

Original article

Mortality of *Varroa jacobsoni* Oudemans during or soon after the emergence of worker and drone honeybees *Apis mellifera* L

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Summary — In a naturally infested colony a strong correlation between levels of falling mites and the emergence of honeybee brood was found. When comparing the number of mites falling between emerging worker and drone brood with known infestation levels, the mite fall was 2–3 times higher from worker than from drone cells. It was estimated that around half of the falling mites originate from mites that died within the sealed cell with the other half dying shortly after bee emergence. About 50% of the fallen mites were still alive and found to be able to reproduce when artificially introduced into sealed brood cells. The implications of mite mortality associated with brood emergence on the mite population dynamics and of using numbers of falling mites as a monitoring tool are discussed.

Apis mellifera / *Varroa jacobsoni* / mite drop / mortality

INTRODUCTION

Varroa jacobsoni Oudemans is a parasitic mite that lives exclusively on honeybees, feeding on the host haemolymph and reproducing within sealed brood cells. Due to the mite being a serious pest of *Apis mellifera* L, beekeepers have attempted to monitor mite

levels within their hives by a variety of techniques, of which a census of the number of mites falling to the hive floor each day is one of the more widely used.

Although the majority of the natural mite fall occurs within 2 days of brood emergence (Liebig, 1994; Boot et al, 1995) it

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remains unclear whether these mites arose from those dying within the sealed cells (Martin, 1994, 1995) or shortly after bee emergence, when the mites often transfer between hosts (Kovac and Crailsheim, 1987; Le Conte and Arnold, 1987).

This study investigates the association between worker and drone brood emergence and natural mite fall. Also the origin and viability of these fallen mites were studied.

MATERIAL AND METHODS

All colonies used in this study were either naturally infested with *Varroa* mites or considered to be uninfested, since they had been treated with an acaricide (Bayvarol[®]) 2 months previously. Inspection of 100 randomly chosen sealed brood cells from each uninfested colony prior to the study revealed no mites, confirming that the levels of infestation in these colonies were very low. The fallen mites were collected on a white vinyl sheet placed on the hive floor. A wire gauze prevented the bees from removing the mites from the sheet.

Fallen mites were classed as live or dead, depending on whether they responded to tactile stimuli or not.

All the observations were carried out using honey bee brood of a known age. This was achieved by recording on transparent sheets, temporarily placed over the frames, the time and position when cells were sealed.

After the final moult the cuticle of the adult female mite is initially almost white with the edge of the dorsal shield and legs a light brown colour. The subsequent process of tanning hardens and darkens the cuticle to a dark red/brown coloration. Although there are no firm data on the duration of this tanning process, our general observations suggest a period of 2–3 days. The degree of cuticle tanning may therefore be used as a relative indicator of how recently a mite has matured. For reference, a slide was prepared containing four mites with the first (I) and fourth (IV) mite having the least (palest) and most (darkest) intense brown coloration, the second and third mites having intermediate levels of intensity. This was used to classify the mites and any that fell between the four classes were rejected.

Association of mite drop with brood emergence

Naturally infested cells

A queen bee was caged on an empty drone comb and released 24 h later when many of the cells contained eggs. Three days later the same procedure was repeated using two queens and two worker combs. This ensured that all the brood would emerge at approximately the same time. The time of cell sealing was also recorded to help predict the time of brood emergence. The three frames were then placed into the middle of a hive which contained four broodless (empty) frames and approximately 15 000 heavily infested bees. A one frame gap separated each frame type and the area under each frame type was separated using barriers. A thin layer of petroleum jelly smeared on the vinyl sheet kept live mites from wandering into other sections. The number of emerged cells, recorded on acetate sheets, and the natural mite fall were monitored every 6 h over a 3-day period, starting approximately 24 h prior to the predicted emergence of the first bees.

