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Short communication

RE-INSEMINATION OF EGG LAYING QUEEN HONEY BEES

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Abstract

Instrumental insemination of queen bees that have started laying eggs is used extremely rarely. It is one of the methods for obtaining diploid drones, but it is believed that the queen must stop laying eggs for at least five days before insemination, e.g. by placing her in a mesh cage in her colony, in order to deactivate the ovaries. According to unpublished information, re-insemination of queens with active ovaries causes their death. The aim of this work was to verify this information. The results of our experiments show that re-insemination does not affect the survivability of queens with active ovaries and can be performed without prior interruption of egg laying.

Keywords: honey bee queen, instrumental insemination, interruption of egg laying, oviposition, re-insemination of laying queen

INTRODUCTION

The re-insemination of egg-laying queens is used only in special cases and thought not to be a viable practice, although this has been used is some studies. A specific queen, which has mated naturally and is laying eggs, can be re-inseminated using instrumental insemination with semen from specific drone or drones. Some of the queen's offspring may come from the particular drones used for re-insemination and will inherit the specific desired traits. This is important for such recessive traits as eye colour mutations. Instrumental insemination of laying queens is also one of the methods for obtaining diploid drones (Polaczek et al., 2000) in which virgin queens are treated twice with carbon dioxide resulting in rapid activation of the ovaries and the laying of only unfertilized eggs, from which drones develop. The queen is then inseminated with semen from her sons; half of the fertilized eggs laid by such a queen hatch into worker larvae and the other half into diploid drone larvae. However, Polaczek believes (personal information not included in the aforementioned publication) that the queen must stop laying eggs for at least five days before insemination in order for the ovaries to be deactivated. This can be achieved, for example, by placing the queen in a mesh cage in her colony, or otherwise she will die after insemination. This information was also provided by Jasiński, a well-known scientist and queen inseminator in Poland, but was not published. The aim of the study was to investigate whether brood interruption is necessary before instrumental insemination of egg-laying queens.

MATERIAL AND METHODS

The research was conducted from May to July 2024 at Warsaw University of Life Sciences. Queens established in mating nucs with about thousand workers were investigated. A total of sixty-seven queen bees were instrumentally inseminated. Two experiments were performed. In the first one, the mortality of queens was examined depending on the time of interruption of egg laying and filling of the spermatheca before insemination. All queens were inseminated with 3 μl of semen. Five experimental groups were created:

I group - Queens were naturally mated and laying eggs. Queens were re-inseminated without interrupting egg laying (8 queens).

II group - Queens were naturally mated and laying eggs. They were caged in Zander cages in their colonies six days to stop egg laying before insemination (8 queens).

III group - Queens were naturally mated and laying eggs. They were caged in Zander cages in their colonies three days to stop egg laying before insemination (8 queens).

IV group - Queen excluders were placed on the mating nuc entrances to prevent mating flights. Virign queens were anaesthetized twice with carbon dioxide to induce egg laying and were inseminated without interrupting egg laying of unfertile eggs (8 queens).

V group - Control, queen excluders were placed on mating nuc entrances. Virgin, non-laying queens were inseminated. They were additionally treated with carbon dioxide one day before insemination (7 queens).

Queens in groups I-III laid eggs for 2-3 weeks prior to re-insemination and were re-inseminated at approximately 1 month of age, queens in group IV laid eggs for 2-3 weeks prior to insemination and were inseminated at approximately 1 month of age and queens in group V were inseminated at 7 days of age.

Based on the results of the first experiment, a second set of experiments were performed. In this set, all queens were naturally mated and laying eggs for 2-3 weeks. The queens were divided into three groups and inseminated without interruption of egg laying. The following semen doses were used: 3 µl (8 queens), 6 µl (10 queens), and 9 µl (10 queens). Two days after insemination, the survival rate of the queens was monitored, and after another four days, queens were observed for egg laying.

RESULTS AND DISCUSSION

All queens in both sets of experiments were alive two days and six days after insemination. Of the seven queens from the control group in the first experiment, which did not lay eggs before insemination, four started laying eggs within six days from insemination and the remaining three within the next five days. Out of all sixty queens that laid eggs before insemination, only one did not start laying eggs again within six days after re-insemination, but was alive. The next inspection revealed that the nuc had been robbed and consequently the queen died but not due to injury or infection during insemination procedure. The overall level of survival of re-inseminated queens was surprising, especially because in routine instrumental insemination of queens a percentage tend not to survive the procedure. The mortality rate of queens after insemination reported by others was 3-8% (Bieńkowska & Panasiuk, 2006; Bieńkowska et al., 2008; Chuda-Mickiewicz et al., 2012; Gąbka et al., 2016; Gąbka & Cobey, 2018; Gąbka, 2022; Gąbka et al., 2022) or even 16-35% (Kaftanoglu & Peng, 1980, 1982; Moritz & Kühnert, 1984). The results of this experiment indicate that in very rare cases, re-insemination of an egg-laying queen can be performed without previous interruption of oviposition and with good queen survival. The assumption that laying queens must be caged to stop egg laying before re-insemination is not confirmed.

It should be noted that queens in our experiment were re-inseminated at approximately 1 month of age. With age, changes occur in the reproductive system of the queens, especially in the valve-fold (Fyg, 1966), which is believed to hold the egg micropyle against the orifice of the spermathecal duct directing the sperm to the micropyle and permitting the fertilization (Camargo & Mello, 1970, Kozii et al., 2022). The valve-fold also plays an important role during natural mating or instrumental insemination by preventing sperm escape towards the opening of the genital chamber (Fyg 1966, Mackensen & Tucker, 1970). According to Fyg (1966), the valve-fold serves as a seal for the vaginal orifice only in young queens, because with age it degenerates and calcifies. This indicates that re-insemination of older queens may be problematic. However, Dziechciarz et al. (2024) demonstrated that three-year-old drone-laying queens, when re-inseminated, had semen in the oviducts, and the valve-fold functioned properly. In these authors' studies, several old queens that were placed back in their colonies after re-insemination died. Thus, the instrumental insemination of egg-laying queens at different age should be investigated in more detail. In future studies, attention should also be paid to progeny testing of re-inseminated queens. This is needed to verify the sperm migration and fertilization of eggs resulting from the re-insemination.

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