

## **Effect of carbon dioxide on the onset of oviposition in instrumentally inseminated honeybee queens**

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**Early onset of oviposition by instrumentally inseminated honeybee queens decreases the cost of their production. Under intensive management the break in egg-laying that occurs between the time of the removal of the old queen and initiation of egg-laying by the new one has a negative impact on the utilization of successive flows because of a reduced number of foragers. The aim of the study was to investigate if the additional treatment of carbon dioxide affects the onset of oviposition by instrumentally inseminated honeybee queens. Queens were inseminated at the age of 8 days. After 2 days 20 out of 45 queens were treated with carbon dioxide for 3 minutes. Queens additionally treated initiated oviposition at the age of 11-19 days (mean 13.6). Queens untreated additionally started to lay eggs at the age of 10-29 days (mean 16.6). It was found that the additional treatment of carbon dioxide accelerates significantly the onset of oviposition in instrumentally inseminated honeybee queens.**

**KEY WORDS: honeybee / honeybee queen / carbon dioxide / instrumental insemination / oviposition**

During the requeening in the bee colony, there is a break in egg-laying, which occurs between the removal of the old queen and the onset of oviposition by the new one. Under the intensive itinerant management, usually there are no periods without honey flow and oviposition intervals have a negative impact on the utilization of successive nectar flows, due to the reduced number of forager bees. Early initiation of oviposition in instrumentally inseminated queens allows breeders of queens to shorten the production cycle, which subsequently reduces the costs of queens production.

Mackensen [9] reported that honeybee queen can be instrumentally inseminated 2 days after emerging from the queen cell. According to Woyke and Jasiński [15] the survival of queens inseminated at the age of 2, 3, 4, 5, 6 and 7 days amounts 14, 23, 60, 75, 82 and 100% respectively.

The experiments of Moritz and Kühnert [10] indicated that the initiation of oviposition in instrumentally inseminated queens depends on season of the year. According to these

authors, the queens inseminated in April initiated egg laying on average 5.7 days after the insemination, while the queens inseminated in September 14.3 days after the insemination. According to Woyke and Jasiński [16] the number of days from instrumental insemination to the onset of oviposition is affected by the number of bees attending the queen. Queens in colonies with 9500 workers began the oviposition 6.9 days after the insemination and queens in mating hives with 750, 350 and 150 workers began the oviposition 9.9, 12.1 and 12.6 days after insemination, respectively.

Mackensen [8] reported that double treatment with carbon dioxide accelerates onset of oviposition of honeybee queens. Instrumentally inseminated queens untreated with CO<sub>2</sub> began oviposition at the age of 47 days, queens treated with CO<sub>2</sub> once began to lay eggs at the age of 36 days and queens treated twice started the oviposition at the age of 15 days. According to Ebadi and Gary [4] instrumentally inseminated queens treated with a mixture of air and carbon dioxide (50-90% CO<sub>2</sub>) initiated oviposition 12.7 days earlier than queens treated with pure carbon dioxide. Woyke et al [18] reported that additional CO<sub>2</sub> treatment of queens before or after instrumental insemination significantly accelerates the onset of oviposition. Ebadi and Gary [4] stated that queens treated for 10 minutes with carbon dioxide, one day after instrumental insemination, initiate oviposition significantly earlier than queens untreated additionally. Virgin queens after double CO<sub>2</sub> treatment (for 10 minutes each) begin egg laying at a similar time as the instrumentally inseminated queens [10]. In the research of Woyke and Jasiński [17] there was no significant influence of the CO<sub>2</sub> treatment, 2 days before and 2 days after insemination of queens, on the beginning of oviposition. According to Konopacka [7] the initiation of oviposition in instrumentally inseminated queens depends primarily on the duration of the carbon dioxide treatment. Honeybee queens treated twice for 5-6 minutes began the oviposition significantly earlier than queens treated for 2 minutes. This author also concluded that the dual 10-minute treatment with CO<sub>2</sub> shortens the life of honeybee queens. Konopacka [7] recommends double treatment for 3 minutes. Also Skowronek and Jaycox [11] report that treatment with carbon dioxide shortens the life of bees, in proportion to the time of treatment. Carbon dioxide treatment of honeybee queens increases their attractiveness to worker bees [12].

