

## Response of *Apis cerana* Fabr towards brood infested with *Varroa jacobsoni* Oud and infestation rate of colonies in Thailand

W Rath<sup>1</sup>, W Drescher<sup>2</sup>

<sup>1</sup> *Institut für Landwirtschaftliche Zoologie und Bienenkunde der Universität, Melbweg 42, 5300 Bonn 1, FRG;*

<sup>2</sup> *Ministry of Agriculture and Cooperatives Department of Agricultural Extension, Beekeeping Subdivision; Regional Agricultural Extension Office, Arruk Rd Soi 5, Amphoe Muang, 50000 Chiang Mai, Thailand*

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**Summary** — *Apis cerana* F, colonies responded with effective removal behavior towards sealed worker brood that was artificially infested with vital or dead *Varroa jacobsoni* Oud. The bees removed artificially infested drone brood to a very low extent. *A. cerana* workers showed a differential hygienic behavior towards freeze killed sealed brood, consisting of fast removal of dead worker and slow removal of dead drone brood. The *V. jacobsoni* populations infesting *A. cerana* colonies in South Thailand were determined by acaricide treatment. A maximum infestation with 798 mites was ascertained.

***Apis cerana* / *Varroa*-resistance / hygienic behavior / natural infestation / Thailand**

### INTRODUCTION

*Varroa jacobsoni* Oud is believed to originally be a parasite of *Apis cerana* Fabr colonies. As usual in host-parasite relations that have evolved over a long period of mutual adaptation, the host is not able to eliminate its parasite nor is the parasite likely to endanger the survival of its host species. It has been reported that *A. cerana* bees performing body cleaning have a high chance of catching and killing the ectoparasites with their mandibula (Peng *et al*, 1987a). Furthermore, it has been observed that *V. jacobsoni* is rare in worker brood (Koeniger *et al*, 1981; Koeniger *et al*, 1983). Mites reproducing in *A. cerana* worker brood were reported by DeJong

(1988) who found one deutonymph, one nymph and one egg in 3 out of 720 evaluated cells. Reproduction of *V. jacobsoni* with adult offspring has been reported from drone brood only (Koeniger *et al*, 1981, 1983; DeJong, 1988). Infested drone larvae can be damaged to such an extent that they are unable to uncup their cells (Koeniger, 1987). The dead drones are not removed and the mites die within the cell. Finally, absconding behavior, which may be a response to adverse environmental conditions, helps *A. cerana* bees to leave diseased organisms and parasites on the deserted combs (Woyke, 1976). Together, these factors indicate that reproduction of *V. jacobsoni* in *A. cerana* colonies is limited.

The present study reports on the above – mentioned regulative factors in an effort to further understand the host – parasite relation in *A cerana* colonies.

## MATERIALS AND METHODS

### **Artificial infestation of *A cerana* worker brood cells with live and dead *V jacobsoni* mites and artificial infestation of *A cerana* drone brood cells**

Investigating the observation that *V jacobsoni* are rarely to be found in sealed *A cerana* worker brood cells, we artificially infested worker and drone brood cells containing spinning 5th instar larvae with female *V jacobsoni* mites collected from newly sealed *A mellifera* worker brood cells. For the artificial infestation, the cell caps were partially opened below the cap border using a fine surgeon's knife. In the first experimental sequence, live *V jacobsoni* were placed into worker and drone cells. The openings in the cell caps were closed by gently pressing the wax into the original position. In a second experimental series, dead mites collected from hive debris of *A mellifera* colonies were used for the artificial infestation of worker brood cells. In order to remove strong odors and fungus germs from the dead mite bodies, they were washed in water and 97% ethanol before being placed into the brood cells. The cell caps of the control group were opened and closed in the same way, but no mites were placed inside. For re-identification the position of the cells was marked on a clear plastic sheet that could be readjusted to the same position (De Ruijter, 1987). The condition of the cells was examined at daily intervals and the status of sealed cells, uncapped cells and removed brood recorded.

