupae accumulated in the tissue during the larval stage[28]. This, and the transfer of viral particles, contribute to malformation and weakening of individual bees (Plate 12) and will eventually lead to colony collapse. Reproduction occurs in both the drone and the worker brood, but the drone brood is often 5-9 times more infested. This preference may be due to a number of reasons; (1) the chemical stimuli produced by the developing larva, (2) drone cells are larger in size, (3) cells are unsealed for a longer period and (4) cells are capped for longer, thus allowing more daughter mites to reach sexual maturity. The effect of parasitism during the pupal stage is a reduction in the body weight, in the life expectancy and in the survival of the emerging bee. Infested drones also produce fewer sperm and have a reduced flight duration, which is of critical importance during the multiple mating sequences exhibited by the queen. The infested drones may also be a source of viral infection and a possible sexual transmission route for viral particles during mating (*see viral diseases*).

(a) (b)

Plate 12(a-b): *Varroa on developing larvae*

## Varroa and the Colony

Symptoms of varroa mite infestation in the honeybee colony depend on the degree of infestation. Low levels of infestation have no obvious effect, while the appearance of a spotty brood pattern and malformed drones and workers on the comb is an indication of medium to high infestation (Plate 13).

(a) (b)

Plate 13: *Symptoms of varroa infestation in honeybee colony: (a) spotty brood pattern; (b) malformed bees on the comb*

Eventually, the mite population reaches a level that the colony can no longer tolerate and thus loses its social organization and disbands. This is referred to as **colony collapse** (Plate 14). Typical symptoms of colony collapse are; a sudden decrease in the adult bee population, dead bees in the hive, various abnormalities of the brood (*see bald and chilled brood*) and numerous varroa mites on the remaining bees and in the brood cells.

Plate 14: *Colony collapse as a result of varroa infestation*

## Detection

Early detection, careful monitoring, prompt treatment and good beekeeping practices are essential for varroa control. Mite levels may be determined in the hive by monitoring **natural mite fall, drone brood sampling** or by using the ether roll test.

### Monitoring Natural Mite Fall

Place an insert under a **varroa floor** (Plate 15) for 3-5 days and count the number of mites that fall naturally, that is, without any anti-varroa treatment. If the average daily mite fall exceeds the following, treatment is necessary: (mites/day) Jan-Mar-2; Apr-May-7; Jun-Aug-8; Sept-Dec-8. Colony collapse is imminent at the following infestation levels: (mites/day) winter-spring-0.5; May-6; Jun-10; Jul-16; Aug-33; Sept-20 (www.irishbeekeeping.ie).

Plate 15: An *insert is placed underneath brood box to estimate the mite population in the colony using natural mite fall*

## Drone Brood Sampling

During the active season, drone brood is removed at the purple eye stage with an uncapping fork. If <5% of the brood is infested, the colony can be considered lightly infested, while >25% indicates a severe infestation and possible colony collapse (Plate 16).

Plate 16: *Estimating mite infestation using drone brood sampling*

## Ether Roll Test

Brush approximately 500 bees into a jar and spray briefly with engine starter fluid (Plate 17). Shake the jar vigorously for 15-20s, turn the jar on its side and gently roll it. If present, the mites will adhere onto the sides of the jar. Because the ether roll test only samples a portion of the population, the total colony mite population has to be calculated. *No brood*: multiply 100; *plenty of brood*: multiply 600. Colony collapse in imminent when mite population is >1000.

(a) (b) (c)

Plate 17: *Ether roll test: (a) sample bees; (b) add ether-based aerosol starter fluid; (c) count mites*

## Prevention

The prevention of this pest is difficult, however regular monitoring of the mite population and consequently managing/treating accordingly may reduce the adverse effects which the feeding mite has both on the developing larvae and the adult bees. Co-ordinated treatment within an area is also important as it not alone reduces the risk of re-infestation, but also the transfer of viral infection between apiaries. Finally, monitoring for resistance is becoming increasingly important (*see method in Appendix 4*).

## Treatment

*Varroa destructor* can be controlled by a number of different treatments which may be subdivided into four main categories: (1) chemical, (2) biotechnical (3) organic acids and (4) biological. Specific products in each group are given in Figure 3.

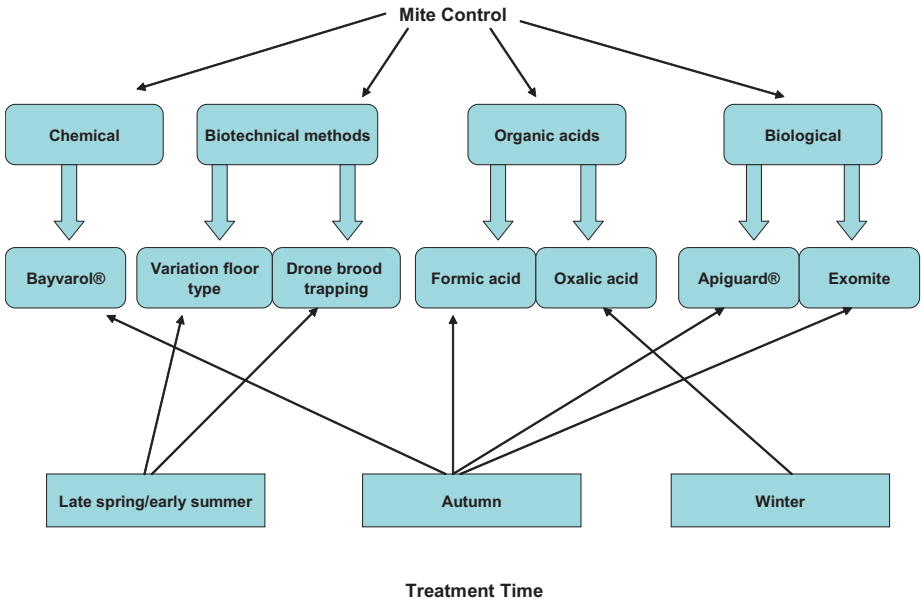



Figure 3: *Varroa treatments and treatment time*

*Note: Bayvarol® and Apiguard® are the only products registered for use in Ireland, but biotechnical methods should be incorporated into bee management*

In general, the efficacy of chemical products is greater than non-chemical based products. In Ireland only two products, Bayvarol® and Apiguard® are registered and legally available for use in honeybee colonies. However, biotechnical methods such as drone brood trapping and open mesh floors incorporated into hive management reduces mite populations especially during honey-flows and when no other treatment is feasible. In the United States, Canada and many parts of Europe, the organic acids, such as oxalic and formic acids, are being used extensively either as a sole treatment or part of an integrated system.

## Bayvarol®

This is a spring or autumn chemical treatment which should not be applied during foraging or before honey harvesting. It is a contact pesticide, killing mites only in the phoretic stage. The treatment period is six weeks, thus incorporating two brood cycles. Bayvarol® strips are plastic strips impregnated with flumethrin. When treating a colony in a single brood box four strips should be suspended in the spaces between the combs in the centre of the brood area (Plate 18). Two strips are sufficient when treating a nuclei.

(a)


(b)

Plate 18: *(a) Bayvarol®; (b) strips distributed over the brood area*

Bees crawl over the strips distributing the active ingredient throughout the hive. This product has >95% efficiency, however, the varroa mite like most mites will develop resistance to the pesticide. This occurs because individual mites differ in their susceptibility to a given substance. If a population of mites is exposed to a varroacide dose that only kills the more susceptible ones, the resistant mites will survive and reproduce and thus overtime develop a resistant population. This process of **resistance** is accelerated by the fact that **acaracides** are **lipophyllic,** and thus mites are continuously being exposed to non-lethal doses of the chemical in the wax. Misuse of strips also encourages resistance.

## Apiguard®

Apiguard® is a natural product (Plate 19(a-b)). It has a slow release gel matrix, ensuring the correct dosage of the active ingredient thymol. The latter is a naturally occurring substance derived from the plant thyme (*Thymus* spp.). It has a proven high efficacy against the varroa mite and is also active against both tracheal mite (*see tracheal mite*) and chalkbrood (*see chalkbrood*). It is distributed around the hive by inhalation and contact. The percentage efficiency is estimated at 93%, but at ambient temperatures <15°C, efficiency is significantly reduced. Prior to administration additional bee space should be created above the brood frames using an **eke** (Plate 19c). Apiguard® is administered by placing a 50g container over the brood area (Plate 19b) and two weeks later adding a second. Treatment should continue until the containers are empty or supers are about to be placed for the spring flow.


(a) (b) (c) (d)

Plate 19: *Apiguard® treatment method: (a) apiguard®; (b) application method; (c) eke; (d) upturned feeder*

An upturned feeder (Plate 19d) may also be used to provide bee space and hive roofs should be insulated to conserve heat. It is not recommended to apply Apiguard® and feed simultaneously and mesh floors should be replaced with solid floors or at least closed off using an insert.

## Drone Brood Trapping

Large number of mites can be removed from an infected colony, without affecting the worker population using drone brood trapping. Mites are attracted to the drone brood for reproduction and thus a disproportionately large number of mites will be associated with the drone brood. During May-July, a shallow frame may be placed in the brood box, close to the brood area (Plate 20).

Plate 20: *Drone brood trapping: shallow frame showing sealed drone brood*

In a **queen right colony**, the bees will naturally build drone comb and mites will be attracted to the developing larvae. Once the cells are sealed, the mites are trapped inside and the comb can be cut off and removed from the hive. The shallow frame is placed back into the brood box so that the cycle may be repeated. Drone removal does not adversely affect colony health or honey production[12], thus may serve as a valuable component in an integrated pest management programme. However, it is possible that the large scale removal of drones from a colony could have an adverse affect on the colony's social structure and further aggravate the problem of reduced number of drones available for mating already caused by viral infections (*see viral infections and brood diseases*). Thus, drone brood trapping is only recommended in colonies with high mite infestation in early summer when no alternative treatments are feasible.

## Mesh Floors

In infested colonies, 39-50% of the mites which fall naturally from bees are alive and mobile and capable of re-infesting the colony[65]. This live proportion of mitefall increases in warmer weather. Mesh floors, often referred to as varroa floors comprise of #8 hardware screen (3mesh/cm). The device can either be a standard bottom board whose solid floor has been replaced, or a rim (at least 10-20mm high) with screen made to fit between the brood box and standard floor (Plate 21).

Plate 21: *Mesh floor underneath brood box*

This screen floor placed underneath the brood box prevents mites, knocked off by grooming, from returning to the hive and also eliminates any contact between returning foragers and the expelled mites, thus preventing re-infestation. Additionally studies have shown that mesh floors lower the percentage of the mite population residing in the brood cells[32] and significantly increases the amount of sealed brood in a colony[51]. Colonies may be placed on open mesh floors throughout the year.

## Alternative Treatments

Among the substances used for varroa control in Europe and other parts of the world are organic acids, primarily formic and oxalic acid. **These products are not licensed by the Irish Medicines Board (Appendix 5) and thus are illegal for use in honeybee colonies in Ireland.**

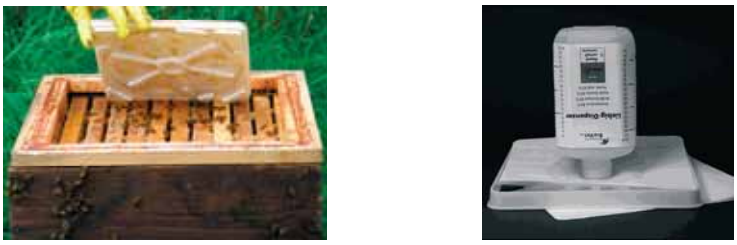
### Formic Acid (not registered for use in Ireland)

Formic acid is found as a natural component in honey and is effective against varroa and tracheal mite. There is no maximum residue level agreed for formic acid, but the **taste threshold** is between 150-600 mg/kg. Effective and reliable control with formic acid has proven elusive[21]. Consequently, in the US, formic acid is registered as a suppressant for varroa mites, rather than a stand alone single application method. Formic acid fumigation as an alternative control has many advantages in that it is inexpensive, there is no documented resistance and it does not leave residues above natural levels in the honey. However, the effect of formic acid treatment on colony health and development is controversial. Although many studies have shown that formic acid has no adverse effects on brood, young bees or colony development[59, 67], Calderone reported a reduction in the amount of brood reared when formic acid was applied in spring[11]. Other adverse affects include toxic effects on capped and uncapped brood[23], poor physiological development of immature and young workers[11] and queen losses[35]. Prolonged treatment with formic acid

may result in the abandonment of nurse bees from their larval feeding duties, resulting in brood death[7]. The efficacy of formic acid in the literature ranges from 29.6%[5] to >90%[39], depending on the doses, modalities of application and experimental and environmental conditions.

## Application

Formic acid may be applied as a liquid poured onto an absorbent pad or as a gel matrix. Typical examples of liquid dispensers are the FAM dispenser, Liebig dispenser and Mite-away II (Plate 22). **Due to the high corrosive nature of this acid, irrespective of application method, it is essential that the user adheres to the recommended health and safety instructions.**



(a) (b)

Plate 22: *Different application methods of formic acid: (a) FAM dispenser; (b) Liebig dispenser*. *Note: not registered for use in Irish honeybee colonies*

The FAM dispenser consists of an absorbent pad encased in a plastic dispenser. 130ml of 70% acid is poured onto the sponge; the dispenser is inverted and placed over the brood area. The release of formic acid is controlled by an adjustable opening on the upper surface of the dispenser. It may be used as a 7 day treatment in August or a 14 day treatment in September.

