

TESTING HONEY BEES FOR HYGIENIC BEHAVIOUR

Beata Panasiuk, Małgorzata Bieńkowska, Dariusz Gerula
 Research Institute of Pomology and Floriculture, Apiculture Division, Puławy, Poland
 e-mail: beata.panasiuk@man.pulawy.pl

INTRODUCTION

Hygienic behaviour of the honey bees is one of the mechanisms for disease resistance. It involves the detection of diseased or dead brood in sealed cells, uncapping, and removing it from the nest. Hygienic behaviour of bees eliminates the source of infection and limits disease transmission within the colony and sometimes leads to the colony self-healing. The behaviour depends on: genetic factor (Rothenbuhler 1964), strength of bee colony and age of workers (Spivak and Gilliam 1993), manner of brood killing (Kefuss et al. 1996), natural flow and weather conditions (Spivak and Reuter 1998).

Many authors have demonstrated various signals provoking dead brood removal in worker bees. There are olfactory cues (Masterman et al. 1998) or differences in the temperature of healthy and infected or physically damaged brood (Gramacho et al. 1997). Worker bees uncap and remove brood from the cell when there is a *Varroa destructor* mite (Aumeier and Rosenkranz 2001).

The aim of the research was to study bee response to dead brood inserted into the colony regarding genotype of the bees and the method of brood killing.

MATERIAL AND METHODS

Studies on hygienic behaviour of bees were carried out in the Department of Bee Breeding in the Institute of Pomology and Floriculture, Apiculture Division in Puławy in the years of 2004-2005.

Two populations of carnica bee colonies were tested

- ▶ *car* GR1- selected for chalkbrood resistance (hygienic)
- ▶ *car* Zosia- selected for high honey production and observed as susceptible for the brood disease (non-hygienic)

Brood killing methods applied in the test

- ▶ freezing a section of brood comb (12hours, temp. -18°C) and returning it to the colony
- ▶ inoculating with spores of *Ascosphaera apis* (3-4 days-old bee larvae fed with polen candy with macerated mummies, Taber and Gilliam 1987)
- ▶ pinning brood cells with an entomological needle

The experiment was repeated three times every year. The pieces of combs with experimental brood killed with three methods were moved into tested bee colonies. They were placed in the centre of bee nest, between two brood combs. The effect of the honey bee genotype, and of the method of brood killing, was estimated after 12, 24, 48, 72 hours and then once a day up to all cells with dead brood were cleaned.

The average rate of cleaned dead brood was calculated and given as percentages. Data were calculated according to the Bliss' transformation. Two-way ANOVA was used for statistical calculation, and Duncan's multiple range test was applied to determine significant differences between the means.

RESULTS

Significant differences were found in the rate of removing dead brood. In both years of the research pin-killed brood was significantly faster recognized and removed by bees (Fig. 1). Within the first 12 hours bees cleaned on average 73% of cells and during 3 days of observation only few unclean cells left in tested colonies. Non-hygienic bees had an approximately 10% lower rate of cleaning than hygienic bees for the whole experiment

Regardless of the tested colony, pin-killed brood was removed the most quickly by bees (Fig. 2). Bees cleaned an average of 73% of the cells with pin-killed brood within 12 hours. Only a few unclean cells were left after 3 days of observations.

Significantly lower rate of cleaning cells with *Ascosphaera apis* inoculated and freeze-killed brood was observed. Bees removed about 30% of *A. apis* inoculated and only 10% of freeze-killed brood within 12 hours. During 3 days of research bees removed 83% of brood inoculated with *A. apis* and 65% of freeze-killed brood. In our experiment, bees removed pin-killed brood significantly faster than *A. apis* inoculated or frozen brood. The highest differences in cleaning rate were found within the first 12 hours of cells cleaning

Both analyzed factors: the genotype of bees and the method of brood killing differently influenced the rate of cells cleaning in particular time intervals. There was no interaction between these two factors (Table 1).

