PREVENTION OF NATURAL MATING OF INSTRUMENTALLY INSEMINATED QUEEN HONEYBEES BY PROPER METHOD OF INSTRUMENTAL INSEMINATION

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S u m m a r y

Black coloured queens were instrumentally inseminated with semen from yellow coloured drones, and were allowed to mate naturally in an area containing black drones. Since yellow body colour is dominant over black, any black coloured worker progeny would indicate additional natural mating of the mother-queen. Together 308 queens were investigated, and body colour of 188 300 workers was determined.

Nine groups of queens were investigated. Queens of the control group mated solely naturally. The others were instrumentally inseminated either with 8 µl semen and treated with CO_2 or not, or were inseminated twice, with 4 µl of semen each time.

The results show, that among queens inseminated once with 8 µl of semen at the age of 6, 8 and 14 days, 69, 53, 29% respectively mated also naturally, and among those treated additionally with CO_2 two days later, only 14%. Queens treated with CO_2 two days before insemination with 8 µl of semen, as well as those inseminated twice with 4 µl of semen did not mate naturally in addition.

The highest losses occurred within the three groups of queens inseminated exclusively with 8 µl of semen, - 14.0, 13.6 and 10.0% respectively. Within all groups, as more queens flew out for natural mating, more of them were lost ($r = 0.96$). Three times more queens were lost within those which mated solely naturally, or were inseminated exclusively with 8 µl of semen, than within queens inseminated twice with 4 µl of semen.

Queens of the control group, which mated solely naturally, started to lay eggs 3 days after the queen excluders were removed from the entrances. Queens inseminated with 8 µl of semen and treated with CO_2, or inseminated twice with 4 µl of semen started oviposition significantly later (6 - 8 days), and those inseminated exclusively with 8 µl of semen started to lay eggs latest of all (10 - 11 days).

Queens of the control group produced exclusively black worker progeny. Queens inseminated instrumentally exclusively with 8 µl of semen at the age of 6, 8, and 14 days produced 20, 18, and 19% of black coloured worker bees respectively. Queens treated with CO_2 two days after insemination produced 11% of black progeny. Thus queens of the three first groups mated on average also naturally probably with 2 drones, and of the last group with 1 drone.

Due to low losses and absence of additional natural mating within high number of queens inseminated instrumentally twice, the method of double instrumental insemination of queens with 4 µl of semen with subsequent open entrances can be recommend for practical beekeepers.

Keywords: queen honey bees, natural mating, instrumental insemination, CO_2 treatment.

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INTRODUCTION

Instrumental insemination of queen bees is widely applied by beekeepers in Poland. About 30,000 queens are inseminated instrumentally per year. Honey production of colonies headed by queens of particular crosses of breeding lines is much higher than that of colonies headed by sister queens mated naturally. The wide use of instrumentally inseminated queens by beekeepers requires simplifications of dealing with instrumentally inseminated queens. The practice of fixing queen excluders before the entrances of nuclei, in order to prevent natural mating of instrumentally inseminated queens is very inconvenient. Worker bees have difficulties going through the entrances, they loose pollen loads, ventilation is reduced and so on.

Roberts (1944) showed, that queens which mated naturally in one mating flight do not stop to fly out of the hive. Out of 110 observed queens 55 flew for their next mating flights and mated again. Similarly, S. and F Ruttner (1953-54) and Alber et al (1955) proved that queens which mated in one flight mated again in the following flights. Those authors thought that the queens mate with only one drone in one flight. However, Triasko (1951), Taber (1954) and Woyke (1955) showed that queens mate with several drones during a single mating flight. According to Woyke (1956), queens returned from mating flights with 1.13 to 22.39 µl of semen in their oviducts. They had to mate with from 1 to at least 13 drones. Woyke (1958, 1964) showed that queens which stored 5.6 mil spermatozoa in their spermatheca after the first mating flight did not fly out of the hives. Those which stored 4.2 mil flew out but did not mate, and those which stored 3.9 mil spermatozoa mated again in a second mating flight. Thus, the next flights depended upon the number of spermatozoa stored in the spermatheca after the first mating flight. According to Woyke (1960) the queens mate with up to 17 drones, and on average with 8 to 9 drones during one mating flight.

Mackensen (1947) showed that CO_2 treatment of both, virgin and instrumentally inseminated queens accelerated beginning of egg laying. Woyke (1963, 1966) showed that additional CO_2 treatment of queens inseminated once with 4 µl of semen accelerated onset of oviposition, however, did not accelerate when queens were inseminated with 8 µl of semen. But Ebadi and Garry (1980) and Konopacka (1991) reported that queens inseminated once with 8 µl and treated additionally with CO_2 also began oviposition earlier then those untreated additionally. The first authors concluded that 75% CO_2 would be better than 100% for narcotising queen honeybees.