The Liebig dispenser is an 85% formic acid solution, which evaporates from a paper wick. The evaporation rate is controlled by the size of the paper wick area. Two treatments are required per colony. In a single chambered colony, the August treatment is 50ml over 3-4 days followed by a second treatment in September, when 100ml is applied. Absorption rate should be maintained at 10-15ml/colony per day.

The Mite-Away II™ pad is pre-packed and contains 250 ml of 65% food grade formic acid soaked into a fiber board pad inside a perforated plastic pouch. The number, size, and placement of holes in the pouch have been tested, documented and proven effective over three years of trials. These are critical for efficacy and minimizing damage to the brood. Treatment period is 3 weeks. During this period the vapor will be mixed in the hive air and travel down to the bottom of the hive because the formic acid vapor is heavier than the air. This turns the hive into a fumigation chamber. As varroa mites are exposed to the formic acid, they die.

Formic acid gel matrix is designed to liberate the acid slowly over a longer period. Two typical products include Apicure and Beevar. The former has been withdrawn from the market due to problems with leakage during transport. Beevar consists of 200g of formic

acid in a gel (68% formic acid and 32% carboxipolimetilen), placed over the brood chamber. The gel pack is replaced after 2 weeks and the total treatment time is 4 weeks.

### Oxalic Acid **(not registered for use in Ireland)**

Oxalic acid like formic acid is a naturally occurring acid. Residues do not accumulate in wax, and in honey are limited and toxicologically insignificant[38], assuming the beekeeper uses the acid according to the recommended health and safety instructions. To minimize the risk to the apiarist protective clothing must be worn[54]. The European Union's agency for evaluating medical products (EMEA) has determined the **Maximum residue level (MRL)** of the active ingredient allowed in the final product according to Council Regulation (EEC) 2377/90[48]. According to European honey standards, honey may have up to 50 milliequivalents of free acid (http://www.alp.admin.ch). In 2003, oxalic acid was listed in Annex II of Council Regulation (EEC) 2377/90 and thus it is now considered safe to use in bee hives. However, each European country must now apply for legal approval. Oxalic acid is most effective as a winter treatment when the colony is broodless. It is thus a follow-up treatment, rather than a sole treatment. It can be administered in three ways, trickling, evaporation and spraying.

#### *Trickling method*

Trickling oxalic acid dihydrate is a simple application method, especially for large apiaries. The beekeeper only needs a syringe, gloves and protective glasses to apply the substance. The recommended oxalic acid dihydrate concentration and dosage rate varies across Europe[54]. The recommendations for Northern Europe according to The European Group for Integrated Varroa Control (2000) are: 4.5% oxalic acid dihydrate in a sugar-water solution (1:1) (= 45g oxalic acid dihydrate/litre) at a dosage rate of 20-25ml for a small colony, 25-30ml for a medium size and 30-35ml for a large colony (http://www.apis.admin.ch). In winter, when the colony is broodless, a single treatment of approximately 5ml per bee space is trickled directly onto the bees in the spaces between the combs (Plate 23). The estimated **efficacy** is >90%, however in the presence of brood this efficacy is significantly reduced. Higher oxalic acid concentrations 5-8% not alone reduce efficacy**,** but increase stress within the colony and often impairs spring development. Mutiple autumn treatments also have adverse effects on colony survival and development[33]. Recently a new product 'Oxuvar' has been developed in order to simplify the application of oxalic acid (Andermatt BioVet AG.).

Plate 23: *Application of oxalic acid using the trickling method*
*Note: not registered for use in Irish honeybee colonies*

## Evaporation/ sublimation

Oxalic acid, which occurs in three forms; crystals, gelatine capsules or tablets can be **sublimed** using different types of evaporators, the most common being the Varrox vaporizer (Plate 24). This small electrical device can be inserted into the hive and allows the evaporation of oxalic acid in the closed hive[55]. During heating, approximately half of the oxalic acid disintegrates into harmless carbon dioxide, while the remainder forms an oxalic acid precipitate which settles on the bee, causing the mites to become dislodged. This application device, with a dose of 1-2g in a single storey hive, provides an efficiency of 90-95%. A higher dosage does not increase efficacy[55] and the percentage efficacy is reduced in the presence of brood. Oxalic acid administered using this device has minimal impact on bee mortality, colony over-wintering or queen losses. However, to ensure the health and safety of the beekeeper, it is important to follow the user instructions carefully and to wear protective clothing including the specified protective mask (**EN149: 2001 FFP 3**).

(a) (b) (c)

Plate 24: *Oxalic acid application using Varrox vaporizer: (a) vaporizer pan with OA crystals; (b) vaporizer in a colony, (c) sublimation of OA crystals*
*Note: not registered for use in Irish honeybee colonies*

## Spraying

In central Europe, oxalic acid is applied by spraying. The dosage rate is approximately 2.5-4.0ml per comb side and efficacies of 97.3-98.8% have been reported [15]. Single treatments are well tolerated by bees, but multiple treatments may cause high bee mortality. Spraying oxalic acid on bee larvae also affects the columnar cells of the mid-gut leading to necrosis[30].

### Exomite™ *(not registered for use in Ireland)*

This may be used as an autumn or spring treatment. The product is applied at the hive entrance and delivers a very low application of Entostat™ powder and thymol (Plate 25). As bees arrive at the hive entrance, they pass over the powder, collecting it on their bodies and carrying it into the hive. As they encounter other individuals, the preparation is disseminated throughout the hive by the bees themselves. Two consecutive treatments, each lasting 12 days are required for the control of the mite. The percentage efficacy is estimated at approximately 80%.

(a)


(b)

Plate 25: *(a) Treating a honeybee colony with Exomite™; (b) exomite™ dust on a honeybee after treatment. Note: not registered for use in Irish honeybee colonies*

## Tracheal Mite (*Acarapis Woodi*)

Acarine is caused by the tracheal mite *Acarapis woodi* Rennie. The adult female quests on a hair of its old host, transfers to the thoracic hair of a young bee (<5 days old), and crawls through the **spiracle.** It enters the trachea and starts to lay eggs. Mites may occasionally be found in the air sacs in the thorax, abdomen and head, but they mostly reside in the prothoracic trachea. The larvae and adults pierce the breathing tubes and feed on the haemolymph of their **host.** Tracheal mites are associated with the death of a honeybee colony in late winter/early spring period, typically where more than 30% of the bees in a colony are infested.

### Biology

The tracheal mite is very small; females are 143-174µm (µ=**micrometer)**, which is approximately 1/7mm in length, while males are 125-136µm. The body is oval in shape, white in color, with a smooth cuticle. It has a long beak-like mouthpart and a few long hairs protruding from the body and the legs (Plate 26).

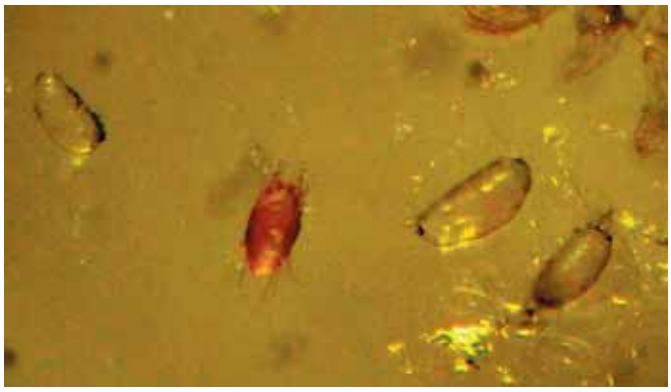


Plate 26: *Adult tracheal mite (Acarapis woodi) (centre) and larval instars*
(©jmcm; photography Trinity College Dublin)

## Life Cycle

The entire life cycle of the tracheal mite is spent within the respiratory system of the honeybee, except for a brief migratory period. During the reproductive period, the female mite lays 5-7 eggs. After 3-4 days, the eggs hatch and develop into larvae which feed and then molt forming a non-feeding nymph. The adult mite emerges after a final molt. Males develop from egg to adult in 11-12 days, while females take 14-15 days. In order to re-infect, **gravid** females emerge from the trachea through the spiracle and attach themselves to the tip of the bees' hairs which allows easy transfer between bees (Figure 4).

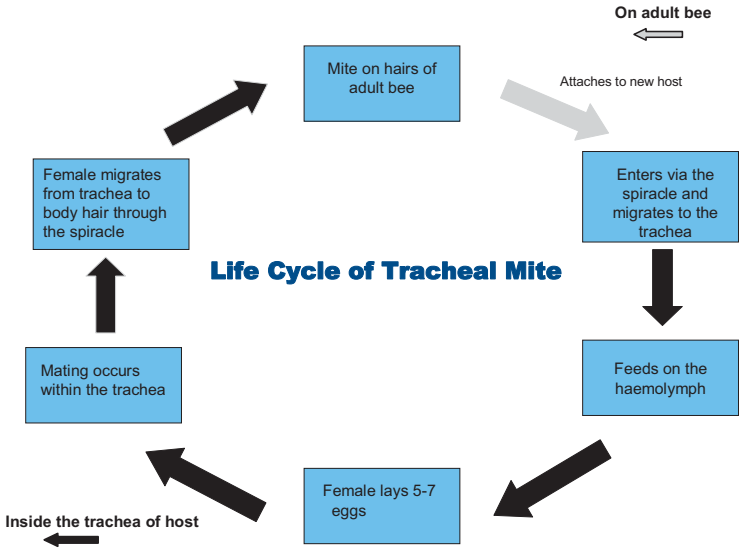
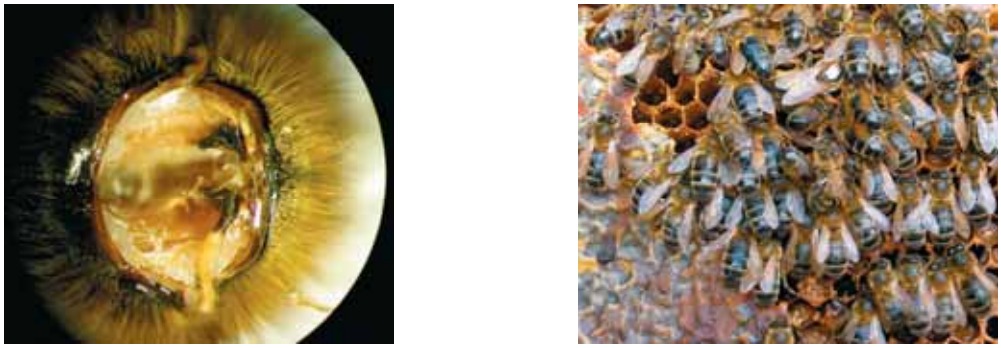



Figure 4: *Life cycle of the tracheal mite (Acarapis woodi)*

## Detection

Positive identification of tracheal mites can be most readily determined by dissecting recently dead bees, however, in cool winter conditions, it is possible that bees in colonies which have died can be dissected for the presence of tracheal mite even if they have been dead for a few weeks. The trachea of a healthy bee has a uniform, smooth white or fleshy appearance. In contrast, the blackening of the trachea due to mite activity in both the left and right tracheal trunks occurs in heavily infested colonies (Plate 27a). Early and intermediate stages of infestation are indicated by the bronzing of the trachea along part of its length, which is followed by black specks or streaks which eventually coalesce.



(a) (b)

Plate 27: *(a) Blackening of the trachea indicating tracheal mite infestation; (b) clump of bees with queen in the centre (marked white) and workers with signs of dysentery on their wings* (©jmcm; photography Trinity College Dublin)

Tracheal mite infestations may last for many years without noticeable effect. However, mite infestation shortens the lives of adult bees and the ability of bees to thermoregulate. Thus, when a colony is near death, large numbers of bees can be seen crawling out of the hive, clinging to stalks of grass and occasionally vibrating their wings. Another typical sign of tracheal mite infestation, at the terminal stage is when pockets of bees are huddled together on the comb, with the queen in one of them, plenty of stored honey, some brood and traces of dysentery (Plate 27b). Although many similar symptoms are displayed by bees suffering from chronic bee paralysis virus (CBPV), infested bees (CBPV) tremble all over, may have a shiny and greasy appearance and in general look sickly. In many incidences more than one disease is present in a colony, which results in a synergistic effect and thus rapid colony mortality.

## Prevention

Close contact between bees facilitates mite transfer. Dispersion is primarily at night[53] and only bees less than 5 days old are susceptible. In short-lived summer bees only one generation per host is possible, but in the winter multiple generations can develop in each bee[52]. Infestation between colonies is caused by drifting, **swarming,** crawling bees and **robbing**. Suppression of foraging in bee colonies increases the rate of infestation by the tracheal mite in young bees. In foraging colonies, part of the mechanism of separating migrating mites from young bees is that mites become dislodged from foragers during foraging trips and are lost in the field. This increases the chance for newly emerged bees escaping infestation during the brief period of susceptibility. Varroa infested colonies are also more likely to be infected than non-infested colonies. The presence of varroa decreases colony activity and thus facilitates the transfer of the migratory tracheal mites[20]. Moderate infestation also reduces the capability of honeybees to use their fight muscles to generate thoracic heat to maintain brood nest temperatures at 30-35°C. Reduced brood temperature increases the vulnerability of newly emerged bees to tracheal infestation. Nest development will be restricted and thus the ability of the colony to adequately replace old winter bees in spring will be limited. The infected colony will dwindle, causing a further reduction in nest temperatures and thereby increasing susceptibility to the tracheal mite[41]. Autogrooming is suggested as a mechanism by which

bees resist infestation by the parasitic tracheal mite[50]. Genetically resistant strains of honeybees groom more persistently and have lower response thresholds to stimulation (i.e. perceived movements, etc) by migratory tracheal mite[19]. Honeybees from the Primorsky region of far eastern Russia show strong resistance to the tracheal[31] mite, possibly due to this mechanism.

