EFFECT OF DIFFERENT CARBON DIOXIDE GAS CONCENTRATIONS USED DURING THE INSEMINATION OF HONEY BEE QUEENS ON STARTING OVIPOSITION

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S u m m a r y

The experiment was conducted in 2004, 2005 and 2007 at the Research Institute of Pomology and Floriculture, Apiculture Division in Puławy, Poland. Carniolan sister queens at the age of 7 days were inseminated with an 8µl dose of semen. Queens were anesthetized once during the insemination with different concentrations of carbon dioxide and air gas mixtures. It took queens a shorter time to be narcotized when CO_2 was given at higher concentrations. The timing was from 6.1 s when 100% CO_2 was used to 95.5 s when 50% CO_2 was used. Semen injection took longer in queens anaesthetized with CO_2 at the lower 50% and 75% concentrations. The queens remained anesthetized significantly longer when higher CO_2 concentrations were used.

Among 276 instrumentally inseminated queens, 88% started laying eggs before the end of the experiment and 12% did not start laying eggs, or died before the end of the experiment. The highest percentage of queens that did not start laying eggs or died was noted in the group anaesthetized with 75% and 80% of CO_2 (16.4% and 14.5%). In the other groups, the percentage of queens who did not start laying eggs or died ranged from 7.4% to 14.5%. Different CO_2 gas concentrations used for immobilization of bee queens during instrumental insemination significantly influenced oviposition of queens. Instrumentally inseminated bee queens began laying eggs 4 to 55 days after the insemination. The significantly shortest time from insemination to oviposition was noted in queens that were narcotized with 50, 100 and 90% of CO_2 (17.4, 17.6 and 19.9 days respectively). The longest time was noted in queens treated with 75-80% of CO_2 (after 22 days).

Keywords: *Apis mellifera*, anesthesia, carbon dioxide, nitrogen, oxygen, instrumental insemination.

INTRODUCTION

In natural conditions, after bee queens copulate with drones, the unknown stimuli triggers the activation of the queen's ovaries and of egg laying (Alber et al., 1955; Koeniger, 1976, 1981; Koeniger et al., 1979). The carbon dioxide used for queen immobilization during instrumental insemination causes egg laying to start earlier in inseminated queens. According to Mackensen (1947), double CO_2 treatments lasting 10 minutes each, significantly shortened the period from the insemination to oviposition. Fischer (1990) stated that bee queens which were anaesthetized on the day before insemination and then during the treatment were characterized as having better sperm migration into the spermatheca. Previous studies have also shown that anaesthesia of queens the day before instrumental insemination inhibits them from performing post-insemination mating flights (Woyke et al., 1995, 2001). But it was shown that the effect of carbon dioxide is not neutral for both the queens and the workers. The effect of carbon dioxide changes both

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queen and worker behavior (Austin, 1955; Skowronek and Jaycox, 1974; Skowronek, 1976, 1982; Ebadi et al.,1980, Wilde and Sobiechowski, 2002). Czekońska (2009) found that worker bees treated with low concentrations of CO_2 lived significantly longer than those treated with a gas concentration that was higher than 80%. Negative consequences of narcosis are greater, the longer the duration of anesthesia and the older the treated individual. Attempts to shorten the anaesthesia duration and dividing it into two gas applications for a total of six minutes, gave the same effect as a double 10-minute anesthesia treatment (Konopacka, 1989). Ebadi and Gary (1980) used a mixture of 50-90% CO_2 gas and air gases. The queens immobilized with this mixture began laying eggs faster than the queen anesthetized with pure CO_2. Queens treated twice for 10 minutes with a 75% CO_2 narcosis, began laying eggs after an average of 3.7 days. This was almost as soon as the naturally mated ones. It was also found that instrumentally inseminated queens begin oviposition 8-12 days after insemination without a second CO_2 treatment (Woyke, 1962, 1966). According to Harbo (1986 a,b), virgin queens, which were kept in the queen banks for about 2 months before insemination, started laying eggs without anesthesia.

