http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (17): 1476-1481, 2003
ISSN 1028-8880

Performance Evaluation of Naturally Mated and Instrumentally Inseminated Honeybee (*Apis mellifera* L.) Queens in Field Colonies

Abdulaziz S. Al-Qarni, [1]Brian H. Smith and [1]Susan W. Cobey
Department of Plant Protection, College of Agriculture, King Saud University
Riyadh 11451 PO Box 2460, Saudi Arabia
[1]Department of Entomology, Ohio State University, Columbus, Ohio 43210, USA

Abstract: *Apis mellifera* queens were reared to evaluate the performance of Naturally Mated (NM) and instrumentally inseminated (II) queens, using Doolittle grafting method. A group of twelve queen cells was introduced to five frames nucleus colonies and virgins allowed to mate naturally. A second group of 14 queen cells was confined to cages and instrumentally inseminated once with 8 μl of semen and placed back to their assigned nucleus colonies. At oviposition, nucleus colonies for both groups were transferred into a standard Langstroth hive filled with empty combs and all colonies were allowed to build up naturally into full-size colonies. A perusal of the data presented a slightly higher survival rate in NM queens but binary logistic regression test revealed no significant differences between the survival rates of both NM and II queens that ended up with 5 NM (41%) and 3 II (21.4%) queens after 23-months. A detail probe of ANOVA also indicated no significant difference in brood production which were recorded 1274.4 and 1304.3cm^2 2-months after colony establishment, 1585.2 and 1534.6 cm^2 during April 1997 and 1674.9 and 1541.5cm^2 during spring 1998 for NM and II queens respectively. Likewise, honey production for NM remained statistically at par with that of II queens and was recorded 251.7 and 241.1 lbs. during fall season 1996 and 314.2 and 297.5 lbs. during fall 1997 for NM and II queens respectively.

Key words: Naturally mating, instrumental insemination, honeybee queens, survival rate, brood production and honey production

Introduction

Instrumental insemination is a valuable breeding technique that offers a way to utilize the desirable traits. It allows types of mating that are not possible with natural mating such as mating a queen to a single drone or to a few specific drones, mating mutant queens and drones and mating a queen to her own male offspring (Harbo, 1986a). Naturally, mated queens could mate with drones from unknown origins that could result in bees with undesirable characteristics. Instrumental insemination enables bee breeders to design breeding programs in which complete genetic isolation is maintained with the ability to produce consistent, high quality queens selected for a specific trait and high brood viability (Page and Laidlaw, 1982 and Page *et al.,* 1983).

Beekeepers replace their queens annually to maintain high performance and low levels of Africanization in commercial colonies. Requeening is also required as colonies led by young queens are more productive than those led by old queens (Kostarelou-Demianidou *et al.,* 1995). Progeny of old queens could also have undesirable characteristics, e.g., high susceptibility to diseases and strong defensive behavior (Free, 1987).

Instrumentally inseminated queens have been reported to have problems with initial introduction and acceptance and with early supersedure (Smith *et al*., 1993; Wilde and Loc, 1997; Tew, personal communication). II queens were also reported to have lower rates of oviposition (Harbo, 1986b), survival, brood production, honey production and initial number of stored spermatozoa (Harbo and Szabo, 1984). In contrast, Roberts (1946) and Mackensen and Roberts (1948) reported no significant differences in brood production and honey production between NM and II queens. Nelson and Laidlaw (1988) produced similar findings in queen weight, brood and honey production. They explained differences between NM and II queens to the lack of some special beekeeping treatments given to II queens during rearing and insemination techniques that influence their performance.

Instrumental insemination technique is widely employed in queen breeding programs for the improvement of honeybee races to have best colony performance. Because of conflicting reports in the literature, research is still needed to study factors that affect rearing, insemination and introduction of II queens that would lead them to have better evaluations. The present study

has been undertaken to compare the performance of naturally mated and instrumentally inseminated queens for survival, brood production and honey production.

