

CORRECT QUEEN MAINTENANCE BEFORE AND AFTER INSTRUMENTAL INSEMINATION, TESTED IN EGYPT

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Summary

A common Egyptian method of storing several queens in one bee colony, and caging them after instrumental insemination, was compared with a method recommended by the author. When queen cells were introduced into nuclei instead of virgin queens, the acceptance increased from 40-60% to 100%. Storage of virgins in a queen bank resulted in injuries to the queens, especially to their legs. When the storage period was shortened from 10 to 4 days after emergence, the percentage of non-injured queens increased from 46 to 100%. Legs of accepted, freely moving virgin queens were not injured. When queens were caged after instrumental insemination, only 1.8 million spermatozoa entered the spermatheca, but when newly inseminated queens were released, 5.1-5.2 million sperms entered. Thus a 2.8- to 2.9-fold increase was achieved.

Introduction

The method of instrumental injection of serum into the oviducts of queen bees is well developed. Therefore, the conditions in which queens should be maintained before and after insemination were investigated during recent years in order to improve insemination results. The main concerns are: (a) virgin queens should not be kept in queen banks before or after insemination, because they are injured by workers (Woyke et al., 1956; Jasiński, 1987; Woyke, 1988); and (b) queens should not be caged after instrumental insemination, because semen is retained in the oviducts (Vesely, 1970; Woyke, 1979) and spermatozoa enter the spermatheca in low numbers (Woyke, 1979; Woyke & Jasiński, 1979). After instrumental insemination, the queen must be in direct contact with worker bees (Woyke, 1979).

Still, storage of queens in queen banks, as well as caging them after instrumental insemination, is recommended in recent publications (Harbo, 1986) and is widely practised. Therefore, a test was conducted to see whether the results obtained with *Apis mellifera mellifera* in Poland were also valid for other honeybee subspecies under different agricultural conditions.

Materials and Methods

The test was conducted in the apiary of the Bee Division of the Ministry of Agriculture in Cairo, Egypt. *A. m. ligustica* bees, as well as those of *A. m. carnica*, were present in the apiary. Methods of queen maintenance practised in Egypt, which are in accordance with the latest international recommendations, were compared with those recommended by the author.

Queens were reared in hives divided by vertical queen excluders into three unequal parts. Each outside compartment contained a normal bee colony on 10 Langstroth combs. The central compartment had room for 5 combs. Here, queen cell cups with transplanted larvae were inserted and left for further queen rearing. A few days before the queens emerged, the queen cells were removed and attached to a comb with unsealed honey. Each queen cell was protected by a hemi-spherical wire-screen, push-in cage (Fig. 1). The comb, with the protected queen cells, was placed between two broodcombs in the 'super' of another queenless or queenright colony. The queens emerged and remained here until they were sold as virgins or until they were introduced to queenless nuclei or larger colonies for natural mating. For instrumental insemination, queens about 1 week old were taken from the queen bank colony. After insemination, each queen was introduced to a queenless nucleus, and was protected on a comb with the same push-in screen cage. Two days later, the queens were treated for a second time with CO₂ and were then released. A problem occurred with the acceptance of these queens (Ibrahim, 1982). Therefore, the method of introduction was changed. After the nuclei

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FIG. 1. Push-in cages used for storing queens in Egypt.

had been queenless for 1–2 days, mature queen cells, but not queens, were introduced without any protection.

Queens were also introduced to bees and kept according to a method worked out in Poland (Woyke & Jasiński, 1979). Small boxes, $12 \times 12 \times 4.5$ cm, were constructed and supplied with pieces of comb, candy and water. About 250 workers were added. Queens taken from a queen bank colony were introduced after insemination to these boxes. To avoid killing queens, the workers were treated with CO_2 , and the queens were added while the workers were unconscious. The boxes, with bees and a queen, were left in the laboratory. Queens stored in different ways were examined for injuries. The legs were examined under a stereomicroscope.

One-week-old queens were instrumentally inseminated with 8 mm^3 of semen. Three methods of queen maintenance after insemination were compared.

1. Queens were maintained by the established method; i.e. they were collected from the push-in cages in the queen bank colony and, after insemination, each was introduced into one nucleus in a push-in cage.

2. Queens collected from the queen bank colony were introduced after insemination to small boxes, while the workers were still unconscious following CO_2 treatment.

3. Queens moving freely in nuclei were collected, and after insemination were returned into the same nuclei in cages plugged with a little candy. The queens were released by workers within 15–30 min.

All queens were killed 2 days after instrumental insemination. They were dissected, and the number of spermatozoa in their spermathecae were counted by the standard method (Woyke, 1979).