### Treatment

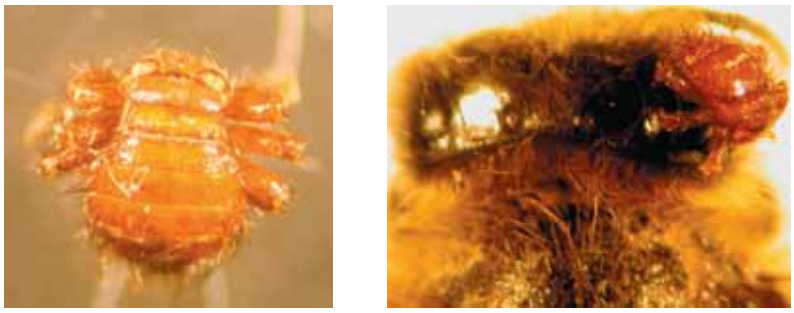
There is currently no approved propriety product registered for the control acarine in the UK and Ireland. However, Apiguard® *(see varroa treatment),* though primarily marketed as a varroacide is effective against the tracheal mite infestation. Requeening with a local colony that has demonstrated resistance to the tracheal mite is also recommended, since within an area there can be a wide variation in grooming ability of bees[19].

## Bee-Louse (*Braula Coeca*)

The bee-louse, or braula (*Braula coeca*), is a wingless fly, once common in Irish honeybee colonies. The braula fly does not damage or parasitize any stage of the honeybee life cycle. However, adults do steal food from bees and larvae and when large numbers are found on a queen, they may reduce the food availability to her and impair her egg-laying ability.

### Biology

Adult braula are small (1.0-1.5mm long), robust, reddish-brown flies (Plate 28a). They have no wings or halters. Their legs-tarsi are specially adapted with comb-like structures to firmly attach to the bees. Braula can move quickly over the bee's body surface, but generally settle on the dorsal surface between the bee's abdomen and thorax. When hungry they crawl to the bee's head and feed on regurgitated nectar directly from the bee's mouth (Plate 28b).



(a) (b)

Plate 28: *(a) Adult braula; (b) braula on the head of the worker honeybee*

### Life Cycle

The eggs are very small, measuring 0.42-0.84mm. They can be deposited in many locations, including empty cells, brood cell cappings, in wax debris on the bottom board and on capped honey comb. Only eggs deposited on capped honey comb will hatch. Eggs hatch in 2–7 days, depending on the temperature. The larvae hatch and tunnel under the cappings, leaving narrow tracks about 1mm wide across the surface of the honey comb. The larvae progress through three instars (stages of development) before pupation. The larval stage can vary from 7–11 days. The pupa is creamy-white in color, measuring 1.4–1.7mm long x 0.50–0.75mm wide. This stage only takes 1–3 days, before the adult hatches. The development from egg to adult can range from 10-23 days, depending on the temperature (time of year) and, presumably, the availability of capped comb honey in the hive. Adult braula overwinter on adult bees.

## Detection

Braula is superficially similar to varroa in colour and size. However, braula is more mobile and has only three pairs of legs attached to either side of the body *(see varroa)*. The tunnels created by the larvae give honey cappings the appearance of being intersected with fine fractures and thus can damage the appearance of combed honey.

## Prevention/Treatment

In Ireland today, braula is rarely seen in colonies because of the regular use of miticides for varroa control. Furthermore, most beekeepers practice mechanical control unknowingly when they remove cappings before honey extraction, thus eliminating braula larvae.

## Wax Moths (*Achroia Grisella & Galleria Mellonella*)

Two species of wax moth are normally associated with bee colonies and comb. Adults of the greater wax moth (*Galleria mellonella*) (wingspan 29-40mm) are greyish-brown with wings that fold over the body like a roof and are active from June-October. The lesser wax moth (*Achroia grisella*) is more common. It is smaller (wingspan 16-24mm) with sheened brown-grey wings that appear silvery in recently emerged adults (Plate 29). Wax moths normally require comb to survive, although larvae of the lesser wax moth are known to also feed on dried fruit and dead insects and are important in the breakdown and decomposition of old, disused comb in the wild. In Ireland, the wax moths are not serious pests and although they may occasionally cause problems when they invade a weakened colony, they generally are only troublesome when the comb is not in use.

Plate 29: *Lesser wax moth (Achroia grisella) adult*

## Biology

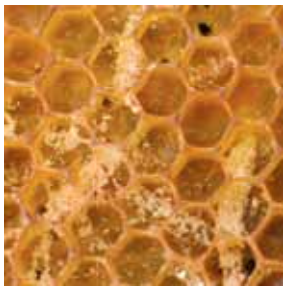
Adults are active at night when they enter hives, slipping past guard-bees, or locate abandoned or dead hives. They lay there eggs in batches in dark crevices: the lesser wax moth may lay between 300-600 eggs, whereas the greater wax moth is reported to lay as many as 1800 eggs. These eggs hatch about a week later and the tiny larvae burrow into the wax, where they eat voraciously. The color of wax moth larvae varies with region and diet. Lesser wax moth larvae are usually described as being pinkish in color and greater wax moth larvae as white (Plate 30(a-b)). The larvae have three pairs of legs behind the head and a series of pseudo-legs. In contrast to the name, wax moths do not digest the beeswax, but live on impurities in the comb. They prefer comb that has been used for brood rearing and seldom damage foundation or new comb. The wax moths create obvious galleries within the comb, often laying down silken webbing. After some months of feeding (temperature dependent), fully grown larvae will spin a silk cocoon in the comb, in the debris on the hive floor (Plate 30c) or attached to a frame or hive body. Some weeks later, young adults will emerge from the cocoons and the life cycle begins again. Larvae of the greater wax moth may damage wooden hives at the time of cocoon formation[47].

(a) (b) (c)

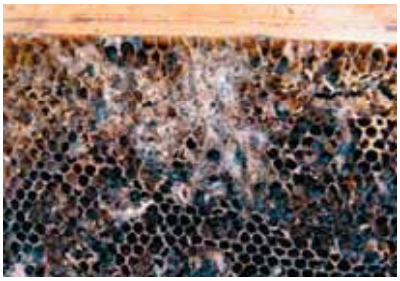
Plate 30: *Wax moth larvae: greater wax moth (Galleria mellonella); (b) lesser wax moth (Achroia grisella); (c) lesser wax moth cocoon*

## Detection

Wax moths are present in many hives at low numbers, kept in check by vigilant bees. It is only in colonies that are weakened through disease or food shortage that wax moths become a problem. However, in abandoned or dead hives during the summer months, they may build up to relatively high populations. Infestation can be recognized by galleries through the comb and webbing on the frames and supers (Plate 31a). Cocoons are often attached to the hive frame and body, wrapped in silk-webbing. The adult moth may be seen running quickly over the frames and comb before alighting. In severe infestations, comb may crumble and become discolored with moth faeces (Plate 31b).



(a)



(b)

Plate 31: *Wax moth damage: (a) galleries through the comb; (b) crumbled and discolored comb*

## Prevention/Treatment

Wax moth rarely causes problems in honeybee colonies in Ireland and in general no treatment is required as bees will remove eggs, larvae and adults during normal cleaning duties. However, they can cause serious damage to stored brood and 'super' comb. 'Supers' can be stored 'wet' (with honey) or 'dry' (cleaned by bees after honey extraction), but in general wax moth causes the least damage to 'wet' supers, but it is necessary that the storage area is bee-proof. Brood comb is much more attractive to the wax moths as it contains wax, pollen, larval skins, faeces and propolis. In late Spring, brood frames can be treated by placing a cloth soaked in acetic acid (100ml per brood box ) over the frames and sealing the entire box with a black refuse bag. After, 1 week the acid will have evaporated and combs should be aired well before reuse. A natural microbial bacterium, *Bacillus thuringiensis* offers a highly effective protection against the wax moth. 'Certan' also known as B 401 (Vita Europe Ltd.) is a concentrated solution of *B. thuringiensis* which offers up to 100% efficacy.

# Tropilaelaps Mite

The *Tropilaelaps* mite is a *quarantine* pest and has not been reported in Ireland to date. Two species of the mite have been identified, namely *Tropilaelaps clareae* and *Tropilaelaps koenigerum*. The mite *T. clareae* occurs in Asia, from Iran in the northwest to Papua New Guinea in the south east[43], while *T. koenigerum* is known only from Sri Lanka and Nepal. The natural host of both species is the giant honeybee *Apis dorsata*, but *T. clareae* has been collected from *A. florea*, *A. mellifera* and *A. laboriosa*. The only other addition host found for *T. koenigerum* has been *A. laboriosa.*

## Biology

*Tropilaelaps clareae* females are light brown in color and are approximately 1.0mm long x 0.6mm wide (Plate 32).

Plate 32: *Physical features of Tropilaelaps* spp.

The males are almost as wide, but are less sclerotised. *T. koenigerum* is slightly smaller, 0.7mm x 0.5mm, oval in shape and brown in color. Both species can be distinguished from varroa using a magnifying glass. Varroa is wider than it is long and moves slowly on the comb. In contrast, *Tropilaelaps* are elongated, with a heavily sclerotised holoventral shell and is a fast running mite.

### Life Cycle

The colonizing female lays 1-4 eggs on mature larvae just before it is capped (Figure 5).

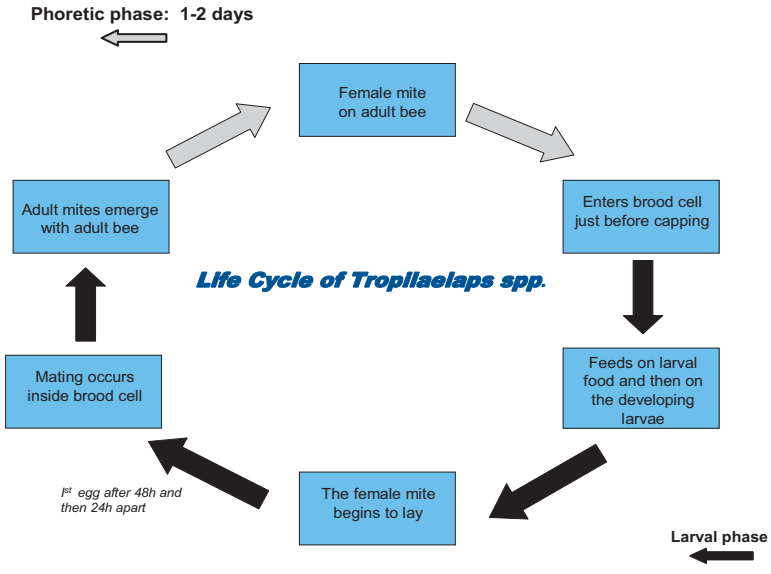



Figure 5: *Life cycle of the Tropilaelaps* spp.

Both worker and drone brood can be infected, but mites show a preference for drone brood. The eggs hatch after 12hrs and the larvae undergo a number of nymphal changes before reaching adulthood. All stages of both males and females feed on the haemolymph

of the developing bee causing brood malformation. Development from egg laying to the adult stage takes approximately one week. Once the young bee emerges, the original invading mother mite and the young males and females exit the cell. *Tropilaelaps* mites cannot pierce the integuments of adult bees, thus **phoretic** survival on bees is quite short (1-2 days). It is thought that these mites are unable to survive in broodless colonies and gravid female mites will die within two days unless they deposit their eggs. The short life combined with the brief stay on adult bees explains why populations of *T. clareae* increase faster than those of the varroa mite. When both mites infect the same colony, *T. clareae* out-competes the varroa mite[9].

### Detection

Infestation by *Tropilaelaps* mite causes death to many larvae and emerging young bees are deformed with symptoms similar to those caused by the deformed wing virus (*see deformed wing virus*). Infested colonies generally have an irregular brood pattern and many cappings may be perforated as a result of sanitation activities by worker bees.

### Prevention/Treatment

In regions where the *Tropilaelaps* mite is a pest, fluvalinate (Apistan) and formic acid are being used effectively as a control. Infestations may also be reduced using non-chemical methods such as queen caging, as the mite cannot feed on adult bees and will only survive a few days outside sealed brood[72]. *Tropilaelaps* mites are mobile and can readily move between bees within a colony. However, movement between colonies is generally caused by the beekeeper moving infested colonies into new areas or by natural processes such as drifting, robbing and swarming.

## Small Hive Beetle (SHB)

The small hive beetle *Aethina tumida* Murray, a *quarantine* pest belongs to the coleopteran family, Nitidulidae. They are endemic to sub-Saharan Africa, where they exist as both scavengers and symbiotes. In its native range, the beetle feeds on pollen, honey and bee brood, but can also complete its life cycle on fruit. Although it may act as a parasite that destroys weakened and diseased hives, it is generally only considered a minor pest in the African honeybee subspecies. In contrast, in European honeybee colonies, SHB infestation has a deleterious effect. The beetle and its larvae can multiply to huge numbers within an infested colony, where they consume bee eggs, brood as well as honey and pollen. Beetles do not necessarily wait for a recently abandoned nest, but can reproduce and destroy existing colonies and utilize their food. Weakened and stressed colonies may even succumb to the beetle in two weeks.

