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A COMPARISON OF INSTRUMENTALLY INSEMINATED AND NATURALLY MATED QUEENS[1]

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Summary

Sister queen honeybees (*Apis mellifera*), 59 instrumentally inseminated (IIS) and 59 naturally mated (NM), were produced in Baton Rouge, Louisiana, and put into field colonies at 3 locations in Alberta, Canada and at 1 location in Louisiana, USA. Survival of the IIS queens was lower than that of the NM queens; 37 IIS queens and 50 NM queens survived from August 1981 to May 1982, and 18 IIS and 34 NM queens survived until August 1982 ($P<0{\cdot}01$). At all 4 locations, the colonies with NM queens had 21–73% more capped brood in May (February in Louisiana) than did colonies with IIS queens ($P<0{\cdot}01$). Probably because of this difference in brood production, the colonies with NM queens produced more honey. Over a 12-month period, the mean loss of spermatozoa from IIS queens was 0·9 million (28% of the original number in the spermatheca), and the mean loss for NM queens was 1·5 million (27%).

Introduction

When evaluating instrumentally inseminated queens there are 2 major questions to be answered: (1) do they survive as long as naturally mated queens and (2) do colonies with instrumentally inseminated queens produce as much honey and brood as those with naturally mated queens. Roberts (1946) compared honey production of colonies with IIS and NM queens and found no difference. The queens he tested were produced in Baton Rouge in the spring of 1945, shipped to Wisconsin, united with packages in May and evaluated in the autumn of the same year. Mackensen and Roberts (1948) stated that the IIS queens tested in 1945 had a lower survival rate than the NM queens but presented no data. Our plan was to compare IIS and NM queens over a 12-month period at 4 locations. In addition to queen loss and honey production, we compared brood production and the depletion of spermatozoa from the spermathecae.

Methods and Materials

Queens tested in this experiment were super-sisters (their mother was a non-inbred, wild-type queen mated to a single drone from an inbred (*Ka*) line). Larvae were grafted into queen cups in Baton Rouge on 29 May, 5 June, 19 June, 26 June and 2 July, 1981.

The NM queens were put into small colonies with about 2000 workers and allowed to mate at any time. Before and after insemination, the IIS queens were stored in groups of 20–40 per colony in colonies that contained no laying queen, about 1 kg bees, and combs of brood and of honey. Each queen was inseminated twice, 48 h apart, when 2–3 weeks old. The semen came from drones collected at colony entrances in areas where NM queens were flying. About 22 million spermatozoa (2·7 µl of semen) were given to each queen at each insemination, using about a 2-min exposure to concentrated CO_2. When IIS queens were 3–4 weeks old, each was put into a small nucleus to lay eggs. Both NM and IIS queens were allowed to lay eggs for 3–7 days, and were then caged and stored in a queenless colony until needed.

About one-half of the left wing was removed from each queen before 6 August, 1981 when the queens were sent to Canada in an air parcel. They reached Beaverlodge, Alberta on 7 August, and were put into colonies on 8 August in Beaverlodge, on 15 August in Falher, and on 17 August in Scandia. Queens at St. Gabriel, Louisiana were put into colonies on 20 and 24 August. Queens were introduced to large colonies by removing the old queen about 5 days before the new queen was introduced under a 'push-in' cage (*c.* 12 × 8 cm) with a candy release.

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Queen survival

Queens were checked 2 weeks, 1 month, 9 months and 12 months after introduction. Queen cells were removed on 18 March and 15 April in St. Gabriel, but were never removed at the Aberta locations. The reason for this difference was that swarming does not occur in Alberta if ample space is available in the colony. Queen losses between all possible intervals were analysed by the χ^2 test. Differences at the 5% level of probability were considered to be significant.

Brood and honey production

Total capped brood was measured at the Alberta locations in May and at St. Gabriel, Louisiana in February. Colonies with supersedure queens were not measured. Measurements were made with a sheet of acrylic that was marked in square centimetres. Both worker and drone cells were included in each colony total, which was rounded to the nearest 10 cm^2.

