Apidologie 31 (2000) 377–385

Original article

Removal of *Varroa jacobsoni* infested brood in honey bee colonies with differing pollen stores

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(Received 9 March 1999; revised 18 October 1999; accepted 1 November 1999)

Abstract – The effects of high or low pollen storage on *Apis mellifera* L. brood removal behavior and *Varroa jacobsoni* reproduction were examined. High pollen storage colonies removed 49% of the infested larvae compared to 33% removal by the low pollen storage colonies. No difference was found in the proportion of fertile mites between those reared in high or low pollen storage colonies, although mite fertility appeared to decrease from mid to late summer in British Columbia, Canada. These findings indicate that the presence of pollen stores increases the rate of cell removal, and warrants further investigation into colony management as a potential means of *V. jacobsoni* infestation control.

***Varroa jacobsoni* / honey bee / pollen / brood removal**

1. INTRODUCTION

Varroa jacobsoni Oudemans has become the most serious threat facing beekeepers today. Honey bee (*Apis mellifera* L.) colonies typically die 1–2 years after an infestation begins, although infestation growth rates vary between colonies [1]. Numerous studies have identified potential behavioral mechanisms that may reduce *V. jacobsoni* population growth in colonies (reviewed in [5]), but to date these proposed mechanisms for *V. jacobsoni* tolerance have not proven viable as management tools.

Worker removal of infested brood is one identified defense mechanism of honey bee colonies. For example, colonies that engage in a high degree of infested brood removal behavior have a lower incidence of the bacterial disease, American foulbrood [21]. Similarly, *A. mellifera* workers remove some brood infested by *V. jacobsoni*, and this behavior could play a role in the development of stock resistant to the effects of

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V. jacobsoni [3, 25]. The brood removal rate is known to be greatly influenced by environmental factors, such as nectar flow [15]. However, the impact of disturbances to the colony's internal state, such as changes in pollen area, requires further investigation.

Under suboptimal colony conditions, disease effects may be amplified due to a decrease in hygenic behavior. For example, the incidence of the fungal pathogen chalkbrood (*Ascosphaera apis*) increases under nutritional stress, such as little stored honey or low pollen collection [11]. Suboptimal colony conditions may affect hygenic behavior through an alteration of worker response thresholds to dead or diseased brood. Workers may be less likely to respond to cell removal cues in the presence of high pollen foraging cues when space demands are low. Furthermore, suboptimal colony conditions may lead to the production of compromised workers that are less able to perform colony tasks. Therefore, overall colony condition needs to be considered when studying behavioral defense mechanisms.

There is considerable variation in the reproductive success of *V. jacobsoni* mites in honey bee colonies [14], and ecological conditions, especially pollen availability, are thought to play a role in determining mite reproduction [27]. This reproductive ability directly affects the growth rate of the infestation and its impact on the host colony [10]. Changes in brood removal rate and mite reproduction need to be considered simultaneously to determine the impact of either factor on the growth of the parasite population. The objective of the present study was to determine if colony brood removal behavior and *V. jacobsoni* mite reproduction are affected by the level of the host colony's pollen stores.

2. MATERIALS AND METHODS

Three trials were conducted in July and August 1996, and in July 1997 at Simon Fraser University, Burnaby, British Columbia, Canada. For the two trials in 1996, two brood-rearing colonies, with sister queens (requeened two months prior to the experiment), were established and equalized in brood area, adult population and honey stores (approximately 16 000 bees, 3 500 cm^2 sealed brood). One colony received additional pollen stores for a total of 3 000 cm^2 of pollen, and was designated the high-pollen brood rearing colony. The low-pollen brood rearing colony contained 430 cm^2 of pollen, and was fitted with a pollen trap to collect incoming pollen. In 1997, six unrelated colonies were established and equalized in brood area, adult population and honey stores (16 000 bees, 2 300 cm^2 sealed brood). Three colonies received additional pollen for a total of 3 600 cm^2 of pollen, and were designated as the high-pollen brood rearing colonies; the remaining three colonies were fitted with pollen traps, and all the pollen stores were removed. Each colony was contained in one Langstroth box with 10 frames of comb to supply the colonies with ample comb.

