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Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance*

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Abstract – Instrumental insemination, a reliable method to control honey bee mating, is an essential tool for research and stock improvement. A review of studies compare colony performance of instrumentally inseminated queens, IIQs, and naturally mated queens, NMQs. Factors affecting queen performance are also reviewed. The collective results of the data demonstrate that the different methodologies used, in the treatment of queens, has a significant affect on performance rather than the insemination procedure. Beekeeping practices can optimize or inhibit performance. The competitive performance of IIQs is demonstrated when queens are given proper care. The advantage of selection and a known semen dosage can result in higher performance levels of IIQs.

***Apis mellifera* / queen honey bees / instrumental insemination / colony performance**

1. INTRODUCTION

Honey bee queens mate in flight with numerous drones from diverse genetic sources. The ability to control mating has been one of the most challenging aspects of honey bee breeding. Attempts to mate honey bees in confinement date back to the 1700's and remain unsuccessful today. The technique of instrumental insemination, developed in 1920's and perfected in the 1940's and 1950's, provides a method of complete genetic control, as reviewed by (Cobey, 1983; Laidlaw, 1987). Today, with improvements in instrumentation, the technique is highly repeatable and highly successful.

Instrumental insemination also enables the creation of specific crosses that do not occur naturally, providing significant advantages to research and stock improvement. For example, a single drone can be mated to one or several queens, isolating and amplifying a specific trait. Varying degrees of inbreeding can be created to produce different relationships, including "selfing"; the mating of a queen to her own drones. Specific backcrosses can be created by extracting semen from the spermatheca of one queen to inseminate another (Harbo, 1986b).

The ability to pool and homogenized sperm cells from hundreds of drones and inseminate a portion to a queen or batch of queens enables unique mating system designs and simplifies stock maintenance. Within a closed population breeding system, the effective breeding population size, viability and fitness are increased using this technique (Page and Laidlaw, 1985). Another advantage of instrumental insemination is the ability to store and ship honey bee semen. Short term storage has been perfected (Collins, 2000b). The ability to ship semen, rather than live bees, minimizes the risk of spreading pests and diseases.

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Over the past 50 years instrumental insemination has been utilized in bee research institutions, worldwide, although the commercial industry has been slow to adopt the technique. A factor in the reluctance of beekeepers to take advantage of this tool is the perception of poor performance of instrumentally inseminated queens. The ambiguity in reported differences, compared to naturally mated queens, can be explained in the varying treatments and methodology used. The objective of this article is to review comparison studies and the factors affecting queen performance.

Comparison studies, of instrumentally inseminated queens (IIQs) and naturally mated queens (NMQs), date from 1946 to the present. Various aspects of queen performance are reviewed and compared across studies: colony productivity, queen longevity, onset of oviposition, the number of sperm stored in the spermatheca and rate of success. Factors affecting queen performance are also reviewed: rearing conditions, mating age, treatment of queens, semen dosage and handling, pheromone development, effects of CO_2 treatments and environmental conditions.

2. PERFORMANCE COMPARISON STUDIES

The various comparison studies are summarized in Table I and categorized into three groups based on their findings. Group I shows the equal performance of IIQs and NMQs, in six studies, Group II shows higher performance of IIQs in seven studies and Group III shows higher performance of NMQs in one study. The different methodologies used in the treatment of queens across studies are also compared; the age of queens at insemination, semen dosage and methods of queen introduction.

2.1. Colony productivity

To properly evaluate and select colonies for stock improvement, queens must be capable of heading productive field colonies. Several studies compared field performance of IIQs and NMQs based on honey production or weight gain and brood production (see Tab. I).

An early study by Roberts (1946) compared the performance of queens introduced as package bees and reported higher honey production in colonies with IIQs (see Tab. I). Queens were inseminated three times with 2.5 μL of semen and given a direct release introduction. Roberts (1946) attributed the higher performance of IIQs to selection.

In a similar study of queens introduced as package bees, Nelson and Laidlaw (1988) reported no significant difference between IIQs and NMQs (see Tab. I). The IIQs were inseminated with 8 μL of semen at 6 to 10 days old and banked (individually caged in nursery colonies) for 6 days before introduction. Queen weights were compared when the packages were hived and after establishment. Initially the NMQs were heavier than the IIQs and produced more brood, (see Tab. I). These differences were attributed to mating time, queen development and the fact that the NMQs had begun oviposition before shipment. The lower initial weights and brood production of the IIQs were attributed to their not being allowed to begin egg laying before shipment from California to Canada in 2 lb (one kg) packages. As the season progressed, no significant differences between the two groups were observed in queen weights, brood production or honey production. During the peak season brood production of the IIQs was slightly higher than the NMQs (see Tab. I).

Woyke and Ruttner (1976) summarized the results of instrumental insemination stating that most production colonies were headed by IIQs at the Apicultural Research Institute in Oberursel, Germany and performed well. They reported similar performance of IIQs and NMQs, concluding that IIQs can be productive for 3 and 4 years. In a 1970 study reviewed, colonies headed by IIQs were the highest producers. The authors (Woyke and Ruttner, 1976) also reviewed a study by Ruttner in 1961 indicating similar, although slightly lower mean brood and honey production of IIQs compared to NMQs (see Tab. I). In reviews of honey production of colonies headed by IIQs in the Czech Republic from 1961 to 1980, IIQs produced an average of 8% and 12% more than honey NMQs (Vesely, 1984; Woyke, 1989c).

Table I. Summary of Comparison Studies of IIQs and NMQs.

STUDIES			COLONY PREFORMANCE				LONGEVITY		TREATMENT OF IIQs		INTRO. METHOD
PERFORMANCE	No. of QUEENS		HONEY PROD.		BROOD PROD.						
Author, Year	IIQ	NMQ	IIQ	NMQ	IIQ	NMQ	IIQ	NMQ	AGE/II	Semen	
GROUP I											
EQUAL PERFORMANCE											
Pritsch & Bienefeld, 2002	1105	1114	37.9 kg	38.0 kg							
	1656	2025	37.4 kg	37.0 kg							
Gerula, 1999	85	45	45.3 kg	50.0 kg	242.35 dm^2	221.2 dm^2					
Cobey, 1998	14	12	109.8 kg	114.6 kg	8.8 Fr	8.6 Fr	18 mo	18 mo	5 d	8 μL	DR
			127.9 kg	142.4 kg	10.4 Fr	10.7 Fr					
Vesely, 1984	672	1483	8% more				1yr.50%	1yr.58%			
							2yr.27%	2yr.15%			
Nelson & Laidlaw, 1988	19	20	80 kg	70 kg	2074 cm^2	2303 cm^2			6–10 d	8 μL	Bnk 6 d/Pkg
					4145 cm^2	3998 cm^2					
Konopacka, 1987	276	285			1st.yr-3.8 r*	1st.yr-4.0 r*	1yr-94%	1yr-98%			
					3rd.yr-3.4 r*	3rd.yr-3.8 r*	2yr-54%	2yr-87%			
GROUP II											
IIQs HIGHER PERFORMANCE											
Tajabadi et al. 2005	5	10	7.8 kg	7.0 kg	3757 cm^2	2757 cm^2			6–7 d	8 μL	Bnk 10 d/nucs
Čermák, 2004	612	137	21.3 kg	19.4 kg					7–8 d	12 μL	DR/nucs
	233	50					23.4 mo	21.5 mo	7–8 d	12 μL	DR/nucs
Szalai, 1995	24	24 Slct	22 kg	17.8 kg	1011 egg/day	735 egg/day					
		24 Unslct		12.3 kg		631 egg/day					
Boigenzahn & Pechhacker, 1993	186	399 Slct	20.5 kg	19.0 kg							
		46 Unslct		17.9 kg							
Wilde, 1987	16	9	7.0 kg	4.6 kg	18 963 cells	18 343 cells	2yr. II=NM		7–10 d	8 μL	Bnk 8-10 d
	23	10	15.4 kg	11.8 kg	34 413 cells	21 817 cells					Bnk 8–10 d
Woyke & Ruttner, 1976	15	72	54 kg	39 kg							
Roberts, 1946	65	43	95.3 kg	52.6 kg						3 × 2.5 μL	DR/Pkg
GROUP III											
NMQs HIGHER PERFORMANCE											
Harbo & Szabo, 1984	59	59	42.3 kg	75 kg	1840.0 cm^2	2782.5 cm^2	1st.yr.31%	1st.yr.58%	2–3 wk	2 × 2.7 μL	Bnk 2–3wk/Col