According to Woyke (1963, 1966) queens inseminated twice began oviposition earlier than inseminated once with the same total amount of semen. However, Prabucki et al (1987) and Woyke and Jasiński (1990) reported that queens inseminated twice did not begin oviposition earlier than inseminated once. Jasiński (1993) showed, that second insemination of queens which did not lay eggs during 3 weeks resulted in onset of oviposition.

Skowronek (1976) and Kaftanoglu and Peng (1982) reported that virgin queens treated with CO_2 flew out of the hives less frequently than the untreated ones.

The first investigations on natural mating of instrumentally inseminated queens were started by Woyke (1963, 1966). He showed that queens inseminated instrumentally also fly out of the hives and mate naturally. He inseminated queens with 1, 2, 4, 8, 12, and 16 µl of semen. As the dose increased, the percentage of queens flying out and mating naturally, decreased. Further investigations concerned queens insemi-

nated instrumentally with 8 µl of semen. Woyke and Jasiński (1992) and Woyke et al (1995) found that 50 to 60% of queens inseminated instrumentally with 8 µl of semen fly out of hives and mate naturally. However, queens inseminated twice with 4 µl of semen, and those treated additionally twice with CO_2, before and after insemination with 8 µl did not mate naturally.

Therefore we now investigated more differentiated and more numerous groups in order to find the influence of different factors on the behaviour of instrumentally inseminated queens. We hoped to find combinations of procedures which would eliminate natural mating of instrumentally inseminated queens.

MATERIAL AND METHODS

The investigations were conducted in the Apiculture Division of the Agricultural University in Warsaw in the years 1992 - 1995. In order to find out which instrumentally inseminated queens mate afterwards naturally, black coloured queens were reared. They were inseminated instrumentally with semen from yellow coloured drones, and were allowed to mate naturally in an area containing black drones. Since yellow body colour is dominant over black, any black coloured worker progeny would indicate additional natural mating of the mother-queen.

Together 308 queens were investigated of which 267 started to lay eggs. Body colour was determined in 188 300 workers originating from 202 queens which had the possibility to fly. On average, 932 workers per queen were examined.

Hybrid black *Apis mellifera mellifera* and *Apis mellifera caucasica* queens were reared. On 11^{th} day after grafting, queen cells were introduced into trapezoid Kirchhein mating nuclei, already filled with worker bees. Next, the nuclei were kept for 48 hours in a cool room. Afterwards they were placed in an apiary with black drones. Queen excluders have been fixed to entrances of all nuclei.

A control group of 23 queens was created. Those queens were not instrumentally inseminated. They were allowed to mate solely naturally. They served to test the absence of yellow coloured drones in the mating area, and to compare some other characters.

Yellow coloured Italian drones *A. m. ligustica* were used for instrumental insemination of the rest of the queens. The queens were treated as is presented in Table 1. With the exception of queens from two groups (No 3 and 4), all other were instrumentally inseminated or treated with CO_2 at the age of 6 days. The queens were inseminated with 8 µl of semen either once or twice with 4 µl of semen. The CO_2 treatment was applied either before or after instrumental insemination (tab. 1).

At the queen age of 6 days, queen excluders were removed from entrances of the control nuclei. Excluders from entrances of other nuclei were removed after the last queen treatment. However, the excluders were not removed from nuclei with instrumentally inseminated queens of the last group (No 9). Queens of that group, as well as those with cut wings (of group No 8, tab. 1), could not fly for mating flights, and could not mate naturally. They served to test mortality of instrumentally inseminated queens, without interference of losses during flights.

The beginning of oviposition was checked by examination of mating nuclei every 3 days. The exact day start of egg laying was calculated from the age of larvae.

A goodness-of-fit test was applied to compare the frequency distribution of the number of instrumentally inseminated queens with the number of queens mated also naturally, and the number of lost queens. Test-t was used to determine signif-

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Table 1

Way of insemination and number of queens investigated in particular years.
Sposób unasieniania i liczba matek w poszczególnych latach badań.