### Biology

The newly-matured adult beetle is light, yellowish brown and becomes brown, dark brown and finally black at full maturity. The beetles are ovoid in shape and approximately 5-7mm in length. Their antennae are club-shaped and have a short wing case (elytra), which is covered with fine hairs (Plate 33).

Plate 33: *Small hive beetle (Aethina tumida) adult*

During the first day or two after emergence, young beetles are active, take flight readily and orient towards the light. Later they become less active and keep to less illuminated portions of the bee colony. Adult beetles are active fliers and occasionally individuals or swarms can infest a honeybee colony. Stressed colonies can be detected by the SHB at a distance of 13-16km, but this detection mechanism is still unclear.

## Life Cycle

The small hive beetle undergoes four distinct phases, egg, larva, pupa and adult during its life cycle (Figure 6).

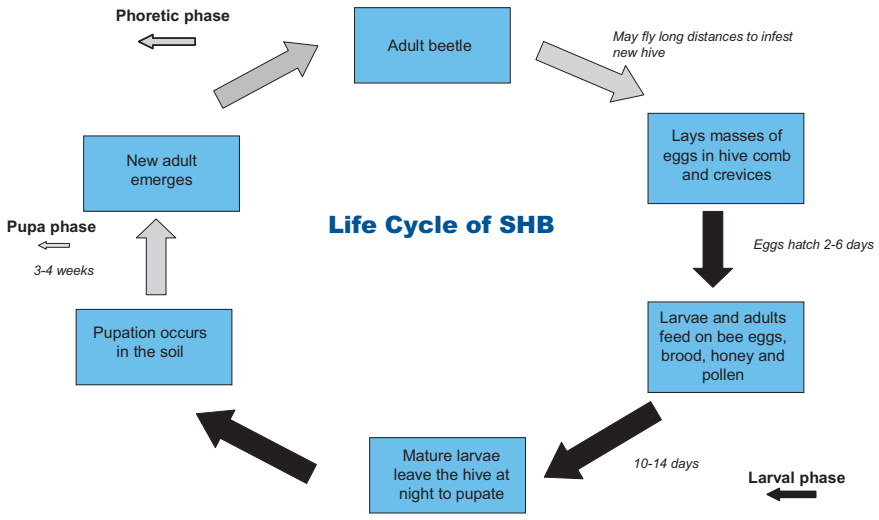



Figure 6: *Life cycle of the small hive beetle (Aethina tumida)*

With the exception of the pupal stage, all others are found within the nest. Eggs are small, approximately 2/3 the size of bee eggs, white in color and are generally found in clusters in grooves and crevices. Occasionally, they are found in brood cells, especially in pollen cells where reproduction is maximized. After 2-6 days, SHB eggs hatch out and young beetle larvae begin to feed, tunneling through honeycombs and killing bee larvae. SHB

larvae are cream in color, 10-11mm long and are similar in appearance to the larvae of the wax moth (Plate 34a). Both species have three sets of legs just below the head, but the SHB lack the series of paired pro-legs that are distinctive of the wax moth larvae (*see wax moth)*.

(a)

(b)

Plate 34: *Small hive beetle larvae: (a) dorsal side; (b) ventral side showing the three pairs of legs*

After 10-14 days, when the larvae have completed their growth, they leave the hive and burrow into the soil beneath or in front of the hive to pupate. Initially, the pupae are pearly white in color, but pigmentation begins when they are transforming to adults, first in the eyes, then the wing base, before encompassing the whole body. Frequent twitching of legs is observed as maturation occurs within the pupal skin. The period spent in the soil varies from 15-60 days. The majority of beetles emerge after 3-4 weeks in the ground.

## Behavioral Adaptations Of Honeybees To The SHB

Africanized and European honeybees adopt a number of tactics to prevent or postpone successful beetle reproduction. These behavioral adaptations include aggressiveness, social encapsulation, patrolling, worker aggregation, absconding and migration. These traits are more pronounced in Africanized sub-species, while in managed European populations, breeding programs have selected against behavioral activities such as absconding, aggression and abundant propolis usage as these are considered undesirable traits in commercial bees.

### *Aggression*

Africanized subspecies protect themselves by active aggression towards both adults and larvae, however, the SHB usually adopts a turtle-like defense position or escapes the attacking bees by running, dropping and hiding.

### *Social encapsulation*

Social encapsulation is an effective means of reducing reproduction of SHB. If beetles successfully enter the hive, they are prevented from moving freely over the comb as bees 'corral' or herd the bees into a particular area. A number of workers propolise around detected, hidden and corralled beetles, while others continuously guard the confinements for up to 57 days[49]. However, occasionally beetles manage to escape encapsulation especially at night, when bees are less active. Within the prison, mating, cannibalism and

behavioral mimicry can ensure their survival. In the latter, the beetles approach the prison guard bee, extend their heads forward and make antennae contact with the guard bees (mimicking normal bee trophallaxis). Although, initially the guard bee make react aggressively, persistence by the beetle usually entices the bees to regurgitate a drop of honey, which the beetle appears to take directly from the mouth parts of the bee.

### *Patrolling and worker aggregation*

SHB is rarely seen on the comb of strong colonies as beetle intrusion is partly restricted to brood areas by the guard bees. This phenomenon is known as patrolling. The latter is more intense in strong colonies as there is a higher density of bees per brood area, but even then, beetles occasionally lay in the outer frames. If beetles intrude, Africanized workers aggregate around, removing the contents of nearby pollen, honey and brood to get access to the hidden beetle, which is then removed. Beetle larvae and eggs are also removed by European workers.

### *Absconding and migration*

Africanized bees are much more mobile than European bees and any form of colony movement will reduce levels of colony infestation by SHB, as the non-phoretic beetles are left behind. Absconding is the abandonment of a colony leaving sealed and unsealed brood and honey, while migration is a seasonally predictable phenomenon and bees generally prepare for migration by reducing egg laying, waiting for brood to hatch and consuming plenty of stores[34]. Although both movements interrupt the life cycle of the beetle, migration has a more serious limiting effect on beetle populations than absconding as less food stores are left behind for exploitation by the beetles.

## Detection

A simple technique used to look for beetles is to remove the lid and place it upside down on the ground. Place the brood box on top of the upturned lid. If beetles are present they will move out of the brood box away from the light and may be seen crawling in the lid. Beetles may also be seen 'surfing' across the comb if the frame is exposed to sunlight.

## Prevention/Treatment

SHB has not been reported to date in Ireland and but is a *notifiable* disease. However, in regions where SHB is considered a pest, a wide variety of controls have been developed. These include prevention through colony management and sanitation, chemical control[22] and insecticide treatment of soil[6]

# Rodents (Mice and Rats)

Mice are the most common and troublesome rodent pest of honeybee colonies. They become a problem during autumn and winter when beehives provide them with food (pollen, honey and bees) and protection from the cold. Mice destroy the frames and comb by chewing them to provide room to build their nest. Not only is the destruction of

equipment a problem, but the odor created by their urine and droppings can cause the bees to abandon a hive. Colonies located near fields or close to woodlands are especially vulnerable. Rat infestation shows similar symptoms, but is more extreme (Plate 35(a-b)). To keep rodents out, restrict the entrance of the bee colony with entrance cleats (Plate 35c). Mice also nest in stored bee equipment resulting in the same kind of damage.

(a) (b) (c)

Plate 35: *Rodent damage in a honeybee colony: (a) rat nest; (b) destruction of comb; (c) mouse cleat*

## Wasps and Bumblebees

Wasps (*Vespula* spp.), bumblebees (*Bombus* spp.) and honeybees from other colonies invade weak honeybee nests and steal their honey, a phenomenon referred to as robbing. It is generally encouraged by one or more of the following factors: (1) weak stocks, which render them unable to guard their stores, (2) careless and unnecessary exposure of comb or feeding syrup when bees are flying, (3) ill-fitting hives which allow access of strange bees by openings other than the entrance, (4) manipulating hives unnecessarily when forage is scarce and (5) queenless stocks which rarely defend their stores rigorously.

### Detection

The presence of wasps and bumblebees, honeybees fighting on the alignment board and bees entering the hive through an opening other than the entrance are typical signs of robbing (Plate 36(a-b)).

(a) (b)

Plate 36: (*a) Honeybees robbing after feeding syrup; (b) wasps (Vespula spp.) and bumblebees (Bombus spp.) robbing a honeybee colony*

### Prevention/Treatment

To prevent robbing, avoid the causes mentioned in the above paragraph. Keep colonies strong and unite weaker ones. Reduce the entrances in hives during periods when robbing is typically present and feed all colonies in an apiary simultaneously. If robbing has commenced, it may be necessary to use a wasp trap.

# Adult Bee Diseases

## Nosema

*Nosema apis* and more recently *Nosema ceranae* are the only two microsporidian parasites identified to date in honeybees (Plate 37). The latter shows none of the dysentery or crawling behaviour usually related with *N. apis* infection, only nonspecific symptoms such as gradual depopulation, higher autumn/winter losses and low honey production. Under a light microscope, the spores from both microsporidian are similar in shape, although *N. ceranae* are consistently smaller. It was originally isolated from the Asian honeybee and further research is needed to establish if *N. ceranae* has different effects on the European honeybee than *N. apis*. Thus, the following information refers specifically to *N. apis*.

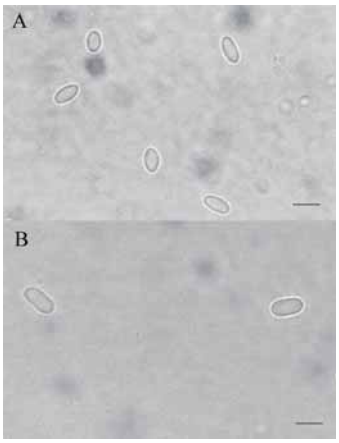



Plate 37: *Nosema spores under a light microscope: (a) Nosema apis; (b) Nosema ceranae Bar=5µm* (©ifries; photograph J Apic Res, 2006)

### *Nosema Apis*

The 6-8µm spore is resistant to environmental degradation and may remain viable in the colony for several months. Within the hive, it occurs in dried spots of excreta on brood comb where it is ingested by the bees when cleaning. It is transmitted via the ingestion of contaminated comb material, water, crushed infected bees and by trophallaxis. It invades the digestive tract of the queen, workers and drones. It may be present at any time of the year, but is most prevalent in spring or after extended periods of cold weather when colonies have been confined to their hives. A healthy colony which receives infected comb in the autumn is likely to develop severe infection, because by this stage of the season, flight activity is decreasing and thus the colony has little ability to suppress infection.

## Biology

The spores germinate in the gut and enter the digestive cells that line the midgut where they rapidly multiply. After multiplying in these cells and consuming the cell contents, the cells rupture and the spores leak into the midgut, small intestine and rectum, passing eventually out of the bee to infect and contaminate other bees in the hive.

## Detection

Damage to the digestive tract may produce symptoms of dysentery (*see dysentery*). Infected workers, unlike healthy ones, defecate in or on the outside of the hive rather than in the field (Plate 38).

Plate 38: *Defecating on the outside of the hive*

Infection of worker bees inhibits digestion of food (pollen) in the stomach and the production of royal jelly. As a result, the productive life of the worker is shortened, and its ability to produce royal jelly decreases. This retards brood production and colony development, a condition often referred to as spring 'dwindling'. Infected queens show a marked reduction in egg laying and are likely to be superseded. The effects of nosema on a colony may be aggravated by the presence of viruses. Black queen cell virus (see viral diseases), filamentous virus and virus Y, though unrelated, all invade the adult bee through the gut. Their close association with nosema may be responsible for the variability in disease symptoms between colonies. Positive identification of this disease is through microscopic examination (*see Appendix 3 for sampling method*).

## Prevention/Treatment

The best defense against nosema is to reduce stress within the colony, thus it is important to over winter strong colonies with plenty of honey and pollen and a vigorous young queen. Nosema is readily transmitted by soiled and infected combs, thus a **Bailey frame change** or replacing combs regularly helps to reduce infection. In general no comb should be left in a hive for more that 3-4 years. If a colony becomes infected and dies, the combs and hive parts should be disinfected with 80% acetic acid (100ml/brood box for a week) as previously described (*see wax moth*). The fumes kill the nosema spores but do not harm the honey or pollen stores in the comb.

# Brood Diseases

## American Foulbrood (AFB)

American foulbrood, is a *notifiable* disease, but is already present in Ireland. It is an infectious disease caused by a spore forming bacterium *Paenibacillus larvae* sensu *(*Plate 39). It is a widespread and destructive brood disease, especially from mid-summer onwards, affecting brood of all three castes. Adult bees are not affected as the action of the pro-ventricular valve may filter the spores from the digestive tract[62]. The pathogen is often present without producing clinical signs visible to the beekeeper, but when clinical disease occur, infected colonies often succumb to the disease.

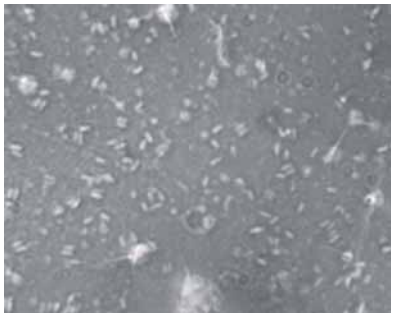


Plate 39: *AFB spores (x100 magnification)*

### Biology

*Paenibacillus larvae* sensu occurs as two forms, vegetative (rod shaped bacterial cells) and spore, the latter is only infectious to honeybees. Spores become mixed with brood food, which is then fed to young larvae by the nurse bees. Larvae 24-28hrs after hatching are most susceptible. The spores germinate within the body of the larva, multiply rapidly and consume the larval tissue. Soon after the larva has been sealed it dies, thus the food supply of the bacterium disappears and the bacterium transforms itself back into the spore stage. This stage is highly resistant to desiccation, direct sunlight, heat and chemicals. Spores are distributed throughout the hive by nurse bees, who attempt to clean out the cells containing the dead larvae. Spores may remain viable for up to 40 years in the hive, in the honey and on other beekeeping equipment.