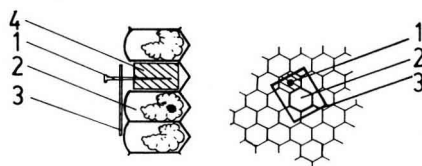
In an additional modified experiment we tried to prevent the removal of the infested brood by the worker bees by protecting the artificially infested *A cerana* worker brood cells with polyethylene cap shelters of 1 cm<sup>2</sup> which covered the infested cell and were attached to the comb (fig 1). After 7 days the protected cells were examined for the condition of the host and mite.

The *A cerana* colonies used in the experiments were local races from Chiang Mai (Northern Thailand) and Chumporn (Southern Thailand) and were kept in Langstroth hives with frames reduced in size by one third.

### **Evaluation of sealed dead drone brood and removal response of *A cerana* bees to freeze-killed sealed brood**

In drone combs from absconded colonies and in abandoned combs sealed drone cells were found containing dead drones which were unable to open the cell caps to emerge or that had died for other reasons. The dead drone brood that had not been removed by the bees was examined for mite infestation and other visible reasons for incomplete development.

The uncapping and removal response of honeybees towards abnormal or dead sealed brood is an important part of the colonies' hygienic nest cleaning behavior (Jones and Rothenbuhler, 1964; Rothenbuhler, 1964). Following the observation that sealed drone cells containing dead brood were not uncapped, nor was the dead brood removed, we investigated the uncapping and removal response of *A cerana* bees towards freeze-killed sealed brood. In 4 separate experiments, sealed worker and drone brood was killed by deep freezing and the dead brood was returned to the colony. Uncapping of cell caps and the removal of dead larvae and pupae were recorded daily for 5 days and, finally, after 13 days.



**Fig 1.** Artificial infestation of protected *A cerana* worker brood cells with vital *V jacobsoni*. 1: needle; 2: infected cell cap protected by plastic sheet; 3: plastic sheet; 4: wax block to hold needle and sheet.

**Determination by an acaricide  
of *V jacobsoni* populations  
infesting *A cerana* colonies**

The experiments were conducted in private bee-yards in Southern Thailand, Chumporn Province. Most colonies were kept with fixed combs in wooden hives (vol 35–45 l).

The beekeepers do not manipulate or influence their colonies until the collection of honey, therefore the colonies provide an almost natural host condition for the mite population. At 5 bee-yards in 4 different locations, 64 colonies were treated with Fluvalinate (Mavric) plywood inserts (Lubinevski *et al.*, 1988). The inserts were either attached to the outer broodcomb by the use of a fine bamboo stick (3 x 70 mm), or placed between the broodcombs in hives with movable frames. On the hive bottom, a plastic coated paper (30 x 31 cm) with a thin film of soya oil was used to trap the disabled mites. The paper inlays were checked after 48 h to determine the number of mites that lived phoretically on adult bees and after 14 days for the mites that had emerged with the bee brood. The presence of drones in the colony and drone cell caps in the debris was recorded and the population of adult bees estimated.

**RESULTS**

**Artificial infestation of *A cerana* worker  
and drone brood cells  
with live and dead *V jacobsoni* mites**

The artificially infested worker cells were detected to a high degree by the *A cerana* workers, uncapped and the infested brood removed (table I). The workers performed brood removal by eating the larvae/pupae in a form of brood cannibalism. Complete discarded bodies were not observed. The control groups remained almost untouched. Fifty percent of the artificially infested brood had been removed by the second and third day of the observation period. After 96 h > 90% of the infested brood was removed by the *A cerana* worker bees. Three cells that remained sealed after 140 h were found not to be infested. In 2 of 3 cells that were uncapped and containing live pupae after 6 days, several mites were found hiding at the cell bottom

**Table I.** Removal response of *A cerana* towards worker brood in cells artificially infested with live *V jacobsoni* mites.

