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Influence of different oxygen and nitrogen mixtures on the survival of worker bees after anesthesia with carbon dioxide

BEATA MADRAS-MAJEWSKA, LUIZA OCHNIO*, MACIEJ OCHNIO, BARBARA ZAJDEL, ZBIGNIEW KAMIŃSKI, TERESA OSTASZEWSKA**, MACIEJ KAMASZEWSKI**

Apiculture Division, Faculty of Animal Sciences, Warsaw University of Life Sciences – SGGW, Nowoursynowska 166, 02-787 Warsaw
*Department of Informatics, Faculty of Applied Mathematics and Informatics
**Department of Ichtyobiology and Fisheries

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Madras-Majewska B., Ochnio L., Ochnio M., Zajdel B., Kamiński Z., Ostaszewska T., Kamaszewski M.

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Summary

Bee anesthesia has long been widely used in scientific research and for practical purposes. Bee anesthesia is usually induced with carbon dioxide. It is required for such procedures as populating mating hives and cages, introducing queen bees and instrumental insemination. The oxygen to nitrogen ratio may play a very important role in the process of awakening. The aim of the experiment was to investigate the survival rate of worker bees after the application of different concentrations of oxygen and nitrogen in their recovery from anesthesia. The general purpose was to determine the optimal gas mixture for the fastest recovery and the longest survival of bees. The results of this study provide a basis for future experiments on queen bees aimed at accelerating the awakening from anesthesia with carbon dioxide during insemination as well as the initiation of laying eggs. The lowest bee mortality in the first days after awakening from anesthesia was observed in the group awoken in a mixture of 48.6% O_2 and 51.4% N_2, but the highest average survival rate was noted in the group awoken in 60% O_2 and 40% N_2.

Keywords: anesthesia, bees, awakening, oxygen, nitrogen

Bee anesthesia has long been widely used in scientific research and for practical purposes. Bee anesthesia is usually induced with carbon dioxide. It is required for such procedures as populating mating boxes and cages, introducing queen bees, and instrumental insemination. Carbon dioxide is easy to use, fast-acting, and safe for humans. The effects of anesthesia are often directly proportional to the time of exposure of worker bees and queens to CO_2 (2, 4, 14, 16, 19). Worker bees anesthetized with carbon dioxide show improved acceptance of queen bees during the population of mating hives with worker bees and the introduction of queen bees (20). However, bee anesthesia with carbon dioxide has been proven to have toxic side effects for the behavior and viability of these insects. Several authors report that, after the carbon dioxide anesthesia, bees aged prematurely and had a shorter lifespan (5, 6, 8, 14), collected pollen less effectively, produced little wax (1), and their pharyngeal glands atrophied faster (13). It was also found that carbon dioxide inhibits the development of the internal organs of worker bees (17). The behavior of bees changes with the increased concentration of CO_2 (13). Prolonged anesthesia (over 2-5 hours) causes changes in their biological processes. After CO_2 anesthesia during insemination, queen bees were less readily accepted by bees (18). Carbon dioxide narcosis performed twice accelerates the onset of bee queen oviposition, but has a negative effect on the queen's organism (2). Carbon dioxide has a positive influence only on drones (3) as it accelerates their maturation. The period of awakening and atmospheric conditions following CO_2 anesthesia are equally important for the health, vitality and lifespan of these insects. The oxygen to nitrogen ratio may play a very important role in the process of awakening. Normal oxygen concentration in the core of the colony is about 15%, but in order to maintain heat during wintering and metabolic slowdown, bees reduce this level (15). Insects adapt very well to temporary shortages of oxygen, and have high survival rates in difficult aerobic conditions (7, 12). However, Madras-Majewska and Jasiński (9) demonstrated that the increase of the proportion of oxygen in the gas mixture used for recovery from anesthesia to 40% increased the survival of worker bees. In another study on different concentrations of oxygen and nitrogen applied during awakening (10), the lowest bee survival rate was observed in a group that recovered in atmospheric air, whereas the highest survival rate was noted in bees recovering in a mixture of 60% of oxygen and 40% of nitrogen. The difference between survival rates for those two groups of bees amounted to 6 days. In another experiment, Madras-Majewska at al. (11) found that recovery from anesthesia in an atmosphere composed of 70% of oxygen and 30% of nitrogen significantly reduced the time of regaining respiratory movements and of total recovery, and also resulted in the highest survival rate 14 days after awakening. By contrast, bees that woke up in an atmosphere of pure nitrogen lived the shortest (22 days).