Legend: Slct is selected; Unslct is unselected; mo is month;wk is week; d is days; DR is direct release; bnk is bank; Pkg is package bees; nucs is nucleus colony; r is a rating system of 1-4.

Harbo and Szabo (1984) reported significantly lower brood and honey production, and reduced survival rates of IIQs (see Tab. I). The treatment of queens in this study varied significantly from other studies. Queens were inseminated past their receptive mating age, given several small semen doses and banked before and after introduction.

In Poland, Konopacka (1987) measured brood production in colonies using a rating system; a low of 1 to a high of 4. During the first and third year of production the IIQs and NMQs averaged similar ratings (see Tab. I).

Cobey (1998) recorded total colony weights and measured brood areas during the active season and found no significant differences between the IIQs and NMQs over two seasons in a study in Ohio, USA (see Tab. I). Colonies were managed as production hives, and divided in spring for swarm control. IIQs were inseminated at 5 days old with 8 μL. of semen and introduced directly into nucleus colonies.

In a study conducted in Iran, Tajabadi et al. (2005) reported no significant differences in and brood production of IIQs and NMQs, measured at five points in time over the season. The IIQ group produced more honey (see Tab. I). The IIQs were given 8 μL of semen at 6 to 7 days old, a second CO_2 treatment two days after insemination and banked for 10 days before introduction into nucleus colonies. A high loss of IIQs at introduction was attributed to an insufficient introduction time.

Two studies compared three groups; unselected NMQs, selected NMQs and selected IIQs, showing higher productivity in the selected groups, regardless of the method of insemination. Boigenzahn and Pechhacker (1993) reported that IIQs in Austria produced the most honey, followed by the NMQs mated in isolated mating stations with select drones, followed by the NMQs mated in non-isolated apiaries with unselected drones (see Tab. I).

Szalai (1995) measured honey production and egg laying rates of unselected NMQs, selected NMQs and selected IIQs, and reported similar findings in Hungary. The selected IIQs were the top performers, followed by the selected NMQs, followed by the unselected NMQs (see Tab. I). The authors (Boigenzahn and Pechhacker, 1993; Szalai, 1995) attribute the higher performance of the selected groups to selection and the ability to control mating.

Over a three year period in Poland, Gerula (1999) observed the performance of IIQs and NMQs of *Apis mellifera caucasica* and *A. m. carnica* and their hybrids. No significant differences were found between the IIQs and NMQs (see Tab. I). Although, slight differences observed between the stocks were attributed to differences in the breeding strains and flow conditions. Caucasian colonies were slightly more productive than the Carniolan colonies.

In another Polish study, Wilde (1987) measured the performance of colonies headed by IIQs subject to varying conditions, over a two year period and reported higher production of IIQs compared to NMQs (see Tab. I). Queens were inseminated at 7 to 10 days old with 8 μL of semen and banked up to 10 days or given a direct release introduction.

More recently, several large data sets collected by bee research institutions that routinely use instrumental insemination for stock improvement programs, report minor or no significant differences in the performance of IIQs and NMQs. Pritsch and Bienefeld (2002) evaluated honey yields of colonies at the Central Breeding Evaluation Program in Germany, and report equal or higher yields in colonies headed by IIQs (see Tab. I).

In the Czech Republic, also reporting on a large data set, Čermák (2004) found similar results, that IIQs had slightly higher honey yields compared to NMQs (see Tab. I). The IIQs were inseminated at the age of 6 to 7 days with 8 μL of semen and banked for 10 days before release into nucleus colonies.

The majority of studies observed similar or higher performance levels of colonies headed by IIQs. The methodology of pre and post insemination care of queens, compared in Table I, was similar among the studies in Group 1 and II. Queens were inseminated at a young age, 5 to 10 days old, given semen doses of 7.5 to 12 μL, and introduced directly or within several days post insemination into small nucleus colonies or package bees.

The treatment of queens varied significantly in the Group III, Harbo and Szabo (1984) study, reporting less competitive performance

of IIQs (see Tab. I). In particular, queens were inseminated at an older age, 2 to 3 weeks, and caged in nursery colonies for another 2 to 3 weeks before introduction. Queens were given two small semen doses of 2.7 μL Queens were caged and shipped at the start of egg laying, and introduced into large colonies or package bees.

Harbo (unpubl. data) recently repeated this study, changing the pre and post insemination treatment of queens. Preliminary results suggest findings similar to the majority of studies reviewed above. The different methodology used in the initial study may have contributed to the reduced performance of IIQs. The affects of the different treatments are discussed in Section 3.

2.2. Queen longevity

The lifespan of the queen must be sufficient to allow time for selection and to ensure breeding stock is available for propagation. The process of evaluation requires field selection at the colony level, over time. Several studies compared queen longevity.

Vesely (1984) presented a large data set on the longevity of IIQs and NMQs recorded from 1961 to 1980 at the Bee Research Institute Dol in the Czech Republic. Survival of IIQs was slightly lower in the first year, and decreased in the second year, compared to the NMQs (see Tab. I). Another data set of queen longevity, reported by (Čermák, 2004), also from the Czech Republic, observed higher rates of survival for both IIQs and NMQs (see Tab. I).

Konopacka (1987) observed the survival rates of IIQs and NMQs over a 12 year period in Poland. She reported no differences in the first year, and a higher rate of survival of NMQs in the second year (see Tab. I). The IIQs were given different treatments and high mortality of queens due to infection was observed in both groups. The reduced survival of IIQs in the second year was attributed to excessive CO_2 treatments, two exposures of 10 minutes.

Several other studies report no statistically significant differences in the longevity of IIQs and NMQs. Cobey (1998) observed that both IIQs and NMQs survived an average of 18 months in production field colonies in Ohio, USA. Woyke (1989b) cited a study in Poland by Wilde in 1986, stating both groups had equal survival rates over a two year period (see Tab. I).

In contrast, Harbo and Szabo (1984) reported a significantly lower survival rate of IIQs, compared to NMQs after one year (see Tab. I). A major difference in this study was the treatment of queens. Queens were inseminated past their prime receptive mating age, held in banks for several weeks and given small semen doses. IIQs stored less sperm cells, starting with a mean of 3.2 million sperm cells, compared to the 5.5 million stored by NMQs. The NMQs had more sperm cells stored after one year, a mean of 4 million, than the number stored initially by the IIQs. The low initial sperm cell counts of IIQs in this study may have contributed to their lower rate of survival.