| Day | 1 mite/cell (n = 105)<br>(%) Brood removed |      | Control cells (n = 107)<br>(%) Brood removed |     |
|-----|--|------|--|-----|
|     | Mean                                       | SD   | Mean   | SD  |
| 1   | 36.6                                       | 14.7 | 9.4  | 2.2 |
| 2   | 48.0                                       | 11.6 | 11.8   | 1.4 |
| 3   | 78.3                                       | 8.5  | 11.8   | 1.4 |
| 4   | 93.5                                       | 4.9  | 12.5   | 2.2 |
| 5   | 97.4                                       | 3.7  | 12.5   | 2.2 |
| * 6 | 98.8                                       | 1.7  | 12.5   | 2.2 |

Mean : Mean (%) of 3 experiments. SD : Standard deviation of 3 experiments. \* : (%) Brood removed including brood with removed mites only.

under the pupae. In one of these 2 cells we found 3 adult *V jacobsoni*, one egg, and 2 female deutonymphs. The control groups remained relatively undisturbed; only 13% of the pupae in the control cells had been removed. After the second day following infestation the removal of infested brood differed significantly ( $P < 0.05$ ) from the control cells; after 3 days the difference became highly significant ( $P < 0.01$ ).

The removal response of *A cerana* bees towards brood artificially infested with dead mites was similar to the removal response towards brood artificially infested with live *V jacobsoni* (table II). In each of 3 experiments we infested 10–15 cells with 2 mites. Within 24 h the bees removed the brood from double infested cells at a higher percentage than the single infested brood (81.7% versus 60.8%, significant at ( $P < 0.05$ )). The *A cerana* workers removed the mite bodies from 37 cells that were artificially infested with dead mites, leaving the brood unharmed and sealing the cells again. The removal rate in the control groups was low, with an average of 12.2% after 5 days.

In 3 *A cerana* colonies a total of 60 drone brood cells were artificially infested with one mite each; 39 manipulated but not artificially infested cells served as a control group. During the 5-day observation period, the *A cerana* workers uncapped 15.8% of the artificially infested and 43.6% of the control cells and removed the drone brood (table III). The high rate of removal in the control groups appeared in 2 colonies that were in a state of low drone acceptance; this probably disturbed the experiment. Five control cells were found to be naturally infested with *V jacobsoni*.

In spite of the protection sheets, the *A cerana* worker bees removed artificially infested brood through the side walls without uncapping the cell cap. In 2 experiments with 31 artificially infested cells with protected cell caps, the brood and mites of 20 cells had been removed through the side wall. The pupae in the 11 cells of this experiment that remained protected from access bees developed well, and the mites remained alive after 8 days. Reproducing mites were not observed. The worker bees accepted sealed brood cells that were not artificially infested but which were located

**Table II.** Removal response of *A cerana* towards worker brood in cells artificially infested with dead *V jacobsoni* mites.

| Day | 1 mite/cell (n = 148)     |      | 2 mites/cell (n = 37)     |     | Control cells (n = 149)   |     |
|-----|---------------------------|------|---------------------------|-----|---------------------------|-----|
|     | (%) brood removed<br>Mean | SD   | (%) brood removed<br>Mean | SD  | (%) brood removed<br>Mean | SD  |
| 1   | 60.8                      | 12.2 | 81.7                      | 7.6 | 5.0                       | 3.8 |
| 2   | 60.8                      | 12.2 | 81.7                      | 7.6 | 10.0                      | 7.8 |
| 3   | 61.4                      | 11.7 | 81.7                      | 7.6 | 10.7                      | 7.7 |
| 4   | 85.3                      | 7.0  | 81.7                      | 7.6 | 10.7                      | 7.7 |
| * 5 | 91.9                      | 8.2  | 95.0                      | 4.1 | 12.2                      | 9.6 |

Mean: Mean (%) of 3 experiments. SD : Standard deviation of 3 experiments. \*: (%) Brood removed including brood with removed mites only.

