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# EFFECT OF DIET ON THE RATE OF BROOD REARING BY NATURALLY AND INSTRUMENTALLY INSEMINATED QUEENS

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## SUMMARY

Small colonies of honey bees were established with ca. 4,000 newly emerged Italian bees and placed in 2 × 2 × 2 m flight cages. The colonies (total of 24) were divided into two groups; twelve were headed by instrumentally inseminated queens purchased from a commercial queen breeder and twelve headed by free flight mated queens reared at Beltsville, Maryland. Each group of 12 colonies was further divided into three treatments of four colonies each. Each group was offered diets containing either Yeaco (43 % protein), Wheast (58 % protein), or soybean protein (90 % protein) as the sole protein source. The amount of sealed brood reared by each colony was measured for 12 weeks. Colonies of bees fed Wheast diets and headed by either instrumentally inseminated queens or free flight mated queens reared significantly more brood than similar units of bees fed either Yeaco or soybean diets. The second greatest amount of brood was reared by bees fed Yeaco diets followed by soybean fed bees. The results of this experiment demonstrated that, overall, instrumentally inseminated queens did as well as free mated queens under controlled conditions. This study also demonstrated that bees fed poor diets regardless of the genetic background of the queens will do poorly.

## LITERATURE REVIEW

The instrumental insemination of queen bees is being used extensively by commercial queen breeders, primarily because of the improvement of techniques and the advantages over natural matings. The technique has been much improved since WATSON (1927) first demonstrated that the idea of instrumentally inseminated queens was practical. Improvements in technology (LAIDLAW, 1944) and instrumentation (WATSON, 1929, NOLAN, 1932, MACKENSEN and ROBERTS, 1948) have made the technique more refined. Also, MACKENSEN (1947) demonstrated that exposure to $CO_2$ stimulated artificially inseminated queens to lay eggs much sooner than unexposed queens. MACKENSEN (1964) determined the volume of sperm in the spermatheca required for successful mating. WOYKE and JASINSKI (1973) studied external conditions that influenced the number of spermatozoa entering the spermatheca. Two

factors which affected the amount of spermatozoa most were : 1) age of grafted larvae, and 2) temperature at which the queens were maintained after insemination.

The variability in performance of free flight mated queens vs. instrumentally mated queens has frequently been a topic of discussion. Researchers have evaluated the quality of instrumentally mated queens by measuring the number of ovarioles, number of sperms in spermatheca (TABER *et al.*, 1977 and ROBERTS, 1946), and honey yields (ROBERTS, 1946). Perhaps an additional measure of queen performance should be a measure of the number of eggs laid and the progeny produced. However, variation in the rate of egg laying by queens fed the same diet if often great. It is presumable that this variation may be the result of genetic différences among queens. To reduce variability arising from genetic differences, we decided to compare the rates of brood rearing by free flight and instrumentally mated queens. Both groups of queens were fed three different pollen substituted to determine the effect of diet on brood production.

## MATERIALS AND METHODS

*Diets :* The three protein sources for the pollen substitutes were Yeaco (43 % protein). Wheast (58 % protein) and soybean protein (90 % protein). Each diet was formulated into patties (50 g each) that contained 23 % protein by varying the amount of sucrose and water. Each type of patty (Wheast, Yeaco or soybean) was made available to four colonies headed by free flight mated queens and to four colonies headed by instrumentally inseminated queens by placing the patty in a plastic petri dish lid (15 × 100 mm) inverted over the top bars of a hive.

*Bees :* The 24 test colonies were each established with 400 g of newly emerged Italian bees and a fertile queen. The instrumentally inseminated queens were purchased from a commercial queen breeder. The free flight mated queen had been reared at Beltsville, Maryland in nuclei containing ca. 4,000 newly emerged bees (4-5 days old). Before the queens were removed to the test cages, they were allowed to mate freely and egg laying and brood pattern were observed.

Bees and queens were maintained in small hives (23 × 19 × 27 cm) normally used for queen mating. Each hive contained five drawn shallow comb (3 × 16 × 24 cm) free of any pollen or honey. Each colony was placed in a 2 × 2 × 2 m screen flight cage. Diet and sugar syrup (50 % w/v) were offered *ad libitum,* and the unused portion of the diet was weighed.