### Detection

The early detection of AFB infection is necessary for its control as it is a contagious disease. Therefore, the beekeeper should make prudent inspections of the brood area and always be on the alert for possible signs of the disease. *Vita® (Europe) Ltd*, has also developed the AFB diagnostic kit which is designed to produce rapid on-site diagnosis of AFB infections in honeybee larvae (Plate 40).


Plate 40: *AFB kit (Vita® Europe Ltd.) which can been use for the detection of AFB in the field*

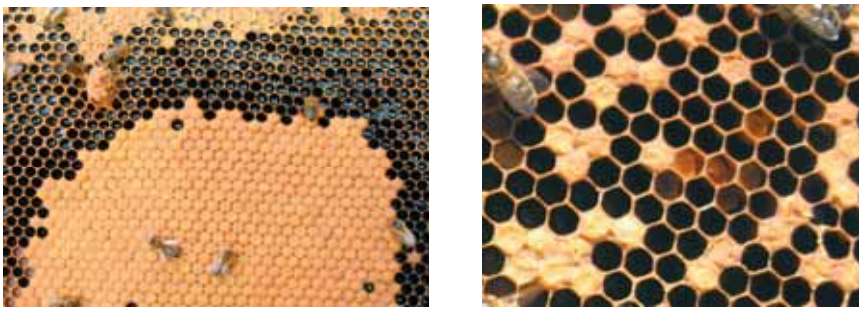
## Hive Inspection

### Symptoms

Symptoms include punctured and sunken brood, larval color change and irregular brood pattern. A good understanding of what constitutes healthy brood will make diagnosis easier (Plate 41a).

### Punctured and Sunken Brood

The collapse of the infected larvae takes place within the cell after the cocoon has been spun. Associated with this collapse are changes in the appearance of the cell cappings. The latter become moist and sunken as the larva continues to shrink. Also, workers nibble holes in the sunken cappings (Plate 41b).



(a) (b)

Plate 41: *Worker brood: (a) healthy brood; (b) nibbled holes and sunken brood cappings*

### Larval Color Change

The collapse of the larvae is accompanied by a change in color from the pearly-white of healthy brood to a creamy brown, light at first, then becoming darker. The consistency of the larval remains is very slimy and if a matchstick is thrust through the sunken capping, twisted around and then withdrawn, the slimy mass will pull out in the form of a mucous-like brown thread (or rope) (Plate 42(a-b)). The ropy condition is succeeded by a tacky stage as the larval remains in the cells gradually dry out and the color changes to a dark brown. Further drying leads to the final stage, which is the dark brown, rather rough scale lying at the lower side of the cell (Plate 42c). House-bees cannot remove the scales and whenever a colony dies out, the combs should be scanned for scales. If death occurs in

the pupal stage, a tongue protrudes from the scale. The scales can be detected if the comb is held facing the light.

### Irregular Brood Pattern

Brood comb in an infected colony have a scattered and irregular pattern of capped and uncapped cells. This is often referred to as a 'pepper-pot' pattern. An unpleasant odor may also be present.

(a) (b) (c)

Plate 42: *Advanced symptoms of AFB: (a) slimy larval remains, (b) mucous-like brown thread or rope when the cell contents are removed with a matchstick; (c) hard brittle scales*

## Prevention

Worker bees become contaminated when removing dried larvae, which is probably an important infection pathway to spread the infectious spores in the colony. Infection may also be transferred horizontally between colonies by robbing of contaminated honey or by drifting of spore carrying adult bees. Swarms from infected colonies may carry infection and become infected when hived. Therefore, new swarms collected should be placed on foundation and not fed syrup for 36hrs. Thus, the honey in the honeysacs is utilized for comb building and it minimizes the risk of infection. Apiary and beekeeping equipment hygiene is also crucial as spores can germinate even after 40 years.

## Treatment

Control of AFB is achieved by regular examination of colonies. Infected colonies and equipment must be destroyed by burning. Hive appliances can be sterilized by scorching with a blow lamp or immersing them for 10mins in paraffin wax, heated to 150°C. Gloves, overalls, footwear and smokers should be washed in hot, soapy water. The incorporation of routine replacement of old combs as part of colony management is an important component of disease prevention[61]. If the presence of AFB is confirmed the following steps should be taken: (1) close the hive as soon as the bees cease flying, (2) smother the infested bees by pouring a half liter of petrol through the feed hole and (3) dig a hole and burn contents of the hive (http://www.irishbeekeeping.ie).

# European Foulbrood (EFB)

EFB is a bacterial disease caused by *Melissococcus plutonius*, originally known as *Melissococcus pluton*. Its development in a colony is complex. It is most prevalent in spring and early summer and is associated with stress within the colony. During this period, there are many larvae relative to the number of nurse bees, thus larvae infected with EFB get less food and as a result die from starvation. However, at other times EFB infested larvae get sufficient food and survive, but contaminate the combs during pupation. *Melissococcus plutonius* does not form spores, but often over-winters in the comb. Although the disease may remain active throughout the entire foraging season, it generally disappears during the nectar flow. All castes of larvae are infected by this bacterium.

## Biology

*Melissococcus plutonius* is an oval rather pointed bacterium, which grows entirely within the gut cavity (Plate 43).

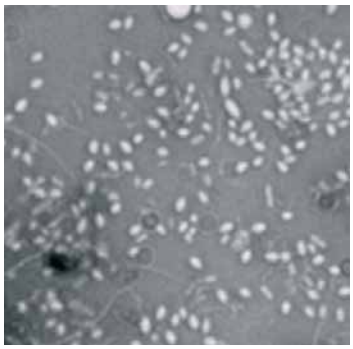


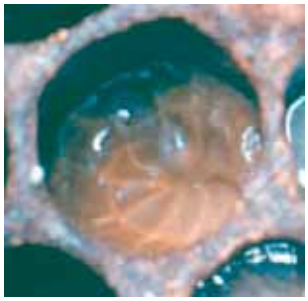
Plate 43: *EFB spores (x100 magnification)*

It does not invade the living tissue and stops growing if the larva dies. When the sac-like mid-gut of the larva becomes joined to the rectum before pupation, the mid-gut contents in which the bacterium has been growing are voided in the faeces. *The Melissococcus plutonius* is deposited in the cell and infected cells may be identified by their dark cappings. These bacteria are alive and can remain so for many years. EFB usually attacks the larva in the early stage of development, which is when it is still curled up at the base of the cell.

## Detection

The earliest indication of the disease is a slight yellow or grey discoloration and an uneasy or wriggling movement of the larvae in the cell. The larvae lose their well rounded opaque appearance and become slightly translucent, so that the trachea may become prominent, giving the larvae a clearly segmented appearance (Plate 44a). Approximately 4 days after hatching, the larvae die and may be found in various positions within the cell, most notably lying across the mouth of the cell, twisted spirally around the walls or stretched out length-wise from mouth to base (Plate 44b). The larvae collapse as though they have been melted, turning yellowish brown and eventually drying

out to form loosely attached brown scales. The consistency of the recently dead larvae varies. They may be sticky or porridge-like, but not ropy. The smell also varies due to variation in secondary infections. The brood pattern presents an irregular appearance, capped and uncapped cells being found scattered irregularly over the frame. EFB rarely kills colonies, but a heavy infection can affect population growth seriously.



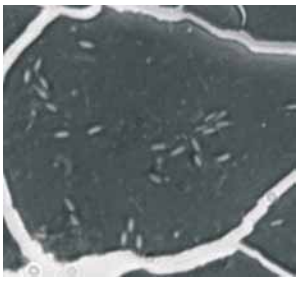
(a)

(b)

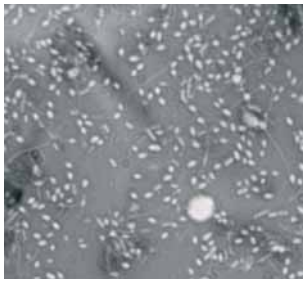
Plate 44: *Symptoms of EFB infection in the brood: (a) larva becomes clearly segmented; (b) uncapped larvae in various positions in the cells*

## Secondary Infections

EFB is caused primarily by the bacterium *Melissococcus plutonius*, but secondary infections from bacteria such as *Bacterium eurydice*, *Paenibacillus alvei* (Plate 45(a-b)) and *Streptococcus faecalis* further complicate the infection. *Bacterium eurydice*, by itself causes no apparent harm, but circumstantial evidence indicates that its presence accelerates the death of larvae already infected with *Melissococcus plutonius*. *Paenibacillus alvei*, once thought to be the cause of EFB grows mostly on larvae that are already dead. The spores produced may remain dormant for many years and eventually become established in colonies chronically infected with EFB. *Bacterium eurydice* may also accelerate death of larvae, but can only survive for short periods in honeybee colonies.



(a)



(b)

Plate 45: *(a) Paenibacillus alvei; (b) Paenibacillus alvei and EFB spores*

## Prevention

EFB is spread by transferring frames or combs from a diseased to a healthy stock, as the bacterium retains its virulence for several months in honey and pollen. Drifting and robbing bees may also infect a healthy colony. Nurse bees, contaminated during the removal of dead brood are the main disseminators of infection within the colony.

## Treatment

EFB rarely occurs in Ireland, but is classified as a *quarantine* species and should be destroyed by burning as described for AFB.

# Chalkbrood

Chalkbrood is a fungal disease of the honeybee larvae caused by *Ascosphaera apis*. It rarely destroys a colony, but can prevent normal population build-up when the disease is serious. Mouldy pollen can easily be confused with chalkbrood in a frame and may be distinguished by the fact that it assumes the hexagonal shape of the cell, while the chalkbrood retains the rounded shape of the larvae (Plate 46 (a-b)).

(a)

(b)

Plate 46: *(a) Chalkbood in the comb; (b) mouldy pollen in the comb*

## Biology

The spores of *Ascosphaera apis* are ingested in the larval food. They germinate in the nearly **anaerobic** environment of the hind gut of bee larvae, but the **mycelial** growth is arrested until the larvae are sealed in the cell. At this stage the larvae are 6-7 days old. After a further 2-3 days, the mycelia elements break through the gut wall and invade the larval tissue.

## Detection

### Chalkbrood 'Mummies'

The affected larvae are transformed into fluffy white or yellow 'mummies', which at first fill the cells completely and have a rubbery consistency. Later these shrink, becoming hard and brittle, turning greenish brown or black in color. The latter is caused by the fruiting bodies (spore cysts) of the fungus (Plate 47).

Plate 47: Chalkbrood 'mummies'

### Chalkbrood in Cells

Diseased brood can be found throughout the brood rearing season, but is most common in late spring when the brood nest is expanding, in weak colonies and in nuclei. Affected cells can be either sealed or unsealed, as honeybees often puncture or remove cappings. Chalkbrood 'mummies', once dry, are loose in the cells and are easily removed by the nurse bees. Occasionally these are visible on the ground at the entrance of the hive or under mesh floors (Plate 48).

Plate 48: *Chalkbrood 'mummies' on the floor of the hive*

## Prevention

Larvae affected by chalkbrood may release millions of spores that all have a sticky coating, enabling them to adhere to combs and adult bees. Spores are the dormant phase and can survive for many years. Both the transfer of combs by the beekeeper and drifting bees can transmit chalkbrood spores between colonies. Recent research indicates that foundation wax may also be a source of *Ascosphaera apis* spores[24].

## Treatment

No treatment is currently available to control chalkbrood. In severe cases, treatment with Apiguard (*see varroa*), the addition of sealed brood, requeening with a more hygienic strain (Plate 49) or generally reducing stress benefits the colony. However, this disease usually disappears in summer when air temperature increases and pollen and nectar become abundant. Sterilization of combs and hive parts with acetic acid (*see wax moth*) kills spores of *Ascosphaera apis*.

Plate 49: *Requeening a colony*

## Stonebrood

Stonebrood is a fungal disease primarily caused by *Aspergillus flavus* and to a lesser extent *Aspergillus fumigatus*. Both larvae and pupae are susceptible and the disease causes mummification of developing larvae. The 'mummies' are covered with a powdery green growth of fungal spores, with the highest concentration of fungal spores near the head of infected larvae and pupae. The diseased larvae are solid 'mummies' and not sponge-like as in chalkbrood. This disease is only considered of minor importance and no treatment is necessary. House-bees normally remove infected brood and the colony recovers naturally. It should be noted that *Aspergillus* moulds can cause respiratory problems in humans and thus it is important not to sniff or inhale infected combs.

# Viral Diseases

A total of 18 viruses have been identified from honeybees and physically characterized. The most commonly observed and best known viruses are 30nm isometric particles containing a single stranded positive **RNA** strand. These viruses include sacbrood virus and deformed wing virus, both which are assigned to the genus Iflavirus, while Kashmir virus, acute paralysis virus and black queen cell virus are members of genus Cripavirus. The chronic bee paralysis virus remains unclassified. Complete or partial sequencing of several RNA viruses of the honeybee has allowed the recent development of highly sensitive methods for viral detection, based on the amplification by reverse transcription-PCR (RT-PCR) of specific viral sequences.