Group No Nr grupy	Way of insemination Sposób unasienienia	Year of investigation - Rok badań				Total Razem
		1992	1993	1994	1995	
1	Naturally mated 6-D* Unasienione naturalnie			23		23
2	8µl** 6-D	27	23			50
3	8µl 8-D	22				22
4	8µl 14-D		20			20
5	8µl + CO_2 6-D				24	24
6	CO_2 + 8µl 6-D				24	24
7	4µl + 4µl 6-D	24	31	19	23	97
8	4µl + 4µl 6-D Cut wing Obcięte skrzydło			24		24
9	4µl + 4µl 6-D Excuder on entrance Okratowany wylot			24		24
Total Razem		73	74	90	71	308

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances.
* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków.
** Amount of semen used for instrumental insemination of queens. - Ilość nasienia użyta do sztucznego unasienienia.

icant differences between two means. Correlation coefficient between percentages of queens which mated also naturally and the lost ones was calculated. Binomial confidence intervals (α one sided = 0.05) were applied to show results which could happen if large number of similar experiments were performed. ANOVA was applied to results of several groups concerning beginning of oviposition and the percentage of worker offspring originating from additional natural mating. The percentages for statistical calculations were transformed according to the Bliss function. Newman-Keuls test was used to determine statistically significant differences between particular means.

RESULTS

Natural mating of queens

Out of 23 queens of the control group of queens mated solely naturally, all produced exclusively black worker offspring. This indicates that yellow drones were absent in the mating area of investigated queens.

Table 2 shows, that among queens inseminated with 8 µl of semen at the ages of 6, 8, and 14 days, 69.2, 52.6 and 29.4% produced black worker offspring, originating from natural mating. As the age of instrumentally inseminated queens increased, the percentages of natural matings decreased. The goodness-of-fit test χ^2 showed that the frequency distribution of the number of laying queens (39 : 19 : 17) and the number of mated in addition natu-

Table 2

Natural mating of instrumentally inseminated queens.
Naturalne dounasienianie sztucznie unasienionych matek.

Group No Nr grupy	Way of insemination Sposób unasienienia	Number of queens Liczba matek			Mated naturally Dounasienionych	
		Instrument. Insemin. Sztucznie unasien.	Laying eggs Czerwiących	Mated natural. Dounas natural.	Percent Procent	#Bin. conf. Interval Dwumian. prz. ufności
1	Natur. Mated 6D* Natur. unasien.	(23)**	18	18**	100**	
2	8µl 6D	50	39	27	69.2	55 - 79
3	8µl 8D	22	19	10	52.6	30 - 67
4	8µl 14D	20	17	5	29.4	23 - 53
5	8µl + CO_2 6D	24	22	3	13.6	4 - 28
6	CO_2 + 8µl 6D	24	22	0	0	0 - 13
7	2 × 4µ 6D	97	87	0	0	0 - 4
8	2 × 4µl 6D cut wing obcięte skrzydło	24	22	0	0	
9	2 × 4µl 6D excluder on entr. krata na wylotku	24	21	0	0	
Total - Razem		308	267			

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances
* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków
** Queens mated solely naturally. Matki unasienione tylko naturalnie
# Binomial confidence interval. Dwumianowy przedział ufności

rally (27 : 10 : 5) differed significantly (χ^2 = 9.13, df. = 2, p = 0.011). This indicates that the ageing of queens significantly decreased the proportion of queens which mated naturally after being inseminated instrumentally.

Comparison of two groups of queens inseminated with 8 µl of semen at the age of 6 days (groups 2 and 5) revealed that additional CO_2 treatment decreased the percentage of naturally inseminated queens from 69.2 to 13.6%. The χ^2 test showed that the distribution of the number of laying queens in both groups (39 : 22) and the number of mated also naturally (27 : 3) differed significantly (χ^2 = 46.05, df. = 1, p = 0.000). Thus, additional CO_2 treatment after instrumental insemination decreased significantly the proportion of naturally mated queens.

Queens treated with CO_2 before insemination did not mate naturally, while 13.6% of those treated after insemination mated naturally. Thus, CO_2 treatment before insemination was more effective then after in decreasing the percentage of naturally mated queens. No one queen inseminated twice with 4 µl of semen, each time, mated naturally.

If a large number of similar experiments were performed, the percentages of natu-

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Table 3

Natural mating of queens inseminated with 8 µl of semen at the age of 6 days during 2 seasons. - Naturalne dounasienianie się matek unasienionych 8 µl nasienia w wieku 6 dni, w dwóch sezonach.

Season Sezon	No of queens Licz. matek		% mated naturally % dounasienionych
	Laying eggs Czerwiących	Mated naturally Dounasienionych	
1992	21	15	71.4
1993	18	12	66.7
Total - Razem	39	27	69.2

Table 4

Losses of insminatad queens. - Straty matek po unasienianiu.

