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Original article

Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*)

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Abstract – The proportion of damaged mites in natural mite fall is considered a useful criterion in the breeding of *Varroa*-tolerant bees. This study, observing about 16 000 mites, tested several modifications of recording this trait. The effects of a predator-proof hive, coating the bottom boards with grease, and the time intervals between recordings were examined. Studies were also conducted to determine the extent of damage by bees to already dead mites, and the influence of storage conditions of the dead mites. The type and the amount of damage to immature and adult mites differed considerably. Protection of the bottom board against predators significantly reduced the extent of damaged mites. Mites should not remain on the bottom board for longer than 2 days, because the extent of damage increases significantly after that, especially when wax moth larvae are present. Conditions under which the mites are stored significantly affect the extent of the damage. Quantity and quality of damage in natural mortality indicate that a proportion of mites died due to specific defensive behaviour of the bees. © Inra/DIB/AGIB/Elsevier, Paris

***Varroa* resistance / defence behaviour / method of evaluation / performance test / breeding**

1. INTRODUCTION

The Asian honey bee (*Apis cerana* Fabr.) is tolerant to *Varroa jacobsoni* Oud. The specific factors that distinguish this species as resistant are regarded as useful selection criteria for the western honey bee (*Apis mellifera* L.). A comprehensive account of these resistance mechanisms is given in two reviews [3, 6]. Defensive behaviours against *V. jacobsoni*, such as auto-grooming, nestmate grooming and removing infested pupae

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from mite-infested cells, among other factors, are presented as promising criteria. Directly recording mite defensive behaviour in observation hives [2, 4, 7, 8, 14, 20] is extremely time consuming and not feasible in practical bee breeding. Therefore, it is useful to find suitable methods that allow an indirect recording of this behaviour. The percentage of naturally fallen mites that are damaged, first described by Wallner [22] and further examined by others [10, 12, 13, 19], could serve as a suitable selection criterion. The defensive behaviour leads to the injury and death of mites [16, 21]. Moosbeckhofer [12, 13] and Hoffmann [10], found significant correlations between this trait and the population development of the parasites.

A problem in quantifying mite damage is that bees may cause damage naturally to already dead mites while transporting them with their mandibles. This clearly is not an expression of active mite defence. Furthermore, already dead mites are prey for ants and wax moth larvae [18], which leads to an overestimation of the percentage of mites damaged by bees. Although some published results support the number of damaged mites as a selection criterion in breeding honeybees tolerant to *V. jacobsoni* [10, 12, 13], this selection criterion is also disputed [15]. The influence of damage not caused by bees is expected to be high, but has not yet been determined. In previous studies, different methods of recording were used which may have affected the results. Before discussing the use of this selection criterion, it is useful to give methological information. Information about possible environmental factors affecting this trait will help in setting up a standard testing procedure. Results of experiments with a somewhat optimised testing procedure should then be the basis of discussing the suitability of the number of damaged mites as a selection criterion in breeding bees tolerant to *V. jacobsoni*.

2. MATERIALS AND METHODS

Twenty-four colonies of *Apis mellifera carnica* Pollmann with different levels of *V. jacobsoni* infestation were used in the experiment. Twelve of the 24 colonies were protected from possible damage by predators, such as ants, by the use of an oil-filled protection under the hive. In half of the colonies within the two groups (i.e. six), a layer of grease was put on the bottom board which was used as a control for mite mortality. All the bottom boards were protected from the bees by a metal screen. The 12-h (two repetitions), 24-h (three), 48-h (three), 72-h (three) and 168-h (one) recording intervals (time from insertion of bottom board to removal) were tested repeatedly during the observation period from the beginning of July to mid September. After the recording interval of 168 h, a very large number of mites were found in some colonies. The total number of mites was recorded but only a sample of 200 mites was examined for injuries.