Differences were found in hygienic behaviour of bees belonging to two populations, regardless of the applied method of brood killing, after 24 and 72 hours of experiment. In the other measurements (after 12 and 48 hours) no differences were found. Differences in the rate of removing brood killed in various manners were stated only in the measurements after 12 and 24 hours of experiment. In

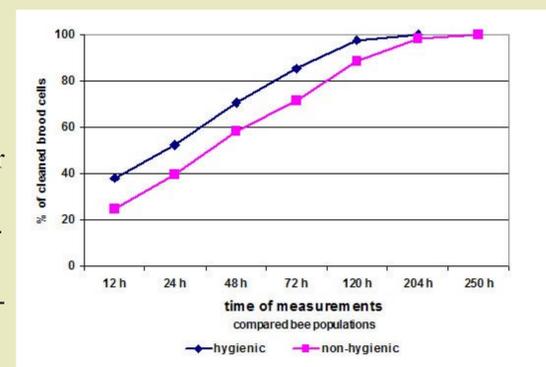


Fig. 1. Dynamics of cleaning cells with dead brood by two populations of bees (regardless of the killing brood method applied)

Average percent of brood killed with three methods and removed by two populations of bees.

| Measurement after | Bee population | Frozen | Inoculated | Pinned | Average |
|-------------------|----------------|---------|------------|---------|---------|
| 12 h | Hygienic | 16.3 e | 35.9 ab | 83.6 c | 45.3 A |
| | Non-hygienic | 5.9 d | 22.9 a | 62.3 bc | 30.4 A |
| 24 h | Hygienic | 37.6 bc | 51.4 c | 96.0 d | 61.7 B |
| | Non-hygienic | 17.4 a | 31.9 ab | 89.7 d | 46.3 A |
| 48 h | Hygienic | 58.9 ab | 67.9 b | 100 c | 75.6 A |
| | Non-hygienic | 43.3 a | 57.2 ab | 96.9 c | 65.8 A |
| 72 h | Hygienic | 74.8 b | 85.6 bc | 100 c | 86.8 A |
| | Non-hygienic | 56.3 a | 80.7 bc | 99.0 c | 78.7 B |

Different small letters indicate significant differences ($p \leq 0.05$) between means of methods of brood killing and bee populations in particular time intervals.

Different capitals indicate significant differences ($p \leq 0.05$) between means of hygienic and non-hygienic populations (column)

Table 1. the next two measurements pin-killed brood was significantly faster removed while there were no significant differences in the rate of removing frozen and inoculated brood. The rate of cleaning pin killed brood did not vary between both bee populations. The rate of removing brood inoculated with *A. Apis* varied only 24 hours after inserting the combs into bee colonies and differences between two populations in the rate of cleaning freeze-killed brood varied during all observation with the exception of the measurement after 48 hours.

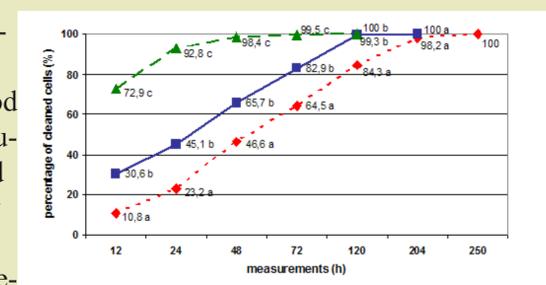


Fig. 2. Dynamics of cleaning cells with brood killed with different methods (regardless of the tested bee population)

CONCLUSIONS

- ▶ Honey bees in colonies selected for chalkbrood resistance clean dead brood from the cells faster in comparison to susceptible colonies
- ▶ Pin-killed brood is removed from the cells in a shorter time than freeze-killed or *Ascosphaera apis* inoculated
- ▶ The dynamics of removing freeze-killed and *Ascosphaera apis* spores inoculated brood are similar

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