Group No Nr grupy	Way of insemination Sposób unasienienia	No queens Liczba matek	Lost queens Zginęło matek		Absconded nuclei Uciekło roików		Total losses Razem straty	
			N**	%	N	%	N	%
1	Natur. Mated 6D* Natur. unasien.	23	3	13.0	2	8.7	5	21.7
2	8µl 6D	50	7	14.0	4	8.0	11	22.0
3	8µl 8D	22	3	13.6	0	0	3	13.6
4	8µl 14D	20	2	10.0	1	5.0	3	15.0
5	8µl + CO_2 6D	24	1	4.2	1	4.2	2	8.3
6	CO_2 + 8µl 6D	24	1	4.2	1	4.2	2	8.3
7	2 × 4µ 6D	97	4	4.1	6	6.2	10	10.3
8	2 × 4µl 6D cut wing obcięte skrzydło	24	1	4.2	1	4.2	2	8.3
9	2 × 4µl 6D excluder on entr. krata na wylotku	24	3	12.5	0	0	3	12.5
Total - Razem		308						

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances

* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków

** Number, Liczba

rally mated queens may fall within the binomial confidence intervals presented in table 2 ($\alpha = 0.05$, one-sided). Thus, (upon the number of investigated queens) it is not excluded that within the CO_2 + 8 µl group, 0 - 13% queens may mate naturally and within the 2 x 4 µl group, 0 - 4 % may mate naturally.

Queens 6 days old were inseminated with 8 µl of semen during two seasons. Table 3 shows that percentages of queens mated also naturally were similar (71.4 and 66.7%) in both years. The frequency distri-

butions of the number of queens laying eggs in both seasons (21 : 18) and the number of mated also naturally (15 : 12) did not differ significantly ($\chi^2 = 0.05$, df = 1, p = 0.830). Thus, the seasons did not interfere significantly with the results.

Losses of queens

Two types of losses occurred. A loss of queens was considered when a dead queen was found in the nuclei, or when a queenless colony was found. The second type concerned the absconding of all bees from the nuclei. In the last case, we were not sure whether the queens died, or absconded together with the worker bees.

Table 4. shows, that among the control group of naturally mated queens, 13.0% were lost. Among 6 groups of instrumentally inseminated queens, which had the possibility to fly out (groups No 2 - 7), 14.0 to 4.1% were lost. The frequency distribution of the number of instrumentally inseminated queens (50 : 22 : 20 : 24 : 24 : 97) and the number of lost ones (7 : 3 : 2 : 1 : 1 : 4) differed significantly ($\chi^2 = 83.71$, df = 5, p = 0.000). Thus, the manner of instrumental insemination affected significantly the proportion of lost queens.

On average 12.5% of queens were lost out of those inseminated instrumentally, at different ages, solely with 8 µl of semen (groups 2 - 4), and only 4.2% out of those treated additionally with CO_2, or inseminated twice (groups 5 - 7). The losses within queens inseminated exclusively once were three times as high as within queens treated twice. The t-test showed highly significantly lower losses within queens of the last groups (t = 6.6, p = 0.003, after Bliss transformation).

The highest loss of 14.0% was found in group of queens inseminated with 8 µl of semen at the age of 6 days (tab. 4) In the same group, the highest proportion of 69.2% of naturally mated queens was noticed (tab. 2). The comparison of results presented in tables 2 and 4 revealed that in all groups, the decrease of the percentage of

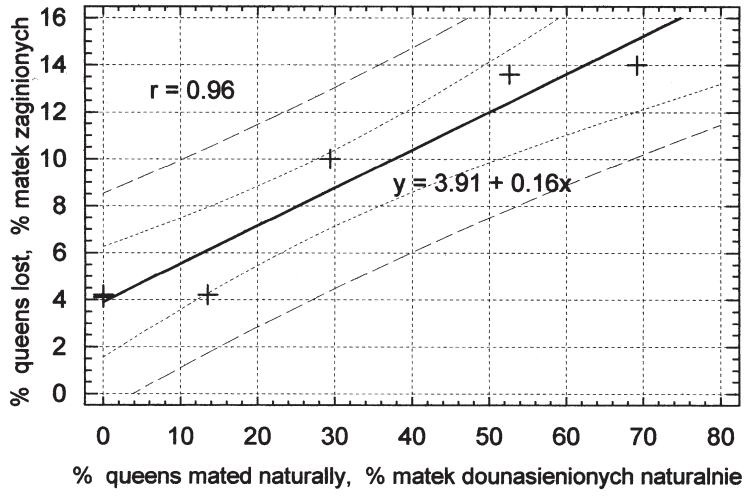

Fig. 1. Regression between percentage of instrumentally inseminated queens, mated in addition naturally, and percentage of queen losses in six groups. Dotted curves indicate 95.0% confidence intervals for the mean of many observations. Dashed curves indicate 95.0% prediction intervals for new observations.
Regresja między procentem sztucznie unasienionych matek dounasienionych naturalnie, a procentem ich strat w sześciu grupach. Krzywe kropkowane oznaczają 95.0% przedziały ufności dla średniej z wielu powtórzeń. Krzywe przerywane oznaczają 95.0% przedziały dla przewidywanych nowych obserwacji.

