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CONTENT OF THE PHEROMONE COMPONENT (*E*)-9-OXO-2-DECENOIC ACID IN MATED HONEY BEE (*APIS MELLIFERA* L.) QUEENS OF DIFFERENT AGE

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Summary

One-, two- and three-year-old mated and egg-laying honey bee, *Apis mellifera* L., queens were investigated using gas chromatography technique.

It was revealed that the amount of (*E*)-9-oxo-2-decenoic acid (9-ODA) in individuals is highly variable independently of bee queen's age. The average amount of 9-ODA in extracts of one-year-old mated queens was 144,73±37,38 μg, in two-year-old ones 153.1±36,28 μg and in three-year-old ones 173±54,76 μg. The differences between the amounts were not statistically significant ($P > 0.05$).

The variation of 9-ODA content differed in queens of different age groups. The range of variation was much less in one-year-old queens (from 84 to 256 μg) than in two-year-old (from 32 to 400 μg) or in three-year-old (from 40 to 380 μg) ones. The coefficient of variation of 9-ODA content was 42,55%, 71.08% and 77.54%, respectively.

The results of the investigations demonstrated that 100 μg of 9-ODA predominated in one-year-old queens, whereas the predominant range is broader in two-year-old queens, reaching from 75 tob 225 μg. No predominat level was present in 3 year old queens.

Keywords. (*E*)-9-oxo-2-decenoic acid, *Apis mellifera* L., queen pheromones.

INTRODUCTION

(*E*)-9-oxo-2-decenoic acid (9-ODA) is the most abundant component of honeybee queen pheromone. Its content varies from 12 μg to 400 μg in the mandible glands of mated queens (Naumann et al., 1991). 9-ODA content depends on the condition of a bee colony, subspecies, and the age of bee queen.

The amount of 9-ODA secreted by less related subspecies of *Apis mellifera* L. differ both in absolute value and in the relative proportion to other components present in the secretion of the mandible gland. The content of 9-ODA in the secretion of mandible gland of *A. m. mellifera* reaches 37,25%, *A. m. adonsoni* - 65,39%, and *A. m. capensis* - 84,83%% (Crewe 1982). Mated European bee queens, inhabiting the North American continent, secrete on average 200 μg of 9-ODA, while Africanised queens secrete 100 μg 9-ODA (Pankiw 1996).

There exists a positive correlation between the content of 9-ODA in the bee queen and honey productivity of a bee colony (Apsegaite, Skirkevicius 1991). In virgin 1-day-old *A. m. caucasica* queens the content of 9-ODA is very low (on average 9.87 ± 2.32 μg). With the queen maturing, the 9-ODA content increases. In the extracts of virgin 8-day-old queens the 9-ODA content is 24.5 ± 4.88 μg on average, after mating in 21-day-old queens it reaches 54.75 ± 7.45 μg and in mated egg-laying 2-year-old queens: - it amounts to 118.27 ± 37.38 μg (Apsegaite, Skirkevicius 1995). Similar results were obtained by Slessor et al., 1991, and by Engels et al., 1997.

However, no data are available on the change of 9-ODA content in queens reaching the age of 2-3 years, i.e. the age when they are substituted by a new queen. The aim of the present work was to reveal the effect of aging on the content of 9-ODA in honeybee queen pheromone.

METHODS

One, two and three year-old mated and egg-laying *Apis mellifera mellifera*, *A. m. carnica* and *A. m. caucasica* x *A. m. carnica* honeybee queens were used for the present study. Our earlier studies (Apsegaite, Skirkevicius 1999) showed that the mean 9-ODA values in mated two-year-old queens of closely related European subspecies (*Apis mellifera mellifera, A. m. carnica* and *A. m. caucasica*) are nearly the same. Therefore in the present work the status of subspecies was neglected.

Bee queens were obtained from the Apiculture Department of the Lithuanian Institute of Agriculture. They were removed from a bee colony in August 1996. Each queen was placed in a dish with ethanol and kept in a refrigerator at a temperature of +4°C.

The ethanol extracts were prepared by the method described by Apsegaite et al. (1995). The extract was analyzed by gas chromatography using a Chrom-5 gas chromatograph equipped with a flame ionisation detector and a glass column 3 m x 3 mm. Stationary phase was 5% SE-30 coated on Chromaton N-AW, with the thermostat program 6°C/min ranging from 80 to 250°C, the injector temperature being 200°C and the detector temperature 250°C. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min.