Since there is a large-scale production of honey bee queens (about 30 thousand per year in Poland), it is important to determine the factors affecting the quality of inseminated queens. Many problems have already been explained in this field but the reason for the significant delay in oviposition starting in instrumentally inseminated queens has not been explained (Bieńkowska and Panasiuk 2006; Bieńkowska et al., 2008, 2011; Gerula and Bieńkowska, 2008; Gontarz et al., 2005; Konopacka, 1989; Laidlaw, 1954, 1981; Mackensen,1964; Woyke, 1960, 1979, 1983; Woyke and Jasiński, 1973, 1976, 1978, 1980, 1982 a,b, 1990).

The aim of this research was to determine the impact on the start of oviposition of the various concentrations of CO_2 used for bee queen immobilization during instrumental insemination.

MATERIAL AND METHODS

The study was conducted in the years 2004, 2005 and 2007 at the Apiculture Division of the Research Institute of Horticulture (previous Research Institute of Pomology and Floriculture) in Puławy, Poland. Carniolan sister queens were reared from one day old larvae. Sealed queen cells, 5-days from larvae grafting, were transferred into incubators. The temperature of the incubators was 35°C. Emerged queens were introduced into mating hives. A total of 276 queens were inseminated with a dose of 8µl of semen at the age of 7 days. Queens were anesthetized only once during the insemination. The following mixtures of carbon dioxide and air gases were used for anesthetizing the queens:

100% CO_2,
90% CO_2 - 2.1% O_2 - 7.9% N_2,
80% CO_2 - 15.82% O_2- 4.18% N_2,
75% CO_2 - 5.25% O_2- 19.75% N_2,
50% CO_2 - 10.5% O_2 - 39.5% N_2.

Factors listed below were estimated during the insemination process:

1. the time it took to immobilize queens when treated with carbon dioxide at different concentrations (100%, 90%, 85%, 75% and 50%) - from the beginning of exposure to CO_2, to full narcosis,
2. the time needed for inseminating the queens - opening of the sting chamber and introducing the semen into the queen's reproductive tract,
3. the time it took for the queens to come to. This includes the time from the end of the CO_2 application till when the queens started moving and walking,
4. the duration of the whole insemination process.

For the statistical data analysis, the nonparametric Kruskal-Wallis test was used. Nucleus colonies were inspected for initiation of oviposition once a week, up to 60 days after insemination. An evaluation of the percentage of egg laying and non-

egg laying queens was done at this time. The differences between averages of studied parameters and interaction between various CO_2 concentrations and days from insemination to oviposition were analyzed using the χ^2 test and the confidence coefficient.

RESULTS

In all of the years of this research study, the higher the concentration of carbon dioxide gas used for anesthesia the shorter the time it took to immobilize the queens (Tab. 1). Queen immobilization time ranged from 6.1 s after application of 100% CO_2 in 2005, to 95.5 s when 50% CO_2 was applied in 2004. The averages obtained during the 3-years of research showed that the moment of immobilization after the application of CO_2 at different concentrations, was significantly different (Kruskal-Wallis test H (4, N=276) = 232.73; p=0.000), but the longest was in the first year of the research (Kruskal-Wallis test H (4, N=276) = 9.04; p=0.0109). The queens treated with 100% CO_2 were immobilized the fastest (after about 7.3 s on average). The queens that were treated with CO_2 at lower concentrations took a significantly slower time to become immobilized (after 16.7 s to 81.5 s on average). There were no differences in the time it took for queens to become immobilized for queens treated with CO_2 at 75 and 80% concentrations, in all the years of the research study.

In our study, the shortest time needed to introduce the semen into the oviducts of queens was for those queens treated with 80% and 90% CO_2 concentrations (average 12.5 and 11.4 s). This tendency repeated each year, but the differences were not always significant.

The whole insemination process was the longest in the first year of the study (Kruskal-Wallis test H (4, N=276) = 35.47; p=0.000). As expected, in all of the study years, the queens treated with CO_2 at higher concentrations were waking up significantly more slowly (average from 18.3 s to 155.7 s; Kruskal-Wallis test H (4, N=276) = 207.5; p=0.000). The queens woke up the most slowly in the first year of the study (Kruskal-Wallis test H (4, N=100) = 75.6; p=0.000).

