

Study on an easy method of hygienic behaviour evaluation in honey bee

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Summary

The aim of research was the assessment of the hygienic behaviour of worker bees. The hygienic behaviour was assessed by means of two test types: the traditional needle test and a test proposed by the authors that consisted in measuring the removal rate of cardboard pieces. The experiments were conducted over three seasons and comprised a total number of 60 colonies. It was proved that the cardboard removal test may replace the needle test in the selection process of bees resistant to brood diseases, but primarily in those bees that have a good expression of hygienic behaviour. The best test results are to be expected in tests which last for 24 hours.

Keywords: honey bee, hygienic behaviour

Hygienic behaviour is an activity which enables worker bees (*Apis m. mellifera* L.) to locate cells containing infected/dead brood, uncap them and remove dead larvae or pupae (12, 15, 18, 19). Results of numerous studies confirm the relation between dead brood removal rate and the resistance of bee colonies to brood diseases. Colonies selected for hygienic behaviour were found to be resistant to American foulbrood (AFB) (12, 15, 20) and chalkbrood (5, 20). Recently, studies have also been conducted concerning the use of hygienic behaviour in breeding bees resistant to *Varroa destructor* (1, 3, 4, 16). Rothenbuhler (15) proved that hygienic behaviour is determined by a pair of recessive genes. One of these genes determines the locating the cells containing dead brood by bees and next uncapping them, whereas the other is responsible for the removal of a dead larva or pupa (cell cleaning). At present, determination and expression of hygienic behaviour are known to be much more complex (1, 11, 18, 21).

The use of acaricides results in the accumulation of their residual amounts in bee products (23) and in developing resistance in these parasites (9). Recently, a considerable increase of AFB resistance to oxytetracyclin has been found in the USA (8, 10) and in South America (8). Therefore, efforts to breed resistant bees may contribute to the reduction or elimination of harmful therapeutics from beekeeping (20, 22) and, what it involves, may minimize the problem of their residual amounts being present in bee products. In this context, the problem of establishing an effective method of hygienic behaviour quantification becomes of crucial importance.

Three methods of hygienic behaviour evaluation are widely used. All of them are based on the assessment of the dead brood removal rate. The most common method, and at the same time the most recommended, is deep freezing of the brood (7). Bekesi and Szalai (2), on the other hand, recommend using the needle test (pin killed brood). This method was also used by Palacio et al. (12). Injection is the least common method of killing the brood. Results obtained by using these three methods are not very highly correlated (6, 17). All these methods are also very laborious and they may raise moral problems (14). Therefore, not many bee-

keepers who breed queens implement these methods. For the same reason, some beekeepers have started seeking easier methods. Known for their professional approach, German professional beekeepers, members of The Gemeinschaft der Buckfastimker e. V., adopted as a criterion of hygienic behaviour the removal rate of pieces of plotting paper or soft cardboard inserted between hive nest boxes. In most studies the term „hygienic behaviour” is limited to the process of cell cleaning, even though bees are undoubtedly able to remove foreign objects introduced into the nest. In this context, however, a question arises whether the ability of the bees to remove paper and cardboard is correlated with the ability to clean the cells. Some German, and also Polish, beekeepers seem to share the opinion that there is the such correlation. There is, however, a lack of scientific reports confirming such a correlation.

Therefore, in the present work, the authors have decided to test the feasibility of a new, simpler method of evaluating hygienic behaviour and compare it with the commonly used needle test.

Material and methods

The studies comprised three seasons. The total number of 60 bee colonies were used. The colonies consisted of Buckfast bees and two types of crossbreeds, Caucasian × Carniolan and Norwegian × Caucasian.

One hundred cells containing pupae (the initial phase of eye pigmentation) were pricked simultaneously with a „brush” made of 100 needles. This particular phase of brood development was selected on the basis of the opinion that older brood is removed more slowly than younger brood. Subsequently, the brood was photographed 6, 12 and 24 hours after it was pricked and the number of cleaned cells was then assessed by means of digital picture analysis (MultiScanBase v. 14.02). In each year of the study, 12 repetitions of the needle test were made in each colony.