A major component of queen longevity is the number of sperm cells stored in the spermatheca. Queens that cannot maintain a large brood nest during the active season are often prematurely superseded. Many factors influence sperm migration into the spermatheca and the number of sperm cells stored, these are discussed in more detail in Section 3.

2.3. Onset of oviposition and rate of success

The criteria for the success of instrumental insemination are often based upon the onset of oviposition and queen survivorship to production of worker brood. Several studies have presented extensive data concerning these aspects. Most studies report similar rates among IIQs and NMQs. There is speculation that the lack of a mating flight may delay queen development, although the data suggests this is insignificant. The influence of seasonal and hive conditions plays a more significant role in physiological developmental differences between the two groups.

Woyke and Ruttner (1976) state that a higher success rate of IIQs, over natural mating, can be achieved with proper insemination techniques. The authors refer to a report by Mackensen who recorded a 92.5% success

rate of 759 queens inseminated from 1964 to 1966. They also report high success rates in Germany. Of the 1480 queens inseminated at the Apicultural Research Institute in Kirchhain 76.8% began laying in 1972 and 83.5% began laying in 1973. Moritz and Kühnert (1984) also report a success rate of 90% of the 3440 IIQs produced from 1972 to 1983 in Germany. Over a 5 year period of an insemination program supporting a breeding project in Australia, Kühnert et al. (1989) report a 85% success rate.

Otten et al. (1998) observed the influence of both queen age and time of day of insemination on the success rate to oviposition of 522 IIQs in Germany. Queens inseminated 10 days post emergence had a rate of 95% success, compared to 82% success rate of 7 day old queens and an 80% success rate of 13 day old queens. The authors suggest physiological factors change with the age of the queens and also during the time of day queens are inseminated. Afternoon inseminations were found to be optimal, 92% of queens inseminated between 2PM and 3PM began oviposition as compared to only 7% inseminated in the morning between 8000h and 9000h.

Onset of oviposition, the time between insemination and the start of egg laying, is generally considered to be somewhat slower and vary more widely among IIQs compared to NMQs. During the peak season, the majority of NMQs begin egg laying within 2 to 4 days post mating. However, Szabo et al. (1987) reported that the onset of oviposition of 1396 NMQs ranged from 4 to 22 days after emergence, with a mean of 10 days in Alberta, Canada.

Wilde (1994b) observed queens over a three year period and reported that of 59 NMQs the start of oviposition ranged from 7 to 26 days post emergence and of 77 IIQs the range was from was 10 to 37 days post emergence. The conditions queens were kept influenced results. The IIQs kept with worker bees laid sooner and stored more sperm cells than those caged without bees.

In another study Wilde and Bobrzecki (1994) observed 79 IIQs and 53 NMQs over a five year period and reported some interesting results. The NMQs produced slightly more brood and began laying earlier, although those beginning earlier, 11.5 days post emergence, produced more brood than those beginning later, 16.2 days. The opposite results were observed among the IIQs, those that began oviposition later produced more brood than IIQs which began earlier.

An extensive data set of 1212 IIQs, reported by Prabucki et al. (1987) from the Bee Research Institute in Poland, observed the start of oviposition of queens inseminated at different ages, from 6 to 16 days post emergence. Queens were given one semen dose of 8 μL or 2 doses of 4 μL. The majority of both groups started egg laying within 14 days, although higher mortality was observed in the group inseminated twice. Queens inseminated when 10 to 12 days old began egg laying in 6 to 8 days. Queen inseminated at 6 to 9 days old began laying in 10 to 13 days. Queens inseminated at 14 to 16 days old began laying in 11 days. The authors suggest seasonal effects and the conditions queens where kept also influence the onset of egg laying.

Skowronek et al. (2002) reported on another large data set of 1289 IIQs in Poland, observed over a six year period. The start of oviposition varied from 3 to 36 days post insemination. Queens inseminated when 5 to 12 days old started the earliest. The majority of IIQs, 67.7%, began oviposition within 6 to 10 days of insemination, 90.4 % within 15 days and 2.9% began within 5 days. The authors also state that seasonal conditions are a contributing factor for determining the onset of egg laying.

In addition, Skowronek et al. (2002) observed that the type of brood in colonies influences the onset of oviposition. Queens established with capped and emerging brood began laying sooner than queens established in colonies with eggs and young brood. Queens introduced during a nectar flow, warm weather and low relative humidity also started laying sooner. Cold nights and humidity above 90% delayed the start of oviposition.

A study by Chuda-Mickiewicz et al. (2003) confirms the observation that the onset of oviposition is influenced by the treatment of IIQs. They observed 79 IIQs and reported that queens confined to small boxes with small

populations were less successful and were slower to start egg laying compared to queens kept in free-flying mating nuclei.

3. KNOWN FACTORS AFFECTING QUEEN PERFORMANCE

The queen undergoes physiological changes in preparation for egg laying. Many factors influence the rate of these changes: from the newly emerged, to the brief receptive mating age; to post insemination and egg laying. The treatment of queens during these stages, before and after insemination, can influence their performance. Beekeeping practices, designed to increase efficiency and reduce labor, do not always provide optimal conditions and can lower queen performance. Factors affecting queen performance are reviewed.

3.1. Rearing conditions

The rearing of quality virgin queens and drones is the first step in the process of producing IIQs. Optimal conditions are essential to assure quality and sufficient numbers of mature drones. Queens may be subject to stress during the procedures and extra handling required. Intended for use as breeding stock, queens must head productive colonies and endure the rigors of evaluation.

The quality of queens is correlated to the age of larvae chosen to rear queens and the diet fed by nurse bees. Studies by Woyke (1966, 1971) demonstrated that increasing larval age, of 1 to 4 days old, results in decreasing body weight, decreasing numbers of ovarioles, and decreasing numbers of spermatozoa stored in the spermatheca of queens.

Queen weight is a recommended criteria to assess queen quality (Kaftanoglu and Peng, 1980; Nelson, 1989). However, Skowronek et al. (2002) found that queens heavier at emergence tend to begin oviposition later than queens with lower weight. Woyke (1962) reported that NMQs with large eggs in their ovarioles tend to have smaller semen volume after their mating flights.

Egg development is also a factor of aging virgin queens. Delayed mating, beyond the optimal receptive age may be due to several factors; poor weather conditions or insufficient drones. The banking of virgin queens, for convenient scheduling of insemination procedures, also has this effect.

A major factor in the success of instrumental insemination is a plentiful supply of healthy, mature select drones. The conditions in which drones are reared and held to maturity affect their quality and survival. Drone production is seasonal, dependent on resource availability, colony health and population, and is subject to a high rate of attrition (Currie, 1987). Stress from pests, disease, and unfavorable weather conditions also impact drone populations. The presence or residues of miticide treatments in rearing colonies reduce the fertility and longevity of drones and queens (Rinderer et al., 1998; Haarman et al., 2002). To rear an abundant supply of mature drones requires attention to these factors. Thus, the rearing environment must be given as equal importance as the genetic makeup of stock.

3.2. Mating age of queens

Virgin queens have a brief, optimal receptive period for mating. Oertel (1940) and Jhajj et al. (1992) reported that the age queens generally take mating flights is between 4 and 13 days post-emergence. Virgin queens can be instrumentally inseminated when they are a few days to several months old (Tarpy and Fletcher, 1998; Collins, 2000a). However, the age at which queens are mated affects their performance.

