

## (Z)-8-Heptadecene reduces the reproduction of *Varroa destructor* in brood cells

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**Abstract** – The alkene (Z)-8-heptadecene, a semiochemical which inhibits the reproduction of *Varroa destructor* was tested in natural comb cells. One-hundred ng of (Z)-8-heptadecene applied under the capping of worker cells, sealed 0–15 h previously, caused a highly significant reduction both in the number of offspring (–12% reduction) and in the number of potentially mated daughters per female (–28% reduction) in single infested cells. No reduction was observed in multiple infested cells. A consistent, but not significant decrease was observed in cells treated at different times after capping. No decrease in mite reproduction was noted in cells treated 0–16 h before capping or 0–6 h after capping. However, infestation of cells treated before capping was reduced by 41%. In single infested cells in the control groups, the number of potentially mated daughters per female decreased from 1.5 in June to 0.78–0.94 in September. The proportion of treated cells emptied by bees was about 30% lower than that of control cells. (Z)-8-Heptadecene may play an important role in the host-parasite relationship.

reproduction / *Varroa destructor* / semiochemical / heptadecene

### 1. INTRODUCTION

Reduced reproduction of *Varroa destructor* Anderson & Trueman (Acarina, Varroidae) is regarded as the most important factor in tolerance of the honey bee towards this parasite. Minor variations in the reproductive rate of the parasitic mite may have a large impact on its population dynamics (Calis et al., 1999) because of the large number of generations per year. A decrease in mite reproduction in cells infested with multiple mites was noted by several authors (Piletskaya, 1982; Moosbeckhofer et al., 1988; Fuchs and Langenbach, 1989; Martin, 1995). Nazzi and Milani (1996) observed this phenomenon under laboratory conditions and showed that it was caused, at least in part, by a substance that could be extracted from infested artificial cells. Among

the substances released in such cells, the unsaturated hydrocarbon (Z)-8-Heptadecene was identified as a compound that reduced the reproduction of *V. destructor* in artificial cells (Nazzi et al., 2002). Repeated tests under laboratory conditions have shown that 100 ng of this compound, applied to the interior of gelatine cells, reduced the total offspring produced by 29%, an effect similar to that observed in cells invaded by three mites.

This inhibitor deserves special interest since it might represent a density dependent factor involved in the population dynamics of *V. destructor*. Density dependent factors are essential for the maintenance of balanced host-parasite relationship. In European bee races, reduced reproduction in multiple infested cells is evidently insufficient to maintain a balance between the host and the parasite at realistic

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infestation levels (Fuchs and Langenbach, 1989), but the situation could be different in other populations of bees and mites.

From a different perspective, heptadecene could be exploited to develop new products that could be applied within bee hives to reduce the population increase of the mite.

The aim of the present work was to assess if heptadecene reduces the reproduction of *V. destructor* under field conditions. In particular, we studied the effects of treatments with heptadecene on the number of female daughters that could reach the adult stage and mate before the bee emerged, and thus could successfully reproduce later. The latter parameter is more relevant for the population dynamics of *V. destructor* than the total offspring generated, since immature offspring die when the bee emerges and unfertilized females remain infertile (Martin et al., 1997).

## 2. MATERIALS AND METHODS

### 2.1. Bee colonies

Bee colonies from the experimental apiaries of the Udine University (north-east of Italy), with naturally mated, unselected queens were used in the experiments, in an area of racial hybridization between *Apis mellifera ligustica* Spinola and *A. m. carnica* Pollman (Comparini and Biasiolo, 1991; Nazzi, 1992).

### 2.2. Experiment 1: treatment 0–15 h after capping

Combs containing brood close to being capped were chosen, and in the evening of the day preceding the treatment the capped cells were marked. The following morning, the comb was brought to the laboratory and groups of worker cells (generally, 5 or 10 cells) capped overnight (thus 0–15 h post capping, 0–15 PC) were treated by injecting 100 ng of (Z)-8-Heptadecene (Fluka Chemie) dissolved in 1 µL acetone. The solution was injected with a microsyringe under the capping, taking care not to hurt the larva. Part of the injected solution often spread over the capping. An equal number of cells was treated with 1 µL of the solvent alone as a control. Groups of control and treated cells were distributed alternately on the surface of the comb and were separated by at least one cell, which was left untouched. The position of the cells used in the experiment was marked on a transparent

sheet. The treatment was carried out within 3 h, then the comb was returned to the hive.