Frames of eggs were exchanged between the colonies in 1996, and additional frames of eggs were placed into each colony from an outside source. Additional frames were added to ensure that there was a large number of newly-sealed cells available for the experimental addition of *V. jacobsoni*. Frames of eggs were exchanged between pairs of colonies in 1997. Frames were exchanged to decrease genetic effects on behavioral observations of emerged bees [12]. After eight days, all capped and partially-capped cells on the manipulated frames were mapped onto clear plastic sheets in the early evening. On the following morning, female *V. jacobsoni* mites were introduced into newly-sealed cells that had been sealed from 0–12 h prior to the introduction. The edge of the capping was cut with a scalpel, a single *V. jacobsoni* mite was introduced through the cut, and the opening was then tamped down [22], a procedure referred to as 'seeding'. Adjacent

larvae on each seeded frame received a sham treatment, in which the capping was opened and closed, but no mites were added. All available newly-sealed cells were manipulated on each frame.

To obtain female mites for introduction to the colonies, frames of emerging bees were taken from colonies heavily infested with *V. jacobsoni* and were kept at 3 °C. After three days, female *V. jacobsoni* mites were removed from the infested bees with powdered sugar [4]. A 48-h fluvalinate (Apistan™) treatment was applied to the brood-rearing colonies two days prior to capping to reduce the likelihood of mite invasion into the larval cells required for the experiment. After 10 days, the mapped frames were removed and placed in an incubator set at 34 °C; the presence or absence of brood in manipulated cells was recorded for each frame. For the trials conducted in 1996, mite reproduction was recorded upon emergence of the adult bees. Reproduction was recorded as the presence of 1 male and 1 female deutonymph.

Both the frequency of cell removal and mite reproduction were analyzed using the CATMOD (categorical data analysis) procedure in SAS Version 6.0, which fits linear models to data represented by contingency tables, and analyzed by χ^2. This procedure was chosen to compare frequencies between treatment groups and populations separated by colony and trial date. In addition to the pollen and *V. jacobsoni* treatment, the colony was included in the cell removal analyses as nested in pollen, and seeding date was included as nested in colony. Only seeding date was included in addition to the pollen and treatments in the mite reproduction analyses.

3. RESULTS

3.1. Removal behavior of manipulated brood

Workers in high-pollen colonies removed more brood from manipulated cells, with or without mites, than workers in low-pollen colonies (see Tab. II for the statistical analysis results). The mean removal rate of manipulated cells in high-pollen colonies

Table I. Percentage cell removal rates for manipulated cells by each high- or low-pollen colony at each trial date. The difference between the number of infested cells removed minus the number of control cells removed is displayed in the last column.

Colony	No. of frames	No. of control cells	Percentage removed	No. of infested cells	Percentage removed	Percentage difference
Trial 1						
High pollen	3	206	46 ± 12	207	63 ± 4	17 ± 11
Low pollen	4	222	16 ± 42	221	35 ± 9	16 ± 9
Trial 2						
High pollen	3	198	31 ± 15	216	39 ± 8	9 ± 11
Low pollen	3	218	26 ± 10	219	29 ± 10	4 ± 2
Trial 3						
High pollen	2	115	29 ± 12	112	44 ± 4	17 ± 9
High pollen	2	145	18 ± 1	145	37 ± 7	17 ± 9
High pollen	1	33	15	39	33	18
Low pollen	2	95	29 ± 32	93	26 ± 8	19 ± 4
Low pollen	1	70	23	47	23	0
Low pollen	2	103	29 ± 62	98	39 ± 4	12 ± 3

was 43.9 ± 15.6%, compared to the removal rate of 28.5 ± 10.2% in low-pollen colonies. The presence of a *V. jacobsoni* mite in a cell increased the removal rate of brood in manipulated cells from 30.5 ± 15% for control cells to 42.7 ± 14% for infested cells. Both high- and low-pollen colonies removed more infested cells than control cells, and the analysis indicated a marginally significant interaction between the pollen treatment and the type of manipulated cell (control versus mite infested). High-pollen colonies removed 15 ± 11% more infested brood than control cells, as compared to a 9 ± 8% difference in the low-pollen colonies (see Tab. I for individual colony differences).

Overall, high-pollen colonies removed a total of 35.6% of the control cells and 48.9% of the experimentally-infested cells, as compared to a removal of 22.7% of the control cells and 32.8% of the infested cells by the low-pollen colonies (see Tab. I for individual colony observations). Both the colony and trial date were found to significantly affect the number of cells cleared (colony: $\chi^2 = 92.6$, df = 1, $P < 0.0001$, and trial: $\chi^2 = 45.7$, df = 1, $P < 0.0001$). These factors were included in the analysis to remove the variation introduced by colony and trial, but were not the main subjects of the investigation.