Mackensen and Roberts (1948) observed that queens instrumentally inseminated, “after the tenth day will be balled and mistreated by the worker bees, and the percentage and quality of laying queen will be reduced”. Queen pheromones are known to play a significant role, as discussed in Section 3.7. Another factor of receptivity may be the change of pH in the spermathecal fluid, which is 7.3 in 3 day old queens and increases to 8.6 in mated queens (Koeniger, 1986).

Woyke (1960) observed that virgin queens older than 14 days tend to mate with fewer

drones and store fewer sperm cells. Zamarliki and Morse (1962) reported that queens prevented from mating for one month did not mate at all or stored fewer sperm cells. To ensure queen quality, commercial producers routinely discard queens that have not started laying within three weeks due to poor weather conditions.

To determine the optimal age of queens for instrumental insemination, Woyke and Jasinski (1976) inseminated 237 queens, from 1 to 47 days old with 8 μL of semen. Queens inseminated at 5 to 10 days had similar numbers of sperm cells stored in their spermatheca and similar survival rates compared to NMQs. Queens inseminated at younger and older ages stored significantly fewer sperm cells. Young queens, inseminated when 1 to 3 days old, had high mortality. Jasnousek (1987) reported that queens inseminated at 7 to 9 days old began laying the soonest.

Seasonal effects influence the timing of natural mating and oviposition. Spring reared queens tend to mate more efficiently and begin oviposition sooner compared to fall reared queens. Moritz and Kühnert (1984) report that the start of oviposition, for both NMQs and IIQs, is delayed late in season. They observed 3440 IIQs produced from 1972 to 1983 in Germany and found that spring queens began laying 5.7 days post insemination, and queens inseminated late in the season began in 14.3 days. Jhajj et al. (1992) confirmed these findings, stating that queens mate about 5 days sooner early in the season, and take longer to begin egg laying, up to 11 days, late in the season.

I have also observed that both IIQs and NMQs reared in the fall season can take up to 3 to 4 weeks to begin laying. Seasonal effects contribute to delayed oviposition and are not solely based on a lack of available drones. Genetic differences also play a role, although Guler and Alpay (2005) reported that environmental conditions have greater effects than the genotype.

3.3. Effects of banking queens

Banking, the practice of confining queens to individual cages and holding these together in a queenless nursery colony, provides convenience. A large number of queens can be held until the proper age for insemination and held after the procedure until new colonies are prepared for their introduction. This allows flexible scheduling and is labor efficient although, does not provide optimal conditions for queens.

In a series of studies, Woyke (1988, 1989a) observed that virgin queens confined to cages in nursery colonies and inseminated past their receptive mating age store less sperm, are delayed in the onset of oviposition and are subject to injury from aggressive worker bees. The practice of banking queens was also observed to negatively effect queen weight and attractiveness. Szabo and Townsend (1974) observed that heavier queens are more attractive. Confined to cages, queens may not receive adequate nutrition. A protein rich diet is essential for egg development and lack of sufficient feeding of protein may contribute to delayed onset of oviposition. Queens confined to cages after insemination and later introduced into individual colonies are slower to begin egg laying and store fewer sperm cells (Wilde, 1994b). This may also delay pheromone development in queens.

Wilde (1994a, b) also observed the effect of different conditions in which queens are kept before and after insemination; free in nuclei and caged, with or without attendant bees, and compared these to NMQs in nuclei. The IIQs kept with worker bees laid sooner and stored more sperm cells than those caged without bees. During the second season, queens that had been kept in boxes with 200 worker bees produced more brood, although the differences were not significant.

Newly mated queens are very active, running on the comb often bending their abdomens, which promotes sperm migration. The movement of sperm cells from the oviducts into the spermatheca is a complex process, involving muscular action of the queen and sperm motility (Ruttner and Koeniger, 1971). Active, free movement of the queen and attendance by workers immediately after insemination helps clear the oviducts of semen and increases the efficiency of sperm

cell migration into the spermatheca (Woyke, 1979).

During mating flights, queens obtain large quantities of semen from numerous drones. Only about 10% of sperm cells collected in the lateral oviducts migrate into the spermatheca and are stored (Koeniger, 1986). Confinement of queens after insemination reduces sperm migration into the spermatheca. Two sets of data of IIQs given 8 μL and caged, stored 2.9 and 2.5 million sperm cells compared to 4.2 million and 4.4 million sperm cells stored by IIQs allowed free movement among bees and held in an incubator (Woyke and Jasinski, 1973, 1976). Woyke (1979) reported that of a total of 96 IIQs inseminated with 8 μL, queens allowed free movement and access to worker bees stored 5.3 million sperm cells while queens screened without bees stored 3 million sperm cells. Woyke (1989b) also reported that IIQs restricted to small push-in cages made of excluder material stored 1.8 million sperm cells, compared to 5.2 million sperm cells stored by IIQs allowed free movement and released directly after insemination The author states that the quality of care given to queens during the critical stage of sperm migration can produce better results than a second insemination.

Queens caged after insemination tend to retain semen in their lateral oviducts which can be harmful and sometimes fatal (Vesely, 1970). Woyke and Jasinski (1978) observed semen in the oviducts of queens after they began laying, under certain conditions. Queens tend to retain semen in their oviducts when: established with small populations unable to maintain brood nest temperatures; inseminated with preserved semen or with semen collected from older drones, and when inseminated late in the season (Vesely, 1970; Woyke and Jasinski, 1978, 1980, 1985; Woyke, 1979, 1983, 1989a).

Interestingly, Rhodes and Somerville (2003) reported that 11.5% of NMQs commercially produced in Australia, observed up to the age of 31 days, had semen residue in their oviducts. They also reported that this could be a factor of small nucleus colony populations and poor drone quality. In this study, sperm cell counts were low in both drones and the spermatheca of queens.

Queens caged and banked often suffer from injuries by aggressive workers bees. Workers bite the arolium (tarsal pads), tarsi, legs, and sometimes the antennae and wings of caged queens (Jasinski, 1987; Woyke, 1988). Of 354 queens stored for one week in queenless colonies, 60% were injured. Queens with damaged or missing arolium do not deposit sufficient foot print pheromones that inhibit queen cell construction and may contribute to supersedure (Lensky and Slabezky, 1981). Furthermore, queens with injured tarsi or legs tend to fall off brood comb when colonies are examined.

A study by Gerinsz and Bienkowska (2002) of 225 IIQs and NMQs showed that 55% of queens in both groups sustained injuries during routine handling. The injury level and supersedure rates were higher among IIQs, although injury did not seem to significantly affect egg laying rates. The rate of supersedure did increase with the extent of injuries; damaged arolia verses a paralyzed or missing leg. The authors emphasize that the different methods of handling of queens, for example the number of times queens come into contact with unfamiliar bees, determines injury levels.

Woyke (1989b) showed that reducing the time virgin queens are caged before insemination, from 10 to 4 days, reduced injury levels from 54% to 0%. The practice of banking queens is a valuable management tool, although the duration of the banking period should be minimized. Provision and maintenance of proper nursery colony conditions is also an important factor in preventing queen injuries that may reduce queen performance and increase supersedure rates.

3.4. Factors affecting the number of sperm stored in the spermatheca of queens

The queen must store a sufficient number of sperm cells to maintain a populous colony over her lifetime. The number of sperm cells stored in the spermatheca is a major factor determining queen longevity. An insufficient

number, less that 4 million sperm cells, results in premature supersedure (Harizanis and Gary, 1984; Woyke, 1989c).