For statistical evaluation the mean (X), the standard error (SE) and the variation coefficient (V) were calculated. Differences were considered to be statistically significant at $P < 0.05$ (Lakin 1973).

RESULTS AND DISCUSSION

Butler and Paton (1962) indicate that older (18 months) honeybee queens secrete lower levels of 9-ODA than do mated egg-laying queens 3 to 6

weeks old. Thus, the oldest queens in the experiment of Butler and Paton (1962) were aged 1.5 years. In our test the age limit was extended to three years.

Our results showed that the average amount of 9-ODA in extracts of one-year-old mated queens was 144,73 ± 37,38 μg, in two-year-old ones 153.1 ± 36,28 μg and in three-year-old ones 173 ± 54,76 μg (Fig. 1).

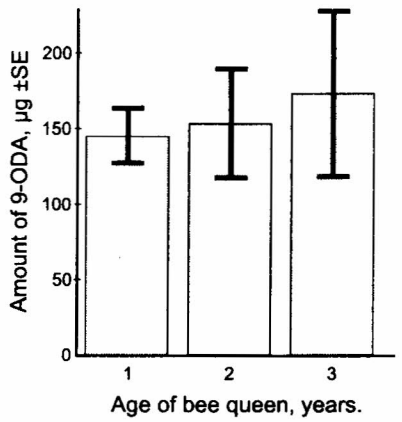

Fig 1. Mean content of (*E*)-9-oxo-2-decenoic acid in mated bee queens of different age: 1 - one-year-old (n=11); 2 - two-year-old (n=9); 3 - three-year-old (n=6)
Średnia zawartość kwasu (*E*)-9-oxo-2-decenowego u różnego wieku matek unasienionych: 1-matki jednoroczne (n=11); 2-matki dwuletnie (n=9); 3-matki trzyletnie (n=6)

The differences between the amounts were not statistically significant ($P > 0.05$). However, the range of variance of 9-ODA content differs depending on queen's age.

The range of variation was much less in one-year-old queens (from 84 to 256 μg) than in two-year-old (from 32 to 400 μg) or in three-year-old (from 40 to 380 μg) ones. Also, in one-year-old queens the coefficient of variation of 9-ODA content (V=42,55%) was significantly lower then in two-year-old (V=71.08%) or in three-year-old (V=77.54%) honey bee queens.

Histograms of 9-ODA amounts secreted by honeybee queens of different age (Fig. 2) show that 100 μg of 9-ODA predominates in one-year-old queens, whereas the predominant range is broader in two-year-old queens, reaching from 75 to 225 μg.

No dominant content of 9-ODA was detectable in three-year-old queens.

When summarising the obtained results, the dynamics of 9-ODA content should be accounted for. The content of 9-ODA secreted by older honeybee queens is not lower, however, its variation is much broader. It is manifested in significantly lower or higher 9-ODR levels then the mean value. The factors that predetermine such variations are not clear. The biological aspects of such variations are also very disputable.

Butler (1957) suggested that the honeybee queens secreting small amount of the pheromone fail to inhibit queen rearing in her own colony. The shortage of queen pheromone is the cause of queen supersedure or swarming. Winston et al. (1998) is of the same opinion. Allen (1965) noted that the proportion of replacements is lower among queens one-year-old than among those two- or three-year-old.

