EXPERIMENTS ON HYGIENIC BEHAVIOUR OF HONEY BEES

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Summary

It has been a long-debated question, if the colony is able to get rid of any agent of disease by themselves, without the help from outside factors. This is the reason, why the researchers follow with keen attention the hereditary hygienic behaviour of immune-mechanism among the bee colonies. Many experts are of the opinion that this form of behaviour has an important role in the defence against infectious and parasitic diseases.

We performed our experiments in the apiary of the Szent Istvan University, Gödöllo, (apiary 1), in a private apiary (apiary 2), and in the apiary of the Institute for Small Animal Research (apiary 3).

The differences in hygienic behaviour between colonies of the three apiaries can be seen in Tables 1-3. The average cleaning success was 74% in the first, and 72% in the second apiary, after 24 hours. In the third apiary, where the cleaning instinct as breeding trait was evaluated, the cleaning success was the highest, 86%, with 19.7% coefficient of variation. The results in the first two apiaries are nearly the same, in the third apiary, due to the freezing method, were significantly lower in the same colonies than the result of the piercing method.

In these experiments the very close results of observation for 24 and 48 hours show that the time factor has a little role in this behavioural pattern. In the case of the freezing method, the hygienic behaviour of colonies was nearly the same.

On the base of our experiments, we recommend the application of the pin piercing method for testing the hygienic behaviour. The systematic testing would be advisable in colonies kept for breeding and for queen rearing.

Keywords: hygienic behaviour, cleaning success, frosty method, pin piercing method.

INTRODUCTION

The new scientific results and experiences in the bee management have elucidated the pathological and epidemic reasons of many honeybee diseases. In the earlier days general view was that one part of the agent, causing illness, was connected to one definite territory, (supposedly had endemic origin), but nowadays, due to the very intensive international traffic, much of them became wide spread. This perception has arisen anxiety throughout the world, and resulted in serious consequences. Defence against the infectious honeybee diseases is going on in many countries using treatment with medicines (chemotherapy), and in many countries there are very strict official prescriptions and blockades against the infected apiaries combined with colony extermination with official compensation.

This defence has resulted in a very different outcome. A perfect solution for preventing the spreading or for defence against the bee diseases have not been developed up to now. The growing number of health problems due to high concentration of

apiaries in Hungary, as well as different methodical troubles, arising from the different types of hives, make the Hungarian apiarists unquiet and disturbed about the honey production in our country.

It has been a long-debated question if the colony is able to get rid of any agent of disease by themselves without the help from outside factors. This is the reason, why the researchers follow with keen attention the hereditary hygienic behaviour of immune-mechanisms among the bee colonies. Many experts are of the opinion that this form of behaviour has an important role in the defence against the infectious and parasitic diseases (Bailey and Ball 1991).

Kefuss et al. (1996) advice to apply a practical method for checking the hygienic behaviour in the colonies. Their work stimulated our research team to test the applicability of this well known, but in Hungary hardly tested method, in our apiaries.

Rothenbulher (1964) was the first who proved that selection could be a tool in breeding much more resistant colonies. He recognised that the hygienic behaviour is checked by two recessive alleles. By one allele the bee recognises the problem, and is able to open the sealed cell, the other allele controls the instinct of clearing away the dead larvae. Both alleles are necessary for a hygienic colony. In heterozygous populations, the appearance of dominant alleles diminishes the success of this character. In this case only one part of bees has hygienic instinct, or follows one or the other behavioural form.

Harbo (1995) found significant connection between hygienic behaviour of three weeks old workers, and the pest.

Spivak (1996) infected larvae of hygienic and non-hygienic colonies by Varroa mite and found differences only by heavier infection. There were differences in the number of chewed or scratched larvae at the bottom of the hives. Among these the hygienic colonies. have an advantage.

MATERIALS AND METHODS

We performed our experiments in the apiary of the Szent Istvan University, Gödöllo, (apiary 1), in a private apiary (apiary 2), and in the apiary of the Institute for Small Animal Research (apiary 3). In apiary 1, and 2. 10 and 11 colonies were chosen by random in July, and from each of them one sealed comb was carved out containing larvae. In an area probably not containing empty cells, a space of 5 5 cm was marked out by help of a model. This fragment contained about 100 cells. By using a sterile injection pin, in the marked fragment each cell was pierced in its centre to kill the larvae, then the comb fragments were marked and taken back to their original place. After 24 and 48 hours, the numbers of cells that were opened and cleaned, opened but not cleaned, and not opened were counted.