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Table 5

Beginning of egg laying by queens, number of days from first treatment.
Początek czerwienia, liczba dni od pierwszego zabiegu.

Group No Nr grupy	Way of insemination Sposób unasienienia	No queens Liczba matek	No of days - Liczba dni		
			Range Od-do	Mean Średnia	Stand. Deviation Odchylenie standardowe
1	Natural. Mated 6D* Natural. unasien.	18	1 ÷ 10*	3C	2.49
2	8µl 6D	39	5 ÷ 29	11A**	4.89
3	8µl 8D	19	3 ÷ 16	10A	4.48
4	8µl 14D	17	6 ÷ 19	11A	3.56
5	8µl + CO_2 6D	22	6 ÷ 10	8B	1.22
6	CO_2 + 8µl 6D	22	5 ÷ 9	7B	1.17
7	2 × 4µ 6D	87	3 ÷ 16	7B	1.89
8	2 × 4µl 6D cut wing obcięte skrzydło	22	3 ÷ 11	6B	1.86
9	2 × 4µl 6D excluder on entr. krata na wylotku	21	5 ÷ 14	6B	2.27

* D Age of queens (in days) at first treatment; in group No 1, queen age at removal of queen excluder from entrances

* D Wiek matek (w dniach) w chwili pierwszego zabiegu; w grupie nr 1, wiek matek w dniu usunięcia kraty z wylotków

** Different letters after means indicate highly significant differences p = 0.01 Różne litery za średnimi oznaczają wysoko istotne różnice p = 0.01

queens which also mated naturally 69.2, 52.6, 29.4, 13.6, 0.0, 0.0 was related to the decrease of the percentage of the lost ones 14.0, 13.6, 10.0, 4.2, 4.2, 4.1. The correlation coefficient between the percentages of naturally mated and lost queens was, r = 0.964, (r = 0.934, after Bliss transformation). The regression equation was y = 3.91 + 0.16 x % mated queens (fig. 1). When no additional natural mating occurred about 4% of queens were lost. The increase of 10% of additional natural mating was related to the loss of 1.6% of queens. Thus, the higher percentage of naturally mated queens was correlated with the higher percentage of lost queens. This indicates that the queens were lost during their flights. It is interesting to note, that 3 times more queens were lost within those mating solely naturally (13.0%) than within those inseminated instrumentally twice (4.1%).

Some queens were lost due to the absconding of whole colonies. Thus the numbers of total losses were higher. However, table 4 shows, that the pattern of total losses in particular groups was similar to the pattern of queen losses. Total losses within group of queens inseminated twice were lower than within queens inseminated only once or mated solely naturally.

Beginning of oviposition

Table 5 shows that queens from the control group of naturally mated started to lay eggs 3 days after being released by entrance opening. Those queens started to lay eggs highly significantly earlier, than any one of instrumentally inseminated.

Table 6

Beginning of oviposition in different seasons, number of days from first insemination.
Początek czerwienia w różnych sezonach, liczba dni od pierwszego unasieniania.

Group No Nr grupy	Season Sezon	No queens Licz. matek	Range Od - do	Mean Średnia	Standard dev. Odchylenie std.
Queens instrumentally inseminated with 8 mm^3 of semen at age of 6 days Matki unasieniane 8 mm^3 nasienia w wieku 6 dni					
2	1992	21	6 ÷ 20	9.9A*	3.58
2	1993	18	5 ÷ 29	11.2A	6.11
Queens instrumentally inseminated 2 x 4 mm^3 of semen Matki sztucznie unasieniane 2 × 4 mm^3 nasienia					
7	1992	21	3 ÷ 9	6.1B	1.42
7	1993	27	5 ÷ 14	7.1B	1.8
7	1994	17	5 ÷ 16	6.7B	2.62
7	1995	22	6 ÷ 11	7.8B	1.34

** different letters after means indicate highly significant differences p = 0.01
Różne litery za średnimi oznaczają wysoko istotne różnice p = 0.01

Table 7

Worker offspring of instrumentally inseminated queens which also mated naturally.
Potomstwa sztucznie unasienionych matek które dounasieniły się naturalnie.