The average time of the whole insemination process, from the start of the CO_2 application till when the queens fully awaken, differed significantly between the groups of queens anesthetized with CO_2 at various concentrations (Kruskal-Wallis test H (4,N=276)=135.9, p=0.000). In each year of the study, the time of the insemination process was significantly shortest after applying 75% CO_2 (average 82.6 s; from 66.5 s in 2005 to 111.1 s in 2004). The queens treated with pure CO_2 remained immobilized the longest (average 132 s; from 162.7 in 2005 to 208.0 s in 2004) (Tab. 1).

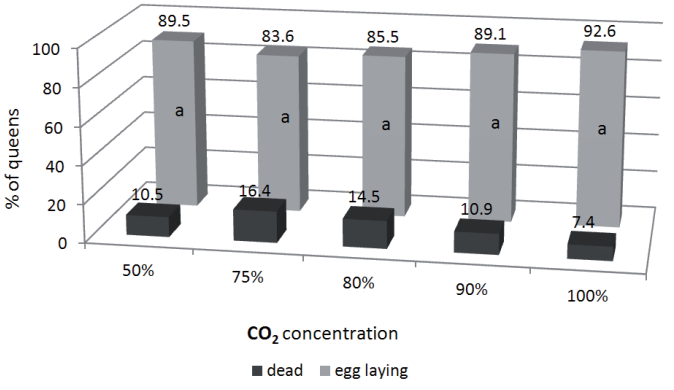

Fig. 1. Percentage of egg laying and dead queens.
a- no statistical differences χ^2=4.22, df = 4, p = 0.377.

Table 1.

Summary of the parameters analyzed during the whole process of instrumental insemination of bee queens

Year	CO_2 concentration	n	Time to immobilize the queen $\bar{x} \pm Sd$ (s)	Median	Time to introduce semen into oviducts $\bar{x} \pm Sd$ (s)	Median	Time to waking up the queen $\bar{x} \pm Sd$ (s)	Median	Time of whole process of insemination $\bar{x} \pm Sd$ (s)	Median
1	2	3	4		5		6		7	
2004	50%	15	95.5±54.8	64.0d	26.3±11.7	24.0c	31.5±9.1	18.0a	153.3±52.4	124.0abc
	75%	15	33.5±4.9	32.0cd	19.3±5.0	18.0c	58.3±36.5	39.0a	111.1±41.4	89.0a
	80%	15	23.5±3.4	23.0bc	16.3±5.3	14.0ab	89.5±35.5	101.0ab	129.3±34.8	137.0ab
	90%	15	20.8±5.9	19.0ab	10.8±2.6	10.0a	138.7±25.4	140.0bc	170.3±25.1	173.0bc
	100%	15	8.7±2.2	8.0a	17.3±6.2	16.0bc	182±48.6	200.0c	208.0±52.2	226.0c
Average 2004		**75**	**36.4±39.1**	**23.0B**	**17.9±8.2**	**16.0C**	**100.0±63.8**	**101.0B**	**154.3±53.5**	**150.0B**
2005	50%	20	84.9±22.2	78.5c	17.8±2.4	17.5b	12.8±7.1	9.0a	115.5±23.1	111.5b
	75%	20	20.7±4.3	20.0b	13.3±2.8	14.0a	32.4±9.5	30.5a	66.5±11.4	67.0a
	80%	20	20.7±4.3	20.0b	11.4±2.9	12.0a	134.3±34.8	131.0b	166.5±35.4	161.5c
	90%	20	13.0±1.6	13.0a	12.1±2.6	11.0a	139.0±45.2	130.5b	164.2±45.4	158.0c
	100%	20	6.1±1.1	6.0a	13.5±2.0	14.0a	143.1±23.9	151.5b	162.7±24.6	169.5c
Average 2005		**100**	**29.1±30.3**	**18.5A**	**13.6±3.3**	**14.0B**	**92.3±63.9**	**110.0AB**	**135.0±49.3**	**142.0A**
2007	50%	22	68.8±17.2	67.5d	14.2±5.9	13.5a	14.4±8.2	12.5a	97.3±18.7	94.5a
	75%	20	27.4±4.7	27.0c	13.5±3.6	13.5a	36.4±15.5	33.0a	77.2±13.5	76.5a
	80%	20	26.5±6.7	26.0bc	10.7±2.3	10.0a	94.8±11.1	93.5b	131.9±11.8	132.0b
	90%	20	17.3±4.7	17.0ab	11.3±2.5	11.0a	102.1±16.1	101.5b	130.7±15.9	130.0b
	100%	19	7.3±1.7	7.0a	11.6±2.1	12.0a	148.2±35.4	158.0b	167.1±35.0	175.0b
Average 2007		**101**	**30.4±23.3**	**24.0AB**	**12.3±3.8**	**12.0A**	**77.1±51.9**	**86.0A**	**119.2±36.8**	**120.0A**
Total years (2004 2005 2007)	50%	57	81.5±33.9	70.0d	18.4±8.4	16.0d	18.3±11.2	16.0a	118.4±38.8	111.0b
	75%	55	26.6±6.8	27.0c	15.0±4.7	15.0cd	40.9±24.1	33.0b	82.6±29.8	72.0a
	80%	55	23.6±5.6	23.0c	12.5±4.2	12.0ab	107.7±34.8	101.0c	143.8±33.2	137.0c
	90%	55	16.7±5.2	15.0b	11.4±2.5	11.0b	125.5±36.0	119.0cd	153.7±35.9	147.0cd
	100%	54	7.3±1.9	7.0a	13.9±4.3	13.0ac	155.7±39.1	158.0d	176.8±41.7	177.5d
Average for all years		**276**	**31.6±30.9**	**21.0**	**14.3±5.7**	**14.0**	**88.9±60.3**	**94.0**	**134.8±48.2**	**132.0**