Eleven days later, when the bees were about to emerge, treated combs were brought to the laboratory. Treated and control cells were identified using a transparent sheet. Intact cells and cells uncapped and emptied by bees were counted, then treated and control cells were opened and inspected. When an infested cell was found, the condition of the bee pupa was noted, and mite females and their offspring were mounted on microscope slides in Berlese's medium (Nannelli, 1996). Developmental stages (egg, protonymph, deutonymph, pharate adult, moulted adult) and sex of the offspring were identified at 30–200 magnification; eggs and protonymphs were not sexed.

In particular, the number of offspring and the number of potentially mated daughters, PMD (i.e., the number of adult daughters in cells containing also an adult male, including pharate adults), were considered. Pharate adults, i.e. adults that have not yet shed the cuticle of the deutonymph, were identified by observing the presence of structures of the adult mite enclosed within the old cuticle; in particular, in pharate adults the long setae of the legs are appressed and parallel to the leg itself rather than spreading out in different directions (Fig. 1).

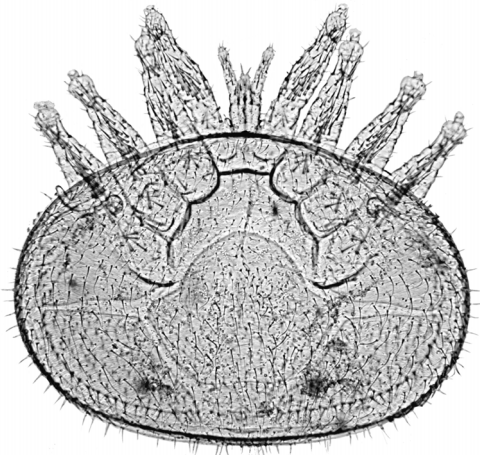
Data on single and multiple infested cells were analysed separately. A few cells (1% of the total), damaged by *Galleria mellonella* (L.) or with abnormal pupae, were not considered; also cells with dead mites were not included in comparisons.

The experiment was carried out four times, in June, July, August and September, in mite infested colonies kept in the experimental apiary of the University. Each time, 100–200 cells on each of two combs were treated (for a total of about 1100 cells) and an equal number of untreated cells were used as controls. The same procedure was also replicated twice (replications 5 and 6), in early and late August, on a population of mites from an apiary maintained without acaricide treatments for three years in an isolated location, on a total of about 225 cells.

The effects of the injection of acetone on the reproduction of *V. destructor* were studied by comparing the reproduction of mites in cells injected with 1 µL acetone and in untouched cells (two replications, with a total of about 240 cells per group).

### 2.3. Experiment 2: treatments at different times after capping

In a second experiment, three combs were marked as in Experiment 1, then brought to the laboratory. Cells capped overnight (0–15 PC) were

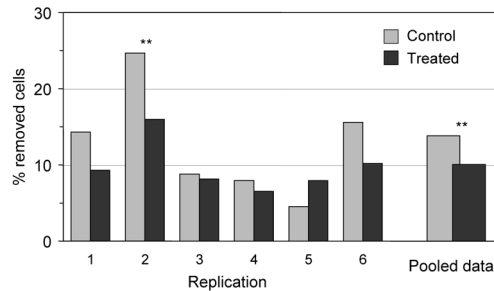


**Figure 1.** Pharate adult female of *V. destructor*. Characters of the adult stage, such as the long setae of the legs, are visible, but these structures are still wrapped in the deutonymphal cuticle.

assigned to one of four groups, C, T1, T2, T3 and treated respectively with 0, 100, 50, 0 ng of (Z)-8-Heptadecene in 1  $\mu$ L acetone. After four days, the combs were brought to the laboratory for a second time and the cells of the groups C, T1, T2, T3 were treated again, with 0, 0, 50, 100 ng heptadecene in 1  $\mu$ L acetone respectively. Each group comprised about 200 cells. Treated and control cells were marked on a transparent sheet and inspected 11 days after capping, as in Experiment 1. The experiment was carried out in August.