For the assessment of the injuries to dead mites caused by bees, dead uninjured mites were marked and placed in empty brood cells. Before introducing the marked mites, they were stored for a day within the respective colonies, protected by a cage to eliminate any odour due to marking. After introducing the mites they were recovered hourly over a period of 5 h. After 24 and 48 h the (few) remaining mites were examined. All three colonies used in this experiment were ant-proof. The experiments were repeated four times. The percentage of damage and individual extent of injuries to marked mites were compared with the corresponding values of naturally fallen mites from the respective colonies. Damage in which more than 50 % of the mites' legs were missing, and large injuries to the idiosoma (larger lacerations) were classified as 'high', and less evident damage as 'low'.

To quantify changes in the mites after natural death, 25 (undamaged) mites which had recently died were examined over a 4-week period. Groups of 25 mites each that were a) stored in a refrigerator, b) kept at normal room temperature, or c) stored in a damp warm cellar, were examined for injuries each week.

All mites used in these experiments were removed with a fine brush and examined for injuries to the legs and idiosoma with a ×40 magnification. According to the approach of Moosbeckhofer [12] we classified mites with a colour brighter than 'ochre brown' as immature. Darker mites were classified as adult. Because the indi-

Table I. Percentage of damage to *V. jacobsoni* in the debris.

Factor	Modification	Extent of damage	Chi^2	*P*
Layer	with grease	34.8	1.8	n.s.
	without grease	35.4		
Predator	yes	31.3	53.8	0.001
Protection	no	38.5		
Wax moths	yes	38.6	20.5	0.001
on bottom board	no	33.4		

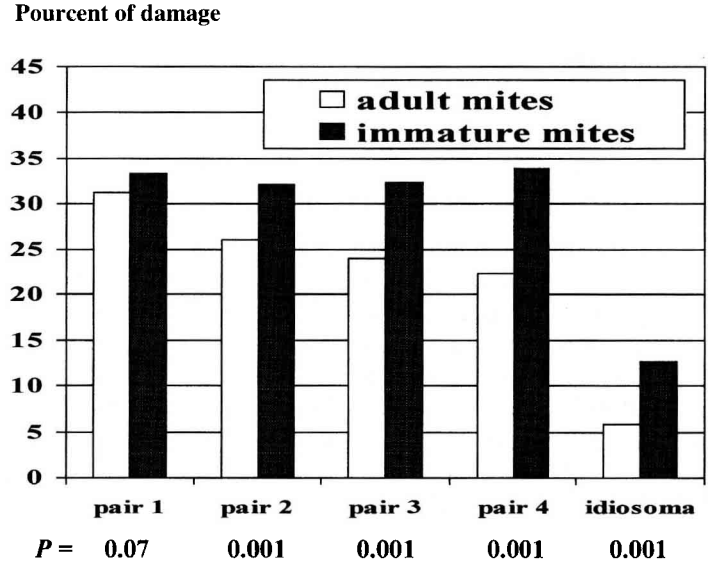

Figure 1. Percentage of damage to the four pairs of legs and the idiosoma of adult and immature *V. jacobsoni*.

vidual damage rate is not normally distributed, these categorical data (injury: yes or no) were analysed with the Chi^2-test [17].

3. RESULTS

From a total of 19 950 mites found on the bottom boards, 5 878 immature and 9 480 adult mites were examined for injuries. The damage to the extremities of immature mites were significantly higher and atypically distributed (*figure 1*). The correlations between the percentage damage to immature and adult mites per colony were r = 0.05–0.11, n.s., depending on the pair of legs, and not significant. Only the correlation between the respective injuries to the idiosoma (r = 0.44, $P = 0.045$) was significant. Due to these results only adult mites were assessed during the following analysis and evaluation.