A, B - significant differences between years at $p \leq 0.05$ (Kruskal - Wallis test);
a, b - significant differences between various CO_2 concentrations at $p \leq 0.05$ (Kruskal-Wallis test).

Table 2.

The number of egg laying, non-egg laying, and dead queens

Year	No of inseminated queens	No of queens			
		Egg laying queens	%	Dead and non-egg laying queens	%
2004	75	68	90.7 a	7	10.3 a
2005	100	90	88.9 a	10	11.1 a
2007	101	85	84.2 a	16	15.8 a
Total	276	243	88.1	33	11.9

a - no differences at $p \leq 0.05$ (Difference test between two proportions);
$\chi^2=0.02$, p = 0.883 between 2004 and 2005;
$\chi^2=1.6$, p = 0.205 between 2004 and 2007;
$\chi^2=1.52$, df = 4, p = 0.217 between 2005 and 2007.

Table 3.

Interactions between various CO_2 gas concentrations used during insemination and initiation of oviposition by instrumentally inseminated queens

Year	χ^2	df	P	Confindency coefficient
2004	141.5	72	0.0000	0.808
2005	192.2	128	0.0009	0.811
2007	75.4	68	0.1978	0.653
Total	215.7	152	0.0000	0.662

Table 4.

Comparison of oviposition period in queens anesthetized with various CO_2 gas concentrations

CO_2 gas concentrations	No of queens	Days to oviposition		
		Min-max	Mean ± Sd	Median
50%	51	4 - (37)	17.4 ± 8.8	18 a
75%	46	9 - 49	23.1 ± 11.8	22 ab
80%	47	6 - 55	24.3 ± 10.9	22 b
90%	49	6 - 54	19.9 ± 11.5	20 ab
100%	50	8 - 43	17.6 ± 8.9	15 a
Total	243	4 - 55	20.4 ± 10.7	20

a,b - different letters indicate significant differences at $p \leq 0.05$;
Kruskal-Wallis test: H(4,N=243)=18.1783, p = 0.0011.