## Sacbrood Virus (SBV)

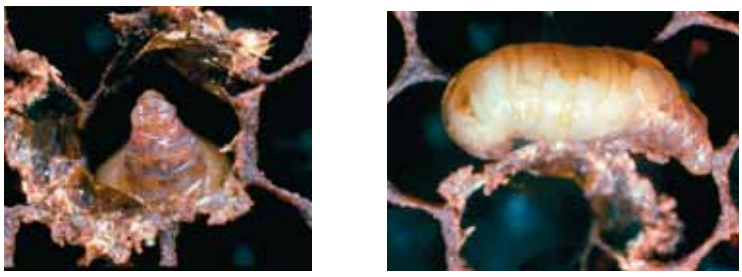
Sacbrood virus (SBV), formerly known as addled brood is a widely distributed disease, but rarely causes serious losses to bees. The disease primarily affects the larvae and pupae, but can also affect adults, a characteristic which enables the virus to persist in bee colonies from year to year. Outbreaks of this disease most commonly occurs in the spring and early summer, when forage is limited.

## Biology

Sacbrood virus particles are 28nm in diameter, non-enveloped, round and featureless in appearance. **Phylogenetic** analysis suggests that at least three distinct genotypes of SBV exists[29]. Each larva killed by SBV contains approximately 1000 viral particles, almost 1% of the body weight. Adult bees less than 8 days old are readily infected by ingesting the virus, which then proliferates in the head and in the fat body tissues[3]. The infectivity of SBV is lost after a few weeks in the larval remains and thus this disease is usually limited to only a few colonies in the apiary. Within the colony, the young larvae are infected by ingesting contaminated larval food. In adult bees, no clinical signs are apparent, but infected bees cease to eat pollen, their main source of protein, and also stop feeding the young larvae. The life span of infected bees is reduced and although they may forage, they rarely collect pollen. The small percentage of infected bees that do forage for pollen, contaminate their loads with glandular secretions during the packing process. Each infected load may contain up to a million virus particles. SBV contamination of nectar is much more dilute and is quickly distributed within the hive.

## Detection

Dead brood cells are scattered around healthy brood. Cappings are generally brown, occasionally punctured or partly removed by the adult bees. Developing larvae usually turn grey in color at the tip (Plate 50a). Larvae infected with SBV fail to pupate and ecdysial fluid, rich in SBV accumulates beneath their unshed skin, forming the sack from which the condition is named (Plate 50b). Infected larvae will eventually die and begin to dry out, turning from white to yellow and later to a black color, giving rise to the characteristic 'Chinese slippers' or 'gondola-shaped scales. These scales are rough and brittle and do not adhere tightly to the cell wall.



(a) (b)

Plate 50*: Symptoms of SBV in a colony: (a) head of SBV killed larva; (b) SBV infected prepupa removed from the cell* (©mvsmith; photograph University of Guelp)

## Prevention/Treatment

Maintaining strong colonies with regular requeening seems to be most effective manner in combating this disease. Frames can be reused as the sacbrood virus becomes non-infectious within a few weeks. Since the disease is caused by a virus, no antibiotic is effective in preventing or controlling it.

## Deformed Wing Virus (DWV)

The frequency of DWV in colonies increases from spring-autumn[63] and is strongly correlated with the detection of DWV in bee haemolymph[8].

### Biology

Adult female varroa mites regularly act as competent vectors of DWV, however, they do not acquire or transfer virus on all possible occasions. DWV may be present in the absence of *Varroa destructor*, indicating that other transmission routes must exists. It is transmitted vertically in honeybee eggs[15], horizontally in food transfer and in the larval food. Oral transmission of DWV is not sufficient to cause crippled wings in honeybees, but a recent study indicates that bumblebees robbing from infested honeybee colonies can become infected. The hypotheses from this observation are that DWV has broader host specificity than originally anticipated or the virulence of the virus increased with the switch of host. Clinical symptoms of DWV infection are not always present in virus positive honeybees, but in general there is a higher **titre** of DWV in bees with deformities compared with **asymptomatic** ones (Plate 51).

Plate 51: *Normal* and d*eformed honeybees exchanging food by trophallaxis*

RT-PCR revealed differences in viral concentrations between castes and honeybee stages of development, with pupae having the highest concentration, followed by deformed bees, larvae, normal workers and finally drones[14]. Crippled and healthy looking bees also differ in the spatial distribution of DWV. Crippled bees were positive for DWV in the head, thorax and abdomen, while healthy looking bees were positive for the virus in the thorax and abdomen only[73]. In queens, DWV was detected in the cytoplasm and plasma membrane of the fat body cells. Viral infection of the fat body cells may impair insect development and physiology and lead to immuno-suppression, an affect so far attributed to varroa parasitism. Also, in queens the fat body cells produce vitellogenin, the yolk protein accumulated during egg maturation. Thus, DWV infection of the queen's **adipose cells** might impair egg production. DWV was also detected in the epithelial cells of the seminal vesicle in drones. These cells play an important role in spermatozoa maturation, thus viral replication in this area may have a negative effect on drone fertility. Mucous

glands and testis epithelia were also shown to be infected and thus an uninfected queen may become infected during mating[15].

## Detection

BEES infected with dwv generally emerge with deformed or poorly developed wings, bloated abdomens and discoloration (Plate 52), which is attributed solely to the feeding activities of varroa on the developing larvae.

Plate 52: *Emerging honeybees showing different degrees of deformity*

The level of mite infestation is not always correlated with the occurrence of crippled, asymptomatic and colony collapse. A possible explanation for this inconsistency is that within a mite population, there is variation in the proportion of DWV-positive individuals. Although a positive correlation exists between virus replication in mites and the development of wing deformities, not all mites facilitate replication of the virus, suggesting the existence of viral subpopulations differing in virulence[73].

## Prevention/Treatment

Mite populations in colonies should be monitored throughout the year using natural mite fall. If infestation is greater than recommended for any time of the year (*see monitoring natural mite fall*), then management strategies should be implemented to reduce the mite population. Uniformity in treatment time may also reduce viral transmission between apiaries.

# Kashmir Virus (KBV)

Kashmir virus (KBV) is a picorna-like virus found in honeybees (Plate 53). It was first isolated from an adult bee of the Asian strain *Apis cerana*. KBV has also been isolated from adult bees of the western honeybee *Apis mellifera* in Canada, USA, Australia, New Zealand, Tasmania, New Guinea and Fiji. Within Europe, it has been reported in Spain, Germany, France and UK. No positive identification of KBV in Irish honeybee colonies has been reported, but as in the UK, it may be present in colonies for a number of years. KBV may be divided into two serological groups; Australian isolate and North American isolate[36]. Bees infected with KBV have no described symptoms, even though Allen and Ball suggested that KBV is the most virulent of all known viruses[1]. KBV may be present as a viral genome, with extremely low levels of viral-capsid proteins. It has been isolated from both queen and eggs, thus suggesting vertical transmission[14]. Horizontal transmission is also likely among adult bees via worker secretions and from adult

workers to larvae through contaminated food resources. The isolation of KBV from adult female mites and their salvia indicates that varroa is also an important vector of this virus and in many incidences KBV and SBV co-infect.

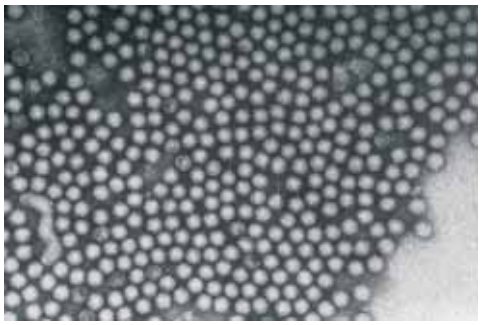


Plate 53: *Kashmir bee virus particles*
(©Rothamsted Research Unit, UK)

## Acute Bee Paralysis Virus (ABPV)

Prior to *Varroa destructor* infestation, ABPV was never associated with disease and colony death in nature. Live adult bees in summer could contain approximately 106 virus particles per bee without showing any signs of paralysis[4]. Thus, the virus appeared to be contained within tissue, not essential for the life of an adult bee. Today, ABPV is considered a common infective agent, present in high proportions of apiaries causing hidden infections[37], but resulting in losses only in colonies heavily infected with *Varroa destructor*. Activation of the virus may be caused by the introduction of foreign proteins such as mite digestive enzymes. Alternatively, the piercing action of the mite causes the body wall to be damaged which enhances the release of the virus, allowing it to replicate. Infection may also be activated by subservient environmental factors such as bacterial infection, pollution and the comprehensive use of chemical and insecticides in agriculture.

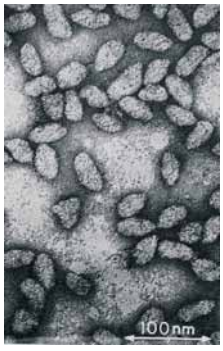
## Black Queen Cell Virus (BQCV)

Black queen cell virus (BQCV) causes death to queen larvae and pupae after cells have been sealed and subsequently the larvae and cell walls turn black in color. Worker and drone brood are also affected, but show no clear signs. The occurrence of this virus in adult bees ensures the maintenance of BQCV within the colony throughout the year, reaching a peak during the summer months[63]. BQCV is transferred both vertically and horizontally. The former was verified by the detection of viral particles in the tissue of the ovaries and in the queen's eggs and larvae. Detection of the virus on the surface of sterilized eggs excludes the possibility of transovarian transmission. Horizontal transmission, primarily by feeding was indicated by the high titre levels of BQCV in the queen gut and in the faeces. This high level of virus in the gut may serve as a reservoir for replication of BQCV and also deformed wing virus[15].

Studies have also shown a close association between BQCV and the microsporidian, *Nosema apis* (*see nosema)* and their co-infection may be implicated in the mortality of bees infected with *nosema*. In general, queens can be co-infected with multiple virus infections and it is not clear how these infections affect the behavior or physiology of the queen[14]. Dissemination of BQCV by varroa mites appear to be improbable as the virus has never been detected in varroa mite samples[63].

## Chronic Bee Paralysis Virus (CBPV)

Honeybees infected with chronic bee paralysis virus show many clinical symptoms which include shivering, shiny/greasy bodies, inability to regulate their flight muscles and crawling onto the ground and up the stems of grass in front of the hive. The virus is spread from bee to bee by unusually prolonged bodily contact or rubbing. This causes many hairs or bristles to break, exposing live tissue. They die within a few days of the onset of symptoms. Plate 54(a-b) shows CBPV particles and the physical appearance of honeybees infected with the virus. Bees vary genetically in susceptibility, thus if symptoms appear, requeening is a good practice.




(a)

(b)

Plate 54: (a) *CBPV particles* (©Rothamsted Research Unit, UK); (b) *adult honeybees infected with CBPV* (©pblanchard, AFSSA, France)

## Parasitic Mite Syndrome (PMS)

The term parasitic mite syndrome is used to describe the complex disease association between *Varroa destructor* and honeybee viruses. In many cases colony losses are due to the association between varroa and the virus rather than the mite acting alone. There are two main hypotheses regarding viral infection: (a) that varroa injects the virus particles into the bee and (b) that the bee already has the virus and the piercing action of the mite activates it. In colonies affected by PMS, varroa is usually present, there is a reduction in bee population, queen supersedure is common and the tracheal mite may also be present. However, infested colonies may not show all symptoms simultaneously. Larvae affected by PMS die in late larval or prepupal stage, stretched out in their cells. They are usually a dull white in color and may turn grey with brownish spots as the infection develops (Plate

55). When the larval remains are stirred out with a toothpick they do not rope, but are globular (*see EFB).* Prepupae die after the cell has been capped, and cappings may be perforated or completely removed by the bees.

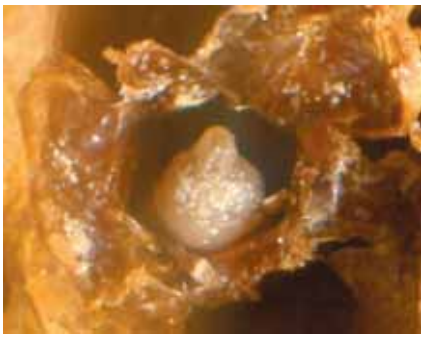


Plate 55: *Globular remains of a larva infected with PMS*

# Other Colony Disorders

## Dysentery

Dysentery is not a disease, but a condition caused by the excessive build up of waste matter in the rectum, i.e. diarrhea. It is often caused by unripe honey, late feeding, granulated stores, fermenting stores or feeding with brown sugar. Bees are unable to wait until cleaning flights are possible and typical signs are fouling on the comb, hive parts and around the hive (Plate 56). Dysentery is also associated with nosema (*see nosema*) .

Plate 56: *A honeybee affected by dysentery fouling on the alighting board*
(©jmcm; photography Trinity College Dublin)

## Starvation

Starvation can occur in a colony if there is a lack of honey or bees are unable to reach the honey in extremely cold temperatures. When colonies are found during the spring to have died in a cluster with their heads first in the cells, a diagnosis of starvation can be determined. Also in late autumn weaker colonies can be robbed by bees from stronger colonies, wasps and bumblebees which inevitability also results in starvation.