Artificially infested cells

Phoretic mites were removed from adult bees using a fine paintbrush and on the same day were artificially introduced into uninfested, freshly capped (within 5 h) cells. Mites were inserted into the cell by making a small hole in the cap with a hot needle and after the mite had been inserted with a fine brush, resealed with the hot needle (de Ruijter, 1987). On the day on which bees were predicted to emerge, the frame was cleared of adult bees and any rejected cells noted before placing it in an observation hive containing no bees, where the ensuing emergence and associated mite drop were observed.

Viability of fallen live mites

Artificial introduction into cells

Twenty live fallen mites of each colour class and 20 phoretic mites (control) were artificially introduced into uninfested, recently capped (within 4 h) worker cells. The location of each brood cell and class of mite introduced were marked on a removable transparent overlay. Ten days

later the infested cells were opened and their contents noted.

Histological examination

Three fallen mites of each colour class were dissected and then stained with Ehrlich's Haematoxylin and Eosin and their reproductive organs examined for the presence or absence of spermatozoa and ova.

RESULTS

Association of mite drop with brood emergence

Naturally infested cells

A total of 1819 workers and 1269 drones emerged during the study. Worker emergence occurred 260–278 h and drone 342–366 h after cell sealing. Figure 1 shows the pattern of falling mites with the emergence of adult bees, with the mite drop level increasing synchronously with emergence of bees to 42.5 and 22.3 mites per 6 h for worker and drone brood, respectively, and returning to the background drop of 7.1 mites per 6 h for worker and drone brood as soon as all the bees had emerged. The

drop of 7.4 mites per 6 h from broodless (empty) combs varied little throughout the study (fig 1) indicating that the mites on the floor derived from the frame directly above them. The proportion of fallen mites that were still alive increased 12% during worker emergence and decreased 10% during drone emergence (fig 1).

Artificially infested cells

Of the 96 worker and 82 drone cells into which mites were artificially introduced, 10 worker and 12 drone cells were rejected by the bees. Both the total number of mites and estimated proportion of the mite population falling were greater from worker than drone cells (table I). Also the mites falling from worker cells were from all colour categories while those from drone cells were predominantly darker (table I).

Viability of fallen live mites

Artificial introduction into cells

Of the 100 mites introduced the bees rejected 31 cells. All colour categories of mites were capable of producing at least one viable offspring when artificially introduced

Table I. Number and colour category of mites falling from emerging brood cells with known levels of infestation in an observation hive without bees. The estimated mite population during one reproductive cycle is calculated by multiplying the number of infested cells by the predicted number of adult female mites emerging, eg. 86 cells \times (1 mother + 1.1 offspring).

Brood type	Number of infested cells	Colour category				Total number fallen mites	Estimated mite population in the cells	Percentage of estimated mite population in the cells to fall (a/b) \times 100
		I	II	III	IV			
Worker	86	26	11	20	12	69	86 \times 2.1	38
Drone	70	0	5	14	9	28	70 \times 3.3	12

into worker cells (table II). Although the palest mites (I) were least successful. There was no significant difference ($\chi^2 = 10$, $P < 0.01$) in ability to reproduce between the darker categories (II–IV) of mites and the control (phoretic) mites.

Histological examination

Spermatozoa and ovaries were found in mites of most colour classifications (table III). Difficulties in the dissection process meant that the negative results were

