



Review

A Veterinary Approach to the European Honey Bee (*Apis mellifera*)

D. L. WILLIAMS

Department of Clinical Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, UK

SUMMARY

The European honey bee (*Apis mellifera*) has the unusual status of being an inherently wild species from which a natural foodstuff (honey) is derived by manipulating its behaviour to deposit this in man-made wooden frames. Bees also produce propolis and Royal Jelly which can be harvested but their most important effect is one not immediately obvious as an economic product: that of pollination. Bee diseases are predominantly infectious and parasitic conditions accentuated by the close confinement in which they congregate, either in man-made hives or in colonies in a natural cavity. Treatment or at least control of some of these conditions can be attempted. In some cases natural bee behavioural traits limit the effect of the disease while in others, such as the notifiable disease American foulbrood, destruction of the colony is the only method of control. The mite *Varroa jacobsoni* can be controlled by the synthetic pyrethroids flumethrin and tau-fluvalinate. The introduction of these products has heightened veterinary interest in this important invertebrate species.

© 2000 Harcourt Publishers Ltd

KEYWORDS: Bee; invertebrate; disease; insect.

INTRODUCTION

The veterinary profession has drifted perilously close to complete neglect with regard to a number of non-mammalian species that should have been afforded due attention. This is certainly true of the bee. Thus, when a disease such as Varroosis strikes, requiring a licensed therapeutic product, the industry has, at least in the early stages, often to rely on research apiarists without a background in disease control across species. While not seeking to fill this gap in a few pages, this paper aims to introduce veterinary surgeons to this fascinating species and its important diseases.

A BRIEF HISTORY

Diseases of the honey bee have been known by man ever since bee keeping was first practised. While the descriptions of Aristotle, Virgil and Pliny

are not sufficiently detailed to identify modern parallels with certainty, Bailey (1981) considers Aristotle's description of a disease of adult bees to correspond closely with one of the two syndromes of bee paralysis. He described a black bee with a broad abdomen very similar to the appearance of chronic bee paralysis type 2, in which bees in the UK are often known as 'black robbers' or 'little blacks' as discussed further below. Bailey also reports a condition *Faux Couvain*, probably either American or European foulbrood described in 1771, and 'dysentery' reported in 1826. Shortly after this, Louis Pasteur elucidated the cause of 'pebrine' a disease crippling the French silk industry and devised a method to reduce the effects of the microsporidian pathogen, later renamed *Nosema bombycis*, on the silkworm *Bombyx mori*. After Pasteur's demonstration, the field was open for similar studies of bee disease. Dzierzon was the first to characterize two types of foulbrood: a 'mild and curable' form of the unsealed larval cell (now known as European foulbrood) and a 'malignant and incurable' disease of the sealed cell (America

Correspondence to: David L. Williams

foulbrood) (Dzierzon, 1882). At the same time, bacteriological investigations were in progress (Cheshire & Cheyne, 1885) and it was presumed that the presence of the appropriate pathogen signalled inevitable development of the disease. However, there is a complex interaction between the pathogen, the bees themselves and the environment in which they are housed; it is not necessarily the case that presence of a pathogen will lead to severe disease. Indeed, it is the understanding of factors influencing development of disease that is central to controlling diseases within the bee population.

BEE KEEPING AS A CONTEXT FOR BEE DISEASE

It is clearly important for veterinarians interested in bee diseases to be conversant with bee husbandry. An important feature of bee keeping is the fact that apiculturists are managing a wild population and not farming a domesticated species. As Bailey notes 'Beekeeping today is still as it has always been: the exploitation of colonies of a wild insect; the best beekeeping is the ability to exploit them and at the same time to interfere as little as possible with their natural propensities' (Bailey, 1981).

In times prior to the 1800s, bees were kept in woven baskets or in hollow logs and the honey harvested only after killing the bees. In the mid 1800s, it was discovered that bees would not wax up spaces around 1 cm wide, through which a bee could pass. Thus hives could contain wooden frames separated by this 'beespace'. In these wooden rectangular frames bees can construct the same vertical combs that they would in the wild. Individual frames can be removed, honey harvested from the comb and the frame replaced with as little disruption to the bees as possible. This also allows brood stages to be inspected and moved to propagate desirable strains of bee. However complex a modern beehive may be, it still conforms to the original concept of Langstroth first developed in 1851 (Langstroth, 1866).

Knowledge of the natural history of the bee (and particularly the worker bee) is important in understanding where different diseases have their predominant effects. The larval worker bee develops through six stages in the comb. First the egg is deposited at the base of an open cell in the comb. It develops there for three days after which it hatches and is fed repeatedly in the open cell by nurse bees. The fully grown larva is sealed in

the cell by the nurse bees and proceeds to spin a cocoon. Fluid deposited from the labium-hypopharynx becomes dry and parchment-like and faeces from the rectum are sandwiched between layers of the cocoon. After a further 2 days the larva sheds its final exuvium to become a pupa. This darkens in colour, finally sheds its skin and emerges as an adult from the cell.

Adult bees eat pollen and honey, the former supplying the protein constituent of the food while the latter, a 30% glucose 40% fructose solution of floral nectar, provides the carbohydrate source. Larval food is a secretion from several glands of the adult insect, 30–50% being carbohydrate from honey while 40–60% is protein from the hypopharyngeal glands. In many cases, a disease agent non-pathogenic to adult bees is passed to larvae through feeding and, thus, the involvement of these hypopharyngeal glands is all-important in several diseases. The food itself is to some degree bacteriocidal – around 10% of larval food by dry weight is 10-hydroxydecenoic acid, a product of the mandibular glands which is bacteriocidal at the acid pH of the larval food. As will be seen below in several diseases, the behaviour of worker bees, quite as much as their physiology, is central in the prevention or limitation of disease in the bee hive.

DISEASES OF BEES

The most important disease is the *Varroa* mite, followed by the bacterial foulbroods and a number of viral diseases such as sacbrood and the bee paralyses.

Varroa jacobsoni

This acarine mite (Fig. 1) was first described by Oudemans in 1904 as a reasonably innocuous parasite of the Asiatic bee *Apis cerana*. In the next 50 years it appears to have increased its Asiatic range bringing it into areas where *Apis mellifera* was farmed commercially; or perhaps it was the introduction of *A. mellifera* into the natural range of *A. cerana* which led to deleterious parasitism of the former species by the mite. By the late 1960s, *Varroa* was reported parasitizing *A. mellifera* and the mite now exists in around 60 countries including Britain. Given that the majority of early reports were from the Pacific rim or the European USSR, there is little literature on Varroosis in the English language.

