ATTRACTION OF *VARROA DESTRUCTOR* TO BEE BROOD CELLS BY CUES FROM LARVAL FOOD

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Abstract
Research has been devoted to the study of the semiochemicals that induce the mite to enter brood cells for reproduction. Since the beginning the research has focused on possible signals released by the bee larva; recently it has been demonstrated that the larval food contained in the brood cells when the varroa mite enters, may play a significant role in the process of cell invasion.
In this study the steps towards the identification of the compounds in larval food that are responsible for attracting the varroa mite to brood cells are reported.

Keywords: *larval food /* Varroa destructor */ cell invasion / semiochemical*

Introduction

To reproduce, the mite *Varroa destructor* Oud. enters a brood cell containing a bee larva before cell capping (BOOT et al., 1992). The idea that attractants possibly involved in this process could be used for the control of the mite, like other attractants used in pest control, stimulated the research on this subject.

Several authors investigated the stimuli that trigger cell invasion by the mite; right from the beginning, research focused on the stimuli released by bee larvae. LE CONTE et al. (1989) showed that the varroa mite is attracted by some esters of simple aliphatic fatty acids found in fifth instar larvae. RICKLY et al. (1992) demonstrated that palmitic acid, which was detected in the air surrounding bee larvae, is attractive to the varroa mite. RICKLY et al. (1994) and AUMEIER and ROSENKRANZ (1995) showed that some saturated and unsaturated hydrocarbons found on the cuticle of the larvae were active on the varroa mite in a bioassay.

During cell invasion the mite leaves the nurse bees to enter the brood cell containing the bee larva. The preference shown by the varroa mite for nurse bees rather than bee larvae in a bioassay (KRAUS, 1993, LEDOUX et al., 2000) suggests that stimuli from a source other than the brood itself are involved in the process of cell invasion.

Apart from the bee larva, the brood cell contains several milligrams of larval food that nurse bees provide for the growing larva; in fact the mite, after entering the cell, reaches the bottom of it and gets trapped inside the larval jelly (IFANTIDIS, 1988).

The attractivity of drone larval food was hypotized as early as 1985 (ISSA et al., 1985); MILANI and CHIESA (1991) showed that larval food influences the reproduction of *V. destructor*.

Here we report the results of a study in which the effect of larval food on the behavior of the mite was tested to verify its role in the process of cell invasion. Some results of this study were reported also in NAZZI et al. (2001).

Materials and Methods

Biological material

Bee larvae and mites used in the experiments came from untreated *Apis mellifera* colonies maintained in Udine (northeastern Italy). The mites and bee larvae were obtained from brood cells capped in the preceding 15 hours (*0-15 PC*). Fifth instar bee larvae before capping (*I5 BC*) were manually extracted from unsealed bee brood cells. Larval food was extracted with a small spatula from drone cells containing either a fourth or fifth instar larva and kept at -20°C in sealed vials until used.

Bioassay

To study, under laboratory conditions, the stimuli involved in the process of cell invasion, a bioassay was used. This consisted in a glass observation arena similar to that used by Rosenkranz (1993) with four wells (7 mm i.d.; 8 mm deep) equidistant (1 cm) from the center (fig. 1). The treatment was applied to two opposite wells while the others were used as controls. One bee larva was put into each well. At the beginning of the bioassay one adult female mite was placed in the center of the arena and the position observed at 5 minutes intervals for 30 minutes. The arenas were kept in an environmental cabinet at 35°C and 75% R.H. for the duration of the bioassay. Twenty arenas were used at a time. Tests were replicated on different days.

