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Original article

Infestation of queen cells by the mite *Varroa jacobsoni*

PC Harizanis *

Technological Education Institute, School of Agriculture, TEI 541 01 Thessaloniki, Greece

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Summary — Infestation of queen cells of the honey bee *(Apis mellifera macedonica)* by the mite *Varroa jacobsoni* was studied. A total of 1 500 larvae were grafted into queenless cell builders: first, when they contained worker brood of all ages; second, when they had only sealed brood; and third, when they had no brood. Ten days after grafting, the sealed queen cells were opened and examined for *Varroa* mites. The percent acceptance of the grafted larvae was not related to the degree of colony infestation, when rearing conditions were otherwise kept similar. No mites entered queen cells in lightly infested colonies. More mites entered queen cells in heavily infested colonies that contained no worker or drone brood.

***Varroa jacobsoni* / queen cell / infestation / grafting**

INTRODUCTION

Moritz (1985) reported that the duration of the postcapping stage of worker brood plays an important role in the successful reproduction of *Varroa* mite and can be a resistance character that might be susceptible to genetic selection. *Varroa* mites are more successful with races of bees like *Apis mellifera carnica* that have a postcapping stage of 12.1 days for worker brood than with races of bees like *A mellifera capensis* that have a shorter postcapping stage (*ie* 9.7 days). In the latter race, for example, the development of *Varroa jacobsoni* is apparently restricted, and only 21% of all mites produce offspring (Moritz and Hänel, 1984). Since the postcapping stage of queen cells is shorter than the developmental time of the mite, experiments were conducted to determine if *Varroa* mites would enter and attempt to reproduce in queen cells.

This paper reports on mite infestation of queen cells under different brood conditions in the same cell builders. Colony acceptance of grafted cells is also discussed.

MATERIALS AND METHODS

This research was conducted in Thessaloniki, Greece, using colonies of *A mellifera macedonica* infested with the *Varroa* mite. All the colonies were started with sister queens and were

* Correspondence and reprints: Idipodos 12, 54632 Thessaloniki, Greece

given no treatment to control *Varroa* mite. The queens were removed and the cell builders were set up as populous, single chamber hives containing 9 frames. Drone brood was removed or destroyed. One-day old larvae, from an additional sister queen colony, were dry grafted into cell cups. Prior to grafting, all cell builders were fed with sugar syrup (50:50 w/w).

Ages of mite progeny were estimated according to the description of Ifantidis (1983). Since the purpose of this study was not to investigate the effect of presence of *Varroa* mite on queen cell development, controls (cell builders without *Varroa* mite) were not included. The research consisted of 2 experiments.

Experiment 1

The aim of this experiment was to examine the effect of colony infestation on queen cell acceptance (development) and queen cell infestation. The experiment included 10 cell builders with different percentage of *Varroa* infestation, which were randomly arranged and used for 2 different graftings.

The cell builders were prepared as described above. One day before grafting, the queens and all open (unsealed) brood were removed. The grafted cells were placed in the middle frame of each cell builder next to a comb with pollen and honey. On one outer side of the cell builder there was a feeder and a comb of honey and on the other outer side, a comb with honey. The rest of the combs contained only sealed brood. A total of 600 larvae were grafted (30 larvae per colony at each grafting) into the cell builders, half of them on April 25th and the other half on May 10th. Ten days after each grafting, the sealed queen cells were opened and examined for the presence of adult and immature mites. Queen cell infestation is defined here as number of mites per 100 sealed queen cells examined per colony. The royal jelly in the queen cells was examined because mites occasionally became trapped there.

Before the second graft, 2 frames of sealed brood were added to each cell builder.