Although, Wilde (1994b) states that queens which stored a range of 3 to 5 million sperm cells did not affect colony productivity.

IIQs are considered to have a tendency to store fewer sperm cells compared to NMQs, although the numbers range widely in both groups. Generally, IIQs store 3 to 6 million sperm cells and NMQs store 4 to 7 million sperm cells (Mackensen and Roberts, 1948; Woyke, 1962; Harbo, 1986b). Mackensen (1964) reported that NMQs store an average of 5.73 million sperm cells with a range of 3.34 to 7.36 million. Woyke (1962) reported similar findings, sperm cell counts averaged 5.34 million with a wider range, 0.69 to 7.92 million sperm cells. Wilde (1994b) reported a range of 4.1 to 5.9 million sperm cells stored by NMQs and a range of 2.1 to 4.8 million stored by IIQs. However, queens commercially produced and mated naturally in the United States and New Zealand, were reported to store an average of 4 to 5 million sperm cells (Harizanis and Gary, 1984; Van Eaton, 1986; Severson and Erickson, 1989).

The efficiency of sperm migration is dose dependent. However, the treatment of queens is a major factor influencing the efficiency of sperm migration into the spermatheca. Most sperm cells enter the spermatheca in the first few hours after insemination, a critical period for the queen. The conditions queens are subject to during this period affect sperm migration.

IIQs are routinely introduced into small nucleus colonies for the purpose of efficiency and to increase acceptance. Activity, temperature and hive conditions can enhance or inhibit sperm migration. In a series of experiments, Woyke and Jasinski (1973) observed the affect of worker populations on the efficiency of sperm migration in IIQs. They demonstrated that queens kept at broodnest temperature, 34 °C, stored more sperm cells than those held at 24 °C. An increase in the population increased the broodnest temperature and the efficiency of sperm migration.

Small populations of attendant bees and the consequential low brood-nest temperatures also reduced the rate of success and delayed the onset of oviposition of IIQs (Woyke and Jasinski, 1980, 1982, 1985, 1990). In a study of 160 IIQs, inseminated with 8 μL of semen and introduced into nucleus colonies with a varying number of attendant bees, Woyke and Jasinski (1980) found that queens with more than 100 worker bees had clear oviducts two days after insemination and most queens kept with fewer than 100 workers still had semen in their oviducts after two days. To produce queens that store a sufficient number of sperm cells, Woyke and Jasinski (1982) recommend a population of at least 350 worker bees is necessary for outdoor nuclei.

Queens introduced into larger bee clusters sizes are maintained at higher and more constant temperatures, and start egg laying sooner. Woyke and Jasinski (1990) observed 70 queens inseminated with 8 μL of semen, 7 to 8 days old and established with varying numbers of attendant bees. Queens established with 150, 350 and 750 attendant worker bees started egg laying in 15, 13.5 and 11.5 days respectively, with a range of 8 to 24 days. Doubling the number of bees attending the queen stimulated oviposition nearly a day earlier. When established with 9500 worker bees, queens started laying much earlier, in 5 to 9 days, averaging 7 days. An increase in the worker population increased the cluster temperature contributing to faster initiation of egg laying. Oviposition was accelerated by 1 to 2 days with a temperature increase of 1 °C.

The size of the colony that new IIQs are introduced into not only affects the number of sperm cells stored, it also affects the rate of acceptance and success. Otten et al. (1998) also reported on the success rate of establishing queens in different size populations and showed results were best when queens were introduced into larger nucleus colonies. The authors reported that queens introduced into weak colonies of 200 worker bees had a reduced success rate: 70% and a 12% queen loss. In colonies with 1200 workers, nearly 95% of IIQs began oviposition with less than a 1% loss.

Gontarz et al. (2005) observed differences in the thermal conditions of queens held in cages with or without attendant bees and

the affect on the efficiency of sperm storage. Queens re-introduced to cages established with attendant bees after insemination had elevated temperatures, sometimes above 35 °C within the first two hours. The temperatures were lower when the workers were newly introduced, or the queens were virgins. The authors suggest that bees caged with the queen before insemination had accepted the queen and adjusted to confinement. Queens held at the higher temperatures stored more sperm cells.

The treatment of virgin queens before insemination appears to play a minor role in performance compared to post insemination treatment, provided queens are inseminated during their optimal receptive period. Wilde (1987) studied the performance of IIQs in which the virgin queens were subject to varying pre- insemination conditions and found no significant differences in performance levels compared to NMQs. Three groups of IIQs were observed: virgin queens established in nucleus colonies with excluded entrances; virgin queens caged with 200 attendant workers bees; and virgin queens caged individually in queenless colonies. The IIQs were given six-minute CO_2 treatments at 5 to 6 days old and inseminated with 8 μL of semen at 7 to 10 days old.

3.5. Semen dosage and sperm quality

The mating frequency of NMQs and the number of sperm cells stored in their spermatheca is highly variable. Among drones, including those from the same colony, the number and viability of sperm is also highly variable (Lodesani et al., 2004). As discussed, environmental conditions as well as the pre and post insemination treatment of queens can inhibit or enhance the number of sperm cells stored. The dosage, quality and handling of semen are also contributing factors.

To obtain results similar to natural mating, the standard semen dosage for instrumental insemination is 8 to 12 μL (Mackensen, 1964; Woyke, 1989c; Vesely, unpubl. data). A slight increase can be gained by multiple inseminations of small doses. Smaller semen doses migrate faster (Bolten and Harbo, 1982). For this reason, Mackensen (1964) recommended two inseminations of 3 μL and Harbo (1986a) recommended 2 or 3 inseminations of 2 to 4 μL of semen, given a day apart. However, Woyke (1989c) stated that the difference between single and multiple inseminations, of up to 8 μL, were not statistically different. Multiple inseminations require extra labor and handling of queens, and may increase the risk of injury and infection. Currently, the standard practice is to give each queen one large semen dose.

The efficiency of sperm migration is dose dependent. Larger semen doses, 4 to 8 μL, generally migrate into the spermatheca over a 40 hour period. Small doses of semen migrate faster, 1 to 2 μL in 4 to 8 hours, and are less affected by conditions (Woyke, 1983; Woyke and Jasinski, 1985). Although, Harbo (1986a) reported variability in the number of sperm cells stored by IIQs inseminated under similar condition and that uniformity increased with larger semen doses and multiple inseminations.

Conditions that cause retention of semen in the oviducts can affect queen performance. Vesely (1970) found that queens inseminated with 6 μL of semen and caged after the procedure often retain semen in their oviducts. Results greatly improved when queens were allowed free movement in colonies, well attended by worker bees, and maintained at brood nest temperatures, as discussed above.

The age of the drones is also a factor of semen retention. Woyke and Jasinski (1978) reported that queens inseminated with semen from older drones tend to retain semen in their oviducts, which can cause the death of queens. Queens inseminated with 8 μL of semen from drones over four weeks old had semen residue in their oviducts more often than queens inseminated with drones two to three weeks old. Drone semen changes in viscosity and color with age. From young drones, less than 2 weeks old, the semen is fluid and light in color. The semen of drones older than 2 weeks is darker and more viscous.

Another factor affecting retention of semen in the oviducts and the number of sperm cells stored in the spermatheca is the size of the insemination tip. Bienkowska and Panasiuk (2006) observed 246 IIQs, in which 72.3%, inseminated with smaller tips, 0.16 mm in

diameter, had cleared oviducts 48 hours after insemination. Of queens inseminated with larger tips, 0.19 mm in diameter, 50.4% had cleared oviducts. Queens inseminated with the smaller tips stored more sperm cells, averaging 3.3 million, compared to queens inseminated with the larger tips, averaging 2.6 million.