#### 2.4. Experiment 3: treatment before and after capping

In a third experiment, cells were treated either shortly before capping or soon after capping. Four combs were chosen and capped cells were marked; 6 h later, the combs were brought to the laboratory. Capped cells (capped 0–6 h before) and cells close to capping were treated with 100 ng (Z)-8-Heptadecene in 1  $\mu$ L acetone; controls received 1  $\mu$ L acetone. The position of controls and treated cells was marked on a transparent sheet. Sixteen hours later, the comb was brought to the laboratory again and the cells that had been capped in the meantime were marked with a different symbol, to identify cells that had received the treatment 0–16 hours before capping. Thus, in this experiment four experimental groups were considered; cells treated 0–16 h before capping and the corresponding controls (respectively TB, CB), and cells treated 0–6 hours after capping and the corresponding controls (TA, CA). The experiment was carried out in September.



**Figure 2.** Experiment 1: Proportion of cells emptied by bees in control and treated groups, for each replication and on pooled data. The asterisks indicate significant differences (\*\*:  $P < 0.01$ ).

Cells were opened and inspected 11 days after the treatment, as above.

#### 2.5. Statistical comparison

Proportions were compared using *G*-tests (with the Williams' correction). Removal of treated and control cells in Experiment 1 was compared with the Mantel-Haenszel test. The number of offspring and that of PMD per foundress female in treated and control groups was compared using a two-sample randomisation test (Manly, 1997, Chap. 6.3). When more than two groups were compared, a one-factor ANOVA (Manly, 1997, Chap. 7.1) was used. The randomization distribution was resampled  $10^6$  times with a computer programme written for this purpose.

### 3. RESULTS

#### 3.1. Experiment 1: treatment 0–15 h after capping

##### 3.1.1. Hygienic behaviour towards treated cells

Bees uncapped and emptied 134 out of 1331 treated cells (10.1%), and 184 out of 1334 control cells (13.8%). This trend was observed in 5 replications out of 6 (Fig. 2). The Mantel-Haenszel test did not reveal heterogeneity among replications ( $P = 0.52$ ), while the difference between control and treated groups was highly significant (Mantel-Haenszel test,  $P = 0.003$ ), with an estimated odds ratio (removal in treated cells/removal in controls) of 0.69.

**Table I.** Average infestation of treated and control cells in each replication.

Replication	Population 1				Population 2	
	1	2	3	4	5	6
<i>V. destructor</i> females per cell	0.10	0.18	0.39	0.36	0.23	0.33

**3.1.2. Condition of the bees**

With a few exceptions (<1% of the total), the bee brood developed normally to the stage of pupa with pigmented thorax, both in the control and treated group, without evident differences.

**3.1.3. Infestation levels**

In intact treated and control cells (in total, 2665 cells), 613 female mites were found. The infestation (Tab. I) was obviously higher in replications carried out later in the year.

**3.1.4. Mortality of mites**

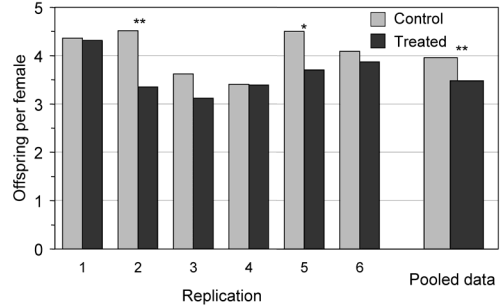
Five out of 296 and 14 out of 317 mites in treated and control cells respectively died. The difference reached the significance level ( $P = 0.05$ ) but it resulted from a scattered record of mortality, without any definite pattern. If the treatment had any effect on the vitality of the mite, the effect was very small and inconstant.

**3.1.5. Effects of the injection of acetone on the reproduction of mites**

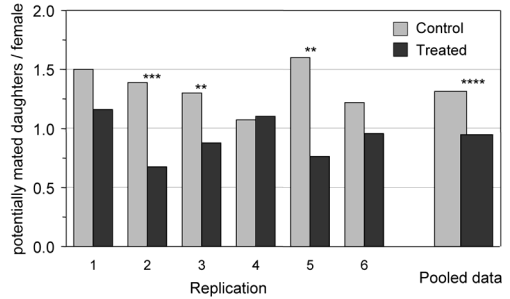
No effect of the injection of acetone on the reproduction of *V. destructor* was observed: in single infested cells, the average number of offspring was 4.35 in acetone injected cells and 4.28 in untouched cells; the average number of PMD was 1.40 and 1.39 respectively. Even when replications were considered separately, the maximum relative difference was less than 4%.