The spread of the mite has also occurred through importation of bees: some countries



Fig. 1. The mite *Varroa jacobsoni* on an adult worker bee.

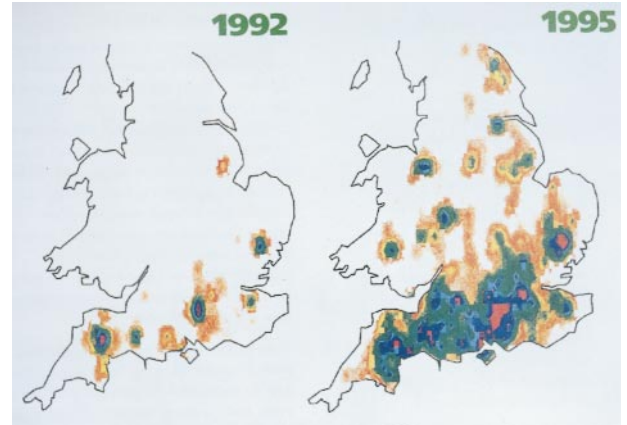


Fig. 2. Spread of *Varroa* across the country in the early years of infestation.

have been successful in preventing importation of the disease while in other cases the donation of bees has been the prime mode of disease spread. Thus, in 1987 an Australian quarantine station discovered *Varroa* in a shipment and was able to prevent spread into the country. Donations of infected bees by Japan to Paraguay in 1969 and by Romania to Tunisia in 1975 were not so well controlled and resulted in the disease spreading to those countries. It has been suggested that *Varroa* entered the UK through a swarm aboard a ship but other possible routes of entry would be through commercial import of queen bees or the illegal importation of a queen bee by an apiarist visiting a foreign apiary. The first outbreak was discovered in Torbay, Devon in 1992, but the speed with which the infestation spread, as shown in Figure 2, has rendered it a nationwide problem.

While several other diseases thrive in weak colonies, *Varroa* infestation is maximal in a strong colony with much available brood. The rapid increase in infestation considerably weakens and reduces the colony. Eggs are laid on bee larvae by the female mite entering the as yet unsealed cell and commencing laying 60 h after sealing. The mite instar undergoes all larval and nymphal stages within the sealed cell and is thus concealed from view; an infestation may escape detection for some time and by the time it is recognized there is widespread infestation of the colony.

As they develop, the mite instars feed on the pupal bees' haemolymph with resulting stunting and deformities. Adult mites also feed on the pupal haemolymph and then, when the adult bee leaves the cell, attach themselves to the emerging bee

before entering an unsealed larval cell to repeat their lifecycle. Some authors suggest that the longer larval stages of *A. mellifera* compared to those of *A. cerana*, account for *Varroa*'s greater success in parasitizing the former species, others consider that the latter's more assiduous grooming behaviour renders the bee resistant to mite challenge. These differences in developmental duration may also account for variation in severity in *A. mellifera* in different geographic areas (Camazine, 1988).

In the initial stages of infestation, few signs will be noted, but as the mite population increases there comes a point, generally at a mite population of several thousand, at which the bee colony loses its social cohesion and begins to disband, a phenomenon known as colony collapse. The increase in damage from mites is a function not predominantly of mite propagation but rather of changes in brood number with nectar production peaking in early summer. A reduction in nectar production leads to reduced brood rearing by worker bees. Mites emerging from sealed cells and being carried by adult bees are thus competing for unsealed cells for their next cycle. Several mites entering one cell cause significant damage to the bee propupa and pupa resulting in deformed bees or adults weakened to the extent that viruses, such as slow paralysis virus, may cause secondary disease. Slow paralysis virus is important in this country associated with varroosis while chronic paralysis virus, though endemic, is more associated with *Nosema apis* and *Acarapis woodi*. In late summer a significant number of drone and worker pupae are infested with *Varroa* and thus there is a failure of replacement of the ageing bee population with young

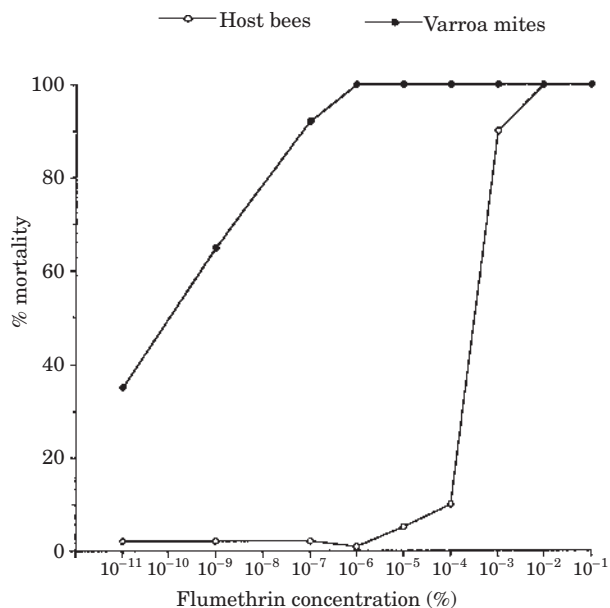


Fig. 3. Bee and mite mortality from flumethrin showing the wide safety margin of the drug (data kindly provided by Bayer plc).

bees. It is at this point that the colony collapses. Bees may leave the collapsing colony and migrate to a nearby apiary, thus facilitating rapid migration of the mite population between colonies.

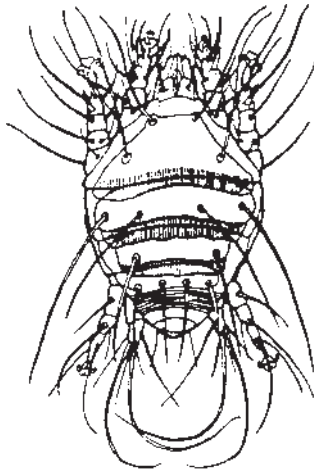
Apiarists should monitor their colonies for mites by sampling the brood, especially the drone brood, and examining frames for percentage of cells infested with mites. Noting natural mite mortality (mite drop) in the debris from a colony or determining number of mites killed by a standard *Varroa* treatment is also valuable. Determining changes in mite numbers can define when treatment should be started. A computer model produced by the National Bee Unit of the Central Science Laboratory (MAFF, 1998) has resulted in a handheld rotary calculator which allows apiarists to define when mite populations will rise to a dangerous level of around 2500 mites per colony during the next several months and thus whether their colony requires treatment.

The only definitive treatments licensed for use in the UK are the pyrethroids flumethrin, in the form of impregnated strips (Bayvarol, Bayer), or tau fluvalinate (Apistan, Vita [Europe] Ltd). Two particularly important features of flumethrin thus formulated are the differential toxicity of the drug to the bees and their parasitic mites and the potential problem of drug residues in honey.

Previously unpublished data concerning flumethrin toxicity are shown in Figure 3. The therapeutic margin is between 1×10^4 and 1×10^5 , with maximal mite mortality occurring at drug concentrations as low as $1 \times 10^{-7}\%$, while bee mortality starts at $1 \times 10^{-5}\%$ and nears 100% only at a drug concentration of $1 \times 10^{-2}\%$. With regard to drug residues in honey, no migration of the acaricide from the plastic strip has been recorded and levels in honey are below the limit of detection of $10^{-9}\%$. The product may, however, be absorbed into the wax of the honeycomb, so further precautions may be required if comb honey is being produced.

Fluvalinate has been used in a number of countries for some time, but uncontrolled use in some has led to resistance (Londzin & Sledzinski, 1996). This is of concern since such resistance acts across all pyrethroids and thus would render mites resistant to either tau-fluvalinate or flumethrin if resistant strains were to enter the country. Such concerns show the importance of restrictions on bee movement. The long-term use of fluvalinate has also been shown to have detrimental effects on brood rearing (Sokol, 1996), but this has not been shown to be the case with flumethrin. Only the trans Z1 and trans Z2 isomers of flumethrin are acaricidal and these compose 94% of the isomers in Bayvarol at levels of $500 \mu\text{g/g}$ of the impregnated plastic of which the strip is manufactured. This may account for the low toxicity of the drug to the bees themselves while being highly efficacious in killing mites. Naturally occurring organic acids such as lactic acid or 60% formic acid have also been used to combat the mite. The former is sprayed at 15% on combs covered with bees, resulting in 90% mite knockdown (Rendall, 1996), while the latter has been used successfully in Germany by soaking an absorbent pad held in a cradle over the brood nest (Rendall, 1996) or with formic acid-soaked pads in Italy with an efficacy of up to 98.8% when used every third day for three weeks (Mutinelli *et al.*, 1994). Increasingly, formic acid is being used in the UK, its application being simplified by the use of vaporizers.

As with other diseases, bee behaviour has a significant effect on the severity of disease. Several factors may be involved in resistance of some strains to *Varroa*. These may include shorter time periods during which larvae stay in cells after capping or may involve differences in behaviour patterns such as cleaning of brood comb or grooming behaviour between bees (Spivak, 1996).



100 μ m

Fig. 4. The tracheal mite *Acarapis woodi*.

Acarapis woodi

While less of a threat than *Varroa*, the tracheal mite *Acarapis woodi* (Fig. 4) has, in the past, been reported as causing severe damage to some colonies. The main effect of infestation is that overwintered bees with the mite die sooner than non-infested individuals but these insects are near the end of their lives and are generally replaced by younger bees which, after a few days old, are resistant to infestation, possibly because of the presence of stiff hairs at the spiracle entrance to the tracheae. This means that in all but the most severely affected colonies little effect is noticed and, in Britain at least, very few colonies are affected to this pronounced degree.

Since an infested bee shows no outward sign of disease, diagnosis has to be by dissection. The mite lives in the thoracic tracheae leading from the first thoracic spiracles and irregular dark stains develop in infested tracheae. While it might be thought that a heavy mite infestation would impede oxygen flow to the bee's flight muscles, supplied with oxygen by these tracheae, there seems to be little alteration in foraging or flying behaviour (Bailey, 1958). The first therapeutic formulation widely used by apiarists to control *A. woodi* consisted of nitrobenzene, petrol and saffrol oil vaporized on top of the brood combs during periods of hive inactivity – either November or February. This mixture has now been



Fig. 5. Brood affected with American foulbrood.



Fig. 6. Viscous ropy thread from a cell affected with American foulbrood.

superceded by safer fumigants such as methyl salicylate or oil of wintergreen. Selective acaricides, chlorbenzilate and bromopropylate, have been used but the latter was withdrawn after accumulation in combs became of concern.

American foulbrood

American foulbrood is, as its name suggests, a disease of the brood, i.e. the developing larvae within the comb. From a diagnostic perspective the appearance of the brood in the disease is pathognomonic: when the larva dies after the cell is sealed the cap over the cell becomes dark, moist and sunken (Fig. 5) or, in a significant proportion of cells, is perforated. If a matchstick is pushed into the cell and withdrawn, a sticky viscous ropy thread is noted (Fig. 6). After about a month the dead larva dries to a hard scale adhering to the side of the cell.

In the past, a diagnostic test useful in detecting infected larvae before the dry stage, relied on the proteolytic action of enzymes liberated by the aetiological agent, the Gram-positive bacterium *Paenibacillus larvae*, (previously known as *Bacillus larvae* but recently reclassified along with several other bacteria of the Bacillus group) (Heyndrickx, 1996). Mixed with a couple of drops of milk on a glass slide, macerated material from an infected cell or scale produces a firm curd in less than 40 s. A cell affected by European foulbrood (see below) takes over 1.5 min to curdle while a normal scale will not produce a curd in less than around 13 min. Microscopic examination of larval remains shows large numbers of oval spores and long threads of the coalesced flagella of the rods once sporulated are characteristic under phase contrast illumination microscopy. *P. larvae* spores will germinate on a 7% blood agar plate at 37°C in 5–10% CO₂ to give rise to grey colonies of catalase-negative Gram-positive rods (Lloyd, 1986). Several million spores may be needed to form colonies with this technique, but a few spores can be germinated using more stringent criteria in a pH 6.6 yeast, glucose, starch and 0.136% KH₂PO₄ semi-solid agar autoclaved to 116°C (Bailey & Lee, 1962). Today a much more readily performed, and considerably more specific, test is that using polymerase chain reaction (PCR) (Alippi & Aguilar, 1998).

American foulbrood is an extremely contagious disease which readily spreads within the apiary by the common technique of moving a comb from one hive to another. The spores of the bacterium are highly resistant to heat, to desiccation for up to 35 years and to the majority, of disinfectants (Haseman, 1961). It is considered by most authorities that destruction of infected colonies is the only workable control measure. Others suggest that a therapeutic option, treating with sulphathiazole or oxytetracycline in sugar syrup, should be used. This latter course of action is illegal in the UK under the Bee Diseases Control Order (1982). Sterilization of combs and equipment used with ethylene oxide has been used as a precautionary measure while a more modern approach seeks to use a bacteriophage specific to *P. larvae* in controlling the organism. Under the 1982 Order, affected frames and combs must be destroyed by burning and hive bodies sterilized by flaming, although gamma irradiation using a cobalt 80 source can be used for bulk loads of equipment.

It is important to understand the impact that the

bees have themselves on both spread of American foulbrood and their resistance to it. Spores are widespread in colonies, even when there is no overt sign of disease. Adult bees which have cleaned out infected cells are important carriers; when they start to feed uninfected larvae the disease is rapidly spread. It takes as few as ten larval spores to infect a larva when it is less than 24 h old, but over a million spores once the larva is over 2 days old. The cleaning behaviour of nurse bees which tends to spread disease within a comb can, however, be turned to advantage. It would appear that particular lines of bees can be bred with resistance to disease associated with workers' behaviour (Thompson & Rothenbuhler, 1957; Rothenbuhler 1964). Nurse bees appear to be able to detect affected cells very soon after infection and remove them before large numbers of spores have been produced. Bees seem to avoid affected cells with remains of dead larvae (Woodrow, 1941). Such behaviour can limit the effect of American foulbrood on a hive and thus a substantial number of cells may have subclinical infection. When over a few hundred larvae in a colony die, the infection appears to take hold, spreads within the hive and the colony dies.

In the UK, American foulbrood is a notifiable disease under the Bee Diseases Control Order (1982) and infected colonies are destroyed without compensation (MAFF 1996). A suspected infected brood comb must be sent to the National Bee Unit laboratory for microscopic examination and infected colonies are destroyed by burning under the supervision of a bee inspector. Antibiotics, used in some countries, are not used in the UK as they suppress but do not eliminate, infection. In some states in the USA oxytetracycline has been approved for prevention or treatment of the disease in bee colonies, but substantial brood losses can occur after its use. Chlortetracycline, similarly caused larval mortality and retarded growth after oral administration (Peng *et al.*, 1992). Laboratory studies of the efficacy of other antibiotics against *P. larvae* showed penicillin and macrolide antibiotics to be much more effective than the tetracyclines (Leighton, 1983) and laboratory and field studies have shown tylosin to be safe and efficacious against American foulbrood (Peng *et al.*, 1996).

European foulbrood

European foulbrood is also a disease of larvae. Unlike American foulbrood, however, which has

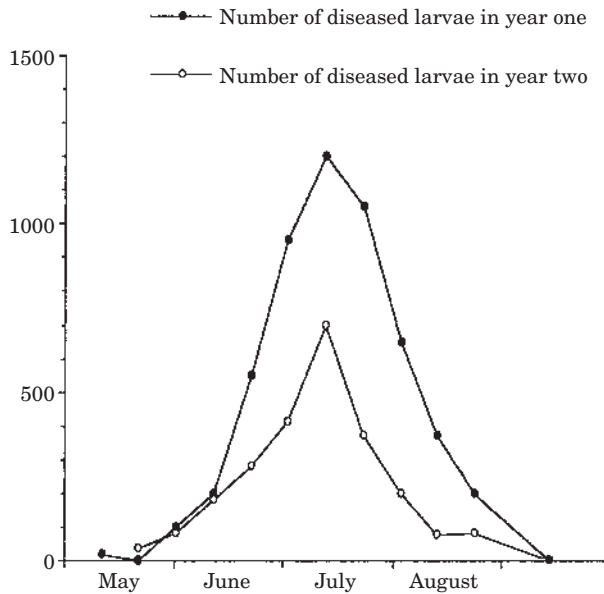


Fig. 7. Numbers of infected larvae in a colony naturally infected by *Mellicococcus pluton* over 2 years, showing the limited duration of disease and the reduction from one year to the next. From Bailey (1981).

no seasonal incidence, European foulbrood kills larvae at the age of 4 to 5 days in the early summer when colonies are rapidly growing (White, 1920, Morgenthaler, 1944; MAFF 1996). After an outbreak, a spontaneous recovery normally occurs (Fig. 7), thus supporting Dzierzon's differentiation between the two foulbroods over a century ago (see above) to be accurate in defining the two diseases. American foulbrood is indeed a 'malignant and incurable' disease while European foulbrood might well be termed 'mild and curable'. The appearance of the two diseases is likewise quite different. Dying larvae in European foulbrood are displaced to the wall of the cell, become flaccid and then turn brown as they decompose after death (Fig. 8). Before decomposition occurs, diseased larvae can be dissected out and examined showing chalk-white clumps in the mid-gut in comparison to the golden-brown colour of the normal larval mid-gut.

The disease is caused by the Gram-positive coccus *Mellicococcus pluton* which may be observed microscopically either singly, in chains or as clusters. The bacterium used to be named *Streptococcus pluton*, but this classification is no longer used since the organism has a DNA content of between 29 and 30.5%, while Streptococci have a higher DNA

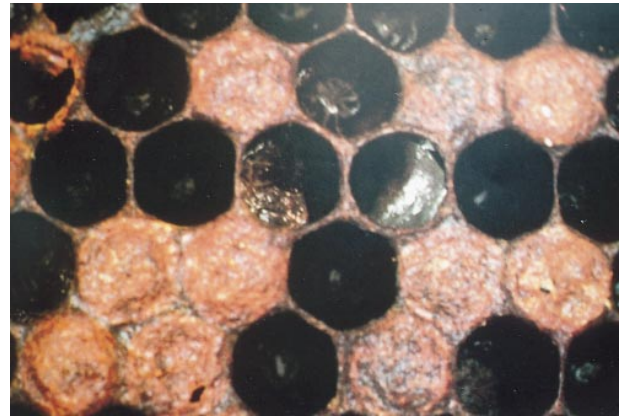


Fig. 8. Brood affected with European foulbrood.

content, ranging from 37 to 41%. In the past, definitive diagnosis was achieved by bacteriological isolation from smears of diseased larval mid-gut (Bailey, 1959) but, as with American foulbrood, culture media had to be made specifically, in this case involving anaerobic incubation in agar with glucose, starch and KH_2PO_4 at pH 6.6. A more specific diagnostic tool is the ELISA test but false negatives may occur through a mutant strain or encapsulated bacteria in which antigens are hidden by the capsule. Today the definitive diagnosis may be reached by characterization of the protein 'signature' of the agent through agarose gel diffusion, by DNA hybridization where a chemiluminescent probe is used or by PCR where specific primers amplify bacterial DNA even in infected but not clinically 'open' cases (Dancer, 1995; Govan *et al.*, 1998).

The bacteria are ingested by larvae with contaminated pollen and multiply within the larval mid-gut, which they may fill almost completely giving the white deposits noted above. Infected larvae may survive long enough to discharge copious numbers of bacteria in the faeces which are deposited on the walls of the brood cells. As with American foulbrood, the hygienic housekeeping activity of adult bees removes a substantial portion of bacteria from larval cells but some inevitably find their way to other larvae. Larvae may be detected or die before the cell is capped in which case they are ejected by nurse adults. They may be capped and fail to pupate in which case they void *M. pluton* in their faeces or they may pupate and then progress to adulthood in which case they leave *M. pluton* in the cell they leave. Infected larvae pupate at a suboptimal weight; this poor growth rate is thought to be caused by the bacteria

assimilating a considerable portion of the food intake. Infected larvae have poorly developed silk glands, which further facilitates bacterial dissemination since bacteria can spread from faeces not encased between layers of the silk cocoon.

In the same way that adult bees can influence the impact of American foulbrood on the colony, so nurse bees clean out cells, ejecting many infected larvae before they are obviously affected. Thus, *M. pluton* infection can occur for several generations without giving obvious disease in the colony or giving a cyclical level of disease severity through successive seasons. As disease severity increases, infected larvae die and are ejected thus reducing the dose of *M. pluton* to the rest of the colony, resulting in an increase in available food and a decrease in disease severity.

Secondary bacteria often occur in the cells of larvae infected with *M. pluton*, the most common being *Bacterium eurydice*, a species similar to *Corynebacterium pyogenes*. Another secondary invader is *Streptococcus faecalis*, again a predominantly vertebrate pathogen. As its name suggests, this organism gives off a putrid smell, (the German term for the disease is *Sauerbrut*). Another secondary organism is *Bacillus alvei* which, while strictly a saprophyte multiplying solely on dead larvae, is most commonly seen in colonies affected by European foulbrood.

As with American foulbrood, European foulbrood is a notifiable disease in the UK but unlike *B. larvae* infection, mild cases of *M. pluton* infection can be treated with oxytetracyclin, under the control of a MAFF bee disease officer (MAFF, 1996).

Chalkbrood

This fungal disease of bee larvae is widespread in Britain but is not a major cause of economic loss for apiculturists. The aetiological agent is *Ascospaera apis*, a heterothallic mycosis, spores of which germinate in the gut, form a mycelium which penetrates the gut wall to produce a white mycelium throughout the body, fruiting bodies on the external surface and killing the larvae after the cells have been capped (Fig. 9). Young larvae are affected, most dying within 2 days of the cell being capped.

Determining that a fungus is responsible for the death of any insect can be difficult: there are several fungi found in a hive but only two are definitively pathogenic, *Ascospaera apis* causing chalkbrood, and *Aspergillus flavus* and *fumigatus* causing

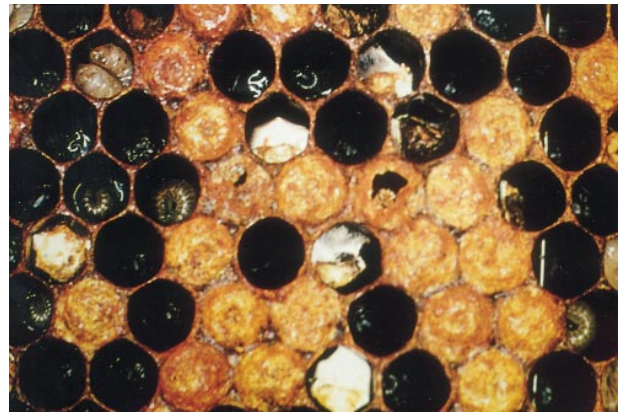


Fig. 9. Brood affected with chalkbrood.

stonebrood. Indeed, the first disease for which an infectious aetiological agent was demonstrated was muscardine of the silkworm larva, shown to be caused by *Beauveria bassiana* by Agostino Bassi in 1834. The difficulty lies in proving that a fungus is the true cause of a disease rather than a saprophytic organism secondarily colonizing a dead insect. Indeed, another *A. apis* subspecies does occur as a secondary saprophytic invader in larvae dying of European foulbrood. *A. apis* has, however, been shown definitively to cause chalkbrood and to fulfil Koch's postulates for the disease (Heath, 1982a, 1982b). Nevertheless, several puzzling aspects of the disease remain. Firstly, outbreaks occurred concurrently in a number of different countries worldwide in the 1970s including North America where the disease had previously been unknown (Gochnauer & Hughes, 1976). This may have been caused by a novel mutation in the organism, but was probably caused by spores germinating when conditions were particularly favourable. Such environmental characteristics include cold weather and damp, poorly ventilated hives. The condition appears to be stress-related. In colonies where there are sufficient adult bees to manage the combs, there is little likelihood of severe disease.

The limited spread of chalkbrood within a colony may be related to the heterothallic nature of the fungus. Formation of fruiting bodies depends on the interaction of mycelia of two different strains of fungus. If a larva is only infected with one strain, no infective spores will be formed (Maurizio, 1934), although it may be possible for larvae to be infected by mycelium rather than spores since combs have been reported in which all dead larvae have been infected by only one

Table I
Prevalence of viruses in *N. apis*-infected and non-infected bees (after Bailey, 1981)

<i>Virus</i>	<i>Bees with N. apis and virus</i>	<i>Bees with virus alone</i>
Filamentous	13/320	0/320
black queen cell	4/180	0/180
bee virus Y	3/180	0/180
bee virus X	28/452	73/452

mycelial strain. The temperature of the brood may be critical in preventing or allowing spread of the disease; a brood chilled from 35°C by as little as 5°C after capping seems the most susceptible (Bailey, 1967) and time of year and size of brood are important: early summer is a key time for such temperature reductions and small colonies are most at risk at this time since they have high surface areas relative to their total volume.

While a large number of chemicals have proved promising in controlling chalkbrood in vitro, spore persistence is a continuing problem which frustrates successful chemotherapy of the disease. The recent introduction of thymol as a gel (Apiguard, Vita [Europe] Ltd) may reduce incidence of chalkbrood, although it is mainly directed towards non-medicinal control of Varroasis.

Stonebrood

In this mycotic infection of the brood, caused by *Aspergillus flavus* or much less commonly *Aspergillus fumigatus*, the organism seems generally to gain access to larvae not through their cuticle but via the gastrointestinal tract (Burnside, 1930). Conidiphores, by which the fungi multiply and spread, are formed wherever the mycelium breaks through the cuticle and is exposed to air. The degree of mycelial involvement in the adult bee does not seem sufficient to account for signs of disease which include sluggish behaviour and flightlessness: it may be that an ether-soluble toxin, which accounts for the deep green-yellow colour of mature conidia, is responsible for these early signs. Stonebrood is not a particularly important infection for two reasons: it is a rare condition in Britain and infections are usually not persistent, thus severe compromise of the brood from the condition is unusual.

Nosema apis

This microsporidian organism produces no obvious signs in infected adult bees except for a

markedly reduced longevity (Beutler & Opfinger, 1949; Bailey, 1955) and reduction in the formation of the hypopharyngeal glands (Wang & Moeller, 1969, 1970). Since it is these structures which produce the proteinaceous secretion fed to larvae and the queen bee, the result is that around 15% of eggs in severely infected colonies fail to develop to produce mature larvae. Infected overwintering bees have reduced amino-acid levels in their haemolymph (Wang & Moeller, 1970) and only about a fifth of the nitrogen stores in their fat bodies when compared with normal adults (Lotmar, 1939). Excessive gastrointestinal fluid loss, termed 'dysentery' by apiculturists, occurs since the overall water content of infected bees is higher than uninfected insects (Lotmar, 1951). *N. apis* should not, however, be seen as the cause of the dysentery. Many of the signs noted in *N. apis*-infected bees are probably associated more with the viruses associated with the protozoan, that is to say black queen cell virus, filamentous virus and bee virus Y. These three viruses, discussed further below, have been shown by Bailey (1976, 1981) to be detected only in bees infected with *N. apis* (Table I).

It may be that the microsporidian has some transmissional influence on these viruses but Bailey (1981) considered it more likely, as discussed further below, that *N. apis* reduces the resistance of adult bees to viruses which are gastrointestinal invaders.

N. apis is treated with the antibiotic fumagillin (Fumidil B, Sanofi) which suppresses infection at between 0.005 and 0.03 mg/mL without detectable side-effects (Katnelson & Jamieson, 1952).

Malpighamoeba mellificae

This protozoan, as with *N. apis*, causes few or no obvious signs in infected bees. Pathologically the malpighian tubules are seen to atrophy (Liu, 1985) but, again in common with *N. apis*, diagnosis rests

on microscopic detection of cysts. *M. mellificae* is not readily spread since it only produces around 500 000 cysts per infected bee in around 20 days, in comparison to *N. apis* which produces over fifty times as many in half the time. Spread of *M. mellificae* thus occurs slowly compared with *N. apis* and is generally associated with severe dysentery. This accounts for the low incidence of infection, reported at 2% of colonies in Britain (MAFF, 1959).

Viral disease

Viral diseases of adult bees have been researched almost exclusively by workers at Rothamsted Experimental Station. Viruses have been detected infecting a significant number of bee colonies in the UK and symptoms previously associated with other pathogens have been found to be linked to these viruses.

Chronic bee paralysis is caused by an RNA virus which is normally not particularly infectious; millions of viral particles are needed to cause paralysis in adult bees when given by mouth while fewer than 100 will cause disease when injected into the haemolymph. The most likely natural route of infection is through pores left by broken bristles which expose cytoplasm into which virus can be rubbed during bee interactions when crowded together. The virus can cause two syndromes. In the first, the abdomen becomes bloated with fluid, gastrointestinal fluid excretion increases giving so-called dysentery and the sick bee dies within a few days. The second syndrome renders worker bees black, almost iridescent and hairless (Rinderer *et al.*, 1974). Attacked by other bees in the colony, these are prevented from re-entering the hive by guard bees, giving them the epithet 'robber' (Drum & Rothenbuhler, 1983). A severely affected colony, especially with the former of these two syndromes, may collapse, with the queen bee left, in the height of summer, with only a few drones in otherwise deserted combs (Bailey, 1969). The propensity of bees to develop either the 'bloated abdomen' or the 'black robber' syndrome, appears to be mediated by genetic influences. While both syndromes may occur in a colony, normally one predominates.

Chronic bee paralysis appears to have been much more common in the earlier part of this century and its decline mirrors the reduction both in number of bee colonies in Britain and in incidence of infestation with the tracheal mite *Acarapis woodi*.

Significant in discussion of these correlations is the fact that the condition is seen commonly in the Black Forest area of Germany where population density of bee colonies is very high. There is no treatment for this viral disease but, as with many other bee diseases, there is evidence that some strains are more susceptible to infection and disease. There is some evidence that the virus may cause up to 30% of what is considered 'a normal level of mortality' in a hive (Bailey, 1976).

Acute bee paralysis virus, so-called because it was first discovered at the same time as chronic bee paralysis virus (Bailey *et al.*, 1963), is probably more of a laboratory phenomenon in experimentally infected bees than a significant cause of mortality or morbidity in bees in Britain. Nevertheless, it has been identified as a cause of disease in mainland Europe, quite possibly carried by *Varroa jacobsoni*. This would fit with the necessity for injection to produce infection, given that the *Varroa* mite could readily inject virus into haemolymph during feeding. In Britain it appears to be naturally suppressed. Although shown to be commonly present in the tracheal mite *A woodi* in the UK, it does not appear to cause significant disease here.

Slow bee paralysis virus (SPV) was dismissed in one sentence by Bailey in 1981 since 'nothing is known of its occurrence and no disease has been associated with it in nature' (Bailey, 1981; 1976). Nevertheless, more recently, it seems that this RNA virus, in association with *Varroa* infestation, is responsible for colony loss. Other viruses, such as cloudy wing virus and deformed wing virus, have increased in incidence and may account for some of the abnormal bees found in an advanced *Varroa* infestation.

Other viruses detected in adult bees include bee virus X, bee virus Y, cloudy wing particle, filamentous virus and black queen cell virus (Bailey *et al.*, 1981, 1983). As their names suggest, these infectious agents are not particularly associated with major disease symptoms, the exception being the last, black queen cell virus. This 30 nm RNA virus causes cells containing larvae destined to be queens to darken with death of the larvae or pupae they contain. It would appear that many of these viruses are associated with *Nosema apis* infection, as discussed above. The reason for this association is unclear. It may be that cytopathic effects of *N. apis* directly facilitate transenteric viral infection or it may be that *N. apis* infection reduces the enteric production of an antiviral protein such as that

Table II
Molecular size and nucleic acid type and molecular weight for bee viruses (from Bailey, 1991)

<i>Virus</i>	<i>Dimensions (nm)</i>	<i>Nucleic acid weight (Da) & type</i>
Chronic paralysis	20 × 45	1.35 × 10 ⁶ Da RNA
Chronic paralysis associate	17	0.35 × 10 ⁶ Da RNA
Cloudy Wing	17	0.35 × 10 ⁶ Da RNA
Acute Paralysis	30	n/a RNA
Arkansas	30	1.8 × 10 ⁶ Da RNA
Black Queen Cell	30	2.8 × 10 ⁶ Da RNA
Deformed Wing	30	n/a RNA
Egypt	30	n/a RNA
Kashmir	30	n/a RNA
Sacbrood	30	2.8 × 10 ⁶ Da RNA
Slow paralysis	30	n/a RNA
X	35	n/a RNA
Y	35	n/a RNA
Iridescent (in <i>A. cerana</i>)	150	n/a RNA
Filamentous	150 × 140	12.0 × 10 ⁶ Da DNA

produced by the gut of the silkworm *Bombyx mori* (Hyashita *et al.*, 1969).

Several other adult bee viruses, as detailed in Table II, have been isolated and characterized at Rothamsted, but are not found in the UK. Sacbrood, on the other hand, is widely distributed with up to 30% of healthy colonies containing a few affected larvae. Sacbrood has been described as one of the few bee viral diseases that produces clear cut symptoms. Larvae do not pupate but rather remain stretched out in the capped cell. Fluid accumulates between the body and the unshed skin and the colour changes to a dark brown (Fig. 10) after which it dies and dries out to a flattened, often keel shaped, scale. This might easily be confused with the scale of the much more important disease American foulbrood, but differs in that the scale can readily be removed from the cell while the darker flatter scale of American foulbrood is often adhered to the cell wall. The virus is carried from generation to generation of brood by adult bees in which it causes no disease. Workers probably transmit the virus to the brood in food. Given that dried larval remains quickly lose infectivity and that workers recognize and remove infected larvae from cells, this disease, although widespread, is not considered a serious disease. This might seem surprising given the huge numbers of viral particles in each infected larva: Bailey has estimated that each larva killed by sacbrood viral disease 'contains



Fig. 10. Sacbrood in which fluid accumulation in the pupal stage is pathognomonic.

around a milligram of virus, enough to infect every larva in more than a thousand colonies' (Bailey & Ball 1991). The relative lack of signs in the bee population indicates the efficacy of hygienic behaviour by workers in removing infected brood.

CONCLUSION

The importance of considering the diseased colony in what might be termed a holistic manner cannot be overestimated. The infectious agent should not be viewed in the context only of the individual

infected bee but of the colony as a whole, including uninfected workers which so often actively root out infected brood. Moreover, disease in one colony must be seen in the context of other colonies locally, nationally and internationally. As shown here, and particularly in the references cited below, veterinarians have had little input into the investigation and treatment of bee disease until recently. It is hoped that this reasonably comprehensive review will, to some extent, remedy that deficiency and encourage veterinary surgeons to have a greater interest in this important species, farmed but still in essence wild.

ACKNOWLEDGEMENTS

Mr Peter Watson is thanked for helpful advice and information as is one anonymous reviewer whose meticulous evaluation of the text ensured that it was as up-to-date as possible.

REFERENCES

- ALIPPI, A. M., & AGUILAR, O. M. (1998). Characterisation of isolates of *Paenibacillus larvae susp. larvae* from diverse geographical origin by the polymerase chain reaction and BOX primers. *Journal of Invertebrate Pathology* **72**, 21–7.
- BAILEY, L. (1955). The epidemiology and control of Nosema disease of the honeybee. *Annals of Applied Biology* **43**, 379–89.
- BAILEY, L. C. (1959). An improved method for the isolation of *Streptococcus pluton* and observations on its distribution and pathology. *Journal of Insect Pathology* **1**, 80–5.
- BAILEY, L. (1967). The effect of temperature on the pathogenicity of the fungus *Ascosphaera apis* for the larvae of the honey bee *Apis mellifera*. In Proceedings of the International Colloquium on Insect Pathology and Microbiological Control (van der Laan P. A. ed) pp. 162–7. North Holland; Amsterdam.
- BAILEY, L. C. (1969). The signs of adult bee diseases. *Bee World* **50**, 66–8.
- BAILEY, L. C. (1976). Viruses attacking the honey bee. *Advances in Virus Research* **20**, 271–304.
- BAILEY, L. (1981). *Honey Bee Pathology*. London: Academic Press.
- BAILEY, L. & BALL, B. V. (1991). *Honey Bee Pathology*, 2nd edn. London: Academic Press.
- BAILEY, L. & LEE, B. V. (1962). The effect of infestation with *Acarapis woodi* (Rennie) on the mortality of honey bees. *Journal of Insect Pathology* **1**, 15–24.
- BAILEY, L. C. & LEE, D. C. (1962). *Bacillus larvae*: its cultivation in vitro and its growth in vivo. *Journal of General Microbiology* **29**, 711–17.
- BAILEY, L. C., GIBBS, A. J. & WOODS, R. D. (1963). Two viruses from adult honey bees (*Apis mellifera* Linnaeus). *Virology* **21**, 390–5.
- BAILEY, L., BALL, B. V. & PERRY, J. N. (1981). The prevalence of viruses in honey bees in Britain. *Annals of Applied Biology* **97**, 109–18.
- BAILEY, L., BALL, B. V. & PERRY, J. N. (1983). Association of viruses with two protozoal pathogens in the honey bee. *Annals of Applied Biology* **103**, 13–20.
- BEUTLER, R. & ÖPFINGER, E. (1949). Pollernährung und Nosemabefall der Honigbee. *Zeitschrift für vergleichende Physiologie* **32**, 383–421.
- BURNSIDE, C.E. (1930). Fungus diseases of the honey bee. Technical Bulletin U.S. Department of Agriculture No. 149.
- CAMAZINE, S. (1988). Factors affecting the severity of *Varroa jacobsoni* infestations on European and Africanized honey bees. In *Africanized Honey bees and Bee Mites*, C. G. Needham, ed. pp. 444–451. Chichester: Ellis Horwood.
- CHESHIRE, F. R. & CHEYNE, W. W. (1885). The pathogenic history and history under cultivation of a new bacillus (*B. alvei*, the cause of a disease of the hive bee hitherto known as foulbrood. *Journal of the Royal Microscopical Society* **5**, 581–601.
- DANCER, B. (1995). Update on European foulbrood. *Veterinary Invertebrate Society Newsletter* **6**, 5–6.
- DRUM, N. H. & ROTHENBUHLER, W. C. (1983). Non-stinging aggressive responses of worker honey bees to hive mates, intruder bees and bees affected with chronic bee paralysis. *Journal of Apicultural Research* **22**, 256–60.
- DZIERZON, J. (1882). *Rational bee-keeping*. Houslston and Sons. London:
- GOCHNAUER, T. A. & HUGHES, S. J. (1976). Detection of *Ascosphaera apis* in honeybee larvae (Hymenoptera: Apidae) from eastern Canada. *Canadian Entomologist* **108**, 985–8.
- GOVAN, V. A., BROZEL, V., ALLSOPP, M. H., DAVISON, S. (1998). A PCR detection method for rapid identification of *Mellisococcus pluton* in honeybee larvae. *Applied and Environmental Microbiology* **64**, 1983–5.
- HASEMAN, L. (1961). How long can spores of American foulbrood live? *American Bee Journal* **101**, 298–299.
- HEATH, L. A. F. (1982a). Development of chalk brood in a honey bee colony; a review. *Bee World* **66**, 9–15.
- HEATH, L. A. F. (1982b). Chalk Brood pathogens: a review. *Bee World* **63**, 130–5.
- HEYNDRIKX, M. (1996). Reclassification of *Paenibacillus* (formerly *Bacillus*) *pulvifaciens* (Nakamura 1984) Ash et al 1994, a later subjective synonym of *Paenibacillus* (formerly *Bacillus*) *larvae* (White 1906) Ash et al 1994, as a subspecies of *P. larvae*, with embedded descriptions of *P. larvae* as *P. larvae subsp. larvae* and *P. larvae subsp. pulvifaciens*. *Int J Sys Bacteriol* **46**, 270–9.
- HYASHITA, K., NISHIDA, J. & MATSUBARA, F. (1969). The production of antiviral substance, a red fluorescent protein, in the digestive juice of the silkworm larva (Lepidoptera, Bombycidae). *Applied Entomology and Zoology* **4**, 154–5.
- KATNELSON, H. & JAMIESON, C. A. (1952). Control of Nosema disease of honey bees with fumagillin. *Science* **115**, 70–1.
- LANGSTROTH, L. L. (1866). *A Practical Treatise on the Hive and the Honey Bee*. Philadelphia: J. B. Lippincott.

- LEIGHTON, T. (1983). Effectiveness and optimal selection of antibiotics for the control of American foulbrood disease. Progress report to Californian Department of Food and Agriculture, December 31.
- LIU, T. P. (1985). Scanning electron microscope observations on the pathological changes of Malpighian tubules in the worker honey bee *Apis mellifera*, infected by *Malpighamoeba mellificae*. *Journal of Invertebrate Pathology* **46**, 125–32.
- LLOYD, J. M. (1986). Simplified laboratory diagnosis of American Foulbrood disease. *Journal of Apicultural Research* **25**, 55–7.
- LONDZIN, W. & SLEDZINSKI, B. (1996). Resistance of the honey bee parasitic mite *Varroa jacobsoni* to varroacides containing tau-fluvalinate. *Medycyna Weterynaryjna* **52**, 526–8.
- LOTMAR, R. (1939). Der Eiweiss-stoffwechsel in Bienenvolke während der Ueberwinterung. *Landwirtschaftliches Jahrbuch der Schweiz* **53**, 34–70.
- LOTMAR, R. (1951). Gewichtbestimmungen bei gesunden und Nosema-kranken Beinen. *Zeitschrift für vergleichende Physiologie* **13**, 195–206.
- MAFF (1996). Foul brood disease of honey bees: recognition and control. Ministry of Agriculture Fisheries and Food and Central Science Laboratory, York.
- MAFF (1998) *Varroa jacobsonii*: monitoring and forecasting mite populations within honey bee colonies in Britain. Central Science Laboratory, York.
- MAURIZIO, A. (1934). Über die Kalkbrut (Pericystis-Mykose) der Bienen. *Archive für Bienenkunde* **15**, 165–93.
- MORGENTHALER (1944). Das jahrezeitliche Auftreten der Beinseuchen. *Beihefte zur Schweizerische Bienen-Zeitung* **1**, 285–336.
- MUTINELLI, F., CREMASCO, S. & IRSARA, A. (1994). Formic acid in the control of varroaosis: a practical approach. *Zentralblatt für Veterinärmedizin [B]* **41**, 433–40.
- PENG, C. Y-S., MUSSEN, E., FONG, A., MONAGUE, M. A. & TYLER, T. (1992). Effects of chlortetracycline on honey bee worker larvae reared in vitro. *Journal of Invertebrate Pathology* **60**, 127–33.
- PENG, C. Y-S., MUSSEN E., FONG A., CHENG P., WONG G & MONAGUE, M. A. (1996). Laboratory and field studies of the effects of the antibiotic tylosin on honey bee *Apis mellifera* L. (Hymenoptera: apidae) development and prevention of American foulbrood disease. *Journal of Invertebrate Pathology* **67**, 65–71.
- RENDALL, G. (1996). The World of the Bee. *Veterinary Invertebrate Society Newsletter* **10**, 5–6.
- RINDERER, T. F. & GREEN, T. J. (1976). Serological relationship between chronic bee paralysis and the virus causing hairless black syndrome in the honeybee. *Journal of Invertebrate Pathology* **27**, 403–5.
- RINDERER, T. F., ROTHENBUHLER, W. C. & KULINCEVIC, J. M. (1975). Responses of three genetically different stocks of honey bee to a virus from bees with hairless black syndrome. *Journal of Invertebrate Pathology* **25**, 297–300.
- ROTHENBUHLER, W. C. (1964). Behaviour genetics of nest cleaning in honey bees. I. Responses of four inbred lines to disease-killed brood. *Animal Behaviour* **12**, 578–83.
- SOKOL, R. (1996). Effects of long-term persistence of Fluwarol (fluvalinate) on honey bee colonies. *Medycyna Weterynaryjna* **52**, 718–20.
- SPIVAK, M. (1996) Honey bee hygienic behaviour and defense against *Varroa jacobsoni*. *Apidologie* **27**, 245–60.
- THOMPSON, V. C. & ROTHENBUHLER, R. C. (1957). Resistance to American foulbrood in honey bees. II Differential protection of larvae by adults of different genetic lines. *Journal of Economic Entomology* **50**, 731–7.
- WANG, Der-I. & MOELLER, F. E. (1969). Histological comparisons of the development of hypopharyngeal glands in healthy and *Nosema*-infected worker honey bees. *Journal of Invertebrate Pathology* **14**, 135–42.
- WANG, Der-I. & MOELLER, F. E. (1970). Comparison of the free amino acid composition in the haemolymph of healthy and *Nosema*-infected female honey bees. *Journal of Invertebrate Pathology* **15**, 202–6.
- WHITE, G. F. (1920). European Foulbrood. U. S. Department of Agriculture Bulletin No 810.
- WOODROW, A. W. (1941). Behaviour of honey bees towards brood infected with American foulbrood. *American Bee Journal* **81**, 363.

(Accepted for publication 1 December 1999)