Pieces of soft cardboard (beer mats) were weighted and placed in the cleft made un top bars. Then they were put between open brood combs, one in every colony. After 6, 12, and 24 hours the pieces were removed and weighted again. The weight of the removed cardboard was calculated on the basis of the weight difference between the measurements. In each year of the study, 12 repetitions of the test were made in each colony.

Results and discussion

Following the terminology proposed by Spivak and Downey (17), the colonies were qualified as hygienic (efficient cleaners) and unhygienic (inefficient cleaners). Colonies which removed over 90% of dead brood within 24 hours were considered hygienic. The results in tab. 1 show that Buckfast bees were a little more efficient cleaners than Norwegian × Carniolan and that Caucasian × Carniolan crossbreeds were the least efficient. In this context, the substantial proportion of hygienic colonies observed among Norwegian × Caucasian crossbreeds corresponds with an opinion that uncapping and dead brood removal are conditioned by a small number of recessive genes (1, 18).

The results of the needle test and the cardboard removal test were not correlated in unhygienic colonies (tab. 2). In hygienic colonies, on the other hand, the coefficients of correlation between both test types were positive and significant. Such results point out to the fact that testing hygienic behaviour by means of a cardboard removal test may constitute a basis of the selection in the colonies whose hygienic behaviour is good developed. One of the most important problems in the breeding programs is maintaining the selection gains after stopping the selection process (13). Therefore, the possibility of using the cardboard removal test as an easy and cheap method of maintaining the selection gains or of continuing the selection which was started by means of the traditional methods seems to be very promising. If the preliminary phase of the selection for hygienic behaviour were made in a professional reproductive breeding facility, the cardboard removal test would facilitate the continuation of the selection process in apiaries. Spivak and Downey (17) proved that in hygienic colonies, the cell cleaning activity is to a great extent genetically determined, whereas in unhygienic colonies it rather seems to be dependent on the environment. This observation combined with the results presented in tab. 2 confirms the fact that selection using the cardboard removal test should render good results especial-

Tab. 1. Results of the needle test

Types of tested bees	H%	T%	TH%	T%-TH%
Buckfast	70	47,0	53	p < 0,216
Caucasian × Carniolan	44	26,5	20	p < 0,074
Norwegian × Caucasian	61	26,5	27	p < 0,890

Explanations: H% – percentage of the hygienic colonies within each of the three genetic bee types, T% – percentage of the colonies of each genetic type in the total colony number, TH% – percentage of the colonies of each genetic type within the hygienic colonies, T% – TH% – statistical verification of the difference significance between T% and TH% (Chi2)

Tab. 2. The correlation coefficients for number of the dead brood removed cells and the removed cardboard weight, monitored after 6, 12 and 24 hours

Cardboard removal rate						After 6 h	Dead brood removal rate
Non-hygienic colonies			Hygienic colonies				
After 6 h	After 12 h	After 24 h	After 6 h	After 12 h	After 24 h		
-0,07	-0,16	0,00	0,20*	0,16*	0,24**	After 6 h	
-0,02	-0,22*	-0,17	0,20*	0,13	0,25**	After 12 h	
0,04	-0,02	0,04	0,17*	0,32**	0,43**	After 24 h	

Explanations: * p ≤ 0.05; ** p ≤ 0.01

ly in those bees which have predispositions for efficient cell cleaning. Therefore, suggestions made by German beekeepers have been confirmed in the present studies. The highest correlation coefficient was found in the case of observations made 24 hours after the brood was pricked and the cardboard placed in the nest. Hence, cardboard removal test in 24 hours may be considered to be the most useful in practice. It is highly probable that high dead brood removal rate observed in Buckfast bees may have resulted from the selection for high cardboard removal rate conducted by German beekeepers, from whom those particular bees were purchased.

It is worth noting that correlations between the results obtained in using various traditional tests (7, 17) were not significantly higher than correlations between needle test and cardboard removal test.

Conclusions

1. The cardboard removal test could be useful for the hygienic behaviour quantification, but rather in the more hygienic colonies.
2. The removed cardboard weight should be assessed 24 hour after the beginning of the test.

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