After examination of the second batch of queen cells the level of colony infestation (number of mites per 100 bees) was determined. Since the cell builders were broodless, the colony infestation was determined from adult bees. The bees were brushed from both sides of every frame through a funnel into a jar containing 25% alcohol. Approximately 400 bees from each cell builder were sampled. These bees were stored in the jar for 24 h and then shaken for *ca* half and hour prior to counting the mites. Each sample was rinsed with tap water on a sieve with a grid large enough to permit the mites to pass through, but which retained the bees. Mites were counted from the wash. For data analysis, the *t*-test was carried out in order to compare the differences between the 2 grafting means of queen cell infestation and queen cell acceptance (Snedecor and Cochran, 1980). Linear regression was also performed to see if colony infestation was correlated to queen cell acceptance for each of the 2 graftings.

Experiment 2

The aim of this experiment was to examine the effect of worker brood presence on queen cell infestation by *Varroa* mite. The experiment included 3 treatments (3 brood conditions) with 6 replicates (cell builders) in a randomized complete block design. Each replicate had 50 queen cells.

Six mite-infested colonies with sister queens were used as cell builders during August. One day before grafting, the queens were removed and the frames of brood were arranged as shown in figure 1. No open (unsealed) brood was removed. This frame arrangement holds true only for the first graft. Three consecutive grafts were given to each cell builder. A total of 900 larvae were grafted (*ie*, 50 larvae per cell builder each time). Ten days after grafting, the sealed queen cells were opened and examined for presence of adult and immature *Varroa* mites. The first graft was introduced one day after removal of the queens. During the development of these queen cells, there was brood of all ages in the cell builders. The second graft was introduced one day after the examination of the first queen cells. During the development of the second set of queen cells, there was only sealed brood in the cell builders. The third graft was introduced one day after the examination of the second queen cells. The queen cells of the third graft developed in cell builders that had no open or sealed brood. At the end of the experi-

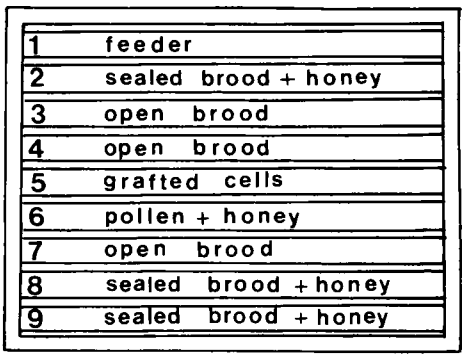

Fig 1. Arrangement of the frames in the one-story cell builders prepared for experiment 2.

ment, the level of colony infestation was estimated as described for experiment 1. Again here queen cell infestation is defined as number of mites per 100 sealed queen cells examined per colony. The data were $\sqrt{x + 1}$ transformed before, the 1-way analysis of variance was performed. Also linear regression was performed to see if colony infestation is correlated to queen cell infestation, or queen cell acceptance of each brood condition separately.

RESULTS

The development (acceptance) of the queen cells was not significantly affected by brood condition and colony infestation.

Excess royal jelly was found in 85% of the queen cells examined in spring and late summer. Of all the mites found in the queen cells, only 2 adult females were trapped in the royal jelly.

Experiment 1

In 401 developed queen cells, 2 adult *Varroa* mites were found in 2 cells from the first graft and 3 from 3 cells of the second graft, and only in the colony with the heaviest infestation (40.3%). No immature mites were seen. Percentage of queen cell development (acceptance), percentage of queen cells infested, and colony infestation are shown in table I. When colony infestation data were regressed against percentage of queen cell development the correlation coefficient found were very low (0.51 and 0.45 for the 2 graftings respectively).

Experiment 2

Percentage of queen cell development, percentage of queen cell infestation, and colony infestation are shown in table II. In the first 2 grafts, *ie* when the cell builders had brood of all ages or when they had only sealed brood, no offspring were detected in queen cells that contained adult mites. In the third graft, *ie* cell builders with no brood, a total of 5 female mites (each in a different queen cell) produced offspring in the 2 most heavily infested colonies. The oldest immature mite was in its first mobile phase (protonymph). In other 4 cases 2 female mites were found in each queen cell in the 2 most heavily infested colonies, but with no offspring.

When colony infestation data were regressed against either queen cell infestation data or queen cell acceptance data, the results indicated that these data were not related (the correlation coefficient found ranged from 0.01–0.63).

