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EFFECT OF RESTRICTED POLLEN SUPPLY TO COLONIES ON THE QUALITY OF REARED QUEEN BEES

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ABSTRACT

The quality of queens reared in colonies with restricted (RP) and not restricted pollen supply (NRP) was evaluated during foraging on false acacia and small-leaved and broad-leaved lime. It was shown that the body weight of queens in both groups immediately after emergence and on the day of instrumental insemination did not differ significantly (221.8 ±15 and 224.3 ±15 and 170.9 ±15 and 177.5 ±14 mg, respectively). The waiting time to start oviposition was similar: 4.7 ±1.8 days in RP group and 5.7 ±2.8 days in NRP group, not being significantly different. There were no significant differences in the diameter and volume of spermatheca and the number of ovarioles in the right ovary between the queens of the tested groups.

Key words: *Apis mellifera* L., queen, pollen, instrumental insemination

INTRODUCTION

The honey bee food is nectar, honeydew and pollen [Haydak 1970]. Nectar and honeydew contain mainly carbohydrates. Pollen, on the other hand, is the main source of proteins, fats as well as minerals and vitamins [Roulston and Cane 2000]. Freshly harvested pollen as well as that stored in comb cells (in the form of beebread) is the food mainly for young nurse bees [Crailsheim et al. 1992].

Pollen is the only source of proteins that guarantee the proper development and functioning of many honeybee organs [Haydak 1970]. The amount and quality of pollen consumed by nurse bees is the basis for the proper development of the hypopharyngeal glands located in the head [Pernal and Currie 2000, Renzi et al. 2016] that produce royal jelly. High-protein food used to feed larvae developing into worker bees and drones up to day 3 of life or until the end of the stage of larvae developing in queens (reared queens). It is also the food of adult queens throughout their entire live [Crailsheim 1992, Fujita et al. 2013]. The protein diet is mainly consumed by 1–8 day old bees, with the highest consumption on day 3. The acini of hypopharyngeal glands attained the age of 5–8 days and then began to shrink afterwards [Deseyn and Billen 2005, Omar et al. 2017].

The decreased availability of pollen as well as its low quality contributes to the development of smaller hypopharyngeal glands [DeGrandi-Hoffman et al. 2010, Renzi et al.2016]. In the rainy season, and during the period of no pollen supply, the frequency of visiting young larvae (1–3 days old) by nurse bees is smaller and correlated with the amount of pollen in the brood chamber and the number of non-capped larvae [Schmickl and Crailsheim 2002]. Bad pollen supply also led to cannibalism of larvae less than 3 days old and to shortening of the time until larvae are sealed [Schmickl and Crailsheim 2001]. Bees rear less brood [DeGrandi-Hoffman et al. 2008] and the life span of bees is shorter [Di Pasquale et al. 2013].

The aim of our study was to evaluate and compare the robustness and onset of oviposition in queens reared in colonies with restricted and not restricted pollen supply.

MATERIAL AND METHODS

The study was conducted at the Department of Zoology and Apiculture, West Pomeranian University of Technology in Szczecin in full beekeeping season (June–July

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2015) during foraging on false acacia and small-leaved and broad-leaved lime. The research material consisted of sister queens of *Apis mellifera* carnica being reared from one-day larvae [Ruttner and Ruttner 1983, Büchler et al. 2013].

Queens were reared in two queenless colonies colonising Wielkopolski hives (360×260 mm frame) with a bottom pollen trap with 5.0 mm diameter hols, collecting about 29% pollen loads [Bobrzecki and Wilde 1990]. In one colony, 10 days before the beginning of rearing, pollen load trapping was started. Queen cells from the colony with restricted (RP) and not restricted pollen supply (NRP) after capping were placed in an incubator at 34.5°C. Staring from day 15 of preimaginal development, the emergence of queens was monitored every six hours. Immediately after emergence, queens were weighed (on a RADWAG WXD 200/2000 laboratory balance for weighing the moving animals) with an accuracy to 1 mg and introduced into plastic mailing cages with 8–10 bees and with candy. On the second day of life, queens were introduced into mini-plus mating nuclei (with 215×163 mm frame), with bees occupaiting 6 combs, including two with capped brood. The nuclei entrances were secured with a queen excluder.

At the age of seven days, queens – after weighing (on a RADWAG WXD 200/2000 laboratory balance, with accuracy to 1 mg) – were instrumentally inseminated once with 8 µl of semen [Woyke 1960, Cobey et al. 2013] collected from drones of the same subspecies aged 14–16 days [Woyke and Jasiński 1978]. Two days after instrumental insemination, queens were anaesthetised with CO2 for 3 minutes [Woyke et al. 2001]. The onset of oviposition by queens was monitored by examining the mating nuclei every two days, until day 14 after instrumental insemination.