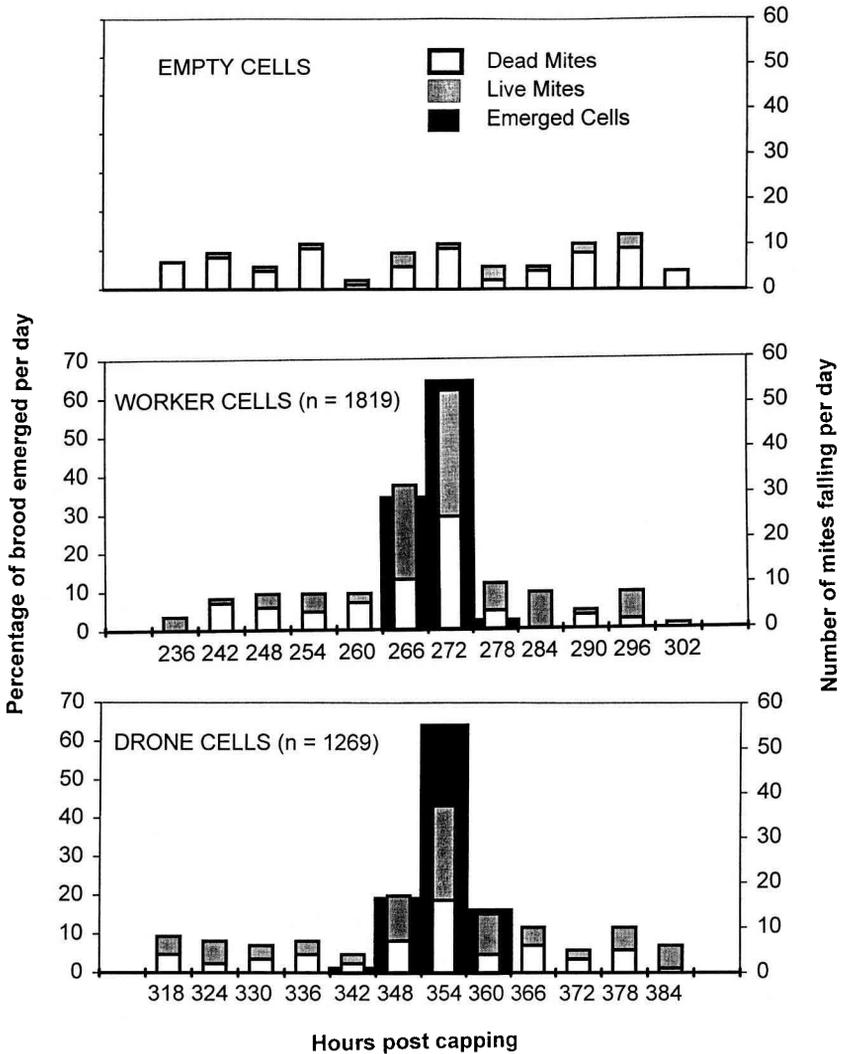


Fig 1. The relationship between brood emergence and natural mite drop during each 6-h period.

Table II. Ability of fallen mites of different colour categories to reproduce.

<i>Mite category</i>	<i>Number of infested accepted cells</i>	<i>Number cells with mite offspring</i>	<i>Percentage of introduced mites reproducing</i>
I	13	3	23
II	15	6	40
III	18	8	44
IV	12	7	58
Control (IV)	11	5	45

Table III. The presence or absence of spermatozoa and ova in fallen mites of different colour categories.

<i>Mite category</i>	<i>Number of mites dissected</i>	<i>Number of mites where spermatozoa found</i>	<i>Number of mites where ova found</i>
I	3	3	3
II	3	1	3
III	3	3	0
IV	3	3	3

unreliable since although ova (eg, mite category III) or spermatozoa may have been present they could have been damaged during the dissection.

DISCUSSION

This study confirms previous field observations (Liebig, 1994; Boot et al, 1995; Martin, unpublished data) that suggested a strong correlation between levels of falling mites and the emergence of honeybee brood. The level of mite drop from naturally infested worker cells was nearly twice as high as that from the drone cells, despite drone cells being more attractive to the mite (Fuchs, 1990). This finding was confirmed using artificially infested cells where again we found that there were twice as many mites falling from emerging worker than drone

cells, which rises to three times if we assume that the introduced mites reproduced normally (table I). Also the majority of the mites falling from worker cells were lighter (younger mites), while predominately the darker (older) mites fell from drone cells (table I). These differences in the number and colour of mites falling can be explained by the shorter developmental time of the worker brood than that of the drone brood. The emergence of the worker bee means that the second and third female offspring become adults only 1 day or several hours, respectively (Martin, 1994), before being released into the colony. However, in drone cells all the female offspring have at least 2 days after they mature before being released into the colony and so have sufficient time to complete tanning.