## Drone Laying Queen

This is also a condition rather than a disease. A drone laying queen is unable to lay fertilized eggs because of a genetic fault, not being mated properly or due to insufficient spermatozoa. Varroa infestation and related viral diseases are also thought to affect the fertility of the queen (*see varroa and viral diseases*). In a colony with a drone layer, the queen is still present and the brood laying pattern is regular. However, drones are produced in worker size cells, with cappings being more pronounced and extending out from the comb surface (Plate 57). Developing drones are small and stunted. During the season, a drone layer may be suspected if there are small areas of drone brood in the middle of large patches of worker brood and on each inspection the amount of drone brood increases, while worker brood decreases. This condition can be treated by requeening or uniting colonies once the drone laying queen has been removed.

Plate 57: *Drone laying queen as indicated by the drones being produced in worker cells*

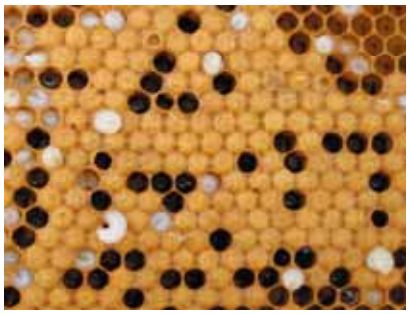
## Laying Worker

If a colony has been queenless for a relatively long time and is unable to requeen itself, a worker bee can begin to lay eggs. The worker's egg laying pattern tends to be scattered and several eggs are placed in each cell (Plate 58). Since the worker's abdomen is considerably shorter than that of a queen, the eggs are usually deposited on the sides of the cell instead of the base. It is difficult to requeen such a colony as the bees usually kill the introduced queen. In this situation very little can be done to save the colony. Occasionally, it can be 'shaken out' close to the apiary, the hive and all its parts are removed from the apiary and the bees return to different colonies in the apiary. The laying worker remains on the ground where the colony has been shook out.

Plate 58: *Multiple eggs in cells, an indicator of a laying worker*

## Colony Over-Heating

The optimal nest temperature for brood rearing is 30-35°C, which is maintained by the thermoregulatory activities of the bee and the colony as a whole. Overheating occurs when there is a loss of control of the temperature and humidity within the hive. This may occur because of a sudden loss of foraging bees due to pesticide poisoning. It may also occur if adult bees and brood are confined to their hive during hot weather without adequate ventilation. Under such conditions adult bees become sticky and greasy and run about noisily fanning their wings. Affected larvae hang out of their cells and turn brown in color (Plate 59a), while pupae turn black and greasy in appearance. Overheating can be prevented by providing shade and plenty of ventilation. Excess supers should be removed from colonies affected by overheating and they may also require feeding internally with water or watery syrup. The addition of frames with mature brood and young bees will also benefit such colonies. Screens should be placed over the brood box when transporting colonies (Plate 59b).



(a)

(b)

Plate 59: *(a) Larvae affected from overheating; (b) hive screen in situ to allow ventilation when transporting colonies*

## Chilled Brood

Chilled brood is not caused by a **pathogen**, but results if the brood area is too large for the nurse bees to maintain brood temperature at 30-35°C. A characteristic of chilled brood is that brood at all stages, sealed and unsealed are affected. Chilled larvae and pupae turn yellow, tinged with black on their margins or become a dull white with brown or black patches (Plate 60). The remains are generally pasty or watery. The outer boundaries of the brood cluster are affected first as the bees retreat to maintain the inner core at optimum temperature. Chilled brood, which is often mistaken for foulbrood differs from the latter in the following respect: the odor normally associated with foulbrood is absent and dead bees in the cells will be found in their natural positions (*see AFB and EFB*). Poor beekeeping practices such as adding brood frames to weak stocks that have not enough bees to cover them or exposing brood frames to cold winds may be responsible for this problem. Other causative factors include the loss of large numbers of bees due to spray poisoning (*see poisoning*) or if simulative feeding in the spring coincides with an exceptional spell of warm weather.

Plate 60: *Chilled brood in the comb*

## Baldbrood

Baldbrood is a condition whereby the heads of the developing pupae and prepupae are visible during the period between capping and emergence of the bee (Plate 61). This may be due to the genetic strain of bee, which results in the incomplete and faulty capping of the larvae by the bees, causing death to some brood. Alternatively, it may be caused by the greater wax moth (*Galleria mellonella*) (*see wax moth)* chewing its way through brood cappings in a straight line. Affected bees may have deformed legs and wings and faecal pellets of the wax moth may be seen adhering to their bodies. There is no specific treatment for baldbrood, but requeening and treating for wax moth are good practices.

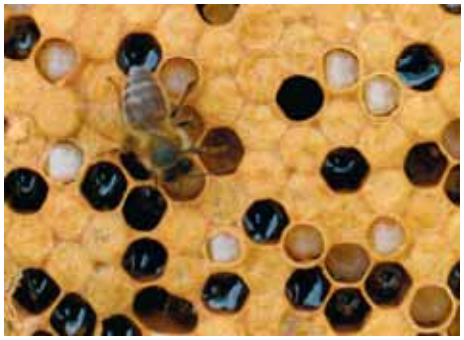


Plate 61: *Baldbrood in situ in the comb*

## Starved Brood

Normally when there is a shortage of food in a colony, larvae are removed and/or consumed by the adult bees. However, if there is a sudden loss in adult bees available to feed the larvae, the larvae starve. Larvae crawling from brood cells over the entire brood area are a typical sign of this condition. Although starved brood is almost restricted to the larval stage, emerging bees may starve if stressed as pupae due to chilling, overheating or too few nurse bees available to feed them just before emergence. These bees usually die with only their heads out of the cells and their tongues extended (Plate 62).

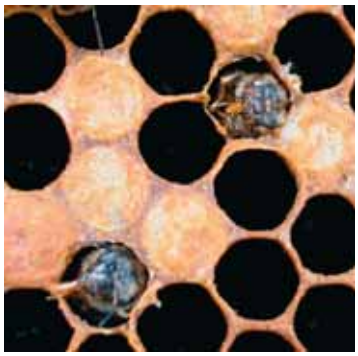


Plate 62: *Starved emerging bees showing tongues extended*

## Neglected Drone Brood

This is a condition rather than a disease. Irregular patches of drone brood are produced by a drone laying queen. In a weak colony, many of the larvae and pupae in the worker cells are undernourished, which leads to the nurse bees abandoning the developing brood, which inevitably becomes chilled and dies. On decomposing, the larvae become soft and are often brownish in color. This eventually dries out to form dark masses (scales) which are removed by the bees. There is no treatment for this condition and the colony is best destroyed.

# Pesticide Poisoning

## Biology

Death of an insect by poisoning is generally caused by the failure of the alimentary system or the poison affects the nervous system leading to complete lack of coordination of the normal bodily functions, which in both cases leads to death and starvation.

## Detection

The most apparent indication of serious poisoning is the sudden loss of adult bees. This loss is characterized by the appearance of many dead or dying adult bees and sometimes pupae at the hive entrance. However, in many instances the bees are lost in the field before returning to the colony. If only the foraging population is affected, the colony will start to recover in approximately 2 weeks, as new brood hatches out and house-bees become guards and foragers by natural progression. If however, the house-bees are also poisoned by feeding on contaminated honey and pollen, the colony will be reduced to the queen, which is generally unaffected due to her royal jelly diet, and a small number of bees. Under such circumstances the brood will exhibit symptoms of neglect and poisoning, and bees often abscond.

### Types of Pesticides

In general, pesticides are classified into five main groups namely organophosphates, chlorinated hydrocarbons, carbamates, dinitrophenyls and botanicals. The symptoms of poisoning exhibited by honeybees may be an indication of the class of pesticide involved. Organophosphorous poisoning causes bees to regurgitate, become disorientated and lethargic. A high percentage of bees die at the colony. Bees affected by chlorinated hydrocarbons and carbamates display erratic movements followed by paralysis. In the former, a large number of bees die in the field and at the colony, while in the latter, the colony becomes aggressive and most bees die at the colony. Dinitrophenyls and botanicals show a combination of both the above symptoms, bees regurgitate, giving them a wet appearance, followed by a display of erratic movements and finally paralysis. Infected bees die both at the colony and away from home.

## Prevention/Treatment

The main source of poisoning is from agricultural sprays. Bees are caught in sprays in three ways: (a) when the bees are foraging on the sprayed crop, (b) when the crop is sprayed for weeds which are been utilized by the bees and (c) when bees are flying over a crop which is being sprayed to reach a crop further away. Thus, application method and time of spray is critical to minimize damage done by spraying. Tractor mounted sprayers are less harmful than air craft and helicopter spraying. Also, if a crop needs to be sprayed, it should be carried out before 8.00am or after 8.30pm or when flying activity is minimal. Greatest spray damage occurs in field bean or crucifers such as rape and mustard (Plate 63). Rape crops are sprayed for seed weevil, pod mites and pollen beetle. The latter attacks oilseed rape crops just before first flowering, but occasionally the crop is sprayed when it is in full bloom, a practice which maximizes bee losses.

Plate 63: *Oilseed rape (Brassica napus) crop*

Beekeepers' can minimize damage to colonies by collaborating with farmers within flying distance of the hives, partially or completely closing colonies and moving colonies more than three miles from the area to be sprayed. Closing colonies is fraught with danger, particular larger colonies as these tend to overheat, causing brood loss, suffocation and melting of wax. Moving colonies is also impractical especially for beekeepers with relatively large numbers of hives. Thus, collaborating with farmers in the area is often the only option to the beekeeper. In UK, bee poisoning is part of the Wildlife Incident Investigation Scheme (WIIS), which investigates possible pesticide poisoning of wildlife. The Scheme enables post-registration surveillance of agricultural and other chemicals to be conducted, allows validation and improvement of the risk assessment processes used in the registration of products and can be used to enforce legislation. Over the past number of years the number of incidents reported to the Scheme has declined and most incidences are the result of the misuse of products, rather than the products themselves.

## Colony Collapse Disorder (CCD)

Colony collapse disorder is the phenomenon used to describe the loss of large numbers of colonies in America in 2006. Research indicates that affected colonies display a number of colony threatening conditions simultaneously. Typical symptoms are an insufficient work force to maintain the brood present, the workforce consists mainly of young bees, the queen appears outside the hive, adult bees are absent, but are not found near the hive and the colony has plenty of stores. The exact causes of CCD are unknown, but Figure 7 gives some of the factors which may contribute.

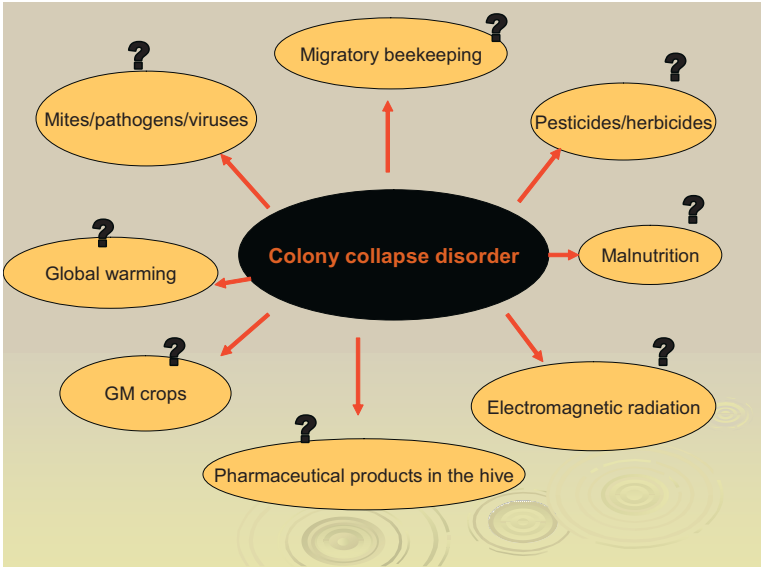



Figure 7: *A list of some of the factors which may contribute to colony collapse disorder*

Presently, the research findings are inconclusive, but preliminary findings indicate that nearly all beekeepers experiencing CCD noticed that colonies were stressed prior to their demise.

# Glossary

**Acaracide**: a chemical agent used to kill mites.

**Africanized bee**: often known as killer bees and were produced when the African subspecies (*Apis mellifera scutellata*) hybridized with the one or a number of European subspecies (for example: *Apis mellifera ligustica*)

**Anaerobic**: an organism that does not require oxygen for growth.

**Andipose cell**: a type of connective tissue that contains stored cellular fat.

**Angiosperm**: is a flowering plant.

***Apis***: is the genus which honeybees belong.

**Asymtomatic**: when infection shows no physical signs or symptoms.

**Bailey frame change:** moves the whole colony onto clean comb in a clean brood box

**Brood**: the young of bees are collectively called brood.

**Caste**: form of a social insect having a particular function.

**Chelicerate:** is the first pair of limbs of the body and is small pincer-like appendage.

**Colony collapse**: a mite infestation level which causes a colony to lose its social structure and disband.

**Comet**: is a group of drones in a drone congregation

**Complete metamorphosis**: is the development of an insect from egg, larva, pupa and adult.

**Deleterious recessive allele**: a deleterious allele can affect fitness at any stage of development. Some alleles cause abnormal phenotypes, while many others are morphologically cryptic. Recessive mutations that do not cause clear cut phenotypic variations but can be assayed through inbreeding depression.

**Diploid**: having two of each type of chromosome except the sex chromosomes.

**Diploid drone**: is a male from fertilized eggs and is the result from the match-mating of a queen and drone with identical sex alleles.