Among 276 instrumentally inseminated queens, 243 (88.1 %) individuals started laying eggs but no significant differences were found between the different years of the study (Tab. 2). Oviposition was started by 89.5% (50% CO_2), 83.6% (75% CO_2), 85.5% (80% CO_2), 89.1% (90% CO_2) and 92.6% (100% CO_2) of the queens. The groups did not differ significantly in the percentages of queens starting oviposition ($\chi^2=4.22$, df = 4, p = 0.377), (Fig.1).

A high dependency between the concentration of CO_2 used during instrumental insemination of bee queens and oviposition was noted (Tab. 3). A high dependency was not noted only in the last year of the research study. Instrumentally inseminated bee queens began laying eggs 4 to 55 days after insemination. The significantly shortest time from insemination to oviposition was found to be in queens that were narcotized with 50 and 100% CO_2 (17.4 and 17.6 days,

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Table 5.

Percentage of queens that started laying eggs after a certain time

Days	n	< 7 days		8 -14 days		15 -21 days		22 - 30 days		>30 days	
CO_2 Concentrat-ion		n	%	n	%	n	%	n	%	n	%
50%	51	8	15.7 a	17	33.3 bc	10	19.6 a	10	19.6 ab	6	11.8 a
75%	46	0	0	10	21.7 abc	17	37.0 ab	8	17.4 ab	11	23.9 a
80%	47	2	4.2 a	5	10.7 a	15	31.9 ab	15	31.9 b	10	21.3 a
90%	49	6	12.2 a	10	20.5 ab	21	42.9 b	6	12.2 a	6	12.2 a
100%	50	0	0	26	52 c	9	18 a	9	18 ab	6	12 a
Total	243	16	6.8	68	28	72	29.5	48	19.7	39	16

a, b, c - different letters indicate significant differences (difference tests between two proportions) $p \leq 0.05$.

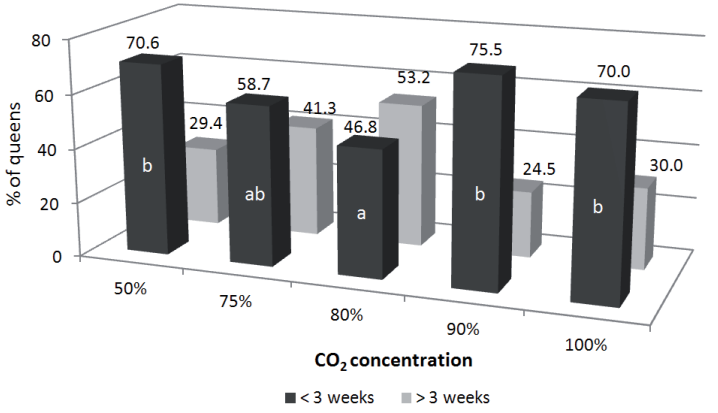

Fig. 2. Percentage of queens that started laying eggs within the first 3 weeks and later
a,b - significant differences (difference tests between two proportions) at $p \leq 0.05$.

respectively). The queens treated with 80% CO_2 began laying eggs significantly later (on average, after about 24.3 days). It was also found that the concentration of CO_2 gas used during the insemination process significantly influences the initiation of oviposition by queens (Tab. 4).

The whole period of time from insemination to oviposition in all of the studied queens was divided into one-week periods. The division showed that within the first week from insemination, oviposition was started by 6.8% of queens, and among them only ones narcotized with 50, 80 and 90% CO_2 (15.7%, 4.2% and 12.2%, respectively) (Tab. 5). Within the second week after insemination, oviposition started in the next 28% of queens. It was noted that in this period, oviposition began in more than half (52%) of queens narcotized with 100% CO_2. However, within the first 2 weeks after insemination almost half of the queens (49%) treated with the lowest CO_2 concentration, started laying eggs.