Materials and Methods

Apis mellifera L. queens were reared in May, 1996 by the Doolittle grafting method (Doolittle, 1889). Young larvae (1-2 days old) were grafted to queen cups supplied with royal jelly, then transferred to a common cell builder colony. The larvae were originally taken from brood nest frames of six different breeders of the New World Carniolan closed population of honeybees maintained by the Rothenbuhler Honey bee Research Laboratory at The Ohio State University, Columbus, OH. After maturation, queen cells were individually placed in five frame nucleus colonies. One group of 12 queen cells was randomly selected and allowed to mate naturally. The second group of 14 queen cells was confined to cages and instrumentally inseminated once with 8 μl of semen at 5 days of age. II queens were kept in an incubator for 24 h (34°C and 75-80 RH), then they were given two minutes carbon dioxide treatment before placing them back to their assigned nucleus colonies. The following day, they were released from their cages and colony entrances were excluded to prevent queens from taking mating flights.

Colonies used to supply larvae for queen rearing and drones for natural mating and instrumental inseminations were derived from the same genetically closed population (Page and Laidlaw, 1985). Two weeks later, the queens in both groups began oviposition, each nucleus colony was transferred into a standard Langstroth hive filled with empty combs and all colonies were allowed to build up naturally into full-size colonies, which then supered and managed for commercial production.

In order to control swarming, all colonies were divided in early spring 1997. One super of bees and brood was removed from each colony and the original queen remained with the parent colony. Three variables were measured to determine if the two groups differ in field performance: supersedure rate, brood production and honey production.

The presence and identity of the queen in each colony was determined at regular intervals to observe supersedure rates and longevity. All queens were marked for easy identification. Natural queen cells, when observed, were not disturbed. The amount of brood produced by each colony was measured using a wire grid that was placed over the brood combs. The wire grid was calibrated in units of 2.5 cm2, representing 23 brood cells. The total brood area of each colony was measured in July of 1996, two months after the queens were established. Measurements were repeated also in April of 1997 and April of 1998.

To determine honey production, colonies were weighed before and after the honey crop was harvested. Each side of each colony was lassoed and lifted with an attached scale. The weights of the two sides of each colony were added to determine the total colony weight (Szabo, 1982). Two honey crops were harvested from each colony in fall 1996 and 1997.

All colonies were monitored for tracheal mite levels, but remained untreated due to low or undetectable levels. Apistan was used in all colonies to control Varroa mites. Minitab statistical software (Version 13.1, State College, Pennsylvania) was used to compare NM and II performance (Minitab Inc., 2000). Binary logistic regression test was used to compare survival rates of queens while One- and two-way analysis of variance (ANOVA) tests were used for the statistical analyses of brood and honey production.

Results

The results of this study showed that colonies of NM and II queens were similar in their field performance. Survival rates were slightly higher in NM than II queens; however, binary logistic regression test did not show any significant differences (P = 0.134). NM queens ended up with 5 out of 12 queens (41.7 %) and II queens ended up with 3 out of 14 queens (21.4 %) by the end of the 23-month study (Table 1).

The status of the queen in each colony was observed at regular intervals to determine supersedure rates and longevity. Colonies of 12 NM and 14 II queens were established in May 1996. By March of 1997, there remained 11 of the 12 NM queens and all 14 of the II queens. Two months later, 9 NM and 10 II queens were present. During the swarm season of the second year, supersedure rates were high in both groups. Six NM and 4 II queens were found by October 1997. By the following spring season, March 1998, 5 NM and 3 II queens remained.

One-way ANOVA for each year's production of brood during July 1996, April 1997 and April 1998 showed no significant differences between NM and II queens in commercially managed colonies (F = 0.2, 0.21 and 2.46, df = 25, 20 and 7, respectively and P > 0.05 for all tests). Brood production of NM and II queens was compared by measuring the brood area on the combs in units of 2.5 cm2, representing 23 brood cell per unit. To determine the number of full brood combs per colony, we estimated 3400 brood cell per standard Langstroth frame.