DISCUSSION

A queen develops more quickly than a worker or drone, and the time that a queen cell is sealed is ≈ 8 days. According to Ifantidis (1983), it takes ≈ 240 h (10 days) for the first adult female *Varroa* to develop

Table I. Acceptance and infestation levels of queen cells from two different grafts into 10 cell builders. No open brood was present. A total of 600 larvae were grafted (30 larvae per colony at each grafting). The colony infestation (No of mites per 100 bees) was estimated at the end of the experiment.

Hive No	*1st graft*		*2nd graft*		
	A	*B*	*A*	*B*	*Colony infestation*
1	56.7	0	56.7	0	3.8
2	73.3	0	76.7	0	5.8
3	66.7	0	60.0	0	5.9
4	50.0	0	46.7	0	6.5
5	60.0	0	60.0	0	6.6
6	63.3	0	63.3	0	7.3
7	53.3	0	50.0	0	10.7
8	93.3	0	96.7	0	11.3
9	66.7	0	76.7	0	12.5
10	80.0	8.3	86.7	11.5	40.3
$\bar{X} \pm SE$	66.3 ± 12.4	1.0	67.3 ± 15.41	1.5	11.03 ± 10.09

A: Percent of queen cells developed (accepted). B: Percent of queen cells infested.

Table II. Acceptance and infestation levels of queen cells from 3 consecutive grafts in 6 cell builders. A total of 900 larvae were grafted (50 larvae per colony at each grafting). The colony infestation (number of mites per 100 bees) was estimated at the end of the experiment.

Hive No	*Cell builders with brood of all ages*		*Cell builders with only sealed brood*		*Cell builders with no brood*		
	A	*B*	*A*	*B*	*A*	*B*	*Colony infestation*
1	88	2.3	82	2.4	82	4.9	19.5
2	74	2.7	76	2.8	72	5.6	23.5
3	78	0	80	5.0	78	2.6	28.5
4	76	2.6	66	6.1	78	2.5	31.0
5	78	2.6	76	5.3	74	24.3	35.0
6	80	2.5	74	2.7	76	15.8	42.1
$\bar{X} \pm SE$	79 ± 4.4	2.1 ± 1	75.7 ± 5.1	4 ± 1.5	76.7 ± 3.2	9.1 ± 8.1	29.9 ± 7.4
Means*		1.733^{a}		2.200^{ab}		2.983^{b}	

A: Percent of queen cells developed (accepted). B: Percent of queen cells infested. * Mean values are $\sqrt{x+1}$, while the rest of the values are real data. Means followed by the same letter are not significant at $P < 0.05$.

(from egg to adult) after the cell is capped. Other researchers reported longer developmental times (Choi and Woo, 1973; Smirnov, 1978; Sakai *et al,* 1979). It is of no evolutionary advantage for *Varroa* mites to try to reproduce in queen cells, because none of their progeny can reach adulthood. Also, it seems logical, from an evolutionary point of view, for female mites to "prefer" drone cells since they will have extra days (compared with worker brood) to lay more eggs and to produce more progeny which will reach the adult stage.

Few female mites utilized developing queens for hosts. Only 1–2% of spring cells and late summer cells from cell builders containing open brood were infested. With the absence of open brood in the late summer, queen cell infestation levels climbed from 4% to 9.1% (sealed brood, no brood, respectively). So in the absence of preferred hosts, at least a portion of the mite population attempts to reproduce in queen cells. This trait appears to be maladapted since Romaniuk *et al* (1988) showed that the development cycle of *V jacobsoni* cannot be completed in queen cells.

The percent development of grafted larvae was not significantly different at the various levels of infestation, when all other queen rearing factors were kept similar. So it appears that *Varroa* mites may not interfere with quality queen production in commercial operations.