**Table III.** Removal response of *A cerana* towards drone brood in cells artificially infested with live *V jacobsoni* mites.

| Day | 1 mite/cell (n = 60)<br>(%) brood removed |      | Control cells (n = 39)<br>(%) brood removed |      |
|-----|---|------|---|------|
|     | Mean                                      | SD   | Mean  | SD   |
| 1   | 8.3                                       | 6.1  | 8.1   | 6.8  |
| 2   | 11.7                                      | 10.5 | 16.2  | 18.0 |
| 3   | 18.3                                      | 14.3 | 32.4  | 23.6 |
| 4   | 20.0                                      | 14.3 | 35.1  | 25.8 |
| 5   | 25.0                                      | 15.8 | 45.9  | 33.6 |

Mean: Mean (%) of 3 experiments. SD: Standard Deviation of 3 experiments.

under the protection cover. It seems that the *A cerana* workers are able to perceive a stimulus from a mite infested worker cell even if the access to the cell cap is prohibited. The removal response towards mite infested sealed worker brood does not necessarily include the uncapping of the cell cap; it can also be performed by dismantling the side wall.

#### **Evaluation of dead sealed drone brood and removal response of *A cerana* to freeze-killed sealed brood**

Most of the drones from the 332 cells evaluated died from a mycosis with symptoms very similar to those of chalkbrood (caused by *Ascosphaera apis*). The fungus species infesting the dead drones was not determined. Chalkbrood has been introduced to Thailand during the last 3 years and is a serious pest infesting *A mellifera* colonies in Northern Thailand. It is possible that we did not detect mites trapped within the mummies' mycelium. Several drones that died in their sealed cells showed no signs of mite infection or disease.

Highly selective hygienic behavior was observed in *A cerana* bees towards dead sealed drone and worker brood. In the removal response to freeze-killed worker brood, *A cerana* workers uncapped 98.3% of the cells and removed 81.5% of the sealed dead worker brood within 24 h (table IV). After 41 h all dead worker brood had been removed. Unlike the fast uncapping and removal response towards dead sealed worker brood the *A cerana* workers took 5 days to remove 25% of the freeze-killed sealed drone brood, and after 13 days only 34.7% of the dead sealed drone brood had been removed.

#### **Determination by an acaricide of *V jacobsoni* populations infesting *A cerana* colonies**

The highest *V jacobsoni* infestation (798 mites) was found in a very strong *A cerana* colony containing adult drones and queen cells. At 4 locations, colonies with low infestations (20 mites) were found in apiaries with colonies infested with more than 150–250 mites (table V). Colonies with adult

**Table IV.** Uncapping and removal response of *A. cerana* towards freeze-killed sealed drone and worker brood.

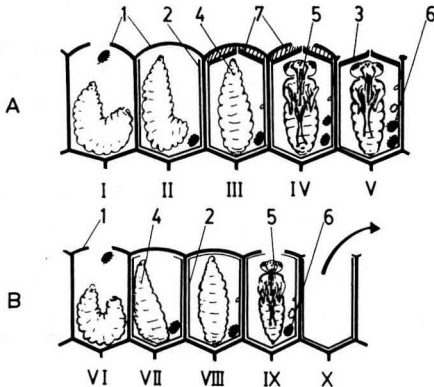
| Hours               | 0   | 24  | 48  | 72  | 96  | 120 | 312 |
|---------------------|-----|-----|-----|-----|-----|-----|-----|
| <b>Drone brood</b>  |     |     |     |     |     |     |     |
| (n) Sealed          | 346 | 281 | 268 | 261 | 257 | 257 | 226 |
| (n) Removed         | 0   | 65  | 78  | 85  | 89  | 89  | 120 |
| (%) Removed         | 0   | 19  | 23  | 25  | 26  | 26  | 35  |
| <b>Worker brood</b> |     |     |     |     |     |     |     |
| (n) Sealed          | 286 | 10  | 0   |     |     |     |     |
| (n) Uncapped        | 0   | 286 | 286 |     |     |     |     |
| (n) Removed         | 0   | 233 | 286 |     |     |     |     |
| (%) Removed         | 0   | 82  | 100 |     |     |     |     |

Data from 2 experiments with drone and worker brood respectively.

