

THE ROLE OF PHYTOHORMONES IN INSTRUMENTAL INSEMINATION OF QUEEN BEES

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S u m m a r y

In total, 164 queen bees were inseminated instrumentally, including 122 ones in mating nuclei being fed with phytohormones-supplemented sugar syrup (cytokinin and epibrassinolide) before and/or after instrumental insemination, with 8 µl of semen. It was found that bee feeding with phytohormones did not have any effect on the percentage of egg laying queens. In groups receiving phytohormones, oviposition was started by 70 to 80.9% of queens whereas in the control group by 76.2 % ($p = 0.14$). Queens started oviposition after an average of 8.1 days. The time of the onset of oviposition by queens in groups receiving phytohormones, was from 7 to 9.5 days. This did not differ significantly from that for the queens from nuclei being fed with pure sugar syrup ($\chi^2 = 5.4386$; $df = 3$; $p = 0.1424$).

Keywords: *Apis mellifera*, honeybee queen, instrumental insemination, oviposition, phytohormones.

INTRODUCTION

In bee breeding, only instrumental insemination of queen bees guarantees a fully-controlled selection of parental pairs. A shortcoming of queens inseminated instrumentally is that their oviposition starts later than those inseminated naturally. Naturally inseminated queens start egg laying 1 to 4 days after the last mating flight, or even the first day after the flight performed on day 3 or 6 of life (Butler, 1954; Koeniger, 1986; Szabo et al., 1987; Woyke et al., 2001). Instrumentally inseminated queens, start oviposition 6.9 to over 20 days on average after instrumental insemination. The beginning of oviposition for instrumentally inseminated queens depends on the age at which they were inseminated, semen dose, and method of keeping (Chuda-Mickiewicz and Prabucki, 1993, 2000; Chuda-

Mickiewicz et al., 2003; Kaftanoglu and Peng, 1982; Konopacka, 1991; Prabucki et al., 1987; Samborski et al., 2008; Wilde, 1994; Woyke and Jasiński, 1990 a, b; Woyke et al., 2001). Mackensen (1947) showed that a single or a two-fold treatment with CO₂ for instrumentally inseminated queen bees shortens the time for initiation of their oviposition. However, it was found that CO₂ is not a neutral gas for bees. The longer they are anaesthetised with CO₂, the higher the harmful effect (Skowronek and Jaycox, 1974; Skowronek, 1976; Czekońska, 2009). Ebadi and Gary (1980) reported that queens flying 10 minutes prior to instrumental insemination with 8 µl of semen and single treated with 75% CO₂ started oviposition 4 days earlier than non-flying ones. Woyke et al. (2008) demonstrated that exposure of queens to

additional treatments before, during and after instrumental insemination (8 μ l of semen) did not accelerate their oviposition. Queens flying 3 min before and/or after instrumental insemination started laying eggs earlier by 0.5 to 1.1 days. Those plugged with mucus directly after instrumental insemination started laying eggs later, on an average of 0.9 day from those inseminated without additional treatments. Similarly, defecation of queens prior to instrumental insemination does not accelerate their oviposition (Czekońska and Chuda-Mickiewicz, 2007). Boytseniuk and Atymirov (2000) and Boytseniuk et al. (2002) found that administration of phytohormones, i.e. epibrassinolide and cytokinin, to bees stimulates queens to more intensive oviposition.

This study aimed to verify whether or not bee feeding with phytohormones-supplemented sugar syrup (cytokinin and epibrassinolide) would shorten the time for initiation of oviposition by queens being instrumentally inseminated.

MATERIAL AND METHODS

The observations were carried out in 2007 and 2008 on queen bees of the Carnolian breed (*Apis m. carnica*) inseminated with semen from drones of the same breed. Queen cells were introduced into 6-comb mating nuclei of the Mini-plus type (215 x 163 mm frame) 1-2 days before queens emerged. Nuclei entrances were secured with queen excluder. Four groups were formed, 10 to 11 nuclei each:

Group 1 - fed with phytohormones-supplemented sugar syrup (1:1) for 3 days before instrumental insemination (Phyt B II).