Table 1: Number and percentage of survival rate of NM and II queens over the period from July 1996 until May 1998

Queen	Jul. 96	Mar. 97	Apr. 97	May 97	Aug. 97	Oct. 97	March98
NM	12(100)	11(91.7)	10(83.30)	9(75)	6(50)	6(50)	5(41.7)
II	13(100)	14(100)	12(85.7)	10(71.4)	6(42.9)	4(28.6)	3(21.3)

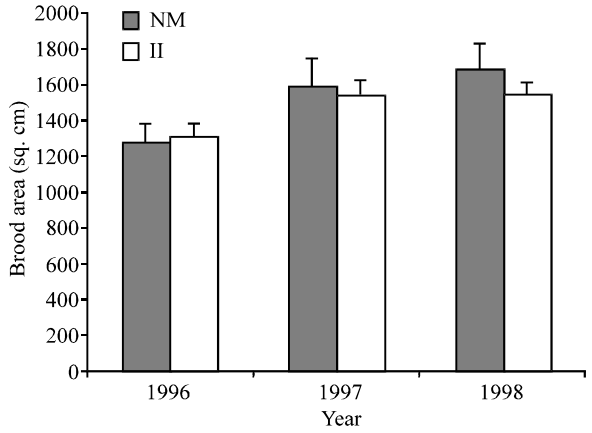

Fig. 1: Means of brood area produced by colonies headed by NM and II queens During Spring seasons of 1996-1998

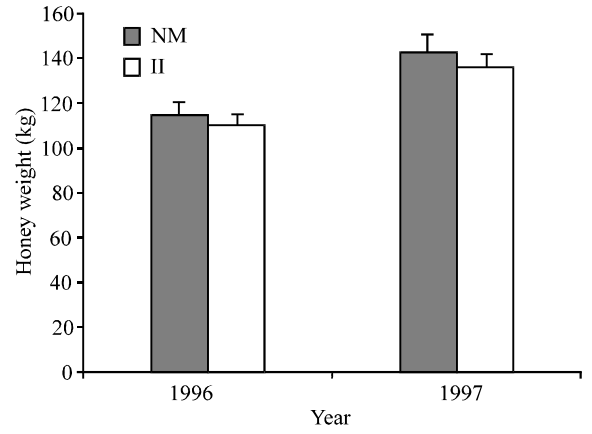

Fig. 2: Honey production (kg) by colonies headed by NM and II colonies in Fall season of 1996 and 1997

In July 1996, two months after queen establishment, colonies headed by NM queens had a colony mean of 1274.4 cm2 (29, 312 brood cells) or an estimate of 8.6 frames and colonies headed by II queens had a mean of 1304.3 cm2 (29,999 brood cells) or an estimate of 8.8 frames of brood. During the second spring season, April 1997, colonies headed by NM queens had a colony mean of 1585.2 cm2 (10.7 frames) and II colonies had a mean of 1534.6 cm2 (10.4 frames). In spring 1998, NM colonies had 1674.9 cm2 (11.3 frames) while II colonies had 1541.5 cm2 (10.4 frames) of brood (Fig. 1).

One-way ANOVA performed to compare differences between NM and II queens in honey production in fall seasons of 1996 and 1997, showed no significant differences ($F = 1.14$ and 0.45, $df = 25$ and 11 respectively, $P > 0.05$ in both tests). For the fall season of 1996, honey production for NM and II colonies measured 251.7 and 241.1 pounds, respectively and for the fall 1997, 314.2 and 297.5 pounds of honey, respectively (Fig. 1 and 2).

Discussion

The results of this study indicate similarity in performance levels of field colonies headed by NM and II honey bee queens. Although NM queens showed higher rates of production in brood area and honey weight, no significant differences were found between the two groups. NM queens showed a slightly higher survival rate than II queens during the 23-month study. Several other studies on NM and II queens have reported similar findings. Nelson and Laidlaw (1988) found that brood area and honey weight were similar in colonies headed by NM and II queens. NM queens were significantly heavier than II queens only on arrival; two months later no differences were found. Roberts (1946) reported that NM and II queens performed equally in honey yield after being shipped in packages. Mackensen and Roberts (1948) stated that II queens compared well with NM queens in brood production and honey production, although survival of II queens was slightly lower. Konapacka (1987) also reported II queens to be slightly lower in survival rate than NM queens. In some cases, II queens were reported to perform better than NM queens. Wilde (1989) found that only in the first year of a two-year study, NM and II queens were similar in their production of brood and honey. In the second year, II queens produced significantly higher brood and honey than NM queens. Szalai (1995) stated that colonies of II queens produced significantly more honey than that of NM queens.

