

Effect of the method of instrumental insemination on the number of spermatozoa in a bee queen spermatheca

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INTRODUCTION AND AIM OF THE RESEARCH

Many factors influence the spermatheca filling of instrumentally inseminated queens. The most important are queens' quality, race, age of queens when inseminated, age of drones used for semen collecting, conditions of keeping queens before and after insemination, technique of insemination and dose of used semen. Woyke (1960) recommended a single insemination with a dose of 8 µl of semen or double insemination with a dose of 4 µl of semen when queens were kept in colonies or mating hives. According to Mackensen (1964) and Harbo (1985), better results are achieved when queens are inseminated 2 or 3 times with smaller doses of semen and after the treatment are kept in cages with worker bees.

Every year in Poland, about 80 thousand of instrumentally inseminated honey bee queens are produced. After insemination they are kept in queen banks in mailing cages with about 25 attending workers (Gontarz et al. 2005). The high percentage of queens inseminated with a higher than 6 µl dose of semen and kept in that way did not clear their oviducts from the excess of semen (Vesely 1969; Bieńkowska, Panasiuk 2006; Bieńkowska et al. 2008).

The aim of the research was to verify the influence of a dose of semen used for insemination on oviducts condition and a number of spermatozoa in spermatheca.

METHODS

The research was carried out in the Apiculture Division in Puławy and in Breeding Apiary in Teodorów in the years 2007-2008. Carniolan sister queens were inseminated at the age of 7 days with semen collected from 1, 2, 3, 4, 6 and 8 drones. Semen collected from more than one drone was divided into smaller doses for multiple inseminations. It was assumed that a single drone produces about 1 µl of semen. Immediately after insemination, queens were kept in "Folchron" cages with 25 attendant bees in queenless colonies. The dead queens were counted 48 hours after insemination. Surviving ones were killed and then dissected. Their oviducts were examined for a residue of semen and the number of spermatozoa in spermatheca was counted. Percentage of queens with residue of semen in oviducts and with cleared oviducts was estimated.

RESULTS

During the two years of research a total of 703 honey bee queens were inseminated and dissected. Among examined queens, 2.8% were dead, 90.6% cleared oviducts and 6.6% had some residue of semen in oviducts (tab.1). Significantly higher percentage of queens (90 to 100%) inseminated once or twice with smaller doses of semen (from 1 to 4 µl) cleared their oviducts in comparison to queens inseminated once with semen collected from 6 or 8 drones (respectively 83 and 84%). But, some queens inseminated with small doses of semen (1x3 µl and 4x2 µl) did not clear their oviducts in a high rate (tab.1).

Table 1.

Conditions of oviduct of queens inseminated with semen collected from different number of drones.

Semen dose from „n” drones	Number of queens	Queens with cleared oviducts		Queens with semen residua in oviducts		Dead queens	
		n	%	n	%	N	%
1 x 1	51	50	98.0 cd	-	-	1	2.0
1 x 2	40	40	100.0 d	-	-	-	-
2 x 1	39	38	95.0 cd	-	-	1	5.0
1 x 3	40	31	77.5 a	9	22.5	-	-
3 x 1	38	33	86.8 ab	3	7.9	2	5.3
1 x 4	97	89	91.7 c	7	7.2	1	1.0
2 x 2	38	36	94.6 c	-	-	2	5.4
4 x 1	77	73	94.8 cd	3	3.9	1	1.3
1 x 6	30	25	83.3 ab	4	13.3	1	1.3
2 x 3	61	57	93.4 c	2	3.3	2	3.3
3 x 2	38	36	94.7 c	-	-	2	5.3
1 x 8	25	21	84.0 ab	4	16.0	-	-
2 x 4	100	90	90.0 c	5	5.03	5	5.0
4 x 2	29	18	62.1 a	10	4.5	1	3.4
average	703	637	906	47	6.6	19	2.8

a,b,c,d – significant differences at $p \leq 0.05$ (using Bliss' transformation)

Table 2.

Influence of dose of semen and multiple insemination on a number of spermatozoa in spermatheca in queens with cleared oviducts.

Semen dose from „n” drones	Number of queens ♀♀	Number of spermatozoa in spermatheca (mln)	Sd	Total	
				N	Number of spermatozoa in spermatheca (mln)
1 x 1	50	1.280 a	0.42	50	1.280 a
1 x 2	40	2.137 b	0.87	78	2.361 b
2 x 1	38	2.596 c	0.67		
1 x 3	40	3.100 de	0.81	64	3.448 c
3 x 1	38	3.765 fg	0.80		
1 x 4	97	2.833 cd	0.94		
2 x 2	37	3.995 g	0.92	205	3.305 c
4 x 1	77	3.489 ef	0.82		
1 x 6	30	3.494 ef	1.08		
2 x 3	61	3.953 fg	1.29	118	3.872 d
3 x 2	38	4.007 g	1.07		
1 x 8	25	3.902 fg	0.78		
2 x 4	100	4.009 g	1.40	121	4.191 e
4 x 2	30	5.269 h	1.56		
average	636	3.319	1.38	636	3.319

a,b,c,d,e – significant differences at $p \leq 0.05$, Sd – standard deviation

Significant differences were found between numbers of spermatozoa filling spermatheca in queens inseminated with semen collected from 1 to 8 drones (tab.2). Dividing a full dose of semen into smaller doses, positively affects spermatheca filling. The lowest number of spermatozoa in spermatheca was among queens inseminated once with 1 µl or 2 µl of semen and twice with 1 µl of semen. The highest number of spermatozoa in spermatheca was found in queens inseminated four times with 2 µl of semen (5.269 mln). The number of spermatozoa filling spermatheca in queens inseminated two and three times with semen collected from 2, 3 or 4 drones (from 3.953 to 4.009 mln) did not differ from the number of spermatozoa filling spermatheca in queens inseminated once with 6 and 8 µl of semen (tab.2). The high number of spermatozoa in spermatheca was found among queens inseminated three or four times with 1 µl of semen (respectively 3.765 and 3.489 mln).

CONCLUSIONS

- Higher percentages of queens clear their oviducts from the excess of semen when inseminated once or twice with small doses of semen.
- Dividing a full dose of semen into smaller doses for insemination positively influences the number of spermatozoa in spermatheca.

REFERENCES

- Bieńkowska M, Węgrzynowicz P., Panasiuk B., Gerula D., Loc K. (2008) - Influence of the age of honey bee queens and dose of semen on condition of instrumentally inseminated queens kept in cages with 25 worker bees in bee colonies. *J. apic. Sci.* 52(2): 23-33
- Bieńkowska M, Panasiuk B. (2006) - Influence of the diameter of the inseminating needle tip on the results of bee queens' fertilization *J. apic. Sci.* (50) 2:137-145
- Gontarz A, Bieńkowska M, Loc K. (2005) - Effect of queen caging conditions on insemination results. *J. apic. Sci.* (49) 1: 5-15
- Harbo J.R. (1985) – Instrumental Insemination of Queen Bees. *Am. Bee J.* (4):282-286
- Mackensen O. (1964) - Relation of semen volume to success in artificial insemination of queen honey bees. *J. econ. Ent.* (57)4: 581-583
- Vesely V. (1969) Retention of semen in the lateral oviducts of instrumentally inseminated honey bee queens (*Apis mellifera* L.) *Acta Entomol. Bohemoslov.* 67: 83-92.
- Woyke J. (1960) – Naturalne i sztuczne unasienianie matek pszczelich. *Pszczeln. Zesz. nauk.*, 4: 183-275