Group No Nr grupy	Way of instrumental insemination Sposób sztucznego unasienienia		No. Queens Liczba matek	% offspring from natural mating % potomstwa z dounasienienia		
				Range Od - do	Mean Średnio	Standard dev. Odchyl.std.
2	8µl	6D*	27	2.05 ÷ 90.43	20.33	20.55
3	8µl	8D	10	1.63 ÷ 59.59	17.85	16.74
4	8µl	14D	5	2.41 ÷ 38.00	18.87	14.87
5	8µl + CO_2	6D	3	9.40 ÷ 12.90	10.70	1.89

* D age of queens (in days) at first insemination
* D wiek matek (w dniach) w chwili pierwszego unasienienia

Queens inseminated with 8 µl of semen at the age of 6 to 14 days, without additional CO_2 treatment, started to lay eggs 10 to 11 days after the first treatment (groups 2 - 4). This is the latest start, highly significantly later than in queens of any other groups. The highest range in the period of the beginning of oviposition was noticed within queens inseminated at the youngest age of 6 days. Queens treated additionally with CO_2, or inseminated twice with 4 µl of semen, started oviposition 6 to 8 days after the first treatment (groups 5 - 9). No significant difference was found between those 5 groups. However, those queens started to lay eggs highly significantly later than those mated only naturally, but highly significantly earlier than those from the 3 groups inseminated exclusively with 8 µl of semen. Thus, three super groups occurred concerning the period of the beginning of oviposition. Queens mated naturally started to lay eggs earlier than commenced queens inseminated once and treated additionally

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Table 8

Effect of season on the ratio of worker progeny from queens instrumentally inseminated with 8 mm^3 of semen at the age of 6 days, which also mated naturally.
Wpływ sezonu na udział potomstwa pochodzącego z naturalnego dounasieniania się matek sztucznie unasienionych 8µl nasienia w wieku 6 dni.

Season Sezon	Number queens Liczba matek	% progeny from natural mating % potomstwa z dounasienienia		
		Range Od - do	Mean Średnio	Standrard dev. Odchylenie std.
1992	15	6.09 ÷ 82.90	20.88	18.80
1993	12	2.05 ÷ 90.43	19.62	23.39
Total - Razem	27		20.33	20.55

with CO_2, or inseminated twice. Queens inseminated exclusively once initiated egg laying the latest. The results of the second super group suggest that additional CO_2 treatment is as valid as second insemination in accelerating the start of oviposition.

Queens inseminated ones with 8 µl of semen at the age of 6 days were investigated during two seasons and those inseminated twice with 4 µl of semen during four seasons (tables 1 and 6). ANOVA showed highly significant variations between those two groups. However, the Newman-Keuls test did not showed statistically significant differences within both groups. Thus, queens inseminated twice with 4 µl of semen started to lay eggs significantly earlier than those inseminated once with 8 µl of semen. However, the effect of season on the beginning of oviposition was not found, even when investigations were repeated during four years.

Worker offspring from naturally mated queens

Table 7 shows that, out of queens inseminated exclusively with 8 mm of semen 5 - 27 mated also naturally and out of those treated afterwards with CO_2 only 3 mated naturally. Those queens produced on average 10.70 to 20.33% of black worker offspring originating from drones which mated the queens naturally. Queens inseminated exclusively with 8 µl of semen produced, on average, twice as many offspring from natural mating as those treated additionally with CO_2. However, ANOVA did not show significant variances between those groups. This was probably due to the very high variation in the percentage of workers originating from natural mating. Table 7 shows that particular queens inseminated with 8 µl of semen at the age of 6 days produced from 2 - 90% of offspring originating from natural mating (group 2). A tendency is visible to decrease the variation in the percentage of offspring from natural mating in queens inseminated at an older age.

Queens inseminated with 8 µl of semen at the age of 6 days mated in addition naturally during two seasons (tab. 8). ANOVA did not show a significant variation between the percentages of queens which mated naturally during both seasons Thus the seasonal conditions did not interfere with the percentages of queens which mated also naturally.

Assuming that a queen mates with 10 drones, the queens inseminated exclusively with 8 µl would mate naturally, on average, with 2 drones, and queens treated additionally with CO_2 would mate additionally with 1 drone. However, taking into account the variation in the percentage of offspring production, it must be accepted that some queens inseminated instrumentally with 8 mm^3 of semen at the age of 6 days, mated in addition naturally with 1 up to 9 drones.

DISCUSSION

Present investigation showed, that none of 22 instrumentally inseminated queens of the CO_2 + 8 mm^3 group mated in addition also naturally. However, the binomial confidence interval indicated that it is not excluded that 0 - 13% queens may mate naturally, if a large number of similar experiments were performed. According to Woyke and Jasiński (1992) 3 (25%) out of 12 queens inseminated this way mated also naturally and Woyke et al (1995) reported that 2 (9.1%) out of 22 mated naturally. Thus this method of instrumental insemination did not exclude additional natural mating of queens.