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Résumé — Infestation des cellules royales par l'acarien *Varroa jacobsoni*. L'infestation par *Varroa jacobsoni* de cellules royales de l'abeille *Apis mellifera macedonica* a été étudiée. Des colonies très peuplées, infestées par le parasite, ont été utilisées comme éleveuses. Les ruches ne comportaient qu'un seul corps, contenant 9 cadres, et les reines étaient sœurs. La disposition des cadres est montrée dans la figure 1. Le couvain de mâles qui était présent a été enlevé ou détruit. Dans les 2 expériences (printemps et fin d'été) 1 500 larves ont été greffées dans des colonies éleveuses sans reine: premièrement, en présence de couvain d'ouvrière de tous âges; deuxièmement, en présence de couvain operculé; et troisièmement, en l'absence de couvain. Dix jours après le greffage, les cellules de reines operculées ont été ouvertes, et les *varroa* ont été recherchés. À la fin de chaque expérience, le taux d'infestation de la colonie a été déterminé sur les abeilles adultes.

Le développement des cellules royales n'a pas été significativement affecté par l'état du couvain et l'infestation de la colonie. Sur les 1 093 cellules royales qui ont été examinées, 5 *Varroa* adultes ont été trouvés au printemps, et 40 à la fin de l'été. Seuls 5 acariens (chacun dans une cellule différente) ont produit des descendants, dont le plus vieux a atteint la première phase mobile (protonymphe).

Très peu de femelles d'acariens ont utilisé des reines comme hôtes. Seulement 1–2% des cellules de printemps et de fin d'été des ruches contenant du couvain ouvert ont été infestées. En l'absence de couvain ouvert, en fin d'été, le niveau d'infestation augmente de 4 à 9,1% (respectivement avec du couvain operculé et sans couvain). On peut donc conclure que l'acarien *Varroa* n'interfère pas avec la production des reines de qualité destinées à la vente.

***Varroa jacobsoni* / cellules royales / infestation / greffage**

Zusammenfassung — Befall von Weiselzellen durch die Milbe *Varroa jacobsoni*. Es wurde der Befall von Weiselzellen der Honigbiene *Apis mellifera macedonica* durch *Varroa jacobsoni* untersucht. Als Zuchtvölker wurden starke, von Milben befallene Völker benutzt. Es handelte sich um einräumige Magazine mit neun Rähmchen, besetzt mit Geschwisterköniginnen. Die Anordnung der Rähmchen wird in Abbildung 1 gezeigt. Vorhandene Drohnenbrut wurde entfernt oder zerstört. In den beiden Versuchen (Frühjahr und Spätsommer, wurden 1500 Larven in weisellose Pflegevölker eingesetzt: erstens, als sie noch Arbeiterbrut aller Stadien enthielten; zweitens, als sie nur noch verdeckelte Brut besaßen; und schließlich drittens, als sie überhaupt keine Brut mehr hatten. Zehn Tage nach dem Umlarven wurden die verdeckelten Zellen geöffnet und auf das Vorhandensein von Varroa-Milben untersucht. Die Entwicklung der Weiselzellen wurde durch den Brutstand und den Volksbefall nicht signifikant beeinflußt. In 1093 untersuchten Weiselzellen wurden im 5 Frühjahr erwachsene Milben gefunden und im Spätsommer 40 erwachsene Milben. Nur fünf weibliche Milben aus verschiedenen Zellen erzeugten Nachkommen, wobei die ältesten das erste bewegliche Stadium erreichten (Protonymphen).

Nur wenige weibliche Milben benutzten Entwicklungsstadien von Königinnen als Wirte. Nur 1–2% der Zellen der Pflegevölker im Frühjahr und der Zellen im späten Sommer in Völkern mit offener Brut waren befallen. Nach Verschwinden der offenen Brut im Spätsommer stieg die Befallsrate von 4 auf 9.1% (verdeckelte Brut, bzw keine Brut). Es hat also den Anschein, daß sich der Varroabefall auf die Erzeugung von Qualitätsköniginnen im kommerziellen Betrieben nicht nachteilig auswirkt.

***Apis mellifera* / *Varroa jacobsoni* / Weiselzelle / Befall / Umlarven**

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