Thirty pollen collecting bees were taken from each colony to be tested for Nosema. The bees were homogenised in a mortar, adding previously a little water to them. Then they were allowed to dry on a slide and were fixed above flame. Dyeing with 0.4% methylene blue lasted 15 minutes, the counter-dyeing with 0.6% fuchsine solution only a few seconds. The assessment was based on the number of spores in three levels of infection.

Testing of the third apiary was in June and August. This testing was different from the previous ones, because 5 5 cm larvae containing, sealed comb fragments were carved out and frozen during 24 hours in a refrigerator. These fragments were subsequently put and fixed into the covered, larvae containing combs of 10 colonies. (Kefuss et al. 1996) The experiment was repeated in the same colonies by piercing with a pin.

Other data were also collected, e.g. of the number of honeycombs with larvae, type of hive, age of queens, signs of illness, etc.

For the comparison of the results, Staatgraf (Advanced Procedures) program analysis was used.

RESULTS

The results of the experiments can be seen in Table 1. This table also contains the results of Nosema testing. For statistical comparison the term of cleaning success was introduced, which was equal to 100 minus the number of the cleaned cells, counted after 24 and 48 hours.

The application of the *chi* test failed to reveal differences between the first and the second apiary in the 24 hour test but a significant difference was between colonies ($P<0.01$) for cleaning success. In the third apiary a significant difference was found ($P<0.05$) between the results from the methods piercing and freezing.

DISCUSSION

The differences in the hygienic behaviour between colonies of the three apiaries can be seen in Tables 1-3. The average cleaning success after 24 hours was 74% in the first, and 72% in the second apiary. In the third apiary, where the cleaning instinct as breeding trait was evaluated, the cleaning success was the highest, 86%, with 19.7% coefficient of variation. The results in the first two apiaries are nearly the same, in the third apiary, results due to the freezing method were significantly lower in the same colonies than the result of the piercing method. ($P<0.05$).

In these experiments the very close results of the observation for 24 and 48 hours show that the time factor has a little role in this behavioural pattern. Fewer hygienic cleaning bees can clean the dead cells but during longer time, and for this reason we accepted the 24 hours observation. Within apiaries the treatments show significant differences, but the differences between apiaries did not reach the level of significance.

In the least hygienic two colonies of one of the apiaries a very serious pest was discovered thus verifying the statement of Harbo (1995). Nosema infection seemed strong in apiaries, tested in the beginning of summer, but there seemed to be no connection between the cleaning instinct and the infection.

In the case of the frosty method, the hygienic behaviour of the colonies was nearly the same. Application of this method is more complicated, needs more time, and the result does not give more information in spite of the fact that the bees should discover the dead larvae in a covered not injured honeycomb. In this method the strange smell of the little fragment of the treated honeycomb could be disadvantageous, as could perhaps be the low temperature, as well as the arising vapour. All this means more work for the bees, because on both sides of the honeycomb dead cells can be found, the evaluation is difficult, cannot guarantee the same number of cells (100-100 cells) on both sides of the honeycomb fragment. At piercing a lot of larvae are injured, which also means work for the cleaning bees.

The number of the injured cells could be diminished by cutting or piercing the honeycomb according to the shape of the cells e.g. by following the shape of rhombus. A freshly disinfected model containing 100 pins can be prepared for piercing,. An important point for the comparison is that the test in an apiary should be performed on the same date because the number of the young bees changes in different periods of the year. The place of testing should also be the same, because the instinct of cleaning is stronger in the middle of the honeycomb than farther away from it.

Based on our experiments we recommend the application of the pin piercing method for testing the hygienic behaviour. The systematic testing would be advisable in colonies kept for breeding and for queen rearing.

Table 1

Result of hygienic behaviour test at 24 and 48 hours (number of cleaned/not cleaned, or not opened cells), and result of *Nosema* test in three apiaries

Apiary 1			
Serial number/ Hive number	After 24 hours	After 48 hours	*Nosema* infection
1	78/18 all OK	98/2	+
2	57/24	90/10	+++
3	58/32	95/5	++
4	98/2	97/3	+
5	56/11	99/1	+
6	86/8	98/2	+
7	85/11	93/7	+++
8	49/5	95/5	+
9	93/7	99/1	+++
10	85/12	99/1	+++

Apiary 2			
Serial number/ Hive number	After 24 hours	After 48 hours	*Nosema* infection
1	72/20 OK	100	+++
2	65/4	100	+++
3	92/7	100	++
4	37/56	99/1	+++
5	76/14	99/1	+++
6	27/31	93/7	+
7	85/3	98/2	+
8	74/21	100	++
9	89/3	100	++
10	98/2	100	+++