**3.1.6. Effects of heptadecene on the reproduction of mites in single infested cells**

The infertility of the females of *V. destructor* was slightly higher in treated cells (11.5%) than in control cells (7.5%), but the difference was not significant ( $P = 0.16$ , *G*-test).



**Figure 3.** Experiment 1: Offspring per female in single infested cells, for each replication and on pooled data. The asterisks indicate significant differences (\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ).



**Figure 4.** Experiment 1: Potentially mated daughters per female in single infested cells, for each replication and on pooled data. The asterisks indicate significant differences (\*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; \*\*\*\*:  $P \approx 0.0001$ ).

The average number of offspring was 3.48 in treated cells and 3.96 in control cells (Fig. 3); the difference was highly significant ( $P < 0.01$ ). In each replication, the reproduction in controls never exceeded that in treated cells; the difference was significant in two replications.

The average number of PMD was 0.94 in treated cells and 1.31 in control cells (Fig. 4). The difference was very highly significant ( $P \approx 0.0001$ ). This difference resulted from a significant reduction both in the number of adult females (1.26 vs. 1.53;  $P \approx 0.002$ ) and in the number of males (0.64 vs. 0.78;  $P \approx 0.001$ ), including pharate adults in both cases. Out of 297 adult males, only 20 were still pharate. The vitality of several of the pharate adult males seemed reduced and sometimes they were abnormally small; however, excluding

pharate males from the counts did not change the results much.

In both populations, a decrease in the reproduction of *V. destructor* in control cells was observed in replications carried out later in the season, but no significant difference among replications in the number of PMD in controls was observed.

### 3.1.7. Effects of heptadecene on reproduction of mites in multiple infested cells

The number of total offspring per infesting female in multiple infested cells did not decrease in treated cells (3.21 vs. 2.96 in controls), nor did the number of PMD (1.40 vs. 1.09 in controls); the differences were not significant ( $P \approx 0.3$  and 0.07 respectively).

## 3.2. Experiment 2: treatments at different times after capping

### 3.2.1. Hygienic behaviour towards treated cells

Cells uncapped and emptied by bees were 14–20% of the total (Tab. II); no significant difference among groups was detected.

### 3.2.2. Infestation levels

Average infestation of intact cells was 50.4%.

### 3.2.3. Effects of heptadecene on the reproduction of *V. destructor* in single infested cells

The decrease both in the total offspring and the number of PMD observed in the treated

**Table II.** Treatments carried out in Experiment 2 and effects of the injection of heptadecene at different times after capping.

	Experimental group			
	C	T1	T2	T3
Treatment (ng heptadecene in 1 $\mu$ L acetone)				
0–15 h PC	0	100	50	0
96–111 h PC	0	0	50	100
Total number of cells	205	195	200	201
Removed cells	33	36	39	28
Infested cells	58	56	61	61
Total mites	76	88	91	80
Single infested cells	45	30	33	47
In single infested cells:				
Offspring per female	3.09	2.4	2.73	2.87
Potentially mated daughters per female	0.78	0.57	0.73	0.81

groups did not reach significance (Tab. II); in both cases, the largest decrease occurred in the group T1, which had received a treatment similar to that of the treated group of Experiment 1. The reproduction was markedly lower than in Experiment 1 in all the groups, including the control one; this effect was observed consistently in each of the three combs used.

### 3.2.4. Effects of heptadecene on the reproduction of *V. destructor* in multiple infested cells

Reproduction in treated cells was somewhat lower than in controls, without significant differences (Tab. III). The numbers of offspring and that of PMD per female were close to those recorded in single infested cells.

**Table III.** Experiment 2: average number of offspring and number of PMD in multiple infested cells. Treatments were the same as in Table II.

	Treatment			
	C	T1	T2	T3
Number of multiple infested cells	10	24	21	13
<i>V. destructor</i> females	25	57	48	32
Offspring per female	2.63	2.21	2.18	2.12
Potentially mated daughters per female	1.23	0.76	0.89	0.76

**Table IV.** Experiment 3: Removal of treated cells by bees; infestation by *V. destructor*; reproduction of the mite in single infested cells of different experimental groups.

