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INFLUENCE OF AGE OF DRONES ON THE RESULTS OF INSTRUMENTAL INSEMINATION OF HONEYBEE QUEENS*

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SUMMARY

Altogether 142 queens were inseminated with 8 mm^3 of semen of drones two to nine weeks old. Inseminated queens were caged in bee colonies. They were killed and investigated 48 hrs later.

As the age of drones increased, mostly a lower number of spermatozoa entered the spermatheca of queens. With the increasing age of drones, the percentage of queens with residue of semen in oviducts increased, from 0-14 % in those inseminated with semen of drones two weeks old, to 43 %-67 % in those inseminated with semen of four week-old drones. Variations occurred in different seasons. Queens which cannot clear their oviducts from the injected semen would die.

INTRODUCTION

Efforts have been made to increase the efficiency of instrumental insemination of honeybee queens. WOYKE (1978) reviewed factors influencing the number of spermatozoa entering the spermatheca of queens. The results of insemination are effected by factors acting as early as in the time of grafting the larvae for queen rearing, as well as by those acting during and after insemination.

Now influence of drones on the results of instrumental insemination is studied. Several papers were published in Polish or Russian and were wrongly interpreted in English or German literature. Therefore the review is made here a little more extensively.

BISHOP (1920) found that the drone is not sexually mature, at the time of emergence of imago, but undergoes a further maturation period of at least nine to twelve

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days. The spermatozoa pass from the testes to seminal vesicles at the age of three days. KURENNOJ (1953) found semen in seminal vesicles of 27 % four day-old drones and in 93 %-100 % of drones nine to thirteen days old. According to MACKENSEN (1955) the number of spermatozoa in the seminal vesicles increased with the age from 0.13 million in drones three to four days old to 9.89 millions in those seven to eight days old. Similarly JAYCOX (1961) and GAROFALO (1972) found that the process of passing of spermatozoa from testes into seminal vesicles is finished at the age of eight to eleven days. The semen remains in the seminal vesicles till the time of mating, or till the death of drones. When the drone is killed, as for instrumental insemination by decapitation, squeezing of thorax or is treated with ether or chloroform then the semen passes from seminal vesicles into the bulb of the endophallus. This phenomenon starts at the age of 6 days, and is found in most of 8 days old drones, but sometimes it does not occur even in 39 days old drones (ZANDER, 1920). The semen is pushed thorough the ejaculatory duct and copulatory apparatus by mucous and epithelium thorn down from the mucous gland (WOYKE, 1958). When the drones are killed for artificial insemination, they evert the endophallus. The process of eversion was described in details by WOYKE (1958) and by WOYKE and RUTTNER (1958). KURENNOJ (1953) found that the percentage of drones everting the endophallus increased from 2 % at the age of 10 days to 25 % at the age of 39 days and to 53 % at the age of 38 days. Older drones everted the endophallus in lower percentage. According to KEPENA (1963) 25 % of seven days old drones everted the endophallus and as many as 100 % of those twenty five days old.

Influence of flight on the eversion process was investigated. According to KURENNOJ (1954) 24 % of drones leaving the hive and 32 % of returning ones everted the endophallus. WOYKE (1955) found that 72 % of departing and 65 % of returning drones everted the endophallus. The 7 % decrease in returning drones was not found to differ statistically, and so almost the same percentage of eversion occurred in departing as in returning drones. But WOYKE made further discrimination between partial and complete eversion of endophallus. He found that among the 72 % of departing drones which everted the endophallus 31 % everted it partly and as many as 41 % completely, and among the 65 % of returning 51 % everted it partly and only 14 % complety. So the endophallus was everted completely three times more frequently in departing drones than in the returning ones. There was found that short flight of drones on the window or wing movement of drones held in hand, by leg or head increased the percentage of drones everting the endophallus over those departing from the hive, but the whole normal flight between 15-16 hr decreased sexual excitement. MINDT (1962) pointed out that before the first flight only 12 % of drones everted the endophallus, after that flight 52 % and before the second flight 38 %. Similar results are visible in WOYKE's (1955) diagrams N° 5, 6 and 7. At 14.15 hr more returning than departing drones everted the endophallus while after some flights at 15.30 hr and 16.00 hr more departing everted it. But complete eversion was always found more frequently among the departing than among the returning ones.