Group 2 - fed with phytohormones-supplemented sugar syrup (1:1) for 3 days after instrumental insemination (Phyt A II)

Group 3 - fed with phytohormones-supplemented sugar syrup (1:1) for 2 days before and 2 days after instrumental insemination (Phyt B&A II)

Group 4 - the control, fed with sugar syrup without phytohormones for 2 days after instrumental insemination (S A II).

Each nucleus colony obtained 600 ml of sugar syrup. The syrup administered to groups 1, 2 and 3 contained 25 mg cytokinin and 0.12 mg epibrassinolide in 1 liter of syrup. Group 4 received pure sugar syrup (without phytohormones). Queen bees were inseminated with 8 μ l of semen at the age of 7 days. Two days prior to insemination, at the age of 5 days, queen bees were anaesthetised with CO₂ for 3 minutes. Onset of oviposition by queens was determined by examining mating nuclei every second day until day 22 after instrumental insemination. Queens not starting oviposition were killed and their reproductive organs were prepared for examination. The oviducts were examined to see whether semen was or was not deposited on them, whereas spermathecas were examined after removal of tracheas to see whether they were filled with spermatozoa. In both study years, the experiment was performed in two replications, with the first one being carried out in June and the second one in July. In total, 164 queens kept in nuclei were inseminated instrumentally, out of which 122 ones were fed with phytohormones-supplemented sugar syrup and 42 with pure sugar syrup. Queen losses after instrumental insemination were evaluated using the G-test with William's adjustment. The percentage of egg laying queens (after Bliss transformation) was compared with the test of differences between two indicators of the structure, while the time for initiation of oviposition with ANOVA (Kruskal-Wallis test). Calculations were made with the Statistica 7 computer software package.

RESULTS

Out of 164 instrumentally inseminated queens, 126 started oviposition. Queen losses in respective groups were similar (Tab. 1), not differing significantly between each other (G-test: $G_{adj} = 1.624$; $df = 3$; $p = 0.654$). Half of these losses were caused by their mortality in the first week after instrumental insemination and half because they did not start oviposition.

In all non-laying queens, semen was not deposited in the oviducts, but spermathecas were filled with spermatozoa. The overall insemination efficiency rate (percentage of egg laying queens) was 76.7%. Differences in insemination efficiency rate between respective groups did not exceed 11%. The least difference, 0.9%, occurred between group Phyt A II and group Phyt B&A II, whereas the greatest, 10.9%, between

group Phyt A II and group Phyt B II. In two groups being fed with phytohormones-supplemented sugar syrup, i.e. Phyt B&A II and Phyt A II, oviposition was started by more queen bees, i.e. 3.8 and 4.7% respectively, than in the group being fed with pure sugar syrup (S A II). Oviposition was started by 6.2% less in groups Phyt B II than in group S A II. The differences observed between groups were not significant ($p = 0.14$).

The time for onset of oviposition from insemination to the finding of the first eggs on combs was 3 to 33 days for all queens, being 8.1 ± 3.71 days on average (Tab. 2). Queens of group Phyt A II started oviposition the quickest, after an average of 7.0 ± 2.77 days, followed by those of group Phyt B II and group Phyt B&A II (after 2.5 and 1.8 days, respectively). In mating

Table 1.

Instrumental insemination efficiency (percentage of egg laying queens)

Group	Queen number	Queen losses after instrumental insemination	% of egg laying queens
1. Phytohormones before II	40	12 a*	70.0 a
2. Phytohormones after II	42	8 a	80.9 a
3. Phytohormones before & after II	40	8 a	80.0 a
4. Sugar syrup without phytohormones after II	42	10 a	76.2 a
Total	164	38	76.7

*Figures in a column followed by the same letter do not differ significantly at $p < 0.05$

Table 2.

The waiting time for oviposition onset

Group	Queen number	Number of days from instrumental insemination to onset of oviposition		
		range	mean value \pm sd	median
1. Phytohormones before II	28	3 - 22	9.5 ± 4.66 a*	8.0
2. Phytohormones after II	34	4 - 15	7.0 ± 2.77 a	6.0
3. Phytohormones before & after II	32	4 - 19	8.8 ± 3.88 a	8.0
4. Sugar syrup without phytohormones after II	32	3 - 15	7.5 ± 2.99 a	7.0
Total	126	3 - 22	8.1 ± 3.71	7.0

*Figures followed by the same letter do not differ significantly at $p < 0.05$

nuclei of the control group, being fed with pure sugar syrup only (S A II), eggs were found on combs after an average of 7.5 ± 2.99 days (Tab. 2). The mean number of days from insemination to starting oviposition by queens between groups did not differ for mean values ranging from 0.5 to 2.5 days, whereas for median values from 1 to 2 days. However, the observed differences did not differ significantly ($\chi^2 = 5.4386$; $df = 3$; $p = 0.1424$). No effect of phytohormones on acceleration of oviposition in inseminated queen bees was found.