The area of brood produced by IIS queens was compared with that produced by NM queens. The statistical design was a randomized-block analysis of variance; each of the 4 locations was a block containing the IIS and NM treatments.

Honey production was measured in Alberta only. Seasonal weight gain of the colony was recorded at Beaverlodge, whereas only the weight of the honey produced by a colony (weight of honey extracted) was measured at Scandia and Falher. For seasonal honey production or colony weight gain we used the same statistical analysis as for brood production.

Sperm depletion

The number of spermatozoa in the spermatheca of each queen was estimated by counting spermatozoa with a haemacytometer. Initial counts were made on 10 NM and eleven IIS queens after the newly-mated queens had laid eggs for 3–7 days in a nucleus colony. Final counts were made on all queens that survived 12 months in a colony and were shipped back to Baton Rouge. One major difference between our IIS and NM queens was the initial number of spermatozoa in the spermathecae. Our experience indicates that had we inseminated the queens 3 or 4 times they would have retained at least as many spermatozoa in their spermathecae as NM queens, but we chose to test IIS queens that had about as many spermatozoa as they receive with the most commonly used dosage, one insemination of 8 µl. A queen inseminated with 8 µl of semen retains about 3·5 million sperm in her spermatheca (Woyke & Ruttner, 1976). We used 2 inseminations of 2·7 µl each instead of one of 8 µl because it gives a similar mean and a smaller variance (Mackensen, 1964).

Brood viability and nosema disease

Colonies headed by IIS and NM queens were compared at the Alberta locations for *Nosema* infection and brood viability. We also compared colonies that superseded with colonies that did not supersede to see if the frequency of nosema disease or brood viability differed before supersedure. Viability of brood, measured in June, was calculated as the number of pupae that developed from 100 eggs in a 10 × 10 cell area. Brood was chosen near the centre of the brood-nest. Incidence of nosema disease was estimated by sampling 25 bees from each colony entrance in autumn and spring. Numbers of spores in the midgut were estimated with a haemacytometer. Analysis of variance was used for brood viability and numbers of *Nosema* spores. The Kolmogorov-Smirnov test compared the frequency of nosema infection.

Results and Discussion

Queen survival

Survival during the 12-month period was significantly higher ($P<0·01$) for the NM than for the IIS queens (Table 1). Survival of IIS queens was 18 out of 59 (31%) and that of NM queens 34 out of 59 (58%). Queen survival during the first 9 months accounted for the major difference between the 2 groups; of the IIS queens, 37 out of 59 (63%) survived the first 9 months (August–May), whereas 50 out of 59 (85%) of the NM queens survived that period. The difference was statistically significant ($P<0·01$). The interval of greatest queen loss was the last 3 months (May–August) when 19 IIS and 16 NM queens were lost. However, loss in May–August was not significantly different between the two groups.

TABLE 1. Survival of naturally mated (NM) and instrumentally inseminated (IIS) queen honeybees in large colonies in Alberta, Canada (Alta) and Louisiana, USA (LA).

Location	*Original no. in Aug. 1981*	*No. surviving in Sept. 1981*	*May 1982*	*Aug. 1982*	*% survival at 12 months*
Beaverlodge, Alta					
IIS	14	13	10	3	21%
NM	15	15	13	8	53%
Scandia, Alta					
IIS	14	12	10	6	43%
NM	15	14	14	8	53%
Falher, Alta					
IIS	16	12	7	3	19%
NM	16	15	13	9	56%
St. Gabriel, LA					
IIS	15	12	10	6	40%
NM	13	12	10	9	69%
Total survival					
IIS	59	49	37	18	31%
NM	59	56	50	34	58%

TABLE 2. Area (cm^2) of capped brood present in honeybee colonies in May (Alberta) or February (Louisiana). Means with standard deviations and numbers of colonies (parentheses) are listed. Abbreviations as for Table 1.