3.2. Mite reproduction

The reproductive rate of *V. jacobsoni* mites placed in the brood cells did not differ if the mites were placed in brood cells from a high-pollen colony versus brood cells from a low-pollen colony ($\chi^2 = 0.51$, df = 1, $P = 0.47$). Of the mites placed in the high-pollen colony, 49.5% reproduced as compared to 46.7% of the mites in the low-pollen colony. However, season had a strong effect on mite reproduction, since 56.2% of the mites reproduced in the first trial, as compared to 39.9% mite reproduction in the second trial ($\chi^2 = 8.7$, df = 1, $P = 0.0032$). A similar decrease in mite reproduction was observed in seeding experiments that were conducted simultaneously (Downey, pers. commun.), further indicating that season was the primary factor affecting mite reproduction.

4. DISCUSSION

This study revealed that *A mellifera* colonies removed brood artificially infested by *V. jacobsoni*, and that this removal behavior varied with the level of pollen storage in the colony. Furthermore, there was a higher removal rate of *V. jacobsoni*-infested cells. Therefore, a colony's behavioral defenses to parasitic infestations were influenced by the colony's nutritional status. Further, the incidence of mite reproduction was not affected by the host colony's pollen state.

4.1. Brood removal behavior

Colonies removed brood infested with *V. jacobsoni* mites at a higher rate than brood receiving the sham treatment, indicating that workers are able to detect the presence of *V. jacobsoni* in a sealed brood cell. These observations agree with those of Rosenkranz et al. [20] on *A. cerana*. These removal rates may be artificially high due to the introduction of foreign mites into cells. Rosenkranz [20] found that workers remove cells seeded with mites foreign to the colony at a higher rate than cells seeded with mites obtained from the colony. Furthermore, the removal rates of control cells are higher than in previous hygienic behavior studies [20, 26], and this high removal rate is most likely due to the length of time (10 days) prior to cell removal observations.

High-pollen colonies removed more of the manipulated brood than low-pollen colonies, indicating that colony removal rates are dependent on internal colony state as well as on variations in resource availability. Both the decrease in pollen stores

[6, 8, 9] and the addition of a pollen trap [29] are known to increase pollen foraging rates, and some low-pollen colonies stored pollen in spite of the presence of the pollen trap (pers. obs.). Therefore, higher pollen foraging rates of low-pollen colonies may have contributed to lower cell removal rates as compared to high-pollen colonies, presumably through differences in the allocation of workers between tasks.

Current research in hygienic behavior has recently begun to examine the role that individual response thresholds play in the expression of hygienic behavior [28]. Previous theories on the division of labor within colonies indicate that the probability that a worker will perform a task is dependent on the magnitude of the task (in this case the degree of infestation or pollen status) and the individual's response to the threshold stimuli [18]. The workers in the colonies with large pollen foraging demands will be exposed to larger foraging stimuli than in the high-pollen colonies, thereby leading to a reduction in the number of workers engaged in cell removal behavior.

Workers often respond to increased pollen demands through a reduction in mean foraging age (unpubl. obs.). The mean age of cell removal bees has been observed to be 15 and 17.6 days in two colonies, where the mean foraging age was observed to be 22 and 20 days, respectively [27]. Therefore, an increase in the foraging demand could feasibly tap into the pool of younger cell removal bees through decreases in the foraging age. Similarly, workers begin foraging earlier in colonies after a worker loss [7], and studies have shown that the cell removal rate is decreased after the population decreases [26]. Therefore, changes in colony state may ultimately alter cell removal rates through changes in task ontogeny. Furthermore, workers in high-pollen colonies may be better able to discriminate between control and mite-manipulated cells, as indicated by the significant pollen-*Varroa* interaction (Tab. II), due to decreased pollen demands.

Table II. Analysis of variance table of the CATMOD analysis of cell removal results. The model includes: high-versus low-pollen treatment groups (pollen), control versus mite-infested cell manipulation (*V. jacobsoni*), colony nested in pollen treatment (colony [pollen]), and trial date, nested in colony (trial [colony]).