*Test :* During the first week of the study, egg viability was measured by caging each queen overnight (4 p.m. to 8 a.m.) on an empty comb enclosed in a queen excluder. The following morning the queens were released, and the number of eggs counted. Then the excluder was replaced over the frame to exclude the queen but not workers, and after three days the numbers of eggs that hatched were counted. The egg counts were determined during the first week of the study.

After the viability tests, the queens were allowed to lay eggs normally for the remaining 11 weeks of the test. As soon as the first sealed brood appeared, the number of sealed cells was estimated weekly by using a wire grid with 6·45-cm$^2$ divisions.

Differences in rate of brood rearing and diet consumption were analyzed by analysis of variance and Duncan's multiple range test. The variation between free flight mated queens and instrumentally inseminated queens was measured by determining the coefficient of variation (CV) of test means.

## RESULTS AND DISCUSSION

Colonies of bees fed Wheast diets and headed by either instrumentally inseminated queens (II) or free flight mated queens (FM) reared significantly more brood than similar units of bees fed either Yeaco or soybean diets (Table 1). Within each diet treatment the four II queens performed overall as well or better than the four FM queens though in some cases the initial brood pattern of the II queens was spotty. The performance of the queens was greatly influenced by the nutritional value of the diets. As in a previous study (HERBERT *et al.*, 1977), bees fed the Wheast diets reared more bees to the sealed stage then bees fed Yeaco or soybean.

Eggs were present in colonies fed Wheast (II and FM queens) two days after the queens were released. After two weeks, three of the units headed by II queens had scattered brood, but the fourth contained only eggs and larvae. However, after this initial lag in one colony the brood rearing in the II colonies fed Wheast continued uninterrupted, and these units contained the greatest amount of brood after 12 weeks. Units headed by FM queens continued brood rearing for 12 weeks although three units contained only eggs when the study was terminated.

The second greatest amount of brood was reared by bees fed Yeaco diets. Units headed by either II or FM queens, were slow in initiating brood rearing, and most units continued rearing for only eight weeks.

The poorest diet tested was soybean. Queens (II and FM) continued to lay eggs during the 12 weeks of the test, and sealed brood was observed for six weeks, but no brood reached the sealed stage after the sixth week.

All three diets were consumed equally well during the first three weeks of the experiment. As the test progressed, differences in consumption became apparent. Wheast diets were consumed in the greatest amount followed by soybean and finally Yeaco (Table 1).

Units of bees fed Wheast diets headed by II queens consumed more diet than any other. Units headed by FM queens consumed the next most. Differences in consumption between units fed soybean (II and FM) and Yeaco (II and FM) were not significant. During the first week of the experiment, the soybean diets were consumed best, but consumption fell off after the second week, and by the end of the experiment this was the poorest consumed diet. HAYDAK (1949) noted that soybean was not an adequate pollen substitute. This deficiency was attributed to low levels of niacin and riboflavin. HERBERT *et al.* (1977) found that bees consumed less Yeaco than pollen or Wheast diet even though Yeaco probably contains all the nutrients required for brood rearing.

As noted, an effort was made during the first week of the experiment to measure egg viability by using a queen excluder to cage the queens overnight on an empty frame. The following morning the queens were released, and the eggs counted. The

TABLE 1. — *Brood rearing* ($cm^2$) *and diet consumption* (g) *by colonies headed by free mated (FM) or instrumentally inseminated (II) queens*

| Diet | Queen | Brood rearing (1) | CV | Consumption (2) | CV |
|---|---|---|---|---|---|
| Wheast | II | 1,517.4 ± 81.6 a | .10 | 733.4 ± 29.0 a | .07 |
| Wheast | FM | 1,306.2 ± 53.7 a | .08 | 632.7 ± 15.2 b | .04 |
| Yeaco | II | 338.7 ± 67.2 b | .39 | 233.8 ± 27.6 d | .23 |
| Yeaco | FM | 372.5 ± 38.5 b | .20 | 236.3 ± 16.8 d | .14 |
| Soybean | II | 167.7 ± 32.4 bc | .38 | 353.8 ± 15.8 cd | .08 |
| Soybean | FM | 38.7 ± 23.7 c | 1.22 | 402.6 ± 10.5 c | .05 |

(1) Mean brood rearing ($cm^2$) ± S.E. by each of four nuclei over 12 week test period. Means in the same row followed by the same letter do not differ significantly at the 5 % level of probability as calculated by Duncan's multiple range test.