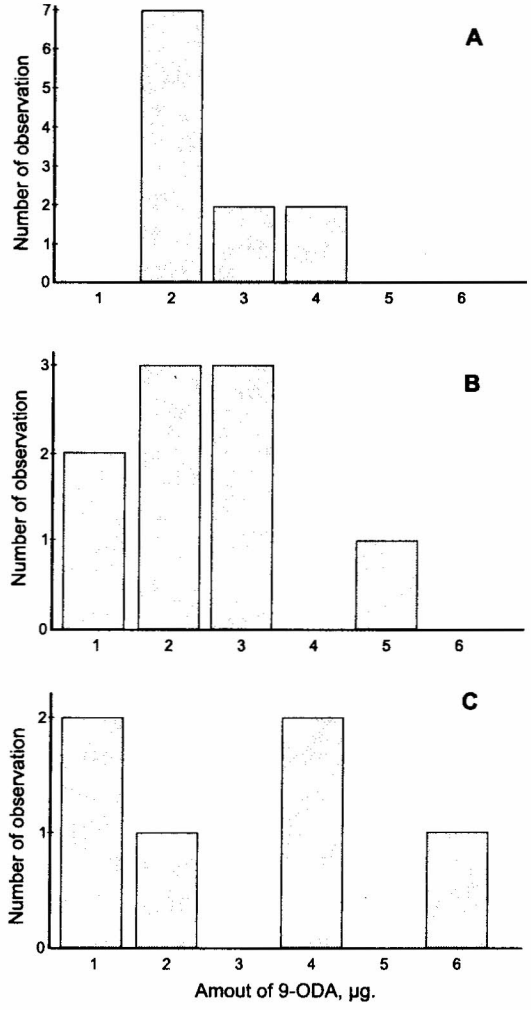

Fig. 2. Histogram of content 9-ODA secreted by honeybee queens of different age: a - one-year-old (n=11); b - two-year-old (n=9); c - three-year-old (n=6); 1 <75 µg; 2 -75-150 µg; 3 - 150-225 µg; 4 - 225-300 µg; 5 - 300-375 µg; 6 >375 µg

Histogram zawartości 9-ODA wydzielanego przez różnego wieku matki pszczele: a-jednoroczne (n=11); b-dwuletnie (n=9); c-trzyletnie (n=6);1 <75 µg; 2 -75-150 µg; 3 - 150-225 µg; 4 - 225-300 µg; 5 - 300-375 µg; 6 >375 µg

However, larger doses of honeybee queen mandible gland synthetic components ((*E*)-9-oxo-2-decenoic acid, (*E*)-9-hydroxy-2-decenoic acid, methyl p-hydroxybenzoate, 4-hydroxy-3-methoxyphenylethanol) produced no effect on pollen foraging (Higo et al., 1992), suppression of queen rearing or swarming (Winston et al., 1990; 1991).

CONCLUSIONS

It was revealed that the amount of (*E*)-9-oxo-2-decenoic acid (9-ODA) in individuals is highly variable independently of bee queen's age. The range of variation was much less in one-year-old queens than in two-year-old or in three-year-old ones.

The results of the investigations demonstrated that 100 μg of 9-ODA predominates in one-year-old queens, whereas the predominant range is broader in two-year-old queens, reaching from 75 tob 225 μg. No predominant level was present in 3 year-old queens.

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ZWARTOŚĆ KWASU 9-OXO-2-DECENOWEGO, SKŁADNIKA FEROMONU U UNASIENIONYCH MATEK PSZCZELICH (*APIS MAELLIFERA* L.) W RÓŻNYM WIEKU

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Streszczenie

U jedno, dwu i trzy letnich unasienionych i czerwiących matek pszczelich badano zawartość kwasu (E)-9-oxo-2-decenowego z zastosowaniem chromatografii gazowej. Stwierdzono, że zawartość kwasu u poszczególnych matek jest bardzo zróżnicowana, niezależnie od wieku. Średnia zawartość kwasu w ekstrakcie jednorocznych matek czerwiących wynosiła 144.73±37,38 µg, dwuletnich 153.1±36.28 µg, a trzyletnich 173±54.76 µg. Różnica w zawartości kwasu nie była statystycznie istotna ($P > 0.05$).

Zmienność w zawartości 9-ODA była różna w różnych grupach wiekowych. Zakres zmienności u matek jednorocznych (od 84 do 256 µg) był znacznie mniejszy niż u matek dwuletnich (od 32 do 400 µg) lub trzyletnich (od 40 do 380 µg). Współczynnik zmienności w tej zawartości wynosił odpowiednio 42,55%, 71,08% i 77,54%.

Wyniki badań zawartości kwasu wskazują, że wśród matek jednorocznych dominowały z zawartością 100 µg 9-ODA, u dwuletnich najwięcej było w przedziale 75 do 225 µg, a u trzyletnich takiej dominacji nie stwierdzono.

Słowa kluczowe: kwas (E)-9-oxo-2-decenowy, *Apis mellifera*, substancja mateczna, feromony matki.