Results

Introduction of queens

According to the records of the Bee Division in Cairo, only 40–60% of all young, non-laying queens were accepted after insemination. Similar results were observed by the author. One of the causes of low acceptance could be robbing. Nuclei were located in an apiary together with other colonies. Due to poor nectar flow, queen rearing colonies as well as others were fed frequently with sugar syrup during the daytime. This resulted in 'silent' robbing.

When unprotected, ripe queen cells were introduced to 1- or 2-day queenless nuclei, none were torn down, and all the resulting queens were accepted.

TABLE 1. Number of spermatozoa (in millions) in spermathecae of queens kept after instrumental insemination in different conditions.

Conditions after insemination	No. of queens	Range	$\bar{x} \pm SE$
Push-in cages	7	0.7 - 3.2	1.8 \pm 0.3
Small boxes	5	4.7 - 5.6	5.1 \pm 0.2
Free in nuclei	6	3.7 - 6.2	5.2 \pm 0.4

One of 8 queens introduced to the boxes with worker bees treated with CO₂ was balled after the bees recovered. But after immediate additional CO₂ treatment, she was released without visible injury.

Injuries to virgin queens in queen bank colonies

Out of 11 queens stored in a colony under push-in screen cages for 10 days, 7 (64%) were injured by worker bees. The footpads of all 7 queens were damaged. Additionally, the pretarsus of one queen was bitten off. Examination of a second series of 15 queens stored in the same way revealed that none of the queens were injured until the age of 4 days. After that, the legs of those queens were also damaged. Thus, shortening the period of storage of several virgin queens in one bee colony increased the percentage of non-injured queens from 36 to 100% (2.8 times).

Queens which emerged from queen cells introduced to nuclei, were not injured, neither were queens accepted in the small boxes.

Number of spermatozoa in the spermathecae of queens maintained in different conditions

Table 1 shows that the average number of spermatozoa in the spermathecae of queens inseminated with 8 mm³ of semen, and kept afterwards in the push-in cages, was 1.8 million, which is very little. Queens released after insemination among worker bees in small boxes or nuclei, had on average, 5.1 or 5.2 million spermatozoa, respectively.

The ranges of the number of spermatozoa in queens caged after insemination and those released freely, did not overlap. The range established for queens in the small boxes was within that for the free-moving queens. Thus, without further statistical calculations, it was obvious that queens caged on their own after insemination had significantly less spermatozoa, than those moving freely among worker bees, but no significant difference was detected between queens released into small boxes and those in the nuclei.

Conclusions

Introduction of queen cells instead of young queens increased the percentage of queen acceptance from 40-60% to 100%. Storage of virgins in a queen bank resulted in queen injuries. Shortening the storage period from 10 to 4 days increased the percentage of uninjured queens from 36 to 100%. Freely moving queens accepted in nuclei or small boxes were not injured. When queens were released among bees after instrumental insemination instead of being caged on their own, the number of spermatozoa entering the spermatheca increased 2.8-2.9 times. Thus the phenomena of injury to stored queens and the low number of spermatozoa entering the spermatheca of queens caged on their own after insemination, which were found for *A. m. mellifera* in Poland, also occurred in other *A. mellifera* subspecies and under different apicultural conditions.

References

- HARBO, J. R. (1986) Propagation and instrumental insemination. *From Bee genetics and breeding*, ed. T. R. Rinderer. Orlando, USA: Academic Press
- IBRAHIM, S. H. (1982) Studies on queen honeybee introduction. *Agric. Res. Rev.*, Cairo 60 : 173-178
- JASIŃSKI, Z. (1987) Injuries of queens caged in queenless colonies. *Abst. 31st int. Congr. Apicult.*, Warsaw : 67-88

- VESELY, V. (1970) Retention of semen in the lateral oviducts of artificially inseminated honey-bee queens (*Apis mellifera* L.). *Acta ent. bohemoslov.* 67 : 83-92
- WOYKE, J. (1979) Effect of the access of worker honeybees to the queen on the results of instrumental insemination. *J. apic. Res.* 19 : 136-146
- (1988) Problems with queen banks. *Am. Bee J.* 128 : 276-278
- WOYKE, J.; GŁOWSKA, Z.; NOWOSIELSKA, B. (1956) Opieka pszczół nad matkami w różnych klateczkach [Worker bees care of queens in different cages]. *Pszczelarstwo* 7 : 4-7. *In Polish*
- WOYKE, J.; JASIŃSKI, Z. (1979) Number of worker bees necessary to attend instrumentally inseminated queens kept in an incubator. *Apidologie* 10 : 149-155