DISCUSSION

The percentage of egg laying queens in our investigation was similar to that obtained by other authors, i.e. 77.2% by Konopacka (1991), 79% by Samborski et al. (2008), 80% by Mackensen (1947), and 80.2% by Woyke and Rutner (1976). However, Moritz and Kühnert (1984) found 92.3% of queens laying eggs after instrumental insemination when applying a 10 min CO₂ anaesthesia two days after instrumental insemination. Otten et al. (1998) demonstrated that onset of oviposition depended on the age at which queens were inseminated; out of the queens inseminated at the age of 10 days, 95% laid eggs, whereas 82 and 80%, respectively, among those inseminated on day 7 and 13. Similar results were found by Chuda-Mickiewicz et al. (1993), when inseminating queen bees twice with 4 µl of semen. Out of the queens inseminated at the age of 11 and 13 days, oviposition was started by 100% of the queens, whereas 87.5% among those inseminated at the age of 8 and 10 days and 50% among those inseminated at the age of 5 and 6 days to day 14 from instrumental insemination.

Administration of phytohormones to bees did not affect acceleration of oviposition onset by queens. Overall mean

time from instrumental insemination to laying first eggs for all queens on day 8.1 was only longer by 0.1 day from that for the queens kept in the same nuclei and also inseminated on day 7 of life with 8 µl of semen (Samborski et al., 2008) and by 0.2 day from those inseminated twice with 4 µl of semen (Woyke et al., 2001). Moritz and Kühnert (1984) demonstrated that queens treated 10 min before instrumental insemination with CO₂ and 5 min during instrumental insemination with 8 µl of semen started oviposition after an average of 7.67 ± 0.07 days. In the present study, queens in groups Phyt B II and S A II started laying eggs at a similar time, i.e. after 7 ± 2.77 and 7.5 ± 2.99 days, respectively. On the other hand, the time from instrumental insemination to onset of oviposition in queens of group Phyt B II (9.5 ± 4.66 days) was similar to that observed in queens inseminated 2 x 4 µl of semen, i.e. 10.7 days (Chuda-Mickiewicz and Prabucki, 1993), as well as to that observed in queens treated two days after instrumental insemination with CO₂ for 3 min, i.e. 10 days (Skowronek et al., 2002), and in those treated two days before instrumental insemination with CO₂ for 3 min, i.e. 10.3 days (Woyke et al., 2001).

CONCLUSION

Administration of phytohormones (cytokinin and epibrassinolide) to queen bees before and after instrumental insemination did not have effect on increase in insemination efficiency rate or shortening of the time for onset of their oviposition.

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FITOHORMONY W INSEMINACJI MATEK PSZCZELICH

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

Ogółem unasieniono 164 matki, w tym 122 w ulikach weselnych dokarmianych syropem z fitohormonami (cytokininą i epibrasinolidem) przed i/lub po inseminacji 8 μ l nasienia. Stwierdzono, że dokarmianie pszczół fitohormonami nie miało wpływu na procent matek czerwiałych. W grupach otrzymujących fitohormony czerwienie rozpoczęło 70 do 80,9% matek w grupie kontrolnej 76,2% ($p = 0,14$). Matki rozpoczynały składać jaja średnio po 8,1 dniach. Okres oczekiwania na rozpoczęcie czerwienia matek w grupach otrzymujących fitohormony od 7 do 9,5 dni nie różnił się istotnie od matek w ulikach weselnych dokarmianych czystym syropem ($\chi^2 = 5,4386$; $df = 3$; $p = 0,1424$).

Słowa kluczowe: *Apis mellifera*, matka pszczela, sztuczne unasienianie, czerwienie, fitohormony.