None of the queens instrumentally inseminated twice with 4 µl of semen mated in addition also naturally during the whole four years of presnted investigations. Also, none of double inseminated queens mated naturally in investigations conducted by Woyke and Jasiński (1992) and Woyke et al (1995). If all those queens, of the six year observations, are aggregated, 12 (1992) + 24 (1995) + 87 (2001) = 123, then the binomial confidence interval is 0 - 2.5%. Thus, if a large number of similar experiments were performed it could not be excluded that 2.5% of double instrumentally inseminated queens would mate also naturally. This does not prove that so many instrumentally inseminated queens will mate naturally, but this is only a very low possibility. Thus it can be assumed that, in practice, queens inseminated instrumentally twice with 4 µl of semen, each time, would not mate in addition also naturally.

Total losses of queens double instrumentally inseminated with 4 µl of semen were similar in groups which had the possibility to fly out of the hives (10.3% - tab. 4, group 7) and those which could not fly (8.3 and 12.5% - groups 8 and 9) due to cut wing or queen excluder on the entrances. Losses in those three groups of queens were half as high as within queens which mated solely naturally (21.7% - group 1).

Thus, due to low losses and absence of additional natural mating within high number of queens inseminated instrumentally twice, during six years, the method of double instrumental insemination of queens with 4 µl of semen with subsequent open entrances can be recommend for practical beekeepers.

CONCLUSION

Queens inseminated instrumentally with 8 µl of semen at age of 6 days mate afterwards also naturally in 70% of cases.

Instrumental insemination of older queens at age of 8 or 14 days decreases the percentage of queens mating also naturally to 53 or 39% respectively.

CO_2 treatment two days after insemination decreases even further the percentage of queens mating also naturally to 14%

CO_2 treatment two days before instrumental insemination as well as double insemination with 4 µl of semen eliminates natural mating of queens.

Binomial confidence intervals show, that if a large number of similar experiments is performed, it cannot be excluded that 0 - 13% queens of the CO_2 + 8 mm^3 group and 0 - 2% of double instrumentally inseminated queens would mate also naturally.

Queens which could fly out of the hives are lost 3 times more often after being inseminated once with 8 µl of semen (12.5%) than after two inseminations with 4 µl of semen each (4.1%).

A high correlation ($r = 0.96$) exists between the percentage of instrumentally inseminated queens which mated also naturally and the percentages of lost queens. This indicates that the losses occur during the flights.

Losses within queens mating solely naturally (13.0%) are 3 times higher than

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within queens inseminated instrumentally twice with 4 µl of semen (4.1%).

Queens mated solely naturally start to lay eggs 3 days after removal of queen excluders from hive entrances. Queens inseminated instrumentally twice, or inseminated once and treated additionally with CO_2, start oviposition significantly later, 7 - 8 days after the first treatment. Queens inseminated solely once with 8 µl of semen start to lay eggs still significantly later, 10 - 11 days after the first treatment.

Queens inseminated once with 8 µl of semen which mated also naturally produce, on average, 20% of worker progeny originating from drones which mated the queens. They probably mated with 2 drones. Queens which after one instrumental insemination were treated with CO_2 and mated also naturally, produce on average 11% of progeny from drones which mated the queens. They probably mate naturally with 1 drone.

Due to low losses and absence of additional natural mating within high number of queens inseminated instrumentally twice, the method of double instrumental insemination of queens with 4 µl of semen with subsequent open entrances can be recommend for practical beekeepers.

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ZAPOBIEGANIE NATURALNEMU DOUNASIENIANIU SIĘ SZTUCZNIE UNASIENIONYCH MATEK PSZCZELICH PRZEZ ZASTOSOWANIE ODPOWIEDNIEJ METODY SZTUCZNEGO UNASIENIANIA

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

Badania prowadzono w Zakładzie Hodowli Owadów Użytkowych, Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie w latach 1992 - 1995. Celem pracy było zbadanie naturalnego dounasieniania się sztucznie unasienionych matek, oraz znalezienie metody, która zapobiegałaby dounasienianiu matek mających swobodę wylatywania z ula.

Do badań użyto ciemno ubarwione matki pszczele, które unasieniano sztucznie nasieniem żółto ubarwionych trutni i pozwalano im na naturalne dounasienianie się w okolicy, gdzie znajdowały się ciemne trutnie. Ponieważ żółta barwa ciała pszczół jest dominującą nad ciemną, potomstwo w postaci ciemno ubarwionych robotnic wskazywałoby na naturalne dounasienienie się matek.