In contrast, other studies reported NM queens to be more productive than II queens in oviposition rate, survival, brood quantity and honey yield. Lodesani *et al.* (1991) reported that colonies headed by NM queens exceeded II colonies in brood production. Both were similar in the number of adult bees and honey production. Harbo and Szabo (1984) found NM queens to be significantly higher than II queens in survival, brood area and honey production in four locations of a two-year study. They also reported a significant difference between the two types of queens in the initial number of spermatozoa in the spermatheca of queens.

This controversy in the performance of the II queens could be related to some factors that might have an impact on the physiology and/ or morphology of the queens. The performance of II queens was found to be affected by banking of queens, age of queens at insemination, amount and dose of semen, colony temperature, $_{CO2}$ treatment and time of year. For example, the performance differences measured by Harbo and Szabo (1984) were confounded by the fact that they stored NM queens in nuclei while II queens were stored in queen banks, which could cause injuries to II queens by aggressive workers. Banking of II queens is a common practice among bee keepers/ breeders. These queens do not receive the same quality

of care as free queens do from workers in colonies. Free movement of queens and the attendance by worker bees after insemination greatly increase the efficiency of sperm migration into the spermatheca of the queen (Woyke, 1979). Semen retention in the oviduct was reported in II queens that had been banked. Semen retention was found to be harmful and sometimes fatal to the queens (Vesley, 1970). Jasinski (1987) and Woyke (1988) reported that workers bit the tarsi, antennae and wings of banked II queens. Injuries were also reported to the ariola (foot pads) of the II queens, ranging from black spots to fully black, dry and shrunken ariola.

Honeybee queens deposit foot-print pheromone while walking on combs. Lensky and Slabezky (1981) suggested that this pheromone in conjunction with the mandibular gland pheromone inhibits the construction of queen cells. If the ariola of II queens are damaged, possibly lower levels of this pheromone may contribute to the lower survival rates reported in these queens. To provide more natural conditions pre- and post-insemination, virgins should emerge individually in nucleus colonies, be removed briefly for insemination and CO_2 treatment and then returned unrestricted. Queens given this treatment generally lay eggs sooner, survive longer and are more productive (Cobey, 1983).

The age of the queen at insemination influences the number of spermatozoa stored in the spermatheca which increases the possibility of early supersedure. Queens naturally mate between 5 and 13 days of age. Studies by Woyke and Jasinski (1976) indicated the optimal age for a queen to be instrumentally inseminated is 5 to 10 days. Fewer sperms enter the spermatheca when queens are inseminated at 14 days or older, which might lead to lower survival rate as reported by Harbo and Szabo (1984). They inseminated queens at two and three weeks of age with small doses of semen (2.7 μl). Woyke (1966) showed little advantage in the insemination of two small doses over one dose of 8 μl, a procedure that also requires more handling of queens, which increases the possibility of injuries and infection.

Queens reared from eggs of 48-72 h old were heavier and had bigger thoraces than queens reared from larvae 0- 24h old (Hatch *et al*., 1999). Also, queens reared from younger larvae (12-24 h) have more ovarioles, larger spermatheca and stored higher number of spermatozoa (Woyke, 1966; Szabo and Townsend, 1974).

Temperature affects the migration rate of semen into the spermatheca. Queens held at brood nest temperature (34°C) immediately after insemination stored 26 % more sperm than queens held at room temperature (24°C) (Woyke and Jansinski, 1973).

Harbo (1986b) reported that oviposition rate of II queens was significantly lower than that of NM queens. The rate of eggs laid per day was correlated to the body weight of queens ($r = 0.73$), but not to the semen stored in the spermatheca ($r = -0.18$). The cause of weight loss in II queens was attributed to the CO_2 treatment given to the queens. A loss of 10 mg per queen was found in NM queens after CO_2 treatment. This makes the use of CO_2 contradictory as it is known to stimulate egg laying in II queens (Mackensen, 1947). Harbo (1986b) calculated the 10 mg weight drop of NM queens treated with CO_2 to represent a reduction of 160 eggs per day. NM queens were heavier than II queens by more than 10 mg. This suggests that other factors, besides CO_2 treatment, either present or absent in II queens might be involved in the weight loss and lower oviposition rates.