Source	DF	Chi-square	*P*-value
Intercept	1	437.37	< 0.001
pollen	1	30.69	< 0.001
Varroa	1	77.39	< 0.001
Pollen × *Varroa*	1	3.93	0.0475
Colony (pollen)	6	92.55	< 0.001
Trial (colony)	2	45.71	< 0.001

Studies conducted by Momot and Rothenbuhler [15] have demonstrated that colonies increased cell removal rates during nectar flow. Since foraging age would be expected to decrease during nectar flow, this result appears to be in contradiction to this study. However, a high demand for comb space accompanies nectar foraging demands [24]. To avoid the effort of comb building, workers will clean cells in the colony [24]. Therefore, hygienic behavior would be expected to increase during nectar flow to meet this high demand for space. However, pollen foraging may be less linked to space demands due to the limited size of the pollen stores in the colony. Furthermore, colonies with experimentally-raised pollen levels will use up additional pollen [8, 13], thereby freeing comb space. In addition, colonies in the present study were equipped with empty frames to decrease space demands. Therefore, the impact of pollen stores on cell removal differs from that encountered during nectar flow.

Considerable research has been devoted to understanding how honey bee colonies regulate the collection of nectar, pollen, and water. Collection of resources is adjusted according to a colony's immediate need, or increased to provide colonies with the

appropriate stores to survive the winter, or periods of inclement weather [24], whereas inspection and removal of brood is intimately tied to the colony's collection of resources and is less dependent on the colony's cell removal needs. These factors indicate that resource demands are of a higher priority to colony survival than cell removal demands. Therefore, the regulation of brood inspection and removal would be an interesting area for future research.

4.2. Mite fertility

There is considerable variation in the number of reproducing mites between colonies, and this variation has been attributed to genetic differences between host colonies [18], environmental conditions [2, 17], and the time of year [17, 18]. The incidence of *V. jacobsoni* mite reproduction directly affects the growth of the mite population, and the resistance of the honey bee colony to the infestation. Colonies of *A. mellifera* in Uruguay were found to be resistant to *V. jacobsoni* infestations due to 70–90% mite infertility in worker brood [23], and Fuchs [10] found a significant correlation between the development of the infestation and the fertility of the mites. If mite fertility increased in response to increased pollen availability in the host colony, the impact of the infestation on the host would increase.

However, the incidence of *V. jacobsoni* mite reproduction did not differ between pollen groups in our study, and decreased as the season progressed. Similarly, the percentage of fertile *V. jacobsoni* females was not found to significantly increase from colonies with a high versus low pollen supply in a study by Blum [2], although this also varied with the season. A significant correlation was found between the percentage of fertile female mites and the amount of pollen stored in colonies in another study [17], but in this experiment the amount of pollen stored varied with the season. Therefore, mite fertility appears to be affected by seasonal physiological changes in brood, adult bees, or in the mites themselves [16] that occur independently of pollen storage levels in the hive. Since mite reproduction does not increase with colony pollen state, it is possible that colonies with high pollen stores may be able to decrease mite population growth through increased removal rates of infested brood. This hypothesis warrants further investigation.

5. CONCLUSIONS

These findings demonstrate that the ability of *A. mellifera* colonies to remove cells varies with colony condition, a factor that can be manipulated by beekeepers through effective colony management. The hygienic behavior of the colonies may be increased through intensive colony management as well as genetic selection, thereby providing beekeepers with a potential method to decrease the impact of the pervasive *V. jacobsoni* mite problem.

Résumé – Élimination du couvain infesté par *Varroa jacobsoni* dans des colonies d'abeilles en fonction des réserves de pollen. L'influence d'un stockage plus ou moins élevé de pollen sur le comportement d'élimination du couvain et la reproduction de *V. jacobsoni* a été étudié. On a pris des colonies qui élevaient du couvain et égalisé leur surface de couvain, leur population d'abeilles adultes et leurs réserves en miel. On a fourni un supplément de pollen au groupe de colonies « à pollen élevé » et placé une trappe à pollen sur celles du groupe « à pollen faible ». Un acarien *V. jacobsoni* a été introduit dans chacune des cellules fraîchement operculées des colonies des deux groupes (cellules tests) et des cellules ouvertes et refermées sans ajout d'acarien ont servi de cellules témoins. Dix jours plus tard, les cadres de couvain ont été retirés des colonies et la présence ou