The higher sexual excitement of drones leaving the hive may probably be explained by considerable greater quantities of fructose, glucose and trehalose in reproduc-

tive organs of leaving drones than in organs of returning ones (BLUM, GLAWSKA and TABER, 1962). BOBRZECKI (1968) found that 10 % more free flying drones everted the endophallus and ejaculated the semen than drones kept all the time on a comb in queen excluder isolator. But the difference was not found to differ statistically.

The drones reared at a different period of the year must probably differ. NOVAK, BLUM, TABER and LIUZZO (1960) found that the relation between the amount of fluid to the solid part of semen varied from 1 : 1 to 1 : 2 depending upon the period of the year. After FRESNAYE (1966) inseminated queens instrumentally he lost 20 % of them in May and June while none in July, and CHWAKOWSKI (1969) lost 5 %-12 % in May, June and July and as many as 30 % to 55 % in the second half of August.

According to WOYKE (1960) a sexually mature drone produced about 11 millions of spermatozoa with a concentration of 7.5 millions of spermatozoa per 1 mm^3. Mostly 1-1.25 mm^3 of semen is collected into the tip of a syringe for artificial insemination. After the queen is inseminated with 8 mm^3 of semen, only 9.6 % of spermatozoa enter the queen's spermatheca, but WOYKE (1963) showed, that the subsequent drones contributed almost in equal proportion the spermatozoa entering the spermatheca.

Influence of the age of drones on the results of instrumental insemination was not investigated till now.

MATERIALS AND METHODS

Altogether 142 caucasian queens were inseminated instrumentally with 8 mm^3 of semen of drones two to nine weeks old. The experiment was conducted during three different years.

Several combs with drone brood were put into the queen excluder isolators a few days before the emerging of the adult drones. The isolators were distributed into brood nests of a few queenless colonies. The colonies were fed all the time with sugar syrup, and some worker brood was added from time to time, to reinforce the colony. Drones of desired age were used for instrumental insemination. The first season they were 2-4 weeks old, the second season 2-5 weeks old and the third one 2-9 weeks old. All the drones used in one season originated from one queen.

The queens emerged in screened nursery cages without workers inside, in queenless colonies, where they were left until insemination. Several series of queens were reared, so that always queens 7-10 days old were inseminated with semen of drones of each age. All virgins inseminated during one season originated from one mother-queen. To make the results more distinct, the queens were returned to the same screened nursery cages and placed into the same colony. Screened cages without accompanying workers, placed in colonies, are known to create severe conditions for queens after insemination (WOYKE, 1978), but this method is commonly used by many persons. The queens were killed 48 hrs after insemination. They were dissected, and their oviducts were examined for the residue of semen in one or two oviducts. Next the spermatheca was taken out and the number of spermatozoa in it was counted by the method described by the author several times (WOYKE, 1978).

Fischer's analysis of variance (test F) was applied to find out whether the differences between means are statistically significant.

TABL. 1. — *Results of instrumental insemination of queens with 8 mm³ of semen of drones of different age*

Age of drones in weeks	No of inseminated queens	No of spermatozoa in spermatheca, millions		% of queens with residue of semen in oviducts		
		Range	Mean	in one	in two	together
		First season				
2	11	1.738-3.932	3.004	9.1	0.0	9.1
3	10	1.540-3.487	2.719	20.0	10.0	30.0
4	6	1.578-3.311	2.307	50.0	16.7	66.7
		Second season				
2	21	2.305-4.730	3.753	14.3	0.0	14.3
3	21	2.338-4.279	3.699	9.5	9.5	19.0
4	14	2.019-4.472	3.667	28.6	14.3	42.9
5	8	2.932-4.466	3.513	12.5	37.5	50.0
		Third season				
2	18	2.965-5.660	4.097	0.0	0.0	0.0
3	12	2.735-4.516	3.367	8.3	0.0	8.3
5	11	2.607-6.171	3.939	9.1	9.1	18.2
9	10	1.771-5.946	3.175	10.0	30.0	40.0

RESULTS

Semen of drones of different ages

Viscosity and colour of semen change with ageing of drones.