The use of smaller tips, for the collection process, are more sensitive to the semen quality of drones (Bienkowska and Panasiuk, 2006). The viscous semen of older drones can be sticky and more difficult to collect. The more fluid semen of young drones readily mixes with mucus which can cause plugs in the smaller tips.

How drones are held until maturity can also affect semen collection. Drones tend to drift between colonies, therefore to ensure the identification of stocks drones are often confined inside colonies until mature. This practice results in the buildup of feces in drones, increasing the risk of contamination and can cause skin irritation.

3.6. Affects of semen handling and storage

Sperm cells remain viable in the queen's spermatheca for several years. The spermathecal fluid contains proteins, sugars and antioxidants, and the surrounding tracheal net provides oxygen (Koeniger, 1986; Phiancharoen et al., 2004). The physical and chemical properties of diluents; pH, osmolarity and nutrients, and the handling methods can enhance or reduce sperm cell survival (Verma, 1978; Moritz, 1984; Harbo, 1986b; Collins, 2000a).

The diluent used is more critical when semen is diluted, mixed or held in storage. A Tris buffer solution with a pH of 8.5, which contains sugars, amino acids and an antibiotic, is recommended (Moritz, 1984). Nutrient rich green coconut water also produces good results (Almeida and Soares, 2002). Further research is needed to formulate a diluent that promotes sperm quality and health.

Honey bee semen can be held in sealed capillary tubes for several weeks at room temperature with good success. Almeida and Soares (2002) observed no significant mortality of semen diluted with green coconut water and held for two weeks. Locke and Peng (1993) reported an initial viability of 87% dropped to 78% after 6 weeks of storage. Collins (2000b) reported 70% to 80% viability of semen diluted with Tris buffer and held at room temperature, 25 °C, or in a refrigerator at 12 °C for up to 6 weeks. Skowronek and Konopacka (1983) reported similar findings of semen held at 12 °C for 3 and 6 weeks.

Temperature is an important factor for in vitro, short term storage of semen. The optimal temperature is 21 °C, with a range of 13 to 25 °C (Harbo and Williams, 1987; Locke and Peng, 1993; Konopacka and Bienkowska, 1995). Temperatures below10 °C or above 32 °C, and exposure to sunlight result in poor success (Taber and Blum, 1960; Harbo and Williams, 1987).

The practice of mixing semen from numerous drones, mechanically or by centrifugation is advantageous for breeding purposes. Sperm tails are long, fragile and processing reduces viability (Collins, 2003, unpubl. data). Several studies have shown that queens inseminated with diluted, mixed and reconstituted semen, were slower to begin oviposition and stored fewer sperm cells compared to queens inseminated with fresh, whole semen (Kaftanoglu and Peng, 1980, 1982; Moritz, 1983; Skowronek and Konopacka, 1983). Lodesani et al. (2004) reported that by stirring and pipetting, rather than centrifuging, the mixing of semen was more efficient and reliable, although queens must be given two 8 μL inseminations of a 1:1 semen/diluent ratio.

Although, queens inseminated with mixed semen may store fewer sperm cells, they are capable of heading productive colonies. Collins (2000a) and Lodesani et al. (2004) showed that sperm cells are selected for quality during migration into the spermatheca. Queens inseminated with 50% live and 50% dead sperm cells produced viable laying queens (Collins, 2000a). Motility, previously assumed as a characteristic to measure semen quality, is not an accurate indicator (Collins, 2006).

Several studies have shown that survival rates of queen inseminated with mixed semen were similar to queens inseminated with whole semen (Kühnert et al., 1989; Harbo, 1990). In

the Kühnert et al. study semen was diluted, centrifuged and reconstituted and in the Harbo study semen was diluted 1:1 and mechanically stirred. Yanke (unpublished data) states that of the 150 to 250 queens produced annually over a fifteen year period and inseminated with diluted, centrifuged and reconstituted semen, many queens are productive for a year, and a few up to three years.

The long term storage of semen using cyropreservation techniques is possible, although is still in the experimental stage. Advances have been made with storage in liquid nitrogen using various cyroprotectants, such as DMSO, (dimethyl sulfoxide) and glycerol, with DMSO providing the best results (Harbo, 1979a). The critical variables are the rate of freezing and thawing and the diluent used. Harbo (1979b) reported queens inseminated with semen stored in liquid nitrogen produced worker progeny, although many queens produced more drone than worker brood and the rate of eggs hatch was lower, compared to the controls. Evidence genetic damage may have occurred in future generations was also reported (Harbo, 1981). Further research is needed develop techniques for the long term storage of honey bee germplasm.

3.7. Effects of carbon dioxide treatments

Carbon dioxide treatments, used to anesthetize the queen during the insemination procedure, also have the beneficial affect of inducing egg laying. CO_2 treatments stimulate the neurosecretory production of juvenile hormone, which contributes to the initiating of oviposition (Mackensen, 1947).

Two CO_2 treatments stimulate young IIQs to begin oviposition in a similar time period as NMQs. One treatment is given during the procedure; another is given either before or after the insemination. The timing and dosage of CO_2 treatments are variable in practice and may influence queen performance.

Mackensen (1947) reported that IIQs lacking a second CO_2 treatment have a delayed onset of egg laying of 40 to 60 days. Although, Woyke (1966) reported that IIQs started egg laying in 8 to 12 days without a second CO_2 treatment. He attributes this to the older age of queens at insemination, which were 8 to 12 days as compared to 3 to 7 days in the Mackensen study. Harbo (1986b) also states that older virgin queens, banked for 2 months before insemination, do not need a second CO_2 treatment to induce egg laying. Banked virgin queens of this age often lay eggs in their cages.

The effects of carbon dioxide treatments on queens are not fully understood. Worker bees exposed to CO_2 have reduced longevity (Austin, 1955) and change their behavior (Skowronek and Jaycox, 1974). Exposure of virgin queens to CO_2 treatments is known to inhibit queen mating flights, cause an initial weight loss and reduce or delay queen pheromone production (Harbo, 1986b; Woyke et al., 1995).

Skowronek (1976) reported that two day old queens, anaesthetized with CO_2 for 10 to 30 minutes, delayed their mating flights from 9 to 25 days, compared to untreated queens which flew on the 6th day post emergence. Treated queens also took fewer flights and stored fewer sperm cells.

The inhibition of mating flights by queens inseminated at different ages, given varied CO_2 treatments was also explored by Woyke et al. (1995). In a series of studies the authors demonstrated that two inseminations or two CO_2 treatments prevented mating when queens were allowed free flight. The age of queens at insemination was a contributing factor. As the age of queens, insemination with 8 μL was increased from 6 to 14 days, the percentage of natural mating decreased from 69% to 29%. Also, queens did not mate naturally when treated with CO_2 two days before insemination with of 8 μL, and when inseminated twice with 4 μL of semen. Of queens inseminated with of 8 μL. of semen and given two CO_2 treatments two days after insemination, 14% mated naturally (Woyke et al., 2001). Of queens inseminated with one dose of 8 μL of semen and not given a second CO_2 treatment, 61% mated naturally (Woyke et al., 1995).