The coating of the bottom board with grease did not significantly affect the damage rate of the parasites (*table I*). The extent of damaged mites was significantly higher in the hives without predator protection (*table I*). Also, the presence of wax moth larvae on the bottom board had a considerable effect on the extent of damage to the mites. Wax moth larvae were found in 15 % of the cases

where the debris remained under the colonies for only 12 h, but increased as the debris was left in for longer periods of time (24 h: 18 %, 48 h: 26 %, 72 h: 32 %, 168 h: 45 %). Consequently, the duration of the recording interval proved to be the most important factor for the extent of mite damage (*table II*).

From the total of 600 marked dead mites introduced into the colony, 397 (66 %) were found on the bottom board within 2 days. The simultaneously observed 430 naturally fallen mites were significantly more injured, and the extent of injury decreased typically from the front extremities to the rear. This tendency was not so obvious in the marked mites (*figure 2*). Additionally, in natural mite fall, 65.5 % of the damages were classified as high, whereas the corresponding value in the marked mites was only 51 %.

Damage after death, however, was not only caused by ants or wax moth larvae. There was a highly significant increase in damage within 4 weeks after mite death without the effect of predators. Whereas storing the dead mites in the refrigerator (2.7 %) or at normal room temperature (1.8 %) had no considerable effect on the extent of damage to the mites, a highly significant increase in changes appeared in the mites stored in a warm damp cellar. The process commenced after several days of storage, and after 4 weeks, 96 % of the limbs displayed injuries.

Table II. Percentage of damage to *V. jacobsoni* in relation to the duration the debris remained under the colonies.

Duration	Extent of damage	Chi^2	*P*
12 h	32.8	105.9	0.001
24 h	27.0		
48 h	34.0		
72 h	38.3		
168 h	40.4		

4. DISCUSSION

Moosbeckhofer [12] exclusively found the (negative) correlation between the percentage of injured adult mites and *V. jacobsoni* infestation to be significant. The corresponding relationship with immature mites was not significant. He combined both age

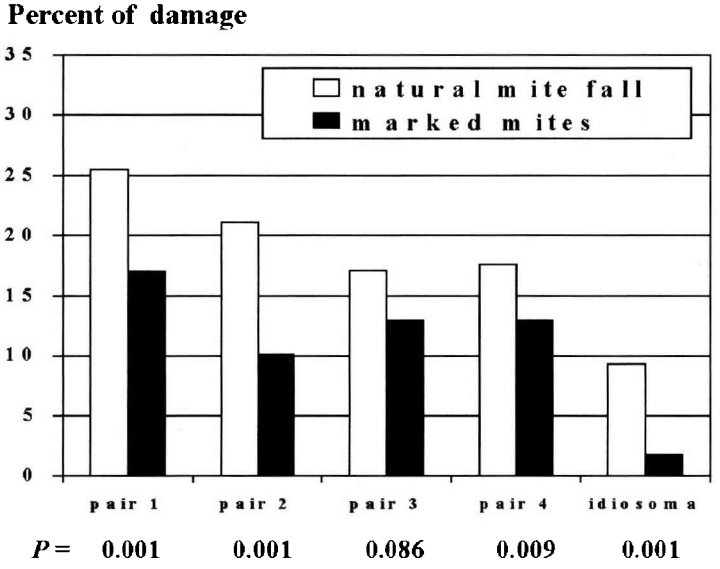

Figure 2. Percentage of damage to the four pairs of legs and the idiosoma of naturally fallen adult *V. jacobsoni* and already dead (marked) mites introduced into the colony.

classes in a later study [13] and found a significant relationship with *V. jacobsoni* infestation. This result was the motive to analyse immature and adult mites separately in the present study. In agreement with the results from Hoffmann [10] and Lodesani et al. [11], the most damage to adult mites is found on the first pair of legs with a decreasing tendency of damage to the rear legs. The first pair of legs is most likely to be damaged in case of an attack, because it is exposed. An almost reverse tendency was found with the immature mites (*figure 1*). Moreover, no significant correlation was found between the extent of damage to the immature and adult mites from the examined colonies. Should the immature mites have died from injuries caused by bees, then the underlying action of the bees is not typical of defensive behaviour against adult mites. By the stability of their idiosoma and more adapted behaviour, adult mites are the more formidable enemies. Damage to immature mites (especially very young mites) will entail a higher percentage of 'damages by chance'. If one accepts the extent of damaged mites as a criterion of the defensive behaviour against this parasite, than the exclusive consideration of adult mites is the more reliable criterion.