(2) Mean diet consumption (g) ± S.E. by each of four nuclei over a 12-week test period.

excluders were replaced over the frames, and each was replaced into its unit. In every case the eggs were always removed by the worker bees. It was therefore not possible to measure egg viability. Again the queens were recaged, and again the eggs were counted. This time all frames of eggs were labeled (type of queen and diet) and placed in a free flying nurse colony. This colony had been prepared by placing the queen below an excluder and placing several frames of young brood in the top super above the queen excluder. The test frames were then placed in the upper super. Nevertheless, the eggs were again removed, and it was not possible to measure egg viability. The only measurement that could be obtained was the amount of sealed brood reared.

The results of this experiment demonstrated that, overall, II queens did as well as FM queens under controlled conditions (CV = 0.94 and 0.98, respectively). The amount of variation (CV) in brood rearing ranged from 0.08 (Wheast FM queens) to 1.22 (soybean FM queens). The variation in the soybean fed bees was exceptionally large because one of the units was not able to rear any brood to the sealed stage though young larvae were always present. Another unit of bees reared only minimal levels to the sealed stage. This variation is probably due to the diet and not the queens since the brood pattern was checked before these queens were placed in the experimental units.

The bioassy used here to study honey bee nutrition is designed so the egg laying performance of caged queens is essential to the outcome. For this reason, one should probably use FM queens reared locally. This would allow us to observe the rate of egg laying and brood pattern of each queen before she is used in our test. Instrumentally inseminated queens are assumed to be adequately inseminated

under controlled conditions, but they may not be tested before distribution. Therefore we can eliminate this variable by using tested queens reared in the Beltsville laboratory.

This study also demonstrated that bees fed poor diets regardless of the genetic background of the queens will do poorly. However, even fed poor diets, queens were able to continue egg laying over a 12-week period although we have no measure in the differences in egg viability. ROBERTS (1946) noted that a number of environmental conditions affect the performance of queens. The number of eggs laid by a queen in a given period was not only dependent upon her inherent capacity to lay eggs, but also upon the food which she receives from the supporting worker population.

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## RÉSUMÉ

INFLUENCE DU RÉGIME SUR LE TAUX D'ÉLEVAGE
DU COUVAIN PAR DES REINES FÉCONDÉES NATURELLEMENT
OU PAR INSÉMINATION ARTIFICIELLE