	Injected before capping			Injected after capping		
	CB (control)	TB (treated)	<i>P</i>	CA (control)	TA (treated)	<i>P</i>
Total	162	162		142	149	
Removed	37	10	<0.0001	20	10	<0.05
Infested	52	36	0.001	44	43	n.s.
Total mites	74	53		53	58	
Mites/cell	0.59	0.35	0.006	0.43	0.42	n.s.
Single infested cells	29	24		31	29	
<i>In single infested cells:</i>						
Offspring per female	3.62	4.21	n.s.	3.74	3.97	n.s.
PMD per female	0.83	0.79	n.s.	0.94	0.93	n.s.

*P*: probability of the observed difference, in the null hypothesis; n.s.: not significant.

### 3.3. Experiment 3: treatment before and after capping

#### 3.3.1. Capping of cells treated before the treatment

Most cells injected before capping were capped by the bees within 16 h, without differences in the proportion of capped cells between the treated and the control group (respectively, 162 out of 199 and 162 out of 189;  $P = 0.25$ ). With a few exceptions, the remaining cells were found to have been capped when the comb was inspected.

#### 3.3.2. Removal of treated brood

Treated cells, either before or after cell capping, were removed less often than control cells (Tab. IV); the difference between CA and TA cells was significant ( $P < 0.05$ ), the difference between CB and TB cells also was highly significant ( $P < 0.0001$ ).

#### 3.3.3. Infestation levels

Infestation levels (Tab. IV) in cells treated after capping and in the corresponding controls were not significantly different ( $P \approx 0.4$ ), while cells treated before capping were less infested than corresponding controls. The differences in the proportion of infested cells (23.7% vs. 41.6% in the controls,  $P \approx 0.001$ )

and in the number of female mites/cell (0.35 vs. 0.59 in the controls,  $P < 0.01$ ) were highly significant.

#### 3.3.4. Effects of heptadecene on the reproduction of mites

In single infested cells, treatments before and after capping did not produce clear effects on the reproduction of *V. destructor* (Tab. IV). As in Experiment 2, the number of PMD was low in all the experimental groups. In multiple infested cells, only small variations in the reproduction among groups were observed; the size of the sample was too small to draw any conclusions.

## 4. DISCUSSION

In Experiment 1, the number of mature offspring in control cells was in the range considered normal for European populations of *V. destructor* mites and European bees.

The injection of 1  $\mu$ L acetone did not affect the reproduction of the mite; thus, cells treated with acetone could be used as controls.

Heptadecene, at the dose of 100 ng applied 0–15 h after capping, influenced the reproduction of *V. destructor* in the hive and reduced both the total number of offspring and the

number of potentially mated daughters, without showing a toxic effect on the mite. This confirms earlier results (Nazzi et al., 2002), obtained in artificial cells under laboratory conditions. In laboratory experiments (F. Nazzi and N. Milani, unpublished data), heptadecene was active at 10 and 100 ng/cell, but not at 1 nor 1000 ng/cell; related hydrocarbons were not active (Nazzi et al., 2002). This rules out the possibility that the effect is an unspecific one, due to overdosing.

On average, the decrease in the number of PMD was 28%, larger than the decrease in the total offspring. There is no way to prove that all the pharate adults would moult and mate before the emergence of the honey bee. Nevertheless the number of PMD 11 days after capping represents a good estimate of the number of female daughters that can reproduce; possible differences would affect the different experimental groups in the same way. A 28% decrease would produce large effects on the population dynamics of the mite, owing to the large number of generations per year.

So far, investigations have concentrated on the infertility of *V. destructor* as the main parameter influencing its population dynamics, since it was implicitly assumed that the number of offspring produced was the same once mite reproduction was triggered. The data presented here show that important differences in the reproductive success may exist even when infertility is low. Such differences can be reliably detected only when mites are mounted on microscope slides; identification under the dissecting microscope was unreliable or impossible.

The larger difference in the number of PMD than the offspring produced may result from delays in the oviposition, slower development of the offspring, loss of the male or disturbances in the oogenesis and oviposition that alter the normal sex ratio.

No clear differences in the response of the mites of the two tested populations were observed. On the contrary, a decrease in the total number of offspring and in the number of PMD in control cells was observed in both mite populations in replications carried out later in the season, when infestation levels increased and availability of brood cells decreased. From the experiment it is not

possible to ascertain what factor produced the observed effect, but it may reveal a density dependent effect on mite reproduction that should be further investigated in depth. Moosbeckhofer et al. (1988) found a negative correlation between infestation level and mite reproduction, but considered both single and multiple infested cells; thus their results could be influenced by the increase in the proportion of multiple infested cells.