Semen of drones 10-14 days old is liquid, of yellow-cream colour and it can be easy soaked into the tip of a syringe for instrumental insemination. Semen of drones older than 4 weeks is thick, of cream-brown colour and is more difficult to soak into the tip of the syringe.

Number of spermatozoa in spermatheca

Table 1 shows, that rather high variation occurred in the number of spermatozoa entering the spermatheca of queens inseminated with semen of drones of the same age. The difference between the highest and the lowest number of spermatozoa in spermatheca from drones of the same age was mostly about 2 millions.

The mean number of spermatozoa in spermatheca of queens inseminated with semen of drones of different ages, decreased in all three seasons (with one exception), as the age of drones increased. The first season there is a negative difference of 0.697 million between the number of spermatozoa entering the spermatheca of a queen inseminated with semen of drones aged 4 weeks and that of a queen inseminated with semen of drones aged 2 weeks. The third season the difference was 0.922 million between a queen inseminated with semen of drones aged 9 weeks and a queen inseminated with semen of drones aged 2 weeks.

But the F test of analysis of variance did not show at the $P < 0.05$ level, significant influence of the age of drones on the number of spermatozoa in spermatheca in any of the seasons. Individual Student t-test, would show significant difference at 94.5 % probability level, between the number of spermatozoa in spermatheca of queens inseminated the third season with semen of drones 2 weeks old (4.097 millions), and of those 9 weeks old (3.175 millions). So the difference is almost significant at the $P < 0.05$ level. It can be thus stated that in the three seasons the number of spermatozoa entering the spermatheca of queens inseminated with semen of older drones tends to decrease.

Residue of semen in oviducts

Table 1 shows, that after queens were inseminated with semen of drones of increasing age, residue of semen in oviducts was found in a different percentage in queens kept after insemination in screened cages without workers in bee colonies. After queens were inseminated with semen of drones two weeks old, the residue of semen was null, or present in one oviduct only, in 9.1 % or 14.3 % of queens. After semen

of older drones was used, residue was found in both oviducts, and the percentage of queens with residue in both oviducts increased as the age of drones yielding the semen increased. Altogether the percentage of queens with residue of semen in one or both oviducts increased from 0 % or about 10 % after they were inseminated with semen of drones two weeks old to 50 % or even almost 70 % after they were inseminated with semen of drones nine or four weeks old. Increasing percentage of queens with residue of semen in oviducts, is very distinctly visible in all three seasons, after the queens were inseminated with semen of older drones. But the particular results differ in the three seasons. The first season, residue was found in as many as 66.7 % of queens after they were mated instrumentally to drones four weeks old, while the last season residue was found only in 18.2 % of queens mated to drones five weeks old and in 40 % of those mated to drones nine weeks old.

Queens with residue of semen in oviducts had a low number of spermatozoa in spermatheca.

DISCUSSION AND CONCLUSIONS

It is obvious that the number of spermatozoa entering the spermatheca of queens inseminated with semen of older drones tends to decrease. There is no doubt that semen of older drones is one of the causes resulting in the presence of a residue of semen in oviducts of instrumentally inseminated queens. Queens which did not clear their oviducts would die. But the results presented in this paper show that some other conditions also influence this phenomenon distinctly.

VESELY (1970) and WOYKE (1978) showed, that the conditions are significantly improved, worker bees have free access to queens immediatelly after instrumental insemination. Nevertheless results presented in this paper indicate, that semen of drones older than 3 weeks should not be used for instrumental insemination of queens kept in screened cages without worker bees. Further investigation will show the age of drones which may be used for instrumental insemination of queens in improved conditions.

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ZUSAMMENFASSUNG

EINFLUSS DES ALTERS DER DROHNEN AUF DIE ERGEBNISSE DER INSTRUMENTELLEN BESAMUNG VON BIENEN KÖNIGINNEN.

Die Faktoren, welche die Zahl der in die Spermatheka der Königin einwandernden Spermatozoen beeinflussen, wurden von WOYKE (1978) in einer Übersicht besprochen. Dabei handelt es sich z.T. um Umweltfaktoren, wie um solche der Königin selbst. In der vorliegenden Arbeit wird der Einfluss der

Drohnen auf die Ergebnisse der instrumentellen Besamung untersucht und eine ausführliche Darstellung der Literatur vorgelegt.