Participation of queens that started, and those that did not start laying eggs after 3-week period from insemination showed that oviposition began in: more than 70% of queens narcotized with 90% and 100% CO_2, more than half of queens narcotized with 50 % and 75% CO_2, and only 46.8% of queens narcotized with 80% CO_2 (Fig. 2).

DISCUSSION

The obtained results show that various concentration of carbon dioxide used for queen narcosis during instrumental insemination influences the immobilization moment and the regaining of consciousness as well as the length of unconsciousness. With the increase of CO_2 concentration, the queens lost consciousness more quickly, which means they were immobilized sooner. The CO_2 concentration also accelerates the introduction of the semen into the oviducts. Queens narcotized with carbon dioxide at lower concentrations (50 and 75%) were able to move their bodies while under anesthesia (the time of CO_2 exposure). This required the utmost attention from the inseminator when putting the needle into the sting chamber. It also increased the time of insemination. Similar observations were reported by Konopacka (1987, 1991). In her studies, queens treated with a short exposure of pure carbon dioxide were still able to move their abdomens, but lived longer than queens who had received two 10 minute CO_2 gas treatments.

In our studies, the various concentrations of CO_2 gas used during the insemination of bee queens did not have an influence the number of lost queens or the number of queens that did not start laying eggs. The rate of dead queens and non-egg laying queens reached, on average, 12%. Similar results were obtained by Woyke and Ruttner (1976), Kühnert et al. (1989) and Otten et al. (1998). These authors used pure CO_2 gas to anesthetize queens during insemination. In our research, slightly less than 7% of the queens treated with 100% CO_2 were lost.

In the present study, the time of carbon dioxide exposure (from the start of narcosis to the completed insemination), regardless of the CO_2 concentration did not exceed 2 min. The percent of queens that started oviposition was from 84.2 to 90.7%. Other authors reached similar results (from 76.8 to 85%), but only when using pure CO_2 gas (Mackensen, 1947; Woyke and Ruttner, 1976; Ebadi and Gary, 1980; Kaftanoglu and Peng, 1982; Konopacka, 1989). The above authors recommended using a double CO_2 narcosis, before and during instrumental insemination or during and after insemination, for 3 or 10 minutes each. According to Janoušek (1987), 1-minute anesthesia is not dangerous for the queens.

In previous research, a relationship was found between oviposition and the carbon dioxide used for queen anesthesia. In our study, 15.7% of queens treated with 50% CO_2, and 12.2% of queens treated with 90% CO_2 started oviposition within the first week after insemination. By the end of the second week, 52% of queens treated for 30 s. with pure CO_2 had started laying eggs. Konopacka (1989) stated that in queens narcotized once with pure carbon dioxide for 1 min, or two times for 0.5 min each, started oviposition, on average, 30 days (from 3 to 50 days) after insemination. According to Gerula et al. (2011), queens given one short treatment of CO_2 oviposited 16-20 days after insemination. In the presented study, this period was much shorter and averaged 17 days (from 8 - 43 days). By the end of the second week after insemination, 49% of queens treated for 90s with 50% CO_2 had begun to lay eggs.

Otto et al. (1998) suggest that the number of queens starting to lay eggs depends on the strength of the colonies they are introduced to. According to Otto et al., in weak bee colonies (with about 200 bees), less than 70% of instrumentally inseminated queens start oviposition, while in strong colonies (1200 bees), almost 95% of queens start laying eggs.

Ebadi and Gary (1980) showed that queens anesthetized twice with 75% CO_2, laid eggs 3.7 days (3-4 days) after insemination. But when their queens were treated once with 75% CO_2, they laid eggs after 12 days (4 to 20 days). The results of our study do not confirm these reports. Queens that were anesthetized once with 75% CO_2 started oviposition, on average, 23 days after insemination (9-49 days). The highest number of queens

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that started oviposition in the shortest time from insemination were in a group of individuals treated once with 50% CO_2. They laid eggs, on average, 17.4 days (4-37 days) from insemination, while queens treated with 100% CO_2, on average, after 17.6 days (8-43 days) and queens treated with 90% CO_2 after 19.9 days (6-54 days). There were differences found in the oviposition between groups of queens treated with 50, 90 and 100% CO_2 concentrations and groups of queens treated with 75 and 80% CO_2.