Location	*IIS*	*NM*[1]
Beaverlodge, Alta	1250 ± 810 (10)	2110 ± 870 (13)
Scandia, Alta	1600 ± 1030 (10)	2770 ± 860 (14)
Falher, Alta	2660 ± 1430 (7)	4020 ± 1070 (13)
St. Gabriel, LA	1850 ± 510 (12)	2230 ± 490 (11)

[1]NM>II. $P<0{\cdot}01$.

TABLE 3. Numbers of spermatozoa in spermathecae of queen honeybees before and after laying in field colonies for 12 months.

Means and standard deviations are for millions of spermatozoa, figures in parentheses are numbers of queens. Abbreviations as for Table 1.

Period and location	*IIS*	*NM*
Before laying	3·2 ± 1·1 (11)	5·5 ± 1·0 (10)
After laying[1]		
Beaverlodge, Alta	2·0 ± 0·01 (2)	4·0 ± 1·4 (6)
Scandia, Alta	2·2 ± 0·7 (6)	4·0 ± 1·0 (8)
St. Gabriel, LA	2·4 ± 0·7 (6)	4·1 ± 0·7 (7)
Overall mean	2·3 ± 0·6 (14)	4·0 ± 1·0 (21)
Net Loss/year	0·9 (28%)	1·5 (27%)

[1]Queens from Falher, Alberta died in transit to Baton Rouge.

Brood and honey production

Brood production was lower in colonies with IIS queens. Surviving NM queens had significantly more capped brood ($P<0{\cdot}01$) in May (measurements were made in February in Louisiana) than surviving IIS queens (Table 2).

Honey production in Alberta is highly correlated with area of capped brood in May, June or early July (Szabo, 1982). Therefore, because colonies with NM queens had more capped brood in May, they would be expected to produce more honey. Weight gain of colonies from June to August in Beaverlodge was 32 ± 13 kg ($\bar{x}\pm$SD) with $n=3$ for IIS and 82 ± 16 kg ($n=8$) for NM queens. Respective values for honey production were 70 ± 34 kg ($n=6$) and 91 ± 16 kg ($n=8$) at Scandia, and 25 ± 24 kg ($n=3$) and 52 ± 24 kg ($n=9$) at Falher ($P<0{\cdot}01$).

Therefore, Alberta colonies with IIS queens did not produce as much honey as colonies with NM queens because they did not build their population through the winter and spring as much as the colonies with NM queens. Colonies that are equalized in late spring may not differ so much. For example, when Roberts (1946) started colonies from packages in May, those with IIS queens produced as much honey as the ones with NM queens.

Sperm depletion

Instrumentally inseminated queens started with $3{\cdot}2\pm1{\cdot}1$ ($\bar{x}\pm$SD) million spermatozoa in their spermathecae, whereas NM queens started with $5{\cdot}5\pm1{\cdot}0$ million (Table 3). Mean numbers remaining after 12 months in colonies were $2{\cdot}3\pm0{\cdot}6$ million for IIS and $4{\cdot}0\pm1{\cdot}0$ million for NM queens, representing losses of 0·9 million (28%) for the IIS queens and 1·5 million (27%) for the NM queens.

Loss of spermatozoa from the spermatheca was less than predicted on the basis of the data of Harbo (1979). However, a later test conducted by Harbo (unpublished) suggested that sperm depletion from IIS queens may be affected by the time of year when queens are reared or mated; the test was done in Baton Rouge in 1981 about the same time that the queens for this experiment were reared. Harbo found that queens reared and mated in May depleted spermatozoa at a higher rate than sister queens reared and mated in August. The May queens had 1·1 million (24%) fewer spermatozoa in their spermathecae after laying 30 000 eggs in June and 1·0 million (22%) fewer after laying their first 30 000 eggs in September (the latter queens were kept caged until September). The August queens had only 0·5 million (10%) fewer spermatozoa after laying 30 000 eggs in September. The queens sent to Alberta and used in St. Gabriel were reared in June and July and mated in July. Thus they were more similar to the August group in timing. Perhaps their lower depletion rate was caused by a seasonal factor.