Material und methoden

Insgesamt wurden 142 kaukasische Königinnen instrumentell mit 8 μl Sperma besamt, das von 2 bis 9 Wochen alten Drohnen stammte. Die Versuche wurden in drei verschiedenen Jahren durchgeführt.

Waben mit Drohnen-Brut wurden wenige Tage vor dem Ausschlüpfen der Drohnen in Wabentaschen mit Absperrgitter isoliert. Diese Wabentaschen wurden auf mehrere weisellose Völker verteilt. Im gewünschten Alter wurden die Drohnen aus diesen Käfigen abgesammelt. Die Königinnen waren bei der Besamung 7-10 Tage alt, sie wurden nach dem Eingriff gekäfigt und in weisellosen Völkern untergebracht. 48 Stunden später wurden die Königinnen getötet und präpariert. Die Ovidukte wurden auf Restmengen von verbliebenem Sperma untersucht und dann wurde die Spermienzahl in der Spermatheka bestimmt.

Ergebnisse

Im allgemeinen gelangte mit wachsendem Alter der Drohnen eine geringere Spermienzahl in die Spermatheka der Königin. Der deutlichste Unterschied wurde in der dritten Saison festgestellt : Bei Verwendung von zwei Wochen alten Drohnen erreichten 4,097 Millionen Spermatozoen die Spermatheka der Königin, Sperma von neun Wochen alten Drohnen hingegen ergab nur 3,175 Millionen Spermatozoen in der Samenblase. Mit steigendem Drohnen-Alter nahm auch der Prozentsatz von Königinnen mit Samenresten in den Ovidukten von 0-14 % bei Verwendung von zwei Wochen alten Drohnen auf 43-67 % bei vier Wochen alten Drohnen zu. In verschiedenen Jahren gab es Unterschiede der Ergebnisse.

Königinnen, die nicht in der Lage sind, ihre Ovidukte völlig von dem injizierten Samen zu reinigen, werden später eingehen.

RÉSUMÉ

INFLUENCE DE L'AGE DES MALES SUR LES RÉSULTATS
DE L'INSÉMINATION ARTIFICIELLE
DES REINES D'ABEILLE

WOYKE (1978) a fait une revue sur les facteurs agissant sur le nombre de spermatozoïdes qui pénètrent dans la spermathèque des reines. Il s'agit de facteurs qui dépendent aussi bien des conditions du milieu que des reines elles-mêmes. On étudie ici l'influence des mâles sur les résultats de l'insémination artificielle et une revue détaillée de la littérature est présentée.

Matériels et méthodes

Cent quarante deux reines caucasiennes ont été au total inséminées artificiellement avec 8 mm^3 de sperme provenant de mâles âgés de 2 à 9 semaines. L'expérience a été menée sur 3 ans.

Des rayons comportant du couvain de mâles ont été placés quelques jours avant l'éclosion des mâles dans des cages d'isolement munies de grille à reine. Ces cages d'isolement furent réparties dans plusieurs colonies orphelines. Les mâles d'âge désiré étaient prélevés dans ces cages. Les reines étaient inséminées à

l'âge de 7 à 10 jours, puis mises dans des cages grillagées placées dans des colonies orphelines. Elles étaient tuées et disséquées 48 h plus tard. On a recherché l'existence de résidus de sperme dans les oviductes, puis dénombré les spermatozoïdes présents dans la spermathèque.

Résultats

En général le nombre de spermatozoïdes pénétrant dans la spermathèque de la reine décroît lorsque l'âge des mâles croît. La différence la plus nette a été trouvée pendant la 3e saison : 4.097 millions de spermatozoïdes ont pénétré dans la spermathèque des reines inséminées avec du sperme de mâles âgés de 2 semaines, et seulement 3.175 dans celle des reines inséminées avec du sperme de mâles âgés de 9 semaines.

Le pourcentage des reines possédant un résidu de sperme dans leurs oviductes augmente avec l'âge des mâles : il est de 0-14 % pour les reines inséminées avec du sperme de mâles de 2 semaines et atteint 43-67 % lorsque les mâles ont 4 semaines. Des variations ont été observées au cours des diverses années. Les reines qui ne peuvent pas nettoyer leurs oviductes du sperme injecté mourront.

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