The assumption that the observed damage of mites is only partly caused by bees [4, 8, 15] is demonstrated by the present results. The influence of predators leads to a significant increase in the damage rate of the mites (*table I*). Predators not only damage dead mites but also remove them. In the group without predator protection, there was a higher degree of damage and fewer mites than in the corresponding control (–13 %). Protection against predators reduces the proportion of injuries not caused by bees, and more mites remain on the bottom board resulting in a larger sample which is favourable for the estimation of the average damage rate. Given the usual extent of damage between 30 and 50 %, a sample size of at least 30 mites is necessary to estimate a reliable average damage rate [1].

The longer the recording interval, the higher is the extent of mite damage (*table II*). A lower extent of damage was found in the 12-h period than in the 24-h period, which may have depended on the method. The bottom boards were inserted in the evening and removed the following morning which meant that all the bees from the colony were in the hive during the whole of the (12-h) recording period. Therefore, the larger number of bees could be responsible for the increased damage rate during the shorter interval. Daytime influences could also be responsible for the underlying behaviour. One reason for the highly significant relationship between the extent of damage and the duration of the recording interval is the increase in wax moth larvae on the bottom board. A further explanation is that decomposition effects appear on the mites without mechanical influence after some time. The longer the mites remain in warm and damp conditions which favour decomposition, the higher the damage rate will be. In addition, the impact of external factors is not yet over when the bottom board is removed. Decomposition processes can affect the damage rate of the examined mites to such an extent that conclusions on the possible effect of the defence behaviour of bees are not reliable.

Transport damage' during the carrying of dead mites by the bees was shown to be important (*figure 2*). Dependent on pairs of legs, 10–17 % of the dead marked mites were damaged, whereas the extent of damage in naturally fallen mites ranged from 17 to 26 %. The increased extent of damage could be due to the defensive behaviour of bees against live mites resulting in more injuries. Aggressive behaviour of bees against *V. jacobsoni* was observed with video recordings [21]. The presence of injured but live mites in the debris of colonies [9, 15, 16] may also indicate a correlation between active defensive behaviour of bees against *V. jacobsoni* and extent of damaged mites in the debris. Also, the results by Moosbeckhofer [12, 13], Büch-

ler [5] and Hoffmann [10] may support this speculation. However, this study demonstrated a considerable influence of factors not associated with the defensive behaviour of bees. Present data may be used to develop a standardised testing procedure to minimised environmental influence. A testing method which considers the factors:

- protection against predators;
- short recording interval (< 2 days);
- exclusive use of adult mites; and
- sufficient sample size (> 30 mites)

could help to reveal genetic differences in the underlying defensive behaviour against *V. jacobsoni*, and, consequently, whether the percentage of damaged mites is a useful selection criterion in honeybee breeding.