The mites that fall shortly after bee emergence may be those that fail to successfully

attach to the bee or transfer between hosts since mites are known to switch hosts rapidly after bee emergence (Kovac and Crailsheim, 1987; Le Conte and Arnold, 1987). These recently matured females may be more easily detected by the host and removed. This could be due to the mites not having had enough time to acquire or synthesise cuticular hydrocarbons which may help integrate the mites into the bee colony (Nation et al, 1992). This may also explain why proportionally more live mites were found to fall from emerging worker than drone cells. This relationship between development time of the bee brood and mite mortality had previously been suggested by Baggio (1994) and shown by Calis et al (1996).

Mites on the hive floor originate from a variety of sources. There is a natural drop of phoretic mites from the adult bees. This drop is almost entirely composed of dead, darker mites although rates will vary according to infestation level and possibly time of year. A possible reason why proportionally more dead phoretic mites were found under the empty (79%) than the brood (58%) frames (fig 1) may be due to the micro-climate on the floor, which may lead to the more rapid death of the mites that are away from the centre of the floor.

When the bees emerge any mites that have died within the sealed cell will fall. The data from 2245 drone and 330 worker cells revealed that 18 and 5%, respectively, of the adult female population died within the cell before bee emergence (Martin, unpublished data). Various studies have found that 18% (Boot et al, 1995), 30% (Martin and Kemp, 1997) and 38% (table I) of the mite population fall in association with the emergence of worker brood and 12% (table I) in association with the emergence of drone brood. This suggests that up to half of the falling mites associated with brood emergence die shortly after the bee has emerged. This is further supported by our observation that 50% of the mites falling

to the floor were still alive. This post-emergence mortality was previously considered only by Calis et al (1996) when calculating mite reproductive success. Results from this study suggest that the overall mites reproductive success will drop from an average of one viable female mite per mother (Boot et al, 1995; Donzé et al, 1996) to around 0.8–0.9 mites per reproductive cycle in worker cells and from 2–2.2 (Martin, 1995) to 1.9–2.1 in drone cells.

None of the live fallen mites collected for the reproductive or histological studies were found to be damaged. These fallen mites are fully fertile and can reproduce if they are introduced into a cell. Donzé et al (1996) showed that mating occurs immediately after the females complete their final moult, which is supported by the current finding that even the youngest mites are mated and able to reproduce, although their success rate increases with time.

From this study we can conclude that the majority of the mites on the floor are associated with the emergence of the brood, especially the worker cells. Therefore, if the number of fallen mites is used to monitor the mite population within the hive the presence or absence of emerging brood will be very influential. Omholt and Crailsheim, (1991) showed that, although Rademacher (1985) found no clear correlation between number of falling mites and total mite population, if the brood rearing pattern was considered the correlation became much stronger. Analysis of the relationship between mite fall and mite population suggests that the ratio of fallen mites to mite population varies dramatically between the times when brood is present or absent (Martin and Kemp, 1997).

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Résumé — Mortalité de *Varroa jacobsoni* Oudemans au cours de ou juste après l'émergence des ouvrières et des mâles d'*Apis mellifera* L. L'étude a porté sur l'origine des acariens *Varroa jacobsoni* Oudemans trouvés sur le plancher de la ruche. Toutes les observations ont été faites avec du couvain marqué de façon à connaître le moment de la naissance des abeilles. Les acariens ont été classés en quatre groupes, en fonction de la couleur utilisée comme critère relatif de leur maturité. Dans une colonie infestée naturellement, dont on connaissait le nombre d'ouvrières et de mâles à naître, on a enregistré toutes les 6 h la mortalité naturelle des acariens. Au cours de l'émergence des ouvrières et des mâles, le nombre d'acariens tombant sur le plancher a été multiplié par six et trois respectivement par rapport à la période où aucune abeille ne naissait (fig 1). La comparaison entre le nombre d'acariens tombant des cellules d'ouvrières et celui tombant des cellules de mâles, après infestation artificielle avec un nombre connu d'acariens, montre que la chute des acariens a été deux à trois fois plus élevée dans les cellules d'ouvrières que dans celles de mâles (tableau I). On a estimé, à l'aide de données antérieures (Martin, 1994, 1995), qu'environ la moitié des acariens tombés étaient des acariens morts dans les cellules operculées, l'autre moitié mourant peu après l'émergence. Ceci est également confirmé par le fait que 50 % des acariens tombés étaient encore vivants. Ces derniers sont capables de se reproduire, quelle que soit leur couleur, lorsqu'ils sont introduits artificiellement dans des cellules operculées (tableau II). La durée de développement plus courte des ouvrières est responsable des différences trouvées dans le nombre d'acariens tombant des cellules d'ouvrières et celui tombant des cellules de mâles. Cette étude a trouvé une forte corrélation entre le taux d'acariens qui