Wilde (1994) confirmed the negative correlation between spermatheca content of semen and colony performance. Within a range of 3-5 million, the number of spermatozoa in the spermatheca had no significant effects on the most important productive characters of the honey bee colony. Further, colonies headed by II queens kept in Woyke boxes had the most brood in spring season. Fresnaye (1966) suggested that season affects the sexual maturity of queens. Early in the season, queens should be inseminated at 10-12 days of age and 5-6 days at the peak season.

Queen tergal secretions are known to function as a close-range sexual attractant in the queen-worker communication (Espelie *et al*., 1990). Natural mating but not instrumental insemination stimulates the production of tergal gland alkenes in honeybee queens. These specific chemicals are not known to have a role in sexual attraction of queens; however, initiation time of production strongly suggests a role of communication within the hive. Virgin and II queens lack tergal gland alkenes until about 40 days post-emergence. It is possible that this has a negative effect on the acceptance and survivability of II queens (Smith *et al.,* 1991, 1993).

In conclusion, field performance of II queens is influenced by many factors. Nevertheless, the present experiments show that successful insemination and high performance levels can be achieved when careful attention is given to queen rearing conditions, pre- and post-insemination treatments, semen doses and quality and technique of insemination utilized. Nelson and Laidlaw (1988) suggested that II queens perform as successfully as NM queens and that findings contrary to this might be due to the lack of some special beekeeping and/ or insemination procedures given to II queens. Conditions that had led to have II queens exceed NM queens in field performance (Wilde, 1989; Szalai, 1995) should be utilized and improved. Controlled mating is essential to improve

honeybee-breeding stock and to maintain high performance in field colonies. We show that instrumental insemination is a viable and insured method that bee breeders and beekeepers can rely on for that purpose.

References

Cobey S., 1983. The development of Instrumental Insemination. Amer. Bee J., 123: 108-111.

Doolittle GM., 1889. Scientific queen-rearing. Thomas G. Newman, Chicago, III.

Espelie KE, V.M. Butz and A. Dietz, 1990. Decyl decanoate: A major component of the tergite glands of honeybee queens, (*Apis mellifera* L.). J. Apic. Res., 3: 15-19.

Free, J.B., 1987. Pheromones of social bees. Ithaca, Comell University Press, NY.

Fresnaye J., 1966. Influence of variations in the age of sexual maturity on queen bees fertilized by artificial insemination. Ann. de l'Abeille English summary, 9: 237-242.

Harbo J. and T. Szabo, 1984. A comparison of instrumentally inseminated and naturally mated queens. J. Apic. Res., 23: 31-36.

Harbo, J.R., 1986b. Oviposition rates of instrumentally inseminated and naturally mated queen honey bees (Hymenoptera: Apidae). Ann Entomol Soc Am., 79: 112-115.

Harbo, J.R., 1986a. Propagation and Instrumental Insemination. in T.E. Rinderer (Ed.) Bee Genetics and Breeding. Academic Press, Orlando, FL.

Hatch, S., D.R. Tarpy and D.J.C. Fletcher, 1999. Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insectes Soc., 46: 372-377.

Jasinski, Z., 1987. Injuries of queens caged in queenless colonies. Proc. 31st intern. Apic. Cong., Warsaw. Apimondia Pub. House, pp: 67-68.

Konapacka, Z., 1987. Biological quality of instrumentally inseminated bee queens. 31st Int. Apic. Cong. Warschau.

Kostarelou-Damianidou M., A. Thrasyvoulou, D. Tselios and K. Bladenopoulos, 1995. Brood and honey production of honeybee colonies requeened at various frequencies. J. Apic. Res., 34: 9-14.

Lensky, Y. and Y.J. Slabezky, 1981. The inhibitory effect of the queen bee (*Apis mellifera* L.) footprint pheromone on the construction of swarming cups. J. Insect Physiol., 27: 313-323.