According to majority of authors [4, 7, 8, 18] and the common opinion, the additional carbon dioxide treatment of instrumentally inseminated queens accelerates the oviposition. However, one of studies [17] stated that additional carbon dioxide treatment delayed onset of oviposition by 1.2 days (on average), while another study [8] declared that it accelerated the oviposition by 21 days.

The aim of the study was to investigate if the additional treatment of carbon dioxide, for 3 minutes, affects the onset of oviposition by instrumentally inseminated honeybee queens.

### **Materials and methods**

The experiments were conducted in July and August 2010 in Apiculture Division at Warsaw University of Life Sciences (SGGW). The research material consisted of 45 queens representing the Italian breed. The rearing of queens was conducted in two rearing

colonies. The breeding frames with 48 queen cells were taken away from rearing colonies when the queen cells reached the age of 14 days, i.e. 2 days before emerging of queens. Queen cells were isolated in 'Zander' cages and placed in an incubator at 35°C. 45 queens were obtained. Queens were introduced into mating hives. Each mating hive was set with about a thousand workers of Carniolan breed. Due to the breed differences, light colored queens of Italian breed were visible among the dark Carniolan bees. The entrances of mating hives had queen excluders that allowed worker bees to exit liberally and restrained the queens inside. This method prevented the queens from flying out the mating hive for nuptial flights and the natural insemination. Queens in the age of 8 days from the emerging were treated with carbon dioxide and instrumentally inseminated. Each queen was inseminated with 8 µl of semen derived from drones of Italian breed. After two days, i.e. at the age of 10 days from emerging, 20 out of 45 inseminated queens were additionally treated with carbon dioxide for 3 minutes. The onset of oviposition of honeybee queens was subjected to control the following day.

The obtained results were analyzed statistically by one-way ANOVA test and the NIR test. Calculations were performed in SPSS 17.0 software [13].

### Results and discussion

44 queens initiated the oviposition, i.e. 20 queens of the additionally treated group and 24 queens of the group, which was not additionally treated. Additionally treated queens started oviposition at the age of 11-19 days, mean 13.6 days. The most of queens (6) in this group began oviposition at the age of 12 days (mode). Queens that were untreated additionally began oviposition at the age from 10 to 29 days, mean 16.6 days. The most of queens (5) in this group began the oviposition at the age of 16 days (Fig.).

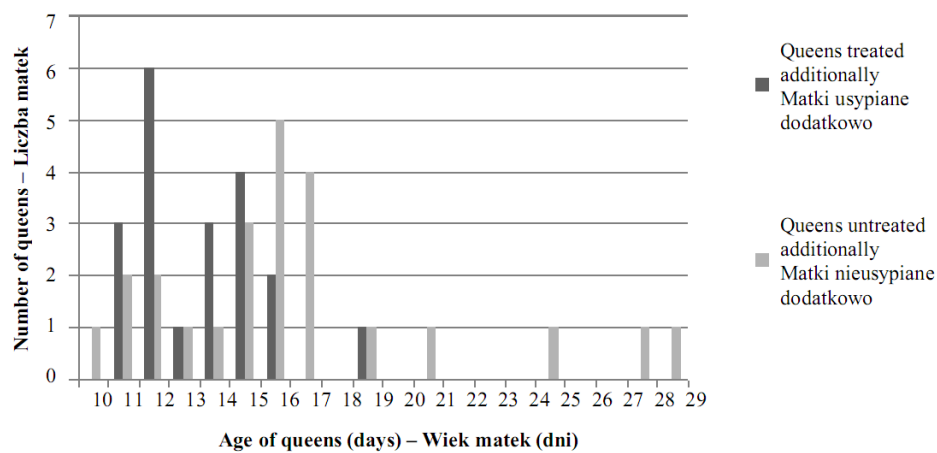


Fig. Age at which queens initiated oviposition (days)  
Rys. Wiek matek rozpoczynających czerwienie (dni)

Additionally treated queens began oviposition three days earlier than untreated queens. This difference was statistically significant ( $p=0.014$ ). These results are consistent with results obtained by other authors [4, 7, 8, 18], who demonstrated that treating the queens with carbon dioxide accelerates the start of their oviposition. Significant effect of  $CO_2$  was not found in studies of Woyke and Jasiński [17], where queens that were treated two days before and after instrumental insemination, began the oviposition 1.2 days (on average) later than the queens that were not additionally treated.