**Table V.** *V. jacobsoni* populations infesting *A. cerana* colonies in South Thailand, determined by acaricide treatment.

| Beeyard   | I    | II   | III  | IV    | V    | Total |
|---|------|------|------|-------|------|-------|
| (n) Colonies treated:                                 | 15   | 19   | 10   | 10    | 10   | 64    |
| (n) Colonies with adult or emerging drones            | 5    | 11   | 10   | 7     | 5    | 38    |
| <b>Mites/colony in the debris after treatment</b>     |      |      |      |       |      |       |
| (n) mites after 48 h:                                 | 10.5 | 39.3 | 67.6 | 155.1 | 78.9 | 61.2  |
| (n) mites after 14d:                                  | 2.4  | 13.5 | 2.9  | 16.2  | 10.7 | 9.2   |
| (n) mites total:                                      | 12.9 | 52.8 | 70.5 | 171.2 | 89.9 | 70.5  |
| SD:   | 16.1 | 51.4 | 77.5 | 239.7 | 63.9 | 117.9 |
| Maximum (n) of mites:                                 | 53   | 157  | 269  | 798   | 235  |       |
| <b>Mites/colony, adult or emerging drones present</b> |      |      |      |       |      |       |
| (n) mites total:                                      | 27.4 | 72.9 | 70.5 | 235.9 | 79.4 | 97.2  |
| SD:   | 20.3 | 48.6 | 77.5 | 260.9 | 40.0 | 140.2 |

SD : Standard deviation



**Fig 2.** Uncapping and removal response of *A cerana* bees towards sealed mite infested drone and worker brood. **A** : Drone brood. **B** : Worker brood. 1: Wax; 2: Cocoon. 3: Drone cocoon cap. 4: Larva. 5: Pupa. 6: Mite and offspring. 7: Fluffy cocoon threads. I-IV: Successive stages of drone brood. I: Fifth instar larva and invading mite. II: Cell sealed with wax cap. III: Cocoon completed. IV: Uncapping of the wax cap. V: Cocoon cap remains; *V jacobsoni* reproduces undistributed. VI-X : Successive stages of worker brood. VI: Fifth instar larva and invading mite. VII: Cell sealed with wax cap. VIII: Cocoon completed. IX: Simultaneous uncapping of the wax and cocoon cap. X: Removal of the worker brood; *V jacobsoni* cannot reproduce.

drones proved to host higher *V jacobsoni* populations. Infestations of > 100 mites were found in weak as well as in very strong *A cerana* colonies. The majority of the evaluated colonies (50 of 64) had *V jacobsoni* populations of < 100 mites. The number of mites per colony counted at the second check (14 days after treatment) was rather low compared to the first check (48 h after treatment). When caps of drone cells were detected in the debris during the second check, the number of mites in several cases surpassed the mites counted 48 h after treatment. Only in one treated colony were no *V jacobsoni* found. Several colonies were highly infested with the mite *Neocyphoelaelaps* sp.

## DISCUSSION

Our observations on the removal response of *A cerana* bees towards sealed infested worker brood support the results of Peng *et al* (1987b). *A cerana* bees even removed sealed worker brood when artificially infested with dead *V jacobsoni*. The brood was accepted when the bees were able to remove the infesting mites from the brood cells. The nature of the stimulus given by a mite or by dead brood within a sealed worker cell is unknown. However, it was found to be strong enough to convert the nursing behavior of bees to an aggressive attitude, including the removal of the brood by cannibalism. Schulz-Langer (1960) investigated the stimulus that causes *A mellifera* bees to remove dead Foulbrood infested larvae and suggested that the nature of this stimulus is physical (vibrations) rather than olfactory. Corresponding to the situation with infested sealed brood, we found highly differentiated hygienic behavior of *A cerana* towards dead sealed worker and drone brood. We suppose that the ability of the *A cerana* workers to detect and remove infested or dead sealed brood is influenced by the structure of the brood cell cap (Hänel and Ruttner, 1985). It seems that the worker bees are not inclined to open the thick drone cocoon cap even when the developing brood has already died (fig 1). The removal behavior towards mite infested worker brood may be genetically determined.