Brood viability and nosema

The brood viability of IIS queens was not different from that of NM queens. Viability was 90·1% for IIS and 90·0% for NM queens in Beaverlodge, and 95·9% for IIS and 93·1% for NM queens in Scandia. Moreover, the brood viability of queens destined to be superseded within 2 months was not different from those not superseded (91·6% for superseded queens and 92·6% for queens not superseded).

Nosema disease was found about equally in colonies having IIS and NM queens. In the fall, some nosema disease was found in 9 of 36 IIS and 8 of 42 NM colonies; spring counts were considerably higher, 21 of 36 and 28 of 42 respectively. At Falher, where all colonies had nosema in the spring, colonies that later superseded averaged 4·1 million spores per bee; those that did not supersede averaged 7·5 million per bee (difference non-significant).

Causes of poor performance by IIS queens

We have documented that IIS queens did not perform as well as NM queens, but the cause of the difference has not been explored. Based on our data, nosema disease and brood viability can be eliminated as likely causes. Possible causes fit into 2 general categories: (1) IIS queens may receive something detrimental during the insemination process such as damaging levels of CO_2, physical damage during handling or insemination, or disease from unsanitary conditions, or (2) IIS queens may lack an essential ingredient that queens receive during normal mating such as flight activity, contact with drones, enough semen in their oviducts, or an adequate number of spermatozoa in their spermathecae.

In the second category, one obvious difference between IIS and NM queens was the initial number of spermatozoa in the spermatheca (Table 3). Harbo (1976) showed that queens with no spermatozoa in their spermatheca (they could thus lay only unfertilized eggs) laid fewer eggs per day than queens with live spermatozoa. However, there is no evidence that brood production increases with the number of spermatozoa in the spermatheca when a queen has enough spermatozoa to produce fertilized eggs. We found no correlation between area of capped brood and numbers of spermatozoa in the spermatheca. Correlations were $r = -0{\cdot}14$ ($n = 8$) and $r = +0{\cdot}06$ ($n = 6$) for NM and IIS queens at Scandia, $r = -0{\cdot}40$ ($n = 7$) and $r = -0{\cdot}24$ ($n = 6$) for NM and IIS queens at St. Gabriel, and $r = -0{\cdot}08$ ($n = 6$) for NM queens at Beaverlodge. Thus brood-rearing does not seem to be affected by numbers of spermatozoa in the spermatheca as long as the queens are producing worker brood. It is possible, however, that supersedure is more likely in queens having fewer spermatozoa in their spermathecae.

Of the possible causes of poor performance of IIS queens, CO_2 narcosis seems most likely. Periods of CO_2 narcosis have been found to reduce the length of life of worker honeybees (Austin, 1955), alter the behaviour of worker bees (Skowronek & Jaycox, 1974), and retard growth in corn earworms (Edwards, 1968). Pain, Barbier and Roger (1967) showed that virgin queens given two 10-min treatments of CO_2 narcosis produced less queen pheromone, 9-oxodec-*trans*-2-enoic acid, than queens not exposed to CO_2. When mandibular glands, the source of this pheromone, were removed from queens, brood production dropped only slightly and the supersedure rate increased (Zmarlicki & Morse, 1964), a result very similar to what we found for IIS queens.

If CO_2 narcosis was the cause of lower brood production in IIS queens, an alternative means of narcosis is needed. Ebadi and Gary (1980) have recommended 75% rather than 100% CO_2 for queen narcosis and have shown that cold narcosis can be used during insemination. Thus they began what may be an important process, that of finding a substitute for CO_2 narcosis.

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