Queens from both groups started the oviposition from 2 to 21 days after instrumental insemination, mean 7.2 days (Table). The mean numbers of days, between instrumental insemination and onset of oviposition, given by various authors were corresponding, yet higher: 6.5-9.7 [17], 7.4-10.3 [5] 7.8-8.6 [6], 8.8-12.0 [7], 8.9 [1] 8.9-9.3 [2], 9.1 [19], 10.1 [3], 11.0 [14].

Within 8 days after instrumental insemination, 95% of additionally treated queens and 62% of additionally untreated queens initiated egg laying. Overall, within 8 days after insemination, 77% of queens started the oviposition. In experiments of Woyke et al [19] 50% of all queens started to lay eggs within 8 days after instrumental insemination and 75% of queens began the oviposition within 12 days after the insemination. The results obtained in the present study are consistent with results of Woyke et al [19], who stated that mean number of days from insemination to the onset of oviposition is greater than the median and mode (Table).

**Table – Tabela**

Number of days between instrumental insemination of queens and onset of egg laying  
Liczba dni od sztucznego unasieniania matek do rozpoczęcia czerwienia

Queens Matki	Number of queens Liczba matek	Min-max Min.-maks.	Mean Średnia	Standard deviation Odchylenie standardowe	Modal Modalna	Median Mediana
Treated additionally Usypiane dodatkowo	20	3-11	5,6 <sup>a</sup>	2,11	4	5,5
Untreated additionally Nieusypiane dodatkowo	24	2-21	8,6 <sup>b</sup>	4,92	8	8
Overall Ogółem	44	2-21	7,2	4,16	4	7

Different letters indicate significant differences ( $p<0.05$ )  
Różne litery wskazują istotne różnice ( $p<0,05$ )

As a result of the present study it was found that treatment with carbon dioxide for 3 minutes, 2 days after instrumental insemination, accelerates significantly the onset of oviposition in honeybee queens. Queens that were additionally treated initiated egg laying three days (on average) earlier than queens untreated additionally.

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## Wpływ dwutlenku węgla na rozpoczynanie czerwienia sztucznie unasienionych matek pszczelich

### Streszczenie

Wczesne rozpoczynanie czerwienia sztucznie unasienionych matek pszczelich zmniejsza koszty ich produkcji. W intensywnej gospodarce pasiecznej przerwa w czerwieniu, powstająca pomiędzy usunięciem starej matki a rozpoczęciem czerwienia młodej, wpływa niekorzystnie na wykorzystanie kolejnych pożytków z powodu zmniejszonej liczby zbieraczek. Celem pracy było zbadanie czy dodatkowe usypianie dwutlenkiem węgla matek pszczelich, po sztucznym unasienianiu, wpływa na rozpoczynanie przez nie czerwienia. Matki pszczele unasieniano sztucznie w wieku 8 dni od wygryzienia z mateczników. Po dwóch dniach 20 spośród 45 matek usypiano dwutlenkiem węgla na 3 minuty. Matki dodatkowo usypiane rozpoczynały czerwienie w wieku 11-19 dni (średnio 13,6 dni). Matki nieusypiane rozpoczynały czerwienie w wieku 10-29 dni (średnio 16,6 dni). Stwierdzono, że matki pszczele, dodatkowo usypiane dwutlenkiem węgla po sztucznym unasienianiu, rozpoczynają czerwienie istotnie szybciej niż matki nieusypiane dodatkowo.

**SŁOWA KLUCZOWE:** pszczoła miodna / matka pszczela / dwutlenek węgla / sztuczne unasienianie / czerwienie