CONCLUSIONS

1. The various carbon dioxide gas concentrations used during instrumental insemination of bee queens influence the time of immobilization (anesthetized) and waking up of queens.
2. With increasing CO_2 concentration, the queens are immobilized faster.
3. The lower the concentration of CO_2 gas, the longer the dosing time (from the start of anaesthesia to completion of insemination).
4. The various carbon dioxide gas concentrations used during instrumental insemination do not influence the rate of queens that start laying eggs.
5. The various carbon dioxide gas concentrations used during instrumental insemination influences the time of oviposition.
6. The queens which were anaesthetized with 50%, 100% and 90% CO_2 started oviposition in the shortest time.

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WPŁYW RÓŻNYCH STĘŻEŃ DWUTLENKU WĘGLA STOSOWANEGO PODCZAS INSEMINACJI NA ROZPOCZYNANIE CZERWIENIA PRZEZ MATKI PSZCZELE

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

Badania prowadzono w latach 2004, 2005 i 2007 w Oddziale Pszczelnictwa w Puławach. Matki siostry rasy kraińskiej w wieku 7 dni unasieniano dawką 8µl nasienia. Matki usypiano jeden raz podczas sztucznego unasienienia. Do usypiania matek zastosowano mieszaninę dwutlenku węgla i głównych gazów powietrza w różnych proporcjach. We wszystkich latach badań wraz ze wzrostem koncentracji dwutlenku węgla użytego do narkozy matki zasypiały szybciej. Czas zasypiania matek wahał się od 6,1 s po zastosowaniu 100% CO_2, do 95,5 s po zastosowaniu 50% CO_2. We wszystkich latach badań istotnie najwięcej czasu (średnio 18 s) poświęcono na wprowadzenie nasienia do dróg rodnych matek usypianych CO_2 o najniższym, 50%-owym stężeniu, a w niektórych latach (2004 i 2007 roku) również 75%-owym stężeniu. Wraz ze wzrostem udziału procentowego dwutlenku węgla w narkozie zastosowanej w czasie inseminacji, matki spały istotnie dłużej.

W badaniach wykazano, że stężenie dwutlenku węgla używanego do usypiania matek pszczelich nie miało wpływu na straty matek i liczbę matek rozpoczynających składanie jaj. Spośród 276 unasienionych matek, 243 (88%) rozpoczęło czerwienie, a 33 (12%) nie zaczęły czerwić lub padły przed zakończeniem doświadczenia. Najmniej takich matek stwierdzono w grupie matek usypianych 100% CO_2 (7,4%), a najwięcej w grupach usypianych 80% i 75% CO_2 (odpowiednio 14,5% i 16,4%). Matki pszczele rozpoczynały czerwienie po upływie 4 do 55 dni od dnia unasienienia. Wykazano, istotny związek między stężeniem dwutlenku węgla zastosowanego w czasie unasieniania matek pszczelich, a szybkością rozpoczynania przez nie składania jaj. Istotnie najszybciej, rozpoczynały czerwienie matki usypiane 50%, 100% i 90% dwwutlenkiem węgla - odpowiednio po 17,4, 17,6 i 19,9 dniach. Matki usypiane 80% CO_2 rozpoczynały składanie jaj istotnie później bo dopiero po upływie średnio około 24,3 dni. Ocena udziału matek czerwiących i nie czerwiących po 3-tygodniowym okresie oczekiwania od inseminacji wykazała że czerwienie rozpoczęło 46,8% matek usypianych 80% dwutlenkiem węgla ponad 70% matek usypianych dwutlenkiem węgla o stężeniu 50%, 90% i 100%, ponad połowę matek usypianych 75% i zaledwie 46,8% matek usypianych 80% CO_2.

Słowa kluczowe: *Apis mellifera*, narkoza, dwutlenek węgla, azot, tlen, sztuczne unasienianie.