The aim of the present experiment was to investigate the survival rate of bees after the application of different concentrations of oxygen and nitrogen during their recovery from anesthesia. The general purpose was to determine the optimal gas mixture for fast recovery and an extended lifespan of bees. The results of this study provide a basis for future experiments on queen bees, aimed at accelerating the awakening from anesthesia with carbon dioxide during insemination and at accelerating oviposition.

Material and methods

The experiments were conducted in July and August 2009 at an experimental apiary of the Apiculture Division of the University of Life Sciences (SGGW) in Warsaw, Poland. The study was conducted on 2,000 worker bees aged 1-3 days. In order to ensure the appropriate age of bee workers, brood, upon emergence, was kept in isolators with a metal mesh in four maternal colonies. Then, the young bees were placed in 30 cages, 100 worker bees in each cage. The bees were kept in wooden cages 17 cm high, 11 cm wide and 6 cm deep. Each cage had two vents in the side walls, a closed plate opening for introducing bees and for providing food in the form of honey-sugar dough, as well as one hole at the bottom of the cage for the removal of dead bees. The front of the cage was glazed for direct observation. A drinker in the form of a syringe was placed in each cage. Inside the cage, there was a piece of honeycomb and a vessel with honey-sugar candy. In these cages, the bees, divided into 5 groups, were subjected to 3-minute carbon dioxide anesthesia, and then woken up for 1 minute in an atmosphere of oxygen and nitrogen at various concentrations (atmospheric air; 48.6% O_2 and 51.4% N_2; 40% O_2 and 60% N_2; 60% O_2 and 40% N_2; 20% O_2 and 80% N_2). Then, the bees were observed, and the time of successive stages of their recovery was recorded. After anesthesia, the bees were kept in cages at room temperature. The number of dead bees was reported daily. Observation ended with the last dead bee in each cage. The awakening rates obtained were analyzed statistically. A covariance model was calculated according to the following formula:

$$E(N_{\text{cumulative number of dead insects}}) = \alpha_0 + \alpha_1 \text{Number\_of\_days} + \begin{cases} \beta_1 & \text{when gas mix} = 1 \\ \beta_2 & \text{when gas mix} = 2 \\ \beta_3 & \text{when gas mix} = 3 \\ \beta_4 & \text{when gas mix} = 4 \\ \beta_5 & \text{when gas mix} = 5 \end{cases}$$

$$N_{\text{cumulative number of dead insects}} = E(N_{\text{cumulative number of dead insects}}) + \epsilon$$

where ϵ has a Poisson distribution (typical distribution at low numbers of counts).

Three hypotheses were tested:

H_0^1: effects of all gas mixtures are equal

H_0^2: effects of all days are equal

H_0^3: effects of all gas mixtures on individual days are equal.

All hypotheses were rejected at $\alpha = 5\%$.

Tukey's test was used to determine significant differences between the groups. All calculations were carried out in SAS software, version 12.3.

Results and discussion

The maximum lifespan (50 days) was observed in bees recovering in a cage with 60% of oxygen in the air mixture, whereas the minimum lifespan (12 days) was noted in the cage with atmospheric air – Fig. 1.

The cumulative number of daily reported dead bees was compared statistically at three points: at the end of the first stage (0-7 days after awakening from

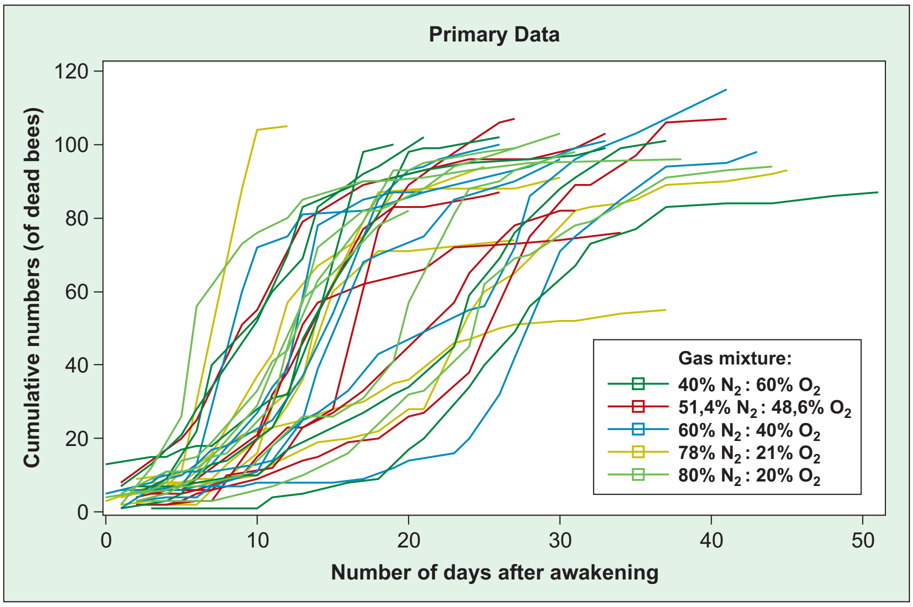

Fig. 1. "Spaghetti" type diagram presenting the primary data shows differences in the number of days after awakening in relation to the gas mixture dose