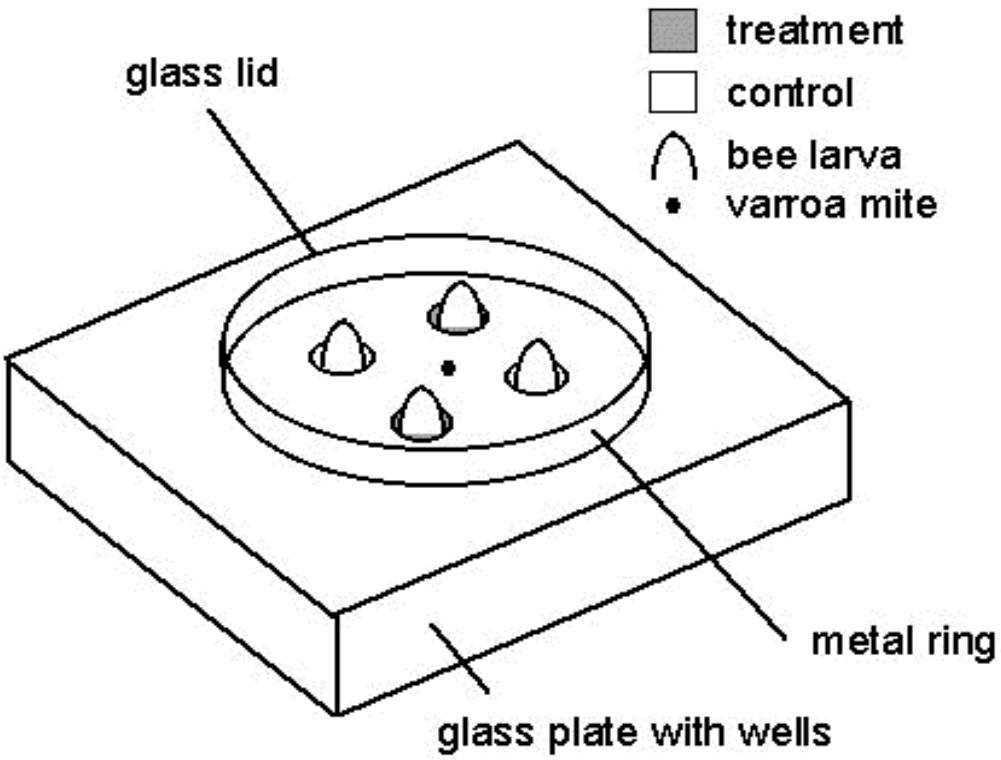

Figure 1 – Arena used for the bioassays

Experiments carried out

a) <u>age of larvae</u>

To check the efficiency of the bioassay and select the best age of larvae to be used in the bioassay a preliminary experiment was carried out in which the attraction of the varroa mite to bee larvae of two different ages was studied. This was done by placing a *I5 BC* bee larva in each of two opposite wells and a *0-15 PC* larva in the others.

b) <u>larval food</u>

The possible effect of larval food on the behaviour of the mite was tested by treating two opposite wells of the arena with ten milligrams of larval food whilst the other wells were left untreated and used as a control. *0-15 PC* larvae were placed in all wells.

c) <u>extracts of larval food</u>

To check the hypothesis that the biological activity of larval food depended on some chemicals contained in this material, larval food was extracted with two different solvents and the extracts tested in the bioassay.

Larval food was extracted with acetone and diethyl ether and assayed at a concentration of 10 mg equivalents of larval food in 10 µl of solvent per treated well; 10 µl of solvent were applied to the control wells. 0-15 PC larvae were placed in all wells.

Statistical analysis of data

For each arena, the number of times the *Varroa* mite was observed in the treated and control wells over the 30 minute period was calculated to use as a score for the statistical analysis. The scores of treatment and control in a given set of data were compared by means of a sampled randomization test (MANLY, 1991; SOKAL & ROHLF, 1995). In this case the randomization distribution was resampled 10^6 times with a computer programme written for this purpose.

Results

a) Age of larvae

More mites were found in wells containing larvae from unsealed cells (*I5 BC*) than in wells containing larvae from sealed cells (*0-15 PC*) (P=0.016) (tab. I).

Table I

Response of *V. destructor* to larvae of different ages. Sum of scores of 20 mites in wells containing a fifth instar larva (I5) or a larva from a sealed cell (0-15 PC). P represents the statistical significance of the observed difference

Replication	I5	0-15 PC	**P**
1	33	8	0.027
2	36	36	0.521
3	37	32	0.418
4	40	16	0.066
5	33	12	0.067
6	32	24	0.333
tot.	211	128	0.016

b) Larval food

The number of varroa mites found in wells treated with larval food was significantly higher (P<0.001) than that observed in control wells (tab. II).

Table II

Response of *V. destructor* to larval food and larval food extracts The sum of scores in treated and control wells is given. P represents the statistical significance of the observed difference

Treatment	replications	Treated	control	**P**
Larval food	5	249	54	<0,001
Larval food - ether extract	8	152	56	<0,001
Larval food - acetone extract	4	110	22	<0,001

c) Extracts of larval food

Both the ether and acetone extract of larval food elicited a significant response from *V. destructor*, in that the number of varroa mites choosing the treated wells was significantly different (P<0.001 in both cases) from the number in control wells (tab. II).

Discussion

Varroa mites used in the bioassay responded more strongly to fifth instar bee larvae before capping than to larvae from sealed cells. This could be due to compounds present on the cuticle of fifth instar bee larvae but may depend also on active compounds present on the larval cuticle because of the contamination of the latter by substances contained in the cell such as larval food. In any case the result of this experiment confirmed the efficiency of the bioassay and prompted the choice of the less active *0-15 PC* larvae in the following experiments, that were designed to test the biological activity of non-larval stimuli.

When larval food was tested in the bioassay a clear response of *V. destructor* was observed; the biological activity of the extracts demonstrates that the observed effect is due to semiochemicals contained in the larval food itself and not to other unspecific cues (e.g. the humidity).

These results show that chemical cues coming from a source other than the host are involved in the process of cell invasion by the Varroa mite; this may appear rather unexpected. In order to carry out their function, chemical cues need to be reliable in that they clearly indicate to the perceiver the presence and suitability of the host; actually, the stimuli identified so far from the larval cuticle lack this requirement being rather widespred inside the hive. On the other hand larval food has a distinctive composition including several typical hydroxyacids (LERCKER et al., 1994).

The extraction of the semiochemical responsible for the biological activity of larval food has opened up the way to their identification. This could contribute to a better understanding of the biology of the mite and suggest novel methods to control the parasite.

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