DeJong (1988) reported on *V jacobsoni* reproducing in sealed worker *A cerana* brood cells but did not find adult offspring of the mites in worker cells; this is probably related to the described removal response. In laboratory experiments not reported in this paper, we reared offspring from *V jacobsoni* mites on *A cerana* worker and drone brood to the adult stage (Rath,

1990). In these experiments and in the observations reported in this paper, we used *A mellifera* colonies as the mite source. It is not clear how far the previous feeding on *A mellifera* bees and brood influenced reproduction. We suppose that under natural conditions successful reproduction of *V jacobsoni* on *A cerana* worker brood is an exceptional case which does not contribute to the population dynamics of the mite population.

Chalkbrood has not yet been reported from Thailand (Bradbear, 1989) but chalkbrood-like symptoms in *A mellifera* colonies have become a very serious problem in the last 3 years. The consequences of the apparently introduced pest to *A cerana* colonies and the interactions with mite infestations are uncertain.

The infestation of *A cerana* colonies with *V jacobsoni*, determined by acaricide treatment was found to vary widely among the colonies within beeyards and between different apiaries. Limitation of *V jacobsoni* reproduction to drone brood would lead to a population peak of *V jacobsoni* during the swarming period of a colony. The dispersal of *V jacobsoni* to other host colonies, with drones serving as vector should therefore occur during the population maximum of the mites. Although the survival of *A cerana* colonies does not seem to be affected by mite parasitism, the drone brood might suffer from some damage or reduced vitality (Schneider and Drescher, 1987).

**Résumé — Réaction d'*Apis cerana* Fabr face à du couvain infesté par *Varroa jacobsoni* et taux d'infestation des colonies en Thaïlande.** L'abeille asiatique *Apis cerana* Fabr passe pour résistante à l'acarien *Varroa jacobsoni* Oud (Peng *et al.*, 1987a). Cette étude faite en Thaïlande présente quelques aspects de l'équilibre

hôte-parasite (*V jacobsoni* – *A cerana*). Des colonies d'*A cerana* dans des ruches à hausses multiples et des ruches fixes situées à Chiang Mai (Nord de la Thaïlande) ont été utilisées. Les expériences ont porté sur les points suivants : la possibilité de survie de *V jacobsoni* et sa reproduction sur du couvain d'*A cerana*, le comportement des ouvrières d'*A cerana* vis-à-vis du couvain infesté et du couvain operculé mort et le niveau d'infestation naturelle des colonies d'*A cerana* par *V jacobsoni*.

La possibilité de survie de *V jacobsoni* dans les cellules d'ouvrières d'*A cerana* a été testée sur des cellules infestées artificiellement et protégées du nettoyage par les abeilles. *V jacobsoni* a survécu plus de 20 j au laboratoire et plus de 6 j au sein d'une colonie sur du couvain d'ouvrières. Au cours de 3 expériences, 105 cellules d'ouvrières fraîchement operculées ont été ouvertes et infestées chacune avec un varroa vivant. Les cellules témoins ont été simplement ouvertes, et dans les 2 cas, les opercules ont été refermés. On a enregistré les jours suivants les modifications apportées aux cellules et au couvain. De la même façon on a infesté 185 cellules d'ouvrières avec 1 acarien mort et 37 cellules avec 2 acariens morts, ainsi que 60 cellules de mâles avec un acarien vivant. Afin de tester le comportement hygiénique d'*A cerana*, du couvain operculé d'ouvrières et de mâles a été tué par congélation, replacé dans les colonies et le nombre de larves mortes évacués compté journellement.