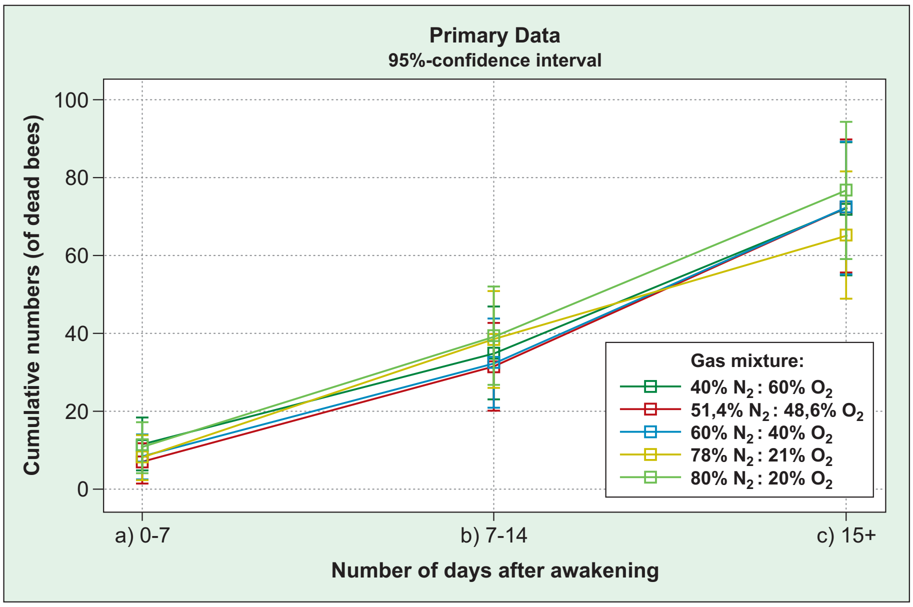

Fig. 2. Cumulative number of dead bees as a function of the number of days after awakening for different gas mixtures (primary data)

anesthesia), at the end of the second stage (7-14 days) and at the end of the third stage (over 15 days). The analysis of primary data shows that the highest average cumulative number of dead bees until the 15th day after recovery from anesthesia was observed in the group recovering in the lowest concentration of oxygen (20% of oxygen and 80% of nitrogen) – Fig. 2.

A comparison of the cumulative number of dead insects for the three stages (0-7 days, 7-14 days and over 15 days after awakening) also reveals differences in the group of bees awoken in atmospheric air (Fig. 3). In this group, the average mortality was higher during 7-14 days after awakening, and decreased later on. In the group awoken in 80% of N_2 and 20% of O_2, mortality was the highest 15 days after the recovery from anesthesia.

A covariance model was fitted to the data. Differences between the mean values for all groups tested by Tukey's test are presented in linear form in Fig. 4. At the stage of 0-7 days, the groups did not differ much. Surprisingly, in the first week after the awakening, the average number of dead bees was the highest and similar for the 80% of N_2 and 20% of O_2 mixture and for the 40% of N_2 and 60% of O_2 mixture. The lowest total mortality in the first week after recovery from anesthesia was observed in the group awoken in 48.6% of oxygen. During the second stage (7-14 days), the cumulative numbers of dead bees observed in particular groups differed and remained the lowest in the group recovering in 48.6% of O_2. The most significant differences were found after 15 days. Two groups differed from the others: the one awoken in atmospheric air and the one awoken in 80% of nitrogen and 20% of oxygen. Discrepancies between these two groups and the others were significant and visible from the 7th day after awakening onwards. The longest average lifespan was noted in the group with 60% of oxygen, which did not differ in this respect from the group with 48.6% of oxygen, but was significantly different from the group recovering in 40% of oxygen.

These results are similar to those obtained in other experiments (9-11), in which the shortest survival time was observed in the group of bees recovering in atmospheric air and in all gas mixtures with the highest proportion of nitrogen.

Conclusions:

1. The lowest average survival was observed in the group awoken from anesthesia in atmospheric air.

2. Every gas mixture with the oxygen percentage lower than 40% results in a lower survival rate after anesthesia with carbon dioxide.

3. The lowest bee mortality in the first days after awakening from anesthesia was observed in the group awoken in 48.6% O_2.