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Résumé – Détermination du pourcentage d'acariens *Varroa jacobsoni* Oud. mutilés dans les débris des colonies d'abeilles, *Apis mellifera* L. La proportion d'acariens mutilés dans la mortalité naturelle des acariens dans les colonies d'abeilles est discutée en tant que critère de sélection significatif dans le cadre de la sélection d'abeilles tolérantes à *Varroa jacobsoni*. Puisque les acariens peuvent être mutilés par d'autres facteurs que les abeilles elles-mêmes, on a voulu tester quelques modifications à la méthode de détermination de ce taux. On a étudié l'influence d'une protection de la ruche contre les fourmis, de la mise en place d'un lange graissé sur le plancher de la ruche et de la durée des intervalles entre les relevés. On a aussi étudié dans quelle mesure les mutilations faites par les abeilles aux acariens morts et l'influence des conditions de conservation des acariens morts pouvaient exercer une action sur le taux de mutilations. On a analysé individuellement environ 16 000 acariens. La nature et la quantité des mutilations faites aux acariens immatures et aux adultes différaient nettement (*figure 1*), si bien que seuls ces derniers ont été pris en compte pour évaluer le taux de mutilation. Une protection du plancher de la ruche contre les fourmis a significativement diminué le pourcentage d'acariens mutilés (*tableau I*). Alors qu'une telle mesure réduit nettement la porportion de mutilations non liées aux abeilles, la pose d'un lange graissé sur le plancher de la ruche ne semble pas améliorer les résultats (*tableau I*). Les acariens ne doivent pas rester plus de deux jours sur le plancher, sinon le taux de mutilation augmente significativement, à cause des larves de fausse-teigne principalement (*tableaux I* et *II*). Les conditions dans lesquelles les acariens morts sont conservés influencent significativement le taux de mutilations. Les acariens sont mutilés post mortem dans le cadre du comportement hygiénique habituel. Néanmoins on peut déduire de la quantité et de la qualité des mutilations trouvées chez les acariens morts que, pour une partie d'entre-eux, la mort est due au comportement défensif spécifique des abeilles.

comportement défensif / tolérance / *Varroa jacobsoni* / méthode évaluation / test performance / sélection

Zusammenfassung – Erfassung des Anteils verletzter Varroa-Milben (*Varroa jacobsoni* Oud.) im Gemüll von Bienenvölkern (*Apis mellifera* L.) Der Anteil beschädigter Milben im natürlichen Totenfall der Völker wird bei der Zucht varroatoleranter Bienen als sinnvolles Selektionskriterium diskutiert. Da die Milben auβer durch Bienen auch durch andere Faktoren beschädigt werden, sollten in der vor-

liegenden Studie einige Modifikationen der Erfassungstechnik mit dem Ziel getestet werden, die Erfassung des Merkmals zu optimieren. Hierfür wurden der Einfluß einer Ameisensicherung der Beute, der Fettbeschichtung der Bodenunterlage und die Dauer des Erfassungsintervalls untersucht. Zusätzlich wurden noch Untersuchungen angestellt, inwieweit Beschädigungen durch Bienen an toten Milben und der Einfluß von Lagerbedingungen toter Milben auf die Verletzungsrate Einfluß nehmen. Circa 16 000 Milben wurden untersucht. Art und Umfang der Beschädigungen von hellen (nicht ausgereiften) und adulten Milben unterscheiden sich deutlich (*Abbildung 1*), so daß nur letztere zur Beurteilung der Beschädigungsrate berücksichtigt werden sollten. Ein Schutz der Bodenunterlagen vor Ameisen (und vergleichbaren Tieren) reduzierte den Anteil von beschädigten Milben signifikant (*Tabelle I*). Während eine solche Maßnahme deutlich den nicht bienenbedingten Anteil an den Verletzungen verringert, erscheint durch die Fettbeschichtung der Bodenunterlagen keine Verbesserung der Erfassung möglich (*Tabelle 1*). Länger als zwei Tage sollten die Milben nicht auf den Bodenunterlagen verbleiben, da sonst die Beschädigungsraten besonders in Folge von Wachsmottenlarven-Frass signifikant zunehmen (*Tabellen I* und *II*). Die Bedingungen, unter denen tote Milben gelagert werden, beeinflussen signifikant die Beschädigungsrate. Milben werden auch postmortal im Rahmen des normalen Hygieneverhaltens der Bienen verletzt. Doch der anderen Quantität und Qualität der Beschädigungen im normalen Totenfall (*Abbildung 2*) ist zu entnehmen, daß ein Teil der Milben durch spezifische Abwehrverhaltensweisen der Biene zu Tode kam.

Varroaresistenz / Abwehr / Testmethode / Zucht

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