In randomly selected egg lying queens from both groups, after anaesthetising, the reproductive system was removed [Dade 2009]. The diameter of spermatheca was determined by a modified method of Woyke [1971]. The removed spermathecae were transferred onto microscope glass slides. After removing the trachea, the photographs of spermathecae were taken using a Zeiss Primo Star microscope with a digital camera (at 20× magnification) and their diameter were measured by means of ScopePhoto software. The number of ovarioles in the right ovary was counted [Carreck et al. 2013]. The removed ovaries were submerged in 95% alcohol for 15 minutes, transferred onto microscope glass slides and cut at half their length [Woyke 1971]. In the cut off thicker, distal part, ovarioles were counted under a microscope, at 25× magnification.

In total, the body weight and the onset of oviposition were evaluated in 61 queens, including 32 in RP group and 29 in NRP group. The spermathecal diameter and the ovariole number in the right ovary were determined in 10 queens in each group.

The figures obtained were analysed statistically using Statistica PL v.12 computer software. Differences between the body weight of queens, diameter and volume of their spermathecae, and the number of ovarioles were evaluated with the Student's *t*-test. Differences in the waiting time to start oviposition by queens was analysed with a χ^2 test. The coefficients of correlation between the physical traits being examined were calculated. The number of queens starting oviposition was analysed with the G-test with Williams's correction [Sokal and Rohlf 1981].

RESULTS

Mean body weight (±SD) of queens immediately after emergence (on day 1 of life) in NRP group was only by 2.5 mg greater than in RP group (Table 1). Similarly, on the day of instrumental insemination, the queens from NRP group had a slightly greater body weight, by 6.6 mg on average, than the queens from RP group (Table 1). The observed differences in queen body weight between the groups both after emergence and on the day of instrumental insemination were not significant (t = –0.632, P = 0.529 and t = 1.789, P = 0.0787). In the period of maturation from emergence to instrumental insemination, the body weight of queens in RP group and NRP group decreased significantly (t = 13.554, P = 0.000 and 12.229, P = 0.000), by 22.9 and 20.9%, respectively (Table 1).

Out of 32 instrumentally inseminated queens in RP group and 29 in NRP group, until day 14 after instrumental insemination, a similar, not significantly different number of queens started oviposition (*G*-test: G_{adj} = 0.001, p_{adj} = 0.974), i.e. 93.7 and 93.2%, respectively. In RP group, two queens were found missing on days 6 and 8 after instrumental insemination, while in NRP group one queen was missing on day 10 and another one did not start oviposition. Her dissection did not show any residual semen in the lateral oviducts, while the spermatheca was filled with sperm.

The waiting time to start oviposition by queens in both groups was similar, not being significantly different (χ^2 = 0.335, P = 0.563). The modal value for the number of days from instrumental insemination to the onset of oviposition in RP group was by 1 day smaller than in NRP group, in which it was 5 days. The evaluation of the reproductive system of queens did not show significant differences in the spermathecal diameter (t = –0.935, P = 0.362), its volume (t = –0.973, P = 0.342), and the ovariole number in the right ovary (t = 0.083, P = 0.935) (Table 1).

No significant differences between the parameters evaluated in queens being reared in colonies with restricted (RP) and not restricted pollen supply (NRP) allowed for a joint analysis of correlations between them. The

Table 1. Average values (±SD) traits investigated in queens from groups with restricted (RP) and not restricted pollen supply (NRP).

Tabela 1. Wartości średnie (±SD) badanych cech matek w grupie z ograniczonym (RP) i nieograniczonym dopływem pyłku (NRP)

Trait – Cecha	N	Restricted pollen supply Ograniczony dopływ pyłku	N	Not restricted pollen supply Nieograniczony dopływ pułku
Body weight immediately after emergence, mg Masa ciała bezpośrednio po wygryzieniu, mg	32	221.8 ±15.1 aA*	29	224.3 ±15.3 aA
Body weight prior to instrumental insemination, mg Masa ciała przed unasienieniem, mg	32	170.9 ±15.0 aB	29	177.5 ±13.8 aB
Number of days from instrumental insemination to starting oviposition Liczba dni od unasienienia do rozpoczęcia czerwienia	30	4.7 ±1.8 a	27	5.7 ±2.8 a
Spermathecal diameter, mm Średnica zbiorniczka nasiennego, mm	10	1.11 ±0.02 a	10	1.12 ±0.03a
Spermathecal volume, µl Objętość zbiorniczka, µl	10	0.714 ±0.04 a	10	0.737 ±0.06 a
Ovariole number in the right ovary Liczba rurek jajnikowych w prawym jajniku	10	127.2 ±16.2 a	10	126.7 ±10.1 a