Low reproduction in all the experimental groups was observed in Experiments 2 and 3. In the control group of Experiment 2, mite reproduction was even lower than in treated group of Experiment 1. This effect could depend on seasonal factors (for example, very high infestation levels) or the manipulation of the combs, which were taken twice from the hive, which may have contributed to obscure differences among different groups, so that effect of heptadecene under these conditions could not be demonstrated conclusively. At this point, how the timing of application of heptadecene influences the biological activity of the mite is not possible to discern.

In multiple infested cells, no decrease in mite reproduction was observed (differences between control and treated cells in Experiments 1 and 2 were not significant and had opposite signs). A possible explanation is that in multiple infested cells, a single injection of heptadecene cannot cause effects much higher than those caused by the same compound, produced within the cell during the development of the pupa, because saturation of effects is reached.

Heptadecene has a high affinity for wax and a low, but not negligible volatility (the vapour pressure is about 0.2 Pa at 35 °C: cf. Kang et al., 2001). These factors made it difficult to evaluate how long minute amounts of heptadecene persisted and remained available in the site of application. On the other hand, its volatility could make it possible that it is active in locations different from the site of release.

In Experiments 1 and 3, bees removed more control cells than treated cells; the difference was small but highly significant. A possible explanation may be that heptadecene might reduce the parasitic activity of the mite and its offspring, thus decreasing the damage caused to the bee larva or pupa and consequently the

release of the compounds that trigger the removal behaviour. Alternatively, heptadecene might interfere with the perception of related compounds (Nazzi et al., 2004) that trigger hygienic behaviour in the honey bee.

Finally, cells treated before capping were less infested than the corresponding controls. The experiment was not designed to assess such effects, and thus this result has to be taken with caution. However, it might indicate that heptadecene interferes with the process of entrance of *V. destructor* into brood cells, without implying that this compound has a repellent action.

These effects suggest that heptadecene might be involved in some way in the tolerance of *V. destructor* by some honey bee strains, and thus its activity in the bee colony deserves further investigation.

#### ACKNOWLEDGEMENTS

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**Résumé – Le (Z)-8-heptadécène diminue la reproduction de *Varroa destructor* dans les cellules de couvain.** Des études précédentes ont montré que la reproduction de l'acarien *Varroa destructor* diminue dans les cellules multi-infestées, que ce soit dans les cellules naturelles des rayons ou dans les cellules artificielles en conditions de laboratoire. Parmi les substances libérées dans les cellules infestées, l'alkène (Z)-8-heptadécène a été identifié comme substance réduisant la reproduction de *V. destructor* en conditions de laboratoire. Nous avons testé cette substance dans les cellules naturelles des rayons.

Dans une première expérience nous avons injecté 100 ng d'heptadécène mélangés à 1 µL d'acétone sous l'opercule de cellules operculées de 0 à 15 h plus tôt ; chez les témoins, seule de l'acétone a été injectée. Les cellules traitées et les témoins ont été inspectées 11 j plus tard. Les acariens trouvés dans ces cellules ont été montés sur des lames de microscope pour une identification précise des stades de développement de la descendance. On a noté en particulier le nombre total de descendants et le nombre de filles adultes potentiellement fécondées (PMD), i.e. le nombre de filles adultes dans des cellules renfermant aussi un mâle adulte. Ce dernier est très important pour la dynamique des populations de *V. destructor*, puisque les descendants immatures

meurent quand l'abeille émerge et que les femelles non fertilisées ne peuvent se reproduire. Dans les cellules mono-infestées, aucune différence significative n'a été notée pour l'infertilité des acariens, mais le nombre de descendants dans les cellules traitées et les témoins était respectivement de 3,48 et 3,96 (Fig. 3), celui des PMD de 0,94 et 1,31 (Fig. 4). Les deux différences étaient hautement significatives. L'heptadécène est donc actif aussi dans les cellules des rayons.

Dans une seconde expérience, l'heptadécène a été appliqué à diverses périodes après l'operculation. Dans les cellules mono-infestées, la reproduction de l'acarien était plus faible dans les groupes traités mais les différences n'étaient pas significatives (Tab. II).