Harbo and Szabo (1984) reported CO_2 treatments cause an initial weight loss in queens, both IIQs and NMQs, 3 to 9 days after the start of egg laying. NMQs were heavier and laid more eggs per day than IIQs. Nelson

and Laidlaw (1988) also reported weight loss of IIQs at the start of egg laying, NMQs averaged 190 mg and IIQs averaged 174 mg. However, after 2 months, both groups of queens were similar in weight and brood production. They attributed the initial difference in weight to the fact that IIQs had not begun egg laying when introduced, whereas the NMQs had been laying before introduction into colonies.

Jasnousek (1987) reported that reducing the CO_2 treatments from 10 to 1 minute had no effect on the initiation of oviposition of queens. However, Konopacka (1991) stated that queens given two, 10 minute treatments laid sooner, in 7 to 8 days, compared to queens given two, 3 minute treatments, which began to lay in 15 days. These differences may be due to other factors such as queen age and seasonal conditions.

Konopacka (1987) attributed a reduced lifespan of IIQs to excessive exposure to CO_2 yet this was only observed after the third year of production. Queens given two strong, ten minute CO_2 treatments lived shorter lives compared to those given a weak, incomplete anesthetization of less than one minute in which queen abdominal movement continued.

Several studies suggest practical recommendations. Fischer (1990) showed that migration of sperm cells is more efficient in queens given a CO_2 treatment the day before insemination, rather than the day after the procedure. Sperm cell migration takes about two days and CO_2 exposure during this time may interrupt or slow migration and chill queens. CO_2 treatment before insemination also inhibits natural mating flights and the need to exclude nucleus entrances (Woyke et al., 2001). Ebadi and Gary (1980) showed that CO_2 treatments diluted with air induced earlier oviposition of IIQs and suggest the use of a 75% CO_2 mixture.

3.8. Pheromone development and queen acceptance

The queen undergoes many changes in physiology and behavior: from a newly emerged virgin, to mating age, mating, egg maturation and production. The composition of queen pheromone changes dramatically between virgin and mated queens. Quantitative and qualitative changes depend on queen status, and are unique to each individual (Apoegait and Skirkevicius, 1995). These complex chemical blends communicate reproductive status of the queen, maintain social cohesion, elicit retinue response and influence the behavior and physiology of worker bees. Pheromone development may be delayed in IIQs compared to NMQs and explain why acceptance of IIQs can sometimes be more difficult.

Workers bees are less attentive to young, virgin queens compared to laying queens. The presence, concentrations and proportions of queen mandibular gland pheromone (QMP) vary with age, reproductive status, genetic makeup and seasonal changes (Pankiw, 2004) QMP produced by young, pre mating age virgin queens 1 to 2 days old, is different from that of virgin queens of mating age, 4 to 7 days, and different from QMP produced by laying queens. Mature, mated queens have the highest levels of QMP compared to newly mated and virgin queens (Slessor et al., 1988, 1990).

Pankiw et al. (1996) found that young, unmated queens produce lower quantity and different proportion of components of QMP compared to mated queens. Drone laying queens have been shown to have intermediate quantities compared to mated and virgin queens. Pheromone production is complex and not only a function of age.

Richard et al. (2005) suggests that how well queens have mated also may affect differences among pheromone profiles. In preliminary studies multiple mated queens attracted a larger retinue response than single mated queens.

Another source of queen pheromone, the tergal gland secretions (TGS) localized on the dorsal surface of the abdomen, plays a role in worker-queen interactions. Workers in the queen's court actively lick and palpate the dorsal abdomen of the queen. The blend of TGS and QMP maintains and stabilizes retinue behavior and communicates to the workers that the queen has mated (Slessor et al., 1988).

TGS are found on mature queens and vary with age. Young virgin queens do not produce TGS until the mating age of 5 to 10 days old.

NMQs, 10 days old or 48 h after mating, produce a full complement of TGS. TGS production is stimulated by mating flights and not by instrumental insemination. Virgin queens and IIQs, prevented from taking mating flights have been shown to have a delayed onset of TGS production, of about 40 days post emergence. Although, no differences were found in the onset of TGS production between IIQs and sister virgin queens (Smith et al., 1991, 1993).

Differences in the development rate of pheromone blends of IIQs and NMQs may influence initial queen acceptance. NMQs may elicit a higher response and acceptance level, compared to IIQs. The lack of a mating flight and the treatment of queens, inseminated at different ages and banked for different periods of time, may influence the rate and blend of queen pheromone development.

Differences in pheromone levels among IIQs and NMQs may become less significant when queens are laying and established. In mated queens, QMP profiles are relatively stable over time. Pankiw (unpubl. data) did not detect any significant changes in the quantity or quality of QMP components between laying NMQs and IIQs. She suggests that brood cues are a more "honest" assessment of the queen's fecundity. Worker bees detect the different pheromone blends of brood and adult bees of varying ages. Pankiw (unpubl. data) hypothesizes that workers respond to changes in the ratio of brood and adult bees, as this relates to the queen's egg laying rate.

IIQs are vulnerable during queen introduction and early establishment, the time when queen pheromone production is developing and maturing. Observing NMQs, aged 7 to 31 days, Rhodes and Somerville (2003) found that the pheromone levels varied between queens of the same age group. The components, of the ten pheromone levels identified, also varied between age groups. The authors suggest a relationship between pheromone level, queen acceptance and survival success.

In another study, Rhodes et al. (2004) reported that young NMQs allowed to lay for several weeks in nucleus colonies before introduction into large colonies had lower supersedure and higher survival rates, compared to queens that were caged and forced to reabsorb eggs during transport soon after the start of egg laying. Free et al. (1992) reported that workers bees pay more attention to queens that have been mated for two months or more.

The complex and changing blends of queen and brood pheromones play an important role in queen acceptance and replacement. Further studies are needed to determine if the treatment of queens, during the process of instrumental insemination and establishment, influences queen pheromone development and production. However, differences in pheromone blends between IIQs and NMQs appears to be insignificant or at least of no importance for queen performance, once queens are established.

4. DISCUSSION

This review provides a level of confidence in the use of IIQs and insight into the methodology to improve queen performance. The collective results of the various studies conclusively demonstrate that IIQs are capable of heading productive colonies and can endure the rigors of performance testing in the field. The demonstrated reliability of instrumental insemination as a tool for selective breeding should encourage increased use of this technique in bee breeding and stock maintenance programs.

Many factors affect queen performance. Beekeeping management practices and the treatment of queens appear to have a more significant effect on queen performance, than the actual insemination procedure. The treatment of queens in the one Group III study reporting lower performance of IIQs, differed significantly from the Group I and II studies showing equal or higher performance of IIQs (see Tab. I).

Common beekeeping practices clearly affect results. The efficiency of sperm cell migration is reduced when queens are: inseminated beyond their receptive mating age, caged in banks or maintained at below brood nest temperatures. Queens with insufficient numbers of sperm cells stored have lower rates of survival. The dosage, quality and handling of semen used for insemination also influences

results. All of these aspects can be controlled and optimized, with proper beekeeping practices, to enhance the performance of IIQs.

Some initial differences may be observed in the establishment of IIQs. Mating flights and the act of mating may stimulate physiological development that may be slower in IIQs compared to NMQs. These differences are minor, and can be minimized with the proper insemination procedures and proper treatment of queens. These differences can be observed during the introduction of queens and become insignificant as queens are established and laying. An understanding and implementation of beekeeping practices that promote efficacy of IIQs, as discussed in this review, will produce competitive performance in such queens, compared to NMQs.