tombent et l'émergence du couvain. La présence ou l'absence d'abeilles naissantes doit être prise en compte lorsqu'on utilise le nombre d'acariens tombés comme outil de suivi de la mortalité. Il faut tenir compte également, dans la dynamique des populations d'acariens, du fait qu'une proportion significative de la mortalité a lieu peu après l'émergence.

***Apis mellifera* / *Varroa jacobsoni* / mortalité / chute des acariens / dynamique des populations**

Zusammenfassung — Sterblichkeit von *Varroa jacobsoni* Oud während und kurz nach Schlupf von Arbeiterinnen und Drohnen der Honigbienen. In dieser Studie wird die Herkunft der toten Milben auf dem Boden der Bienenbeuten untersucht. Alle Untersuchungen wurden anhand datierter Bienenbrut mit vorhersagbarem Schlupfzeitpunkt durchgeführt. Die Milben wurden in vier Farbgruppen eingeteilt, die als Hinweise auf ihre Adultreife angesehen wurden. In einem natürlich befallenen Bienenvolk mit bekannten Anzahlen schlüpfender Arbeiterinnen — oder Drohnenzellen wurde alle 6 Stunden der natürliche Totenfall untersucht. Während des Schlupfes der Arbeiterinnen- oder Drohnenbrut stieg der Totenfall im Vergleich zu Zeiten, in denen keine Brut schlüpfte, um den Faktor 6 bzw 3 an (Abb 1). Bei einem Vergleich des Totenfalls aus künstlich infizierter Brut (dh aus Brut mit bekanntem Befall) war dieser bei Arbeiterinnenbrutzellen 2-3 mal höher als bei Drohnenbrutzellen (Tabelle 1). Unter Einbeziehung früherer Ergebnisse (Martin 1994, 1995) wurde abgeschätzt, daß etwa die Hälfte der abgefallenen Milben bereits in den Zellen, die andere Hälfte kurz nach dem Schlupf der Bienen gestorben waren. Diese Abschätzung wurde auch durch den Befund unterstützt, daß etwa die Hälfte der Milben noch lebten. Diese Milben waren — unabhängig von ihrer Körperfärbung alle repro-

duktionsfähig (Tabelle 2). Der Unterschied in der Zusammensetzung der abfallenden Milben aus schlüpfender Drohnen — und Arbeiterinnenbrut kann auf die kürzere Entwicklungszeit der Arbeiterinnenbrut zurückgeführt werden. In dieser Untersuchung wurde eine klare Korrelation zwischen den Anzahlen abfallender Milben und dem Schlupf der Brut gefunden. Bei der Überwachung des Milbenbefalls in Völkern aus der Anzahl abfallender Milben muß daher die Anwesenheit oder Abwesenheit schlüpfender Brut in Rechnung gestellt werden. Zusätzlich sollte bei Untersuchungen der Populationsdynamik der Milben in Betracht gezogen werden, daß ein erheblicher Teil der Milbensterblichkeit kurz nach dem Schlupf der Bienenbrut stattfindet.

***Apis mellifera* / *Varroa jacobsoni* / Milbentotenfall / Sterblichkeit**

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