l'absence des cellules manipulées a été notée. La reproduction de l'acarien a été considérée comme positive si des deutonymphes mâles et femelles étaient présentes lors de l'émergence des abeilles. Les colonies à pollen élevé comme les colonies à pollen faible ont éliminé une plus forte proportion de cellules et de larves infestées que de cellules témoins. Chaque groupe a éliminé respectivement 42,7 et 30,5 % de cellules. Les colonies du groupe à pollen élevé ont éliminé 49 % de larves infestées contre 33 % pour les colonies du groupe à pollen faible. On n'a pas trouvé de différence dans la proportion d'acariens fertiles entre les acariens élevés dans les colonies à pollen élevé et ceux élevés dans les colonies à pollen faible, bien que la fertilité des acariens ait semblé décroître du milieu vers la fin de l'été en Colombie britannique, Canada. Les ouvrières semblent être capables de détecter la présence de *V. jacobsoni* dans une cellule operculée, puisqu'une plus grande proportion de couvain infesté est éliminé. Parce que, dans les colonies à pollen élevé, la demande en récolte de pollen est plus faible, les ouvrières peuvent être disponibles en plus grand nombre pour inspecter les cellules et éliminer le couvain ; ceci peut expliquer le plus grand pourcentage d'élimination chez les colonies à pollen élevé. Puisque la reproduction de l'acarien n'augmente pas avec les réserves en pollen, la gestion du pollen par les colonies devrait être étudiée comme outil potentiel pour gérer les populations d'acariens.

***Varroa jacobsoni* / abeille / pollen / élimination du couvain**

Zusammenfassung – Das Ausräumen *Varroa* befallener Brut in Honigbienenvölkern mit unterschiedlichen Pollenvorräten. Wir untersuchten den Einfluss von niedrigen und hohen Pollenvorräten von *Apis mellifera* L. auf das Brutausräumverhalten und auf die Reproduktion von *Varroa jacobsoni*. Die zur Bruterzeugung eingerichteten Bienenvölker wurden bezüglich der Brutfläche, Anzahl erwachsener Bienen und Honigbienen ausgeglichen. Die Versuchsgruppe mit hohen Pollenvorräten wurde mit zusätzlichem Pollen versorgt, während in der Versuchsgruppe mit niedrigen Pollenvorräten der Pollen entfernt und die Völker mit Pollenfallen versehen wurden. Frischverdeckelte Zellen beider Versuchsgruppen wurden mit jeweils einer *V. jacobsoni* Milbe infiziert. Die als Kontrolle dienenden Zellen wurden geöffnet und wieder geschlossen, ohne dass eine Milbe eingebracht wurde. Nach 10 Tagen wurden die Brutwaben entnommen und kontrolliert, ob die manipulierten Zellen noch vorhanden waren. Die Milben wurden als reproduzierend gewertet, wenn beim Schlupf der Bienen männliche und weibliche Deutonymphen registriert wurden. In beiden Versuchsgruppen wurde ein höherer Anteil der manipulierten Zellen entfernt, in der Versuchsgruppe mit hohen Pollenvorräten waren es insgesamt 42,7 %, in der mit niedrigen Pollenvorräten 30,5 %. Völker mit hohen Pollenvorräten entfernten 49 % der befallenen Zellen, Völker mit niedrigen Pollenvorräten 33 %. Der Anteil fertiler Milben unterschied sich nicht zwischen den Versuchsgruppen, allerdings schien in Britisch Columbien, Canada, die Fertilität zwischen dem Mittsommer und dem Spätsommer abzunehmen. Die Arbeiterinnen scheinen die Anwesenheit von *V. jacobsoni* in den verdeckelten Zellen wahrnehmen zu können, da infizierte Zellen zu höherem Anteil entfernt wurden. Wegen der geringeren Nachfrage nach Pollen könnte eine höhere Anzahl Arbeiterinnen verfügbar sein, um in den Völkern mit hohen Pollenvorräten Zellen zu untersuchen und zu entfernen. Da die Reproduktion der Milben bei höheren Pollenvorräten nicht erhöht war, könnte die Beeinflussung der Pollenvorräte möglicherweise einen Weg zur Beeinflussung der Entwicklung der Varroose darstellen.

***Varroa jacobsoni* / Honigbiene / Pollen / Brutausräumen**

ACKNOWLEDGMENTS

This study was supported by a Natural Sciences and Engineering Research Council operating grant to MLW, and a post-graduate NSERC scholarship to AFJ. We thank Adony Melathopolous, Danielle Downey, Heather Higo, John Senger and members of the Simon Fraser University Swarm Team for help in maintaining colonies teeming with mites.

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