In the majority of studies, the advantage of controlled mating demonstrates that the ability to select for valued traits can result in higher performance of IIQs compared to NMQs. IIQs have also been known to survive several years and out live NMQs. Yanke (unpubl. data) observed, during years of beekeeping in the northern extreme of New Zealand, that IIQs are more consistent and often live longer than NMQs, especially when weather conditions during the mating period are less than optimal. This confirms my own experience in the annual production of several hundred IIQs for a breeding program from 1981 to the present.

Instrumental insemination offers the advantage of mating queens more uniformly. A review of commercial queen production in Australia reported NMQs had a wide range of sperm counts (Rhodes and Somerville, 2003). Queens mated late in the season, had low sperm counts and began laying with approximately 2 million sperm cells stored, while the average number is 5 to 6 million. During the peak season, 42% of drones had less than 0.5 million sperm, the authors (Rhodes and Somerville, 2003) suggest insufficient drones and low sperm counts may be factors. Sperm cell counts of drones are generally 7 to 11 million per individual (Mackensen, 1955; Ruttner and Tryasko, 1976; Woyke, 1989c).

The insemination of queens with a known and uniform semen dosage eliminates the wide range of mating frequency by NMQs. It has been speculated that queens continue to take mating flights until a sufficient amount of semen is stored. Although, Tarpy and Page (2000) state that there is no control over mating frequency and suggest that the number of available drones, weather and seasonal conditions are the major influences.

The ability to increase genetic diversity within a colony, beyond what is possible through natural mating, is an advantage of instrumental insemination. The impressive array of traits and flexibility in behavior patterns displayed by honey bees are due to their intra-colony genetic variability. Genetic diversity enhances colony fitness, enabling honey bees to exploit and survive in wide ecological ranges, survive extreme climatic conditions and resist pests and diseases. Several studies have shown that adaptability, productivity and survivability tend to be greater in out-crossed stocks (Fuchs and Schade, 1994; Palmer and Oldroyd, 2000; Tarpy and Page, 2002).

Another advantage of instrumental insemination is the ability to isolate a specific trait and eliminate the complexity of genetic background that masks the expression of a particular characteristic. With the sequencing of the honey bee genome, this will become an increasingly valuable research technique.

Applied to stock improvement, instrumental insemination provides an essential tool to develop commercial stocks that have better survival in the presence of parasitic mites and disease, as well as enhance traits such as productivity, good temperament and overwintering ability.

The data presented in Table I overwhelmingly demonstrates that instrumental insemination is a reliable and practical technique. However, beekeeping practices designed to reduce labor, increase efficiency and provide convenience in scheduling, often provide suboptimal conditions for queen development and sperm storage in the spermatheca. The reported lower performance levels of IIQs can probably be attributed to such factors. With the ability to control mating, IIQs generally have been selected for superior genotypes, which may mask the possible disadvantages of instrumental insemination concerning their

performance. The unfounded reputation for poor performance of IIQs has been difficult to dispel, although the scientific community has repeatedly demonstrated their equal and sometimes higher performance compared to NMQs.

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Études comparatives sur des reines d'abeilles après insémination artificielle ou accouplement naturel et facteurs agissant sur leurs performances.

***Apis mellifera* / reine d'abeilles / insémination artificielle / performance de la colonie**

Zusammenfassung – Vergleichende Untersuchungen an instrumentell besamten und natürlich begatteten Honigbienenköniginnen und zu Faktoren, die deren Leistungsfähigkeit beeinflussen. Die instrumentelle Besamung ist eine zuverlässige Methode, um die Paarung bei Honigbienen zu kontrollieren. Sie bietet damit ein unverzichtbares Verfahren für die Forschung und die Bienenzucht. Dieses Review der Forschungsarbeiten von 1946 bis heute vergleicht die Leistung von Bienenvölkern mit instrumentell besamten Königinnen (IIQs) und natürlich begatteten Königinnen (NMQs) sowie Faktoren, von denen die Leistungsfähigkeit der Königinnen beeinflusst wird.
In den Studien wurden verschiedene Leistungsparameter der Königinnen verglichen: Produktivität des Bienenvolkes, Lebensdauer der Königin und Aufbewahrung der Spermien. In Tabelle I sind die Ergebnisse dieser Studien in Gruppen zusammengefasst: Gruppe I mit 6 Untersuchungen zeigt gleiche Leistungsfähigkeit von IIQs und NMQs; Gruppe II mit 7 Untersuchungen zeigt eine höhere Leistung bei IIQs; Gruppe III enthält eine Studie mit höherer Leistung bei NMQs.
Eine detaillierte Analyse dieser Studien zeigt eindeutig, dass die Leistungsfähigkeit der Königin signifikant von der Durchführung der Besamung abhängt. Die Ergebnisse in Gruppe III können demnach auf unterschiedliche Behandlung der Königinnen zurückgeführt werden. Die IIQs in den Gruppen I und II wurden in einem Alter zwischen 5 und 12 Tagen mit einer Samenmenge von 8–12 μL besamt. Diese Königinnen wurden in Jungvölker oder „package bees" eingeweiselt, ohne zuvor über längere Zeit gekäfigt in weisellosen Völkern aufbewahrt worden zu sein. In der Gruppe III wurden die Königinnen dagegen in einem Alter von 2–3 Wochen mit einer relativ geringen Samenmenge (zweimal 2,7 μL) besamt und zusätzlich über 2–3 Wochen in anderen Völkern aufbewahrt, bevor sie in größere Bienenvölker oder „package bees" eingeweiselt wurden.
Die geringe Anzahl an Spermien sowie die geringere Produktivität und Überlebensraten der IIQs in Gruppe III kann ebenfalls mit der verwendeten Methode bei der Besamung erklärt werden. Wenn Königinnen vor dem Zeitpunkt ihrer ersten aufnahmefähigen Paarung besamt werden, speichern sie weniger Samen. Das Käfigen nach der Besamung reduziert ebenfalls die Speicherfähigkeit für die Spermien und führt zudem häufig zu Verletzungen der Königin durch aggressive Arbeiterinnen.
Bienenköniginnen durchlaufen dramatische physiologische Veränderungen während der Vorbereitung zur Eilage. Viele Faktoren beeinflussen diese Veränderungen und damit auch die Leistungsfähigkeit der Königin. NMQs, die sich frühzeitig paaren, sich frei bewegen können und gut von Arbeitsbienen versorgt werden, paaren sich mit mehr Drohnen und speichern mehr Spermien.
Es wurden einige geringe Unterschiede zwischen IIQs und NMQs beobachtet. So können die höhere Variationsbreite beim Beginn der Eiablage sowie eine langsamere Produktion des Königinnenpheromons das Einweiseln von IIQs erschweren. Diese Unterschiede minimieren sich aber bei einer guten imkerlichen Praxis.
Andere vom Imker zu verantwortende Faktoren wie die Art der künstlichen Besamung und die Behandlung des Samens beeinflussen ebenfalls die spätere Leistungsfähigkeit der Königin. Die imkerliche Praxis kann somit die Leistungsfähigkeit der Königin verbessern oder verschlechtern. Es konnte aber klar gezeigt werden, dass bei guter imkerlicher Praxis die Leistungsfähigkeit von IIQs und NMQs vergleichbar sind. Dieses Review soll den Imkern Vertrauen in die instrumentelle Besamung geben und darüber hinaus zeigen, welche methodischen Details dabei die Leistungsfähigkeit der Königinnen verbessern können.

***Apis mellifera* