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Secondary infections and diseases associated with *Varroa jacobsoni*

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SUMMARY - The parasitic mite *Varroa jacobsoni* may directly and indirectly affect the type and prevalence of honey bee pathogens causing mortality in infested colonies. An increase in the incidence of chalkbrood (*Ascosphaera apis*) is probably the result of inadequate brood care due to a depletion of the adult bee population for other reasons. Laboratory studies have shown that the mite is capable of causing septicaemic infection by introducing bacteria contaminating the body surface of honey bee pupae into the haemolymph and it is capable of transmitting infection to healthy pupae during feeding. However, in nature the primary cause of adult bee and brood mortality in severely infested colonies is honey bee virus infections which the mite may first activate to multiply and then transmit. A better understanding of host-parasite-pathogen interactions should provide options for the development of improved control strategies by the better targeting and timing of treatments.

Key words: *Varroa jacobsoni*, honey bee, pathogens, fungi, bacteria, viruses, disease transmission.

RESUME - *"Infections et maladies secondaires associées à* Varroa jacobsoni*". Le parasite acarien,* Varroa jacobsoni, *peut directement et indirectement affecter le type et la prédominance des pathogènes des abeilles mellifères, provoquant la mortalité dans les colonies infestées. Une augmentation dans l'incidence du "chalkbrood" (Ascosphaera apis) est probablement le résultat de soins inappropriés données au couvain à cause de la réduction de la population des abeilles adultes pour d'autres raisons. Des études menées au laboratoire ont montré que l'acarien est capable de causer des infections septicémiques en introduisant une bactérie qui contamine la surface corporelle de la pupe des abeilles à l'intérieur de l'hémolymphe. Cet acarien est aussi capable de transmettre l'infection aux pupes saines lors de l'alimentation. Cependant, dans la nature la cause primaire de la mortalité du couvain et de l'abeille adulte dans plusieurs colonies infestées est constituée par des infections virales de l'abielle. Ces infections sont initialement activées par l'acarien pour ensuite être transmises. Une meilleure compréhension des interactions hôte-parasite-pathogène pourra donner des options pour le développement ou l'amélioration de stratégies de contrôle par un meilleur ciblage et la programmation des traitements.*

Mots-clés *:* Varroa jacobsoni, *abeille, pathogènes, champignons, bactéries, virus, transmission de la maladie.*

Introduction

Over the past twenty years the loss of many thousands of colonies of *Apis mellifera* throughout the Mediterranean region has been attributed to infestation with *Varroa jacobsoni*, and this parasitic mite remains a major cause of concern to beekeepers and to those who rely on honey bees for the pollination of important agricultural crops. The problem is not restricted to the countries of the Mediterranean basin but the damaging effects of infestation may be more prevalent and severe in areas where mite

reproduction can take place throughout most of the year and where colony densities are high. However, these factors alone cannot account for the difference in the impact of the mite in this region when compared with other regions such as south America. Here the same potential for mite reproduction exists but significant differences have been reported in the proportion of non-reproducing female mites in worker brood (Ruttner *et al.*, 1984; Camazine, 1986) and in the development periods of worker pupae (Moritz and Hänel, 1984; Moritz and Mautz, 1990), which limit the number of mite offspring that are produced, or that mature, before the bee emerges. These differences may account to some extent for the variability in the damaging effects of infestation in different areas of the world. However, geographic or climatic factors may also influence mite population growth and the relative importance of genetic or environmental effects remains to be determined.

The discovery of the mite in central Europe in the 1970's prompted a period of intensive scientific investigation. However, the largest proportion of published work in the last 20 years reports the efficacy of a range of chemicals for mite control. This may have been justified as an initial response to the establishment of an exotic and damaging pest but this approach must be viewed as a short term solution. Moreover, the recent development in Italy of populations of mites with resistance to acaricide treatments now makes the search for alternative control strategies more urgent.

Reducing the numbers of mites in colonies by the use of chemicals, by manipulative methods or by exploiting biological traits in the honey bee, provide options for limiting the damaging effects of infestation. However, it is vital to gain a more detailed knowledge of why and how losses result from mite infestation and of the factors determining the outcome in individual colonies if control measures are to be accurately targeted and timed and if management and selection programmes are to be established. The aims of this brief review are to consider the results of observations on the causes of mortality in infested colonies and to examine the changes that have apparently occurred in the prevalence and incidence of honey bee pathogens in association with mite infestation. These observations must be considered in the context of our present knowledge of the natural history and epidemiology of infections and the biology of the mite, as it is apparent that *V. jacobsoni* may play both an indirect and direct role in the establishment of disease outbreaks and colony mortality.

Fungi

There are reports from several countries of an increase in the incidence of chalkbrood (*Ascosphaera apis*) infection in honey bee colonies infested with *V. jacobsoni* (Jenko *et al.*, 1991; Vey, 1991). However the role of the mite in the initiation and spread of infection has not been established and experimental evidence of an association between *V. jacobsoni* and *A. apis* is lacking. The body surface of mites can become contaminated with fungal spores (Liu and Ritter, 1988; Puerta *et al.*, 1990), but for *A. apis* to cause infection many spores would be required to contaminate the larval food and be ingested by the developing honey bee brood. The optimal conditions required for spore germination are found within the larval gut and it is unlikely that infection, other than by ingestion, would be successful. This would preclude effective direct transmission of *A. apis* by the mite.

It has been suggested that the use of chemical acaricides for the control of *V. jacobsoni* reduces resistance to infection by the fungal pathogen (Sulimanovic *et al.,* 1991) but in Britain in 1995 there was no significant difference in the incidence of chalkbrood between treated and untreated colonies (Ball, unpublished observation). Disease outbreaks may simply be the result of inadequate brood care due to the depletion of the adult bee population for other reasons, as brood is most susceptible to chalkbrood infection when chilled (Bailey, 1967). Until more detailed observations and experimental investigations are undertaken the evidence for a link between *V. jacobsoni* and *A. apis* remains circumstantial.

Bacteria

The rapid decline in the adult bee population in severely infested colonies is often accompanied by outbreaks of brood disease, the symptoms resembling those of European foulbrood when dead, unsealed larvae are present or American foulbrood when brood in sealed cells fails to develop and emerge. However, attempts to isolate the pathogenic bacteria, *Melissococcus pluton* and *Paenibacillus larvae* respectively, from diseased material has so far proved unsuccessful. It is unlikely that *V. jacobsoni* could act as an efficient vector of these bacteria even when they naturally occur in the same colony for the same reasons that limit the transmission of *A. apis.* The vegetative cells of *M. pluton* and the spores of *P. larvae* have similar requirements for the near anaerobic conditions and optimal carbon dioxide concentration within the larval gut for growth and germination. Contamination of the mite's body surface with viable bacterial cells is possible, particularly in colonies with overt foulbrood infections (Alippi *et al.*, 1995), but the oral route of infection of bee larvae and the fact that mites alternate between brood and adult bees suggests that *V. jacobsoni* would not play a primary role in transmission. This has recently been confirmed by field experiments designed to test the ability of the mite to transmit American foulbrood from infected to healthy colonies (Alippi *et al.,*1995). Although most of the mites from infected colonies carried *P. larvae* spores on their body surface they were unable to initiate infection in the colonies to which they were transferred.

A number of other bacteria have been isolated from diseased larvae taken from colonies infested with *V. jacobsoni* (Shabanov,1984; Panizzi and Pinzauti, 1988) and *Pseudomonas apiseptica, P. aeruginosa* and *Serratia marcescens* have also been implicated in septicaemic infections of both adult bees and brood (Landerkin and Katznelson, 1959; Liakos and Papadopoulou, 1984; El-Sanousi *et al.*, 1987). However, these bacteria are not specifically associated with honey bees, being common in water and soil. They are probably opportunistic saprophytes that depend upon a variety of primary bee pathogens to establish infections or on parasites such as *V. jacobsoni* to overcome cuticular barriers to gain access to vulnerable tissues.

The ability of *V. jacobsoni* to initiate bacterial infection and transmit *Hafnia alvei* (Strick and Madel, 1988) and *S. marcescens* (Glinski and Jarosz, 1990) to honey bee brood has been demonstrated in the laboratory. Adult female *V. jacobsoni* confined on pupae whose body surface had been contaminated with *H. alvei* induced almost 100% infection and the mites were also able to transmit the bacteria to healthy, uncontaminated test pupae. The efficiency of transmission increased from about 17% when one mite was present to 95% when seven mites were transferred to a healthy

test pupa. This may be analogous to events in nature, as colonies with the largest number of mites show the highest levels of adult bee and brood mortality. However, in these experiments bacterial suspensions containing many millions of cells per ml. were used to contaminate the pupae and half of the control pupae incubated without mites also became infected. In nature the body surface of adult bees, larvae and pupae would rarely become so contaminated. Adult female mites allowed to feed on honey bee brood sprayed or injected with a suspension of *S. marcescens* transmitted the infection to about 20% of healthy test pupae. Mites were found to harbour the bacteria not only on their body surface but also in their salivary glands, intestine and haemolymph. The introduction of bacteria into the haemolymph of honey bees and their transmission from severely infected to healthy individuals by the mite has certainly been demonstrated in the laboratory, but under normal circumstances the possibility of this occurring in naturally infested colonies appears to remain slight.

Further evidence for this is provided by investigations of the bacterial content of haemolymph from adult bees and brood artificially infested with mites which had been collected from severely infested colonies. Although the mortality of caged newly emerged bees infested with one to three mites increased with increasing levels of parasitization, the number of bacteria in their haemolymph did not differ significantly from uninfested controls except at the highest infestation rate (Koch and Ritter, 1988). In similar experiments on brood artificially infested with one to four mites the mortality of pupae increased almost linearly with the degree of infestation. However, again there was no difference between the bacterial content of haemolymph from infested and uninfested brood; no bacteria were isolated from almost 90% of the haemolymph samples examined (Koch and Ritter, 1989). The dead pupae from this study were then tested individually for honey bee virus infection. Large amounts of acute paralysis virus (APV) were detected in over 80% of the pupae infested with one mite and this rose to over 90% when four mites were introduced into the brood cell. The primary cause of mortality of the experimentally infested brood was due to APV infection which was transmitted to the pupae by mites collected from naturally infested colonies. This confirmed earlier observations of an association between *V. jacobsoni* and virus diseases of bees (Ball, 1983).

Viruses

Few detailed studies on the incidence and prevalence of honey bee viruses in colonies infested with *V. jacobsoni* have been undertaken so that much of the information obtained in recent years is from isolated observations in areas where severe colony mortality prompted investigation. This is probably a reflection of the present limited capability in the serological identification of the virus diseases of bees rather than a lack of interest in or awareness of these pathogens. Nevertheless, these observations have yielded some interesting and unexpected results which show remarkable similarity despite the different countries of origin of diseased material and different beekeeping practices.

An investigation of the causes of mortality in infested honey bee colonies in Germany revealed a marked increase in the incidence of both chronic paralysis virus (CPV) and acute paralysis virus (APV) in comparison with uninfested colonies in Britain (Ball and Allen, 1988). In severely infested colonies APV was the primary cause

of the adult bee mortality, being detected in large amounts in over 80% of the samples analysed. This contrasts with findings in Britain where, prior to the detection of *V. jacobsoni*, APV had never been found to be responsible for mortality in nature.

APV and CPV, like many of the viruses of bees, persist as inapparent, sub-lethal infections but whereas CPV can cause sporadic disease outbreaks in the absence of the mite, APV seems to multiply to lethal levels only in association with *V. jacobsoni.* Significantly, APV and CPV are infectious for both adults and pupae by injection into the haemolymph; they might therefore be expected to be readily transmitted by the feeding activities of mites. APV and CPV have also been detected in large amounts in dead adult bees and brood from infested colonies in France (Faucon *et al.*, 1992), and APV has been found as a cause of mortality in infested colonies in Russia (Batuev, 1979), Yugoslavia (Kulincevic *et al.*, 1990), Italy (Carpana *et al.*, 1991) and other countries around the Mediterranean basin (Allen and Ball, 1996). No comprehensive information on honey bee virus incidence prior to the establishment of *V. jacobsoni* in these countries, exists. However, work at Rothamsted since the recognition of APV (Bailey *et al.*, 1963) had never previously detected the virus as a cause of mortality in uninfested colonies anywhere in the world. The fact that overt infection with APV seems to have arisen independently in many countries in association with the mite, strongly suggests that *V. jacobsoni* affects the type and prevalence of honey bee viruses causing mortality.

The study by Ball and Allen (1988) also revealed that a large proportion of unsealed larvae in severely infested colonies were killed by APV. As mites do not enter brood cells until the last larval instar is about to be sealed, it is unlikely that these larvae were infected by mites feeding on them. This suggests that the virus was transmitted to larvae in the food secreted by infected adult bees. APV has previously been shown to spread as an unapparent infection in this way, via the salivary gland secretions of adult bees. Live bees collected from these colonies and assayed individually for APV by ELISA were found to contain large amounts of APV (Ball, 1988); more than bees killed by injection of the virus in the laboratory.

If larvae are fed insufficient virus to cause infection at an early stage of development they survive to the stage when they are sealed in their cells and they continue to develop normally. Less than 1% of pupae from sealed cells in which no mites were present gave positive reactions to APV antiserum in immunodiffusion tests. However, APV was detected serologically in almost half of the dead brood from sealed cells infested with *V. jacobsoni.* This suggests that the virus was introduced into these developing pupae by the feeding activities of mites. In some colonies almost 60% of the mite population entering brood cells to reproduce were apparently acting as virus vectors. The mortality of brood and the release of virus-carrying mites into the adult bee population to spread infection can lead to the rapid decline and death of the colony.

Direct evidence for the transmission of virus by the mite has been provided by laboratory experimentation. Batuev (1979) was the first to demonstrate that *V. jacobsoni* could transfer APV from laboratory injected adult bees to healthy individuals. The mite has also been shown to transmit APV from experimentally infected honey bee pupae to healthy individuals with an efficiency of 50% to 80% (Wiegers, 1988; Ball, 1989). Evidence for the transmission of virus by *V. jacobsoni* in nature is provided

by studies of infested colonies in Germany which were showing symptoms of brood disease or contained many deformed newly emerged bees. Mites were removed from sealed brood cells and incubated in groups of five on 54 healthy test pupae or individually on 70 test pupae (Ball, 1989). Virus was detected serologically in 87% of the extracts of pupae incubated with groups of five mites and four different virus infections were identified. Fewer virus-infected pupae resulted when single mites were incubated on test pupae and two different virus infections were detected. A very high percentage of test pupae incubated with groups of five mites gave positive reactions to the honey bee virus antisera; this may have two explanations. Firstly, increasing the number of mites per pupa increases the likelihood of including a mite carrying virus. Secondly, the feeding activities of several mites would increase the likelihood of an infective dose of virus being introduced into the haemolymph. Nevertheless, 48% of mites incubated individually on test pupae were capable of transmitting virus on one occasion which means that approximately half of the mite population in this colony were acting as virus vectors.

Symptoms of wing deformity in newly emerged bees have been observed in infested colonies in many countries and in each case the damage has been attributed solely to the feeding activities of mites. However, a novel virus has recently been isolated from samples of deformed bees from infested colonies in Japan and the production of an antiserum to the virus has permitted its detection in other samples of bees from infested colonies in France (Chastel *et al.*, 1990), Yugoslavia (Kulincevic *et al.*, 1990), Rumania (Marin *et al.*, 1990) and Iran (Mossadegh, 1990). The virus was provisionally called Japanese strain of Egypt bee virus (JEBV) as there was a distant serological relationship between the two particles. However, more recent work has revealed that strains of the virus are present in bees in many countries and all virus isolates are much more closely related serologically to each other than to EBV. The virus has therefore been renamed deformed wing virus (DWV).

DWV multiplies slowly and pupae infected at the white-eyed stage of development with appropriate dilutions of inocula develop to emergence, but have poorly developed wings. The virus can cause mortality in nature in the absence of the mite but it has probably increased in prevalence due to transmission by *V. jacobsoni* and infection has become more apparent in pupae because of the morphological changes induced during metamorphosis; adult bees infected with DWV after emergence exhibit no outward signs of disease.

A similar increase in the incidence of cloudy wing virus has been observed in infested colonies in England and the detection for the first time of slow paralysis virus (SPV) as a cause of adult bee and brood mortality in infested colonies is further evidence that the mite is directly affecting the type and prevalence of honey bee virus infections.

Discussion

There now seems to be overwhelming evidence that the mortality of colonies of *A. mellifera* infested with *V. jacobsoni* is closely linked to honey bee virus infections which the mite may first activate to multiply and then transmit. The variability in the effect of mite infestation in different areas of the world may therefore partly depend upon the

natural prevalence of these infections. *V. jacobsoni* may directly influence the establishment of infection by the mechanical introduction of microorganisms contaminating its body surface or that of its honey bee host, during feeding. However, the activation of normally unapparent infections, such as APV and SPV, to multiply to lethal levels suggests that other, more subtle mechanisms, are operating. Studies on bee immunology, in particular the host response to parasitism, and an understanding of the early events in virus activation may prove profitable by identifying any differences in host susceptibility. Such knowledge could contribute to breeding or selection programmes aimed at increasing the tolerance of honey bees to mites.

The mite may also indirectly affect pathogen incidence; the loss of adult bees from the colony due to mite vectored pathogens could result in inadequate brood care and lead to an increase in the incidence of honey bee brood diseases. It is therefore important to have a more detailed knowledge of the epidemiology of honey bee pathogens in infested colonies and of the factors affecting their spread and persistence in both honey bee and mite populations. If disease outbreaks are correlated with the degree of infestation it may be possible to establish a threshold level for recommending some form of intervention to reduce the mite population. Such knowledge could contribute to the more accurate targeting and timing of acaricide treatments which would minimize chemical inputs and prolong their useful life.

There are still immense gaps in our understanding of the nature of the association between *V. jacobsoni* and *A. mellifera* and there are numerous factors that will affect the outcome of infestation in individual colonies. However, the knowledge gained by more detailed investigations will contribute greatly to our understanding of the problems, which is essential for devising effective control strategies for the future.

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