Dans une 3<sup>e</sup> expérience, le produit a été appliqué entre 0 et 6 h après l'operculation ou entre 0 et 16 h avant l'operculation. Nous n'avons observé aucune diminution de la reproduction, mais l'infestation des cellules traitées avant l'operculation a été réduite de 41 % (Tab. IV). Dans les cellules multi-infestées, le produit n'a pas réduit la reproduction de l'acarien, peut-être parce qu'une seule injection d'8-heptadécène ne peut avoir d'effets plus importants que ceux dus au même composé produit dans la cellule au cours du développement nymphal. Dans les cellules mono-infestées des groupes témoins, le nombre de PDM/femelle est passé de 1,5 en juin à 0,78–0,94 en septembre, ce qui peut révéler des effets dépendants de la densité.

L'heptadécène pourrait jouer un rôle important dans la relation hôte-parasite et être impliqué dans le mécanisme de tolérance.

***Varroa destructor* / reproduction / composé sémiochimique / heptadécène**

**Zusammenfassung – (Z)-8-Heptadecene verringert den Reproduktionserfolg von *Varroa destructor* in Brutzellen.** Frühere Versuche haben ergeben, dass die Reproduktion von *Varroa destructor* in mehrfach befallenen Zellen abnimmt, sowohl in natürlichen Wabenzellen als auch in künstlichen Zellen unter Laborbedingungen. Unter den Substanzen, die innerhalb befallener Zellen abgegeben werden, wurde das Alken (Z)-8-Heptadecen als eine Substanz identifiziert, die die Reproduktion von *V. destructor* unter Laborbedingungen reduziert. In dieser Arbeit wurde die Wirkung der Substanz unter natürlichen Bedingungen in Wabenzellen untersucht.

Im ersten Versuch wurden 100 ng Heptadecen in 1 µL Aceton unter Zelldeckel injiziert, die zwischen 0 und 15 Stunden zuvor geschlossen worden waren. Bei Kontrollen wurde nur Aceton gespritzt. Behandelte und Kontrollzellen wurden 11 Tage später überprüft; Milben, die in diesen Zellen gefunden wurden, wurden auf einem Objektträger gebracht,



um das genaue Stadium der Entwicklung der Nachkommenschaft zu bestimmen. Besonders die Gesamtzahl der Nachkommen und die Zahl der adulten, eventuell begatteten Töchter (PMD, i.e. die Anzahl der adulten Töchter in Zellen mit ebenfalls adulten Männchen) wurden berücksichtigt. Letztere sind wichtig für die Populationsdynamik von *V. destructor*, denn unreife Nachkommen sterben, wenn die Biene schlüpft und unbegattete Weibchen können sich nicht vermehren.

In einfach befallenen Zellen ergab sich kein signifikanter Unterschied in der Infertilität der Milben, aber die Zahl der Nachkommen in Test und Kontrollzellen betrug 3,48 bzw. 3,96 (Abb. 3) und die Zahl der PMD betrug 0,94 bzw. 1,31 (Abb. 4); beide Unterschiede waren hoch signifikant. Demnach ist Heptadecen in Brutzellen aktiv.

Im 2. Versuch wurde Heptadecen zu verschiedenen Zeiten nach der Verdeckelung injiziert. In einfach befallenen Zellen war die Reproduktion der Milben in der Testgruppe niedriger, aber die Unterschiede waren nicht signifikant (Tab. II).

In einem 3. Versuch wurde Heptadecen zwischen 0–6 h nach der Verdeckelung oder 0–16 h vor der Verdeckelung zugegeben; Es wurde keine Abnahme in der Reproduktion gefunden, aber der Befall der Zellen, die vor der Verdeckelung behandelt worden waren ging um 41 % zurück (Tab. IV).

In mehrfach befallenen Zellen hatte die Behandlung mit Heptadecen keinen Einfluss auf die Reproduktion; wahrscheinlich weil eine einzelne Injektion von Heptadecen keine höheren Effekte auslösen kann als die gleiche Substanz, die während der Entwicklung der Puppe kontinuierlich erzeugt wird. In einfach befallenen Zellen der Kontrollgruppen, nahm die Anzahl der potentiell begatteten Töchter von 1,5 im Juni auf 0,78–0,94 im September ab. Das mag ein Hinweis auf dichte abhängige Effekte sein. Heptadecen könnte eine wichtige Rolle im Verhältnis zwischen Wirt und Parasit spielen und am Toleranzmechanismus beteiligt sein.

#### Reproduktion / *Varroa destructor* / Semiochemikalien

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