4. The optimal composition of the gas mixture was 60% oxygen and 40% nitrogen. The highest average survival rate was observed in the group of bees awoken in this atmosphere (the survival time was extended by

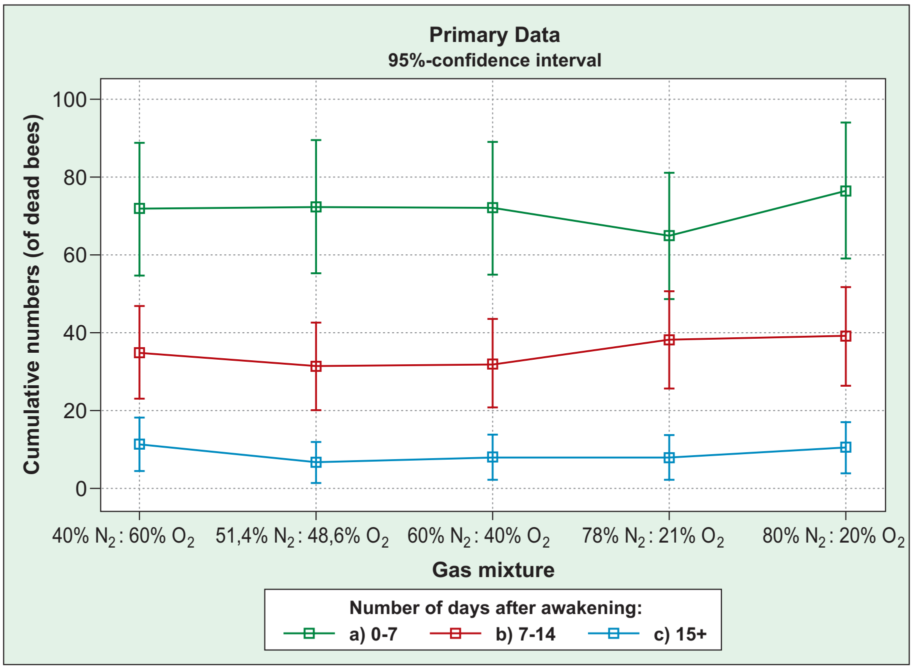

Fig. 3. Comparison of the cumulative numbers of dead bees and the gas composition in time-series segmentation (primary data)

5 days compared to the group awoken in atmospheric air).

5. The maximum survival time (in days) was more than three times as long as the minimal survival time (for bees awoken in atmospheric air).

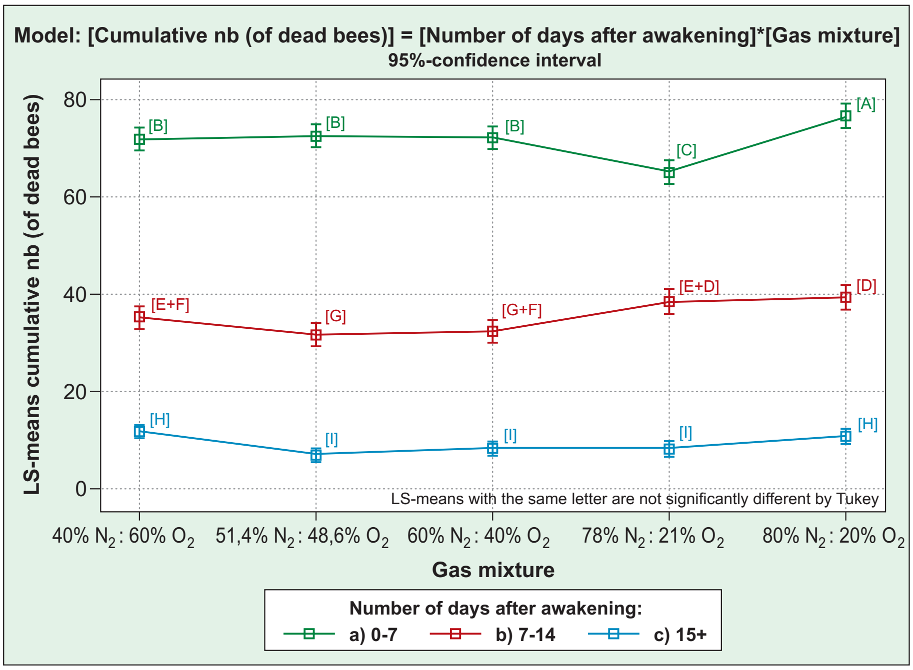

Fig. 4. Mean values of the cumulative number of dead bees and the gas composition. The letters in brackets indicate significant differences between groups (Tukey's test)

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Corresponding author: dr hab. Beata Madras-Majewska prof. nadzw. SGGW, ul. Nowoursynowska 166, 02-787 Warsaw; e-mail: beata_madras_majewska@sggw.pl