On a placé dans des cages de vol de 2 × 2 × 2 m de petites colonies d'abeilles constituées d'environ 4 000 abeilles italiennes nouvellement écloses. Les colonies, au nombre de 24, ont été divisées en 2 groupes; 12 d'entre elles étaient dirigées par des reines inséminées artificiellement, achetées à un producteur de reines, et les 12 autres par des reines élevées à Beltsville (Maryland) et ayant été fécondées naturellement. Chaque groupe de 12 colonies a été ensuite divisé en 3 sous-groupes de 4 colonies, chacun d'eux recevant un régime qui contenait comme seule source protéinique : soit du Yeaco (43 % de protéines), soit du Wheast (58 % de protéines), soit des protéines de soja (90 % de protéines). Le couvain operculé élevé par chaque colonie a été mesuré durant 12 semaines à raison d'une mesure par semaine. Les colonies d'abeilles qui avaient reçu le régime à base de Wheast et étaient dirigées par une reine inséminée artificiellement ou fécondée naturellement, ont élevé une quantité significativement plus grande de couvain que les colonies semblables nourries au Yeaco ou au soja. Pour chaque type de régime les 4 reines inséminées artificiellement se sont comportées dans l'ensemble aussi bien ou mieux que les 4 reines fécondées naturellement, bien que dans quelques cas le nid à couvain des reines inséminées artificiellement se soit présenté au début par plaques discontinues. Les abeilles nourries avec le régime au Yeaco sont arrivées en seconde position en ce qui concerne la quantité de couvain élevé. Les colonies dirigées par des reines inséminées artificiellement, ou par des reines fécondées naturellement, ont été longues à démarrer l'élevage du couvain et la plupart n'ont maintenu l'élevage que pendant 8 semaines. Le régime testé le plus pauvre était celui au soja. Les reines (inséminées artificiellement ou fécondées naturellement) ont pondu durant les 12 semaines du test et du couvain operculé a été observé pendant 6 semaines, mais dans aucun des cas le couvain n'a atteint le stade operculé après la 6e semaine. Les résultats de cette expérience montrent que dans l'ensemble les performances des reines inséminées artificiellement sont aussi bonnes en conditions contrôlées que celle des reines fécondées naturellement. Cette étude prouve aussi que des abeilles nourries avec des régimes pauvres auront des performances faibles, le passé génétique des reines mis à part. Pourtant même des reines nourries avec des régimes pauvres sont capables de pondre pendant une période de 12 semaines.



## ZUSAMMENFASSUNG

EFFEKT DER ERNÄHRUNG AUF DIE BRUTRATE
BEI NATÜRLICH UND INTRUMENTELL BESAMTEN KÖNIGINNEN

Es wurden kleine Völker aus ca. 4.000 frisch geschlüpften Italienerbienen gebildet und in Flugkäfigen von 2 × 2 × 2 m aufgestellt. Die Völker (insgesamt 24) wurden in zwei Gruppen geteilt; 12 erhielten

instrumentell besamte Königinnen von einem kommerziellen Züchter und 12 erhielten frei begattete Königinnen, die in Beltsville, Maryland, gezogen worden waren. Jede Gruppe von 12 Völkern wurde weiterhin geteilt in drei Behandlungsgruppen von je 4 Völkern. Jeder Gruppe wurde eine Nahrung angeboten, die entweder Yeaco (43 % Protein), Weizen (58 % Protein) oder Sojabohnen (90 % Protein) als einzige Proteinquelle enthielt. 12 Wochen hindurch wurde die Menge von verdeckelter Brut wöchentlich bei jedem Volk gemessen. Völker, die mit Weizendiät gefüttert wurden, zogen signifikant mehr Brut auf als solche, die Yeaco oder Sojabohnendiät erhalten hatten, egal ob sie instrumentell besamt oder frei begattete Königinnen enthielten. In jeder Behandlungsgruppe verhielten sich die vier instrumentell besamten Königinnen ebenso gut oder noch besser als die vier natürlich begatteten Königinnen, obwohl in einigen Fällen das Brutmuster der instrumentell besamten Königinnen lückig war. An zweiter Stelle in der Brutmenge waren die Völker, die eine Yeaco-Diät erhielten. Alle Völker, sowohl mit besamten wie mit frei begatteten Königinnen waren langsam mit dem Beginn der Eiablage, und die meisten Einheiten hielten ihre Bruttätigkeit nur 8 Wochen durch. Die schlechteste von den geprüften Diäten war Sojabohnen. Die Königinnen (instrumentell besamt oder frei begattet) legten die ganzen 12 Wochen während des Tests, eine verdeckelte Brut wurde sechs Wochen lang beobachtet, aber nach diesen 6 Wochen erreichte keine Brut das Stadium der Verdeckelung.

Die Ergebnisse dieses Experiments zeigen, dass sich im ganzen instrumentell besamte Königinnen unter kontrollierten Bedingungen gleich gut bewähren wie natürlich begattete. Diese Untersuchung zeigt überdies, dass mit schlechter Diät gefütterte Bienen ein schlechtes Ergebnis liefern, ungeachtet des genetischen Hintergrundes. Aber auch bei schlechter Diät konnten Königinnen ihre Legetätigkeit über eine 12-Wochenperiode aufrecht erhalten.

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