* values marked with different lowercase letters in rows and uppercase ones in columns differ significantly at p = 0.05.
* wartości oznaczone odmiennymi małymi literami w wierszach i dużymi w kolumnach różnią się istotnie przy p = 0,05.

correlation coefficients calculated between the body weight of queens immediately after emergence as well as on the day of instrumental insemination and the onset of oviposition by queens, r = 0.100 and r = 0.108, were not significant (t = 745, P = 0.459 and t = 0.803, P = 0.425). Similarly, no correlation was found between the body weight of queens immediately after emergence and the ovariole number in the right ovary, r = –0.267 (t = –1.172, P = 0.256), and the spermathecal diameter and volume, r = 0.261 (t = 1.150, P = 0.296) and r = 0.262 (t = 1.150, P = 0.265), as well as the ovariole number in the right ovary and the spermathecal diameter and volume, r = –0.03 (t = –0.398, P = 0.696) and r = –0.107 (t = –0.461, P = 0.651).

DISCUSSION

While undertaking the study, it was presumed that a restricted 10-day pollen supply o to a colony before and during the queen rearing (with pollen trapping) would affect the quality of queens being reared. The analysis of the findings did not confirm our assumptions. The body weight of queens immediately after emergence and on day 7 of life, just before instrumental insemination in RP group and NRP group did not differ significantly. The emerging queens in both groups were heavier on average by 28 to 57 mg when compared to the studies by Bieńkowska et al. [2009] and Alqarni et al. [2013], respectively. The body weight of Carniolan queens immediately after emergence, similar to our results, i.e. over 200 mg, was found by Chuda-Mickiewicz and Samborski [2015] and Skowronek et al. [2004]. A significant queen body weight loss in RP and NRP groups, during maturation, before instrumental insemination, on day 7 of life, was on average lower by 4.2 to 8% when compared to the queens emerging from queen cells incubated at 34.5 and 32°C, respectively, and kept until instrumental insemination in mating nuclei in the study by Chuda-Mickiewicz and Samborski [2015]. Similarly, Bienkowska et al. [2009] reported a smaller body weight loss, by 4.7%, in queens in the same period, being kept in cages with 25 bees. However, a larger loss of body weight, by 4.2%, from emergence to instrumental insemination, when compared to the present study and those of earlier cited authors, was stated by Skowronek et al. [2004], who kept queens in nuclei. The body weight of queens on the day of instrumental insemination in both groups was within the range given in the literature. i.e. 160 to 191 mg [Skowronek et al. 2004, Bieńkowska et al. 2009, Chuda-Mickiewicz et al. 2012, Heljanka et al. 2014, Chuda-Mickiewicz and Samborski 2015].

The percentage of queens starting oviposition in our experiment was higher by 8–16% when compared to that being found by Mackensen [1947], Kanfalung and Peng [1982], Czekońska and Chuda-Mickiewicz [2007], and Chuda-Mickiewicz et al. [Chuda-Mickiewicz et al. [2009], Chuda-Mickiewicz et al. [2012]] for the queens inseminated on day 7 of life and anaesthetised with CO2 for 3 minutes two days before or after instrumental insemination. The effectiveness of instrumental insemination, similar to that in our study, was obtained by Bieńkowska et al. [2012], 92.6%, Chuda-Mickiewicz and Prabucki [2000] and Chuda-Mickiewicz and Samborski [2015], 93.48 and 95%, respectively, and Gerula et al.

[2011], 97.4%. A similar percentage of queens who started oviposition received Moritz and Kühnert [1984], i.e. 92.3, who used a longer 10-minute CO_2 anaesthesia two days after instrumental insemination. According to Otten et al. [1998], the onset of oviposition by inseminated queens is to a large extent determined by the number of bees in a mating nuclei. In strong colonies with 1200 worker bees, 95% of queens started oviposition, while in weak ones with 200 worker bees only 70% of queens. Otten et al. [1998] also demonstrated that it depends on the age at which the queens are inseminated; from those being inseminated on day 10 of life, 95% started oviposition, while those inseminated on days 7 and 13 were fewer, 82 and 80%, respectively.