W sumie zbadano 308 matek oraz określono ubarwienie 188 300 robotnic. Mateczniki w wieku 11 dni poddawano do nasiedlonych trapezowych ulików weselnych. Wylotki były zabezpieczone kratą odgrodową. Utworzono dziewięć grup matek: 1./ kontrolna - matki wyłącznie naturalnie unasienione, 2./ unasienione sztucznie 8 µl nasienia w wieku 6 dni, 3./ - w wieku 8 dni, 4./ i - w wieku 14 dni, 5./ unasienione sztucznie 8 µl nasienia i dwa dni później uśpione CO_2, 6./ najpierw uśpione CO_2, a po dwu dniach unasienione sztucznie 8 µl nasienia,

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7./ unasienione dwukrotnie po 4 µl nasienia, 8./ tak samo jak 7, jednak jedno skrzydło przycięte, 9./ tak samo jak 7, jednak krata odgrodowa pozostawała przez cały czas na wylotku. Kraty z ulików weselnych grupy kontrolnej odejmowano gdy matki osiągnęły wiek 6 dni, a w pozostałych siedmiu grupach (nr 2 - 8) po ostatnim zabiegu unasieniania lub traktowania CO_2. Co 3 dni sprawdzano rozpoczęcie składania jaj przez matki. Gdy kryty czerw był bliski wygryzienia, plastry wkładano do izolatora i umieszczano w cieplarce. Po wygryzieniu robotnic, liczono ile było ubarwionych ciemno, a ile żółto.

Uzyskane wyniki wykazały, że wszystkie robotnice od matek z grupy kontrolnej były ubarwione ciemno. Dowodzi to, że w okolicy unasieniania nie było żółto ubarwionych trutni. Spośród matek unasienionych sztucznie wyłącznie 8 µl nasienia w różnym wieku oraz tych potraktowanych CO_2 w dwa dni po unasienieniu, 69, 53, 29, i 14 % produkowało ciemne potomstwo. Oznacza to, że dounasieniły się one naturalnie. Matki traktowane CO_2 w dwa dni przed sztucznym unasienieniem, oraz matki unasienione dwukrotnie po 4 µl nasienia produkowały wyłącznie żółto ubarwione robotnice. Oznacza to, że nie dounasieniły się one naturalnie

Największe straty stwierdzono w trzech grupach matek unasienionych wyłącznie 8 µl nasienia (grupy 2, 3, 4,straty - 14,0 13,6 i 10,0%). Zauważono, iż we wszystkich grupach matek, które miały możliwość wylatywania, procent strat wzrastał w miarę wzrostu procentu naturalnie dounasienionych matek. Stwierdzono wysoką korelację między procentami dounasienionych i zaginionych matek r = 0.96. Wynika z tego, że matki ginęły głównie w czasie wylotów z ulików. Interesujący jest wynik, zgodnie z którym matki unasieniane wyłącznie naturalnie, oraz te które unasieniano tylko 8 µl nasienia ginęły trzykrotnie częściej niż matki unasieniane dwukrotnie po 4 µl nasienia.

Matki grupy kontrolnej, które unasieniały się wyłącznie naturalnie, rozpoczęły czerwić 3 dni po otwarciu wylotka. Później (6 - 8 dni) zaczęły czerwić matki unasienione 8 µl nasienia i potraktowane CO_2, oraz matki unasienione dwukrotnie po 4 µl nasienia. Najpóźniej (10 - 11 dni) rozpoczęły czerwienie matki unasienione wyłącznie 8 µl nasienia.

Matki unasienione 8 µl nasienia w wieku 6, 8 i 14 dni, które dounasieniły się naturalnie produkowały średnio 20, 18, i 19% ciemno ubarwionych robotnic. Oznacza to, że kopulowały one średnio prawdopodobnie z 2 trutniami. Jednak zakres liczby kopulacji wahał się od 1 do 9. Matki potraktowane CO_2 w dwa dni po unasienieniu produkowały 11% ciemnych robotnic, co oznacza, że kopulowały one średnio prawdopodobnie z 1 trutniem. Matki traktowane CO_2 przed sztucznym unasienianiem 8 µl nasienia oraz unasienione dwukrotnie po 4 µl nasienia produkowały wyłącznie żółto ubarwione robotnice. Oznacza to że nie dounasieniały się one naturalnie pomimo otwartych wylotków.

Ponieważ straty wśród matek unasienianych dwukrotnie są najmniejsze, a żadna spośród dużej liczby zbadanych matek nie dounasieniła się naturalnie, można polecać dla praktyki dwukrotne unasienianie matek po 4 µl nasienia, bez kratowania wylotka po ostatnim zabiegu.

Słowa kluczowe: matki pszczele, naturalne unasienianie, sztuczne unasienianie, traktowanie CO_2.