Les abeilles ont désoperculé plus de 98% des cellules d'ouvrières artificiellement infestées avec des acariens vivants en 5 j et évacué le couvain (tableau I). Elles ont écarté le couvain infesté en le mangeant (cannibalisme). Le couvain infesté avec des acariens morts a également été évacué à plus de 91% ou bien les abeilles ont désoperculé les cellules et éloigné les



acariens du couvain (tableau II). Environ 12,5% des cellules témoins ont été nettoyé; la différence avec le couvain infesté est hautement significative ( $P < 0,01$ ) à partir du 3<sup>e</sup> j. Les ouvrières ont réagi aux cellules de mâles infestées artificiellement en les nettoyant à 25%; aucune différence avec le groupe témoin n'a pu être mise en évidence (tableau III). Le couvain mort et operculé d'ouvrières a été totalement évacué en 48 h, tandis que celui de mâles n'était évacué qu'à 34% au bout de 13 j (tableau IV). Dans le Sud de la Thaïlande, 64 colonies d'*A cerana*, réparties en 5 ruchers, la plupart dans des ruches fixes et ne faisant pas l'objet de manipulations apicoles, ont été traitées avec l'acaricide fluralinate (Lubinevski *et al*, 1988) (tableau V). Les acariens tués ont été récoltés sur un lange enduit de graisse et comptés au bout de 48 h et 13 j. Le niveau moyen d'infestation était de 70,5 acariens par colonie, avec un maximum de 789 et un minimum de 0 dans une ruche.

*A cerana* est capable de reconnaître le couvain d'ouvrières operculé et infesté par *V jacobsoni* et réagit en l'évacuant. Les acariens prêts à se reproduire sont limités au couvain de mâles. Le couvain de mâles infesté ou mort est peu éliminé. Nous pensons que la structure particulière de l'opercule du cocon de mâle joue un rôle décisif dans le comportement différent de perception et de réaction des ouvrières d'*A cerana* (Hänel et Ruttner, 1985). Le couvain d'ouvrières d'*A cerana* offre une possibilité de survie suffisante à *V jacobsoni*. Dans des études au laboratoire, qui ne sont pas décrites précisément ici, des acariens récoltés dans des colonies d'*A mellifera* se sont reproduits sur du couvain d'ouvrières d'*A cerana* et les descendants se sont développés jusqu'au stade adulte. Les colonies d'*A cerana* dans le Sud de la Thaïlande ont été presque sans exception infestées par *V jacobsoni*. L'infestation

maximale rencontrée (798 acariens) montre bien la capacité de reproduction de *V jacobsoni* sur son hôte naturel, *A cerana*.

***Apis cerana* / *Varroa jacobsoni* / résistance / comportement hygiénique / infestation naturelle / Thaïlande**

**Zusammenfassung — Reaktion von *Apis cerana* Fabr auf mit *Varroa jacobsoni* Oud infizierte Brut und Infektionsrate bei Völkern in Thailand.** Die asiatische Honigbiene *Apis cerana* Fabr gilt als resistent gegenüber der parasitischen Milbe *Varroa jacobsoni* Oud (Peng *et al*, 1987a). Die vorliegende in Thailand durchgeführte Untersuchung beschreibt einige Aspekte des Parasiten-Wirt Gleichgewichtes (*V jacobsoni* – *A cerana*). *A cerana* Bienenvölker in Magazinbeuten und Stablbau standen in Chiang Mai (Nord Thailand) und Chumporn (Süd Thailand) für die Untersuchungen zur Verfügung. Die Experimente bezogen sich auf die Überlebenschmöglichkeit und Reproduktion von *V jacobsoni* auf *A cerana* Arbeiterinnenbrut, auf das Verhalten von *A cerana* Arbeiterinnen gegenüber milbeninfizierter und abgestorbener verdeckelter Brut und auf das "natürliche" Befallsniveau von *A cerana* Völkern mit *V jacobsoni*.