Similar results were obtained by Chuda-Mickiewicz and Prabucki [1993] who inseminated the queens twice with 4 µl of semen. Out of the queens inseminated at the age of 11 days, oviposition started 100%, of those inseminated at the age of 8 days 87.3%, and of those inseminated at the age of 6 days 50% to day 14 from the first insemination procedure. Mean waiting time to start oviposition in both RP group (4.7 days) and NRP group (5.7 days) was much shorter when compared to the study by Chuda-Mickiewicz et al. [Chuda-Mickiewicz et al. 2009, Chuda-Mickiewicz et al. 2012], 8.1 days, Gerula et al. [2011], 8.3 days, Czekońska and Chuda-Mickiewicz [2007], 9.9 days, and Skowronek et al. [2002], 10 days for the queens – as in the present study – being inseminated on day 7 of life and anaesthetised with CO2 for 3 minutes two days after instrumental insemination. Similarly, queens inseminated with 2×4 µl of semen started oviposition later, according to Chuda-Mickiewicz and Prabucki [Chuda-Mickiewicz and Prabucki 1993, Chuda-Mickiewicz and Prabucki 2000], i.e. after 10.4 and 8.1 days, respectively, and Woyke et al. [2001] – after 6–7 days. The faster oviposition of queens in our experiment was more likely to have been affected by the presence of capped brood and emerging bees in a the nucleus colony [Skowronek et al. 2002].

An indicator of the reproductive potential of a queen is, among others, the size of spermatheca and the number of ovarioles in the ovaries. Mean spermathecal volumen in both groups was lower by 0.1 to 0.31 µl when compared to that being found in Carniolan queens by Woyke et al. [1974], Bieńkowska et al. [2009], Alqarni et al. [2013], and Chuda-Mickiewicz and Samborski [2015]. The number of ovarioles in the ovary was also lower than that determined in this subspecies by Alqarni et al. [2013], 157, Gregorc and Smodiš Škerl [2015], 135 to 161, and Chuda-Mickiewicz and Samborski [2015], 166 to 178. A similar number of ovarioles in the ovary of Carniolan queens to that determined in our experiment is given by Woyke et al. [1974], i.e. 125.7.

The lack of significant correlations between the examined physical traits of queens demonstrated in our study coincides with the study by Corbella and Gencalves [1982], Hatch et al. [1999], and Chuda-Mickiewicz and Samborski [2015]. They did not find a significant correlation between the body weight of emerging queens and the number of ovarioles and the size of spermatheca. Jockson et at. [2011] demonstrated no correlation between the body weight and the ovariole number, while Chuda-Mickiewicz and Samborski [2015] between the ovariole number and the spermathecal diameter and volume. The results different from our ones and those of the cited authors were obtained by Kaya et al. [2008], Bieńkowska et al. [2009] and, Alqarni et al. [2013] who showed a correlation between the body weight and the size of spermatheca. Moreover, Alqarni et al. [2013] showed a correlation between the body weight and the number of ovarioles. Woyke [1971] also demonstrated significant correlations between physical traits, but only evaluated them in the queens being reared from brood at various ages (eggs, one-day, two-day and three-day larvae) together. The lack of a significant correlation between the body weight of emerging queens and the onset of oviposition being also found in our study is consistent with the study by Szabo et al. [1987] and Skowronek et al. [2002].

CONCLUSIONS

Summing up the obtained results, it can be concluded that the short-term restricted pollen supply to colonies in full season did not affect the quality of reared queen bees and the onset of oviposition.

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WPŁYW OGRANICZONEGO DOPŁYWU PYŁKU DO RODZIN NA JAKOŚĆ WYCHOWYWANYCH MATEK PSZCZELICH

STRESZCZENIE

Oceniono jakość matek wychowywanych w rodzinach z ograniczonym (RP) i nieograniczonyn dopływem pyłku (NRP) w czasie pożytku z robinii akacjowej i lipy drobnolistnej oraz szerokolistnej. Wykazano, że masa ciała matek w obu grupach bezpośrednio po wygryzienie oraz w dniu sztucznego unasienienie nie różniła się istotnie i wynosiła odpowiednio (221,8 ±15 i 224,3 ±15 oraz 170,9 ±15 i 177,5 ±14 mg). Okres oczekiwania na rozpoczęcie czerwienia był zbliżony 4,7 ±1,8 dni w grupie RP i 5,7 ±2,8 dni w grupie NRP, nie różniąc się istotnie. Nie stwierdzono również istotnych różnic w średnicy i objętości zbiorniczka nasiennego oraz liczbie rurek jajnikowych w prawym jajniku pomiędzy matkami ocenianych grup.

Słowa kluczowe: *Apis mellifera* L., matka pszczela, pyłek, sztuczne unasienianie

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