Lodesani, M., A. Nanetti and E. Carpana, 1991. A study on the activity of *Apis mellifera ligustica* colonies with instrumentally inseminated or naturally mated queens. Apicoltura English Summery, 7: 101-112.

Mackensen, O., 1947. Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queen bees. J. Econ. Entomol., 40: 344-349.

Mackensen O. and W.C. Robert, 1948. A manual for the artificial insemination of queen bees. USDA Bur. Ent. Quarant. ET-250.

Minitab Inc., 2000. Minitab, Release 13 for Windows (Statistical software). Minitab Inc., State College, PA.

Nelson D.L. and H.H. Laidlaw, 1988. An evaluation of instrumentally inseminated queens shipped in packages. Am. Bee J., 128: 279-280.

Page R.E. and H.H. Laidlaw, 1982. Closed population honey bee breeding. 2. Comparative Methods of Stock Maintenance and Selection. J. Apic. Res., 21: 38-44.

Page R.E. and H.H. Laidlaw, 1985. Closed population honey bee breeding. Bee World, 66: 63-72.

Page R.E., H.H. Laidlaw and E.H. Erickson, 1983. Closed population honey bee breeding. 3. The distribution of Sex Alleles with Gyne Supersedure. J. Apic. Res., 22: 184-190.

Roberts, W.C., 1946. Performance of the queen. Am. Bee J., 85: 185-186.

Roberts, W.C., 1948. Multiple mating of queen bees proved by progeny and flight tests. Glean. Bee Cult., 72: 255-259.

Smith, R., M. Spivak, O.R. Taylor Jr., 1991. Chemical differences between naturally mated and instrumentally inseminated queens. Am. Bee J., 31: 781.

Smith, R., M. Spivak, O.R. Taylor Jr., C. Bennett and M.L. Smith, 1993. Maturation of tergal gland alkene profiles in European honeybee queens, *Apis mellifera* L. J. Chem. Ecol., 19: 133-142.

Szabo, T.I. and G.F. Townsend, 1974. Behavioral studies on queen introduction in the honey bee. 1. Effect of the age of workers on their behavior towards an introduced virgin. J. Apic. Res., 13: 19-25.

Szabo, T.I., 1982. Phenotypic correlation between colonies traits in the honey bee. Am. Bee J., 122: 711-716.

Szalai, E., 1995. Results of instrumental insemination of queen honey bees in Hungary. Pszczelnicze Zeszyty Nnaukowe. English summery, 39: 61-69.

Vesley, V., 1970. Retention of semen in the lateral oviducts of artificially inseminated honey-bee queens (*Apis mellifera* L.) Acta. Entomol. Bohemoslow, 67: 83-92.

Wilde, J. and K. Loc, 1997. The effect of instrumental insemination of queen bees on hive establishment and brood production. Annales Universitatis Marie Curie-Sklodowska Lublin-Polonia, Section DD. English Summery, 52: 305-311.

Wilde, J., 1989. Development and productivity of honeybee colonies with naturally mated and artificially inseminated queens. Proc. 31st Intern. Cong. Warsaw. Apimondia Pub. House, pp: 442-444.

Wilde, J., 1994. Comparison of the development and productivity of honeybee colonies with naturally mated and instrumentally inseminated queens kept in different conditions before and after the insemination. Zootechnica. English summery, 39: 135-152.

Woyke, J. and Z. Jasinski, 1973. Influence of external conditions on the number of spermatozoa entering the spermatheca of instrumentally inseminated honeybee queens. J. Apic. Res., 12: 145-151.

Woyke, J. and Z. Jasinski, 1976. The influence of age on the results of instrumental insemination of honeybee queens. Apidologie, 7: 301-306.

Woyke, J., 1966. Which is the most suitable volume of semen in artificial insemination of queen honey bees. Tech. Rep. PL. 480, USDA Bee Cult. Lab. Warsaw, pp: 12-15.

Woyke, J., 1979. Effect of the access of workers honeybees to the queen on the results of instrumental insemination. J. Apic. Res., 19: 136-143.

Woyke, J., 1988. Problems with queen banks. Am. Bee. J., 128: 276-278.