Apiary 3					
Refrigerated			**Pierced**		
Serial/ Hive number	After 24 hours	After 48 hours	After 24 hours	After 48 hours	*Nosema* infection
1	28/5	93/3	97/3	97/3	-
2	21/7	98/2	91/3	98/2	+
3	62/8	100	87/10	98/2	-
4	93/4	100	94/6	97/3	-
5	74/2	98/2	64/8	88/9	+
6	88/6	100	99/1	99/1	-
7	92/2	100	88/10	91/9	-
8	97/3	100	47/49	82/16	-
9	99/1	100	97/3	99/1	-
10	84/6	95/5	94/6	100	-

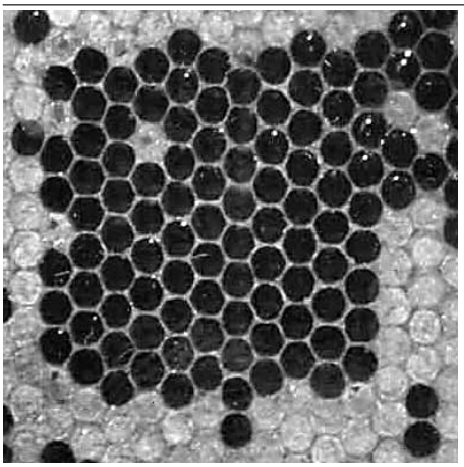

Fig. 1 Testing the hygienic behaviour of bee colonies by the method of pin piercing. The result of testing after 24 hours: in the middle area all cells are cleaned, a few pierced cells remained in the off-centre parts which were not recognised by bees

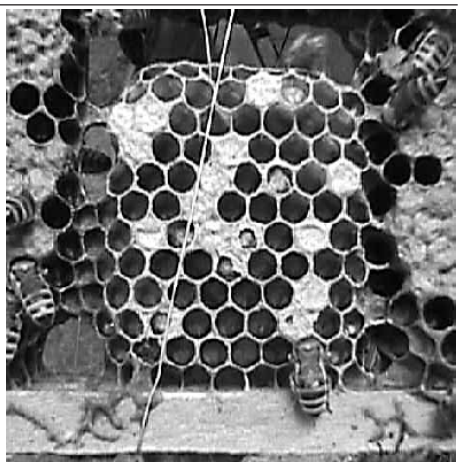

Fig. 2 Testing the hygienic behaviour of bee colonies, by the method of freezing a brood comb section. The piece of comb was fixed by wire. The bees cleaned only small cells after 24 hours. In the majority of the cells they did not recognize the dead larvae, or the cells had already been opened, but the dead larvae were not removed yet

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BADANIA NAD ZACHOWANIEM HIGIENICZNYM PSZCZÓŁ

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S t r e s z c z e n i e

Od dłuższego czasu prowadzone są rozważania czy rodzina pszczela jest w stanie przy pomocy czynników zewnętrznych chronić się przed czynnikami chorobotwórczymi. To jest powodem, dla którego badacze przywiązują wiele uwagi do dziedziczenia się zachowania higienicznego i odporności na choroby pszczół. Wielu ekspertów uważa, że to zachowanie

spełnia ważną rolę w obronie przed infekcją pszczół przez choroby pasożytnicze.

Nasze badania wykonaliśmy w pasiece Uniwersytetu Szent Istvan w Gödöllo, (pasieka 1), w prywatnej pasiece (pasieka 2) i w pasiece Instytutu Drobnego Inwentarza (pasieka 3).

Różnica w zachowaniu higienicznym między rodzinami trzech badanych pasiek (tabele 1-3). W pasiece 3, w której zachowanie higieniczne jest przedmiotem selekcji, częstotliwość oczyszczania komórek była najwyższa (86%). W dwóch pierwszych pasiekach wyniki były zbliżone ale zawsze niższe w przypadku czerwiu zabijanego przez zamrażanie niż przez przekłuwanie. W doświadczeniach tych podobne wyniki uzyskano po 24 i 48 godzinach wskazują, że czynnik czasu ma niewielki wpływ na wynik ostateczny.

Na podstawie naszych doświadczeń polecamy w badaniach behavioru higienicznego zabijanie czerwiu poprzez przekłucie szpilką. Ten sposób powinien być stosowany w pasiekach prowadzących selekcję pszczół.

Słowa kluczowe: zachowanie higieniczne, usuwanie czerwiu, zamrażanie, przekłuwanie szpilką.