**Drone brood sampling**: drone brood is removed at the purple eye stage using a drone brood fork.

**Efficacy**: is a measure of how effective s product is at achieving the acquire effect.

**Eke**: simple rim to allow bee space.

**Enzyme**: protein catalyst produced by a living organism

**Fecundate**: fertilized female.

**Gravid**: pregnant female.

**Groom**: a cleaning mechanism used by bees.

**Haemolymph**: blood

**Haploid**: having only a single set of chromosomes as is present in gametes.

**Haplo-diploid breeding**: when haploid males mate with diploid females.

**Homeostasis**: maintenance of nest temperature and other environment factors at relatively constant levels regardless of external conditions.

**Host**: is the animal having the parasite.

**Hygienic strain**: is a strain that removes diseased larvae effectively from a colony.

**Hymenoptera**: is one of the larger order of insects comprising of sawflies, wasps, bees and ants.

**Hyporpharyngeal gland**: located above the pharynx and under the frons. The secretion of the gland is called brood food or royal jelly.

**Idosoma**: is similar to the abdomen of the insect.

**Incomplete host-parasite**: is a relationship between the host and the parasite which results in the death of the host.

**Lipophyllic**: fat soluble components.

**Match-mating of a queen**: mating of queens and drones with identical sex alleles resulting in diploid drones.

**Maximum residue level**: are designed to protect the consumers of animal foodstuffs and is regulated in the European Community Law.

**Micrometer**: is one millionth of a meter and is noted by the symbol μm

**Molt**: to shed outer covering.

**Mycelial**: belongs to the vegetative system of a fungus made of microscopic filaments.

**Natural mite fall**: is the number of mites that fall naturally from bees per day.

**Nectar**: is an aqueous sugar solution secreted from plant glands called nectaries.

**Ontogeny**: is the developmental history of an organism from its origin to maturity.

**Pathogen**: is a biological agent that causes disease or illness to its host.

**Pheromone**: is a mixture of chemical substances released by an individual into the hive or the environment that cause a change in the physiology or behavior of other bees.

**Phoretic stage**: adult stage.

**Phylogenetic**: is the methods used by biologists to reconstruct the pattern of events that has led to the distribution and diversity of life.

**Pollen**: is a fine coarse powder consisting of pollen grains which produce male gametes of seed plants.

**Propolis**: is a wax-like resinous substance collected from trees by honeybees.

**Pupa (pupal)**: stage between larvae and adult.

**Queen-right colony**: a colony which is headed by a healthy queen.

**Residue**: the remains of a treatment in wax and to a lesser extent honey.

**Resistance**: is when the mite is no longer susceptible to the active ingredient.

**RNA (ribonucleic acid)**: a nucleic acid found in the cytoplasm and nucleus that functions in the synthesis of proteins.

**Robbing**: bees from a strong colony take food from a weak or dying colony.

**Social behavior**: is when a species have a communal nest, cooperate in brood rearing and have a caste system of a queen, worker and drones.

**Sphecid wasp**: is a common name of any of a family of stinging wasps known for their predation and nesting behaviors.

**Spiracle**: the external opening to the trachea in insects.

**Sublime**: is the process of converting a substance by heat into a vapor.

**Super**: is the box containing frames/combs placed over a brood box for the eventual storage of honey which can be extracted by the beekeeper.

**Supersedure**: is the process by which the colony replaces its queen.

**Swarming**: when a group of bees migrate with a queen to establish a new colony.

**Taste threshold**: the level at which the taste of a product is affected by a treatment.

**Thermoregulation**: is the use of energy derived from the consumption of stored honey to generate body heat and keep the nest at adequate temperature for adult survival.

**Titre**: is the unit in which the analytical detection of many substances is expressed.

**Trophallaxis**: the regurgitation of food from one animal to another.

**Varroa floor:** a mesh floor placed underneath the brood box.

**Wax:** is secreted by honeybees of a certain age in the form of thin scales through eight wax producing glands.

**Wax gland**: four pairs of glands that are specialized parts of the body wall, which during the wax forming period in the life of a worker, become greatly thickened and take on a glandular structure. The wax is discharged as a liquid and hardens to small flakes or scales and sits in wax pockets. The worker bee draws the wax scales out with the comb on the inside hind leg. The wax scale is then transferred to the mandibles where it is chewed into a compact, pliant mass. The beeswax is then added to the comb. After the worker bee outgrows the wax forming period, the glands degenerate and become a flat layer of cells.

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# Appendix 1

**Classification of the honeybee**

| | |
|---|---|
| **Kingdom**: | **Animalia** |
| **Phylum:** | **Arthropoda** |
| **Class:** | **Insecta** |
| **Suborder:** | **Apocrita** |
| **Superfamily:** | **Apoidea** |
| **Family**: | **Apidae** |
| **Sub family:** | **Apinae (honeybees/bumblebees/stingless bees)**<br>Euglossi (Orchid bees)<br>Nomadinae (Cuckoo bees)<br>Xylocopinae (Carpenter bees) |
| **Tribe:** | **Apini (Genus *Apis*)**<br>Bombini (Genus Bombus)<br>Euglossini (Five genera of orchid bees)<br>Meliponini (Three genera of orchid bees<br>Others |
| **Species** | ***Apis***<br>*A. cerana*<br>*A. dorsata*<br>*A. florea*<br>*A. loboriosa*<br>***A. mellifera*** *(*Linnaeus*)* |
| **Subspecies** | ***Apis mellifera***<br>*A. m. mellifera* — **Europe**<br>*A. m. carnica*<br>*A. m. ligustica*<br>*A. m. caucasia*<br>*A. m. scutellata* — **Africa**<br>*A. m. capensis*<br>*A. m. macedonica* — **Middle East/ Asia** |

# Appendix 2

The seasonal floral sources of nectar referred to in the text are based on a melissopalynological analysis of fresh nectar sampled at regular intervals throughout the season. In such a study the morphological differences between pollen grains is often so slight that identification to species level in some genera was only carried out for species with distinct pollen. 'Type' was used as a subdivision of a family and Trifolium repens was always referred to as Trifolium repens s.l. (senso lato, in the wide sense) as it incorporates other species of Trifolium with similar pollen types

| **Latin name** | **English name** |
|---|---|
| *Acer* spp. | Maples and sycamores |
| *Calluna vulgaris* | Ling |
| *Crataegus monogyna* | Hawthorn |
| *Epilobium angustifolium* | Rosebay willowherb |
| *Filipendula ulmaria* | Meadow sweet |
| *Hedera helix* | Ivy |
| *Impatiens glandulifera* | Himalayan Balsam |
| *Prunus/Pyrus* type | Fruit blossom which includes apple |
| *Ranunculus ficaria* | Lesser celandine |
| *Ranunculus* spp. | Other species of buttercup |
| *Rosa* spp. | Rose |
| *Rubus* spp | Blackberry/raspberry |
| *Salix* spp. | Sallies |
| *Sambucus* spp. | Elder |
| *Trifolium pretense* | Red clover |
| *Trifolium repens* s.l.. | White clover |
| *Ulex* type | Gorse and broom |

# Appendix 3

## Sampling for Disease DIAGNOSIS (www.irishbeekeeping.ie)

The beekeeper should send samples of bees - in the case of adult diseases, and comb - in the case of brood diseases, for disease diagnosis. This is the only reliable method of disease detection. Currently there is a five Euro fee (per sample) for this service. Cheques or postal order must be made payable to Teagasc. Send the samples to:

**Mr. Pat Maloney**
Bee Diagnostic Service
Teagasc
Malahide Road
Dublin 17

In order to test for adult bee diseases, a sample of 30 bees is required. The sample can be collected in a match box by partly protruding the tray, holding it nearly flat over the bees, on the crown board or at the front entrance, and drawing it back with a sweeping movement. Bees can be killed by placing them in the freezer for 24 hours before posting. Label each sample showing apiary, hive number together with your name and address. On no account should plastic containers be used as the bees decompose rapidly in these containers.

If the beekeeper suspects any of the brood diseases are present in the hives then, the full frame containing suspect brood from the hives should be sent also in a paper container. The sample should contain sealed, dead and/or discolored brood if possible. It would assist diagnosis if the cappings were not damaged (i.e. squashed in the post).

# Appendix 4

## Monitoring for Bayvarol® resistance

### Collection in the apiary

From each colony, carefully pluck 15 mites and 3 pupae from the sealed brood stage using a tweezers forceps.
Transfer to a labeled petri-dish lined with moist cotton wool.
Store dishes in polystyrene box (max 3hrs) until required for testing

### Conducting the test

Line three petri-dishes with moist cotton wool and label A, B and C
Place a drone pupa in each of the petri-dishes
Put on gloves and lay out a fresh Bayvarol strip.
Transfer 5 mites directly onto the pupa in A (control)
For B and C start stop watch while simultaneously placing 5 mites on the strip in a fixed sequence from left to right
Watch mites and use a brush to stop them escaping from the strip.
When the minute has elapsed, transfer the mites to B and C in the sequence in which they were placed on the strip in order to maintain the correct contact time.
Place at room temperature for 5h before assessing for resistance.

### Assessment

Differentiate between the following mite conditions:
Mobile: the parasite crawls away in a coordinated manner in response to a mechanical stimulus
Damaged: there is no coordinated movement even after being touched three times with the brush, some stagger, some tremble or twitch slightly, most stay trembling in one place or show no noticeable movement

### Evaluation

Determine the proportion of damaged control mites
The test is only valid if <10% of the control mites are damaged
If at least 90% of the treated mites (apiary average) are damaged, the colony can be treated with Bayvarol
If <90% are damaged, the parasites are probably resistant and treatment with Bayvarol should be avoided.

# Appendix 5

A list of the products referred to in the text and their legal status for use in Irish honeybee colonies in 2006/2007

**Registered products for use in honeybee colonies in Ireland**

| *Product* | *Description* | *Treatment* |
|---|---|---|
| Acetic acid | Liquid | Disinfection of combs after nosema/wax moth infection |
| Apiguard® | Slow releasing gel | Varroa/tracheal mite/ chalkbrood |
| Bayvarol® | Strips | Varroa/tracheal mite |

**Unregistered products and illegal for use in honeybee colonies in Ireland**

| **Product** | **Description** | **Treatment** |
|---|---|---|
| Exomite | Endostatic dust | Varroa |
| Formic acid | Liquid | Varroa/tracheal mite |
| Mite Away II | Pad | |
| FAM dispenser | Absorbent pad in dispenser | |
| Liebig dispenser | Bottle dispenser | |
| Apicure | Gel matrix | |
| Beevar | Gel matrix | |
| Oxalic acid | Crystal/liquid/tablet | Varroa |

## Please Note:

**IT IS ILLEGAL TO USE UNREGISTERED MATERIALS IN HONEYBEE COLONIES. USE ONLY REGISTERED MATERIALS AND FOLLOW THE LABEL DIRECTIONS CAREFULLY**

***Where trade names appear in this publication, no discrimination is intended and no endorsement by Teagasc is implied***

# List of Tables



# List of Figures

# List of Plates

Plate 26: *Adult tracheal mite (Acarapis woodi) (centre) and larval instars*

Plate 27: (a) *Blackening of the trachea indicating tracheal mite infestation; (b) clump of bees with queen in the centre (marked white) and workers with signs of dysentery on their wings*

Plate 28: *(a) Adult braula; (b) braula on the head of the worker honeybee*

Plate 29: *Lesser wax moth (Achroia grisella) adult*

Plate 30: *Wax moth larvae: greater wax moth (Galleria mellonella); (b) lesser wax moth (Achroia grisella); (c) lesser wax moth cocoon*

Plate 31: *Wax moth damage: (a) galleries through the comb; (b) crumbled and discolored comb*

Plate 32: *Physical features of Tropilaelaps* spp.

Plate 33: *Small hive beetle (Aethina tumida) adult*

Plate 34: *Small hive beetle larvae: (a) dorsal side; (b) ventral side showing the three pairs of legs*

Plate 35: *Rodent damage in a honeybee colony: (a) rat nest; (b) destruction of comb; (c) mouse cleat*

Plate 36: (*a) Honeybees robbing after feeding syrup; (b) wasps (Vespula spp.) and bumblebees (Bombus spp.) robbing a honeybee colony*

Plate 37: *Nosema spores under a light microscope: (a) Nosema apis; (b) Nosema ceranae*

Plate 38: *Defecating on the outside of the hive*

Plate 39: *AFB spores (x100 magnification)*

Plate 40: *AFB kit (Vita® Europe Ltd.) which can been use for the detection of AFB in the field*

Plate 41: *Worker brood: (a) healthy brood; (b) nibbled holes and sunken brood cappings*

Plate 42: *Advanced symptoms of AFB: (a) slimy larval remains, (b) mucous-like brown thread or rope when the cell contents are removed with a matchstick; (c) hard brittle scales*

Plate 43: *EFB spores (x100 magnification)*

Plate 44: *Symptoms of EFB infection in the brood: (a) larva becomes clearly segmented; (b) uncapped larvae in various positions in the cell*

Plate 45: *(a) Paenibacillus alvei; (b) Paenibacillus alvei and EFB spores*

Plate 46: *(a) Chalkbood in the comb; (b) mouldy pollen in the comb*

Plate 47: Chalkbrood 'mummies'

Plate 48: *Chalkbrood 'mummies' on the floor of the hive*

Plate 49: *Requeening a colony*

Plate 50*: Symptoms of SBV in a colony: (a) head of SBV killed larva; (b) SBV infected prepupa removed from the cells*

Plate 51: *Normal a*nd d*eformed honeybees exchanging food by trophallaxis*

Plate 52: *Emerging honeybees showing different degrees of deformity*