Die Überlebenschmöglichkeit von *V jacobsoni* in *A cerana* Arbeiterinnenzellen wurde mit künstlich infizierten und gegen Ausräumen geschützten Zellen überprüft. *V jacobsoni* überlebte im Labor mehr als zwanzig Tage und im Volk mehr als sechs Tage auf Arbeiterinnenbrut. In 3 Experimenten wurden 105 frisch verdeckelte Arbeiterinnenzellen geöffnet und mit je einer lebenden *V jacobsoni* infiziert. Kontrollzellen wurden lediglich geöffnet. Die manipulierten Zelledekel wurden wieder angedrückt und in den folgenden Tagen Veränderungen an den Zellen und der Brut

registriert. Unter Anwendung der gleichen Methoden wurden in je drei Experimenten 185 Arbeiterinnenzellen mit einer und 37 Zellen mit zwei toten Milben sowie 60 Drohnenzellen mit lebenden Milben infiziert. Zur Überprüfung des Nestsäuberungs-Verhaltens bei *A cerana* wurde gedeckelte Arbeiterinnen- und Drohnenbrut durch Tiefgefrieren abgetötet, den Völkern zurückgegeben und die Zahl der ausgeräumten toten Brut täglich ermittelt.

Die *A cerana* Bienen entdeckelten die künstlich mit lebenden Milben infizierten Arbeiterinnenzellen zu über 98% nach fünf Tagen und räumten die Brut aus (Tabelle I). Die Bienen beseitigten die infizierte Brut durch Auffressen (Brutkannibalismus). Auch die mit toten Milben infizierte Brut wurde zu über 91% ausgeräumt oder die Bienen entdeckelten die Zellen und entfernten die Milben von der Brut (Tabelle II). Die Kontrollzellen wurden zu etwa 12,5% ausgeräumt, der Unterschied zu den infizierten Zellen war ab dem dritten Versuchstag hoch signifikant ( $P < 0,01$ ). Die Arbeiterinnen reagierten auf künstlich infizierte Drohnenzellen mit Ausräumen von 25% der infizierten Zellen, ein Unterschied zur Kontrollgruppe war nicht festzustellen (Tabelle III). Tote gedeckelte Arbeiterinnenbrut war nach 48 Stunden komplett entfernt, während tote gedeckelte Drohnenbrut nach 13 Tagen zu 34% ausgeräumt war (Tabelle IV). In Südthailand wurden an fünf Standorten 64 meist in Stablbau und ohne imkerliche Beeinflussung gehaltene *A cerana* Völker mit dem Acarizid Fluvalinat behandelt (Lubinevski *et al*, 1988) (Tabelle V). Die abfallenden Milben wurden mit gefetteten Bodeneinlagen gesammelt und nach 48 Stunden bzw 13 Tagen ausgezählt. Das durchschnittliche Befallsniveau lag bei 70,5 *V jacobsoni* je Volk mit einem Höchstbefall von 789 Milben, lediglich bei einem Volk wurden keine Milben gefunden.

*A cerana* Bienen sind befähigt, *V jacobsoni* infizierte gedeckelte Arbeiterinnenbrut zu erkennen und reagieren mit Ausräumen der Arbeiterinnenbrut. Damit werden reproduktionswillige Milben auf Drohnenbrut beschränkt. Infizierte oder abgestorbene Drohnenbrut wird kaum entfernt. Wir vermuten, daß bei dem unterschiedlichen Wahrnehmungs- und Reaktionsverhalten der *A cerana* Arbeiterinnen der speziellen Struktur des Drohnenkokondeckels eine entscheidende Rolle zukommt (Hänel und Ruttner, 1985). Die *A cerana* Arbeiterinnenbrut bietet ausreichende Überlebenschancen für *V jacobsoni*. In Laborversuchen, die an dieser Stelle nicht näher beschrieben werden, reproduzierten aus *A mellifera* Völkern gesammelte Milben auf *A cerana* Arbeiterinnenbrut und die Nachkommen entwickelten sich zu adulten Milben. *A cerana* Bienenvölker in Südthailand waren fast ausnahmslos mit *V jacobsoni* infiziert, der festgestellte Maximalbefall mit 798 Milben verdeutlicht die Vermehrungskapazität von *V jacobsoni* auf dem natürlichen Wirt *A cerana*.

#### ***Apis cerana* / Varroa-Resistenz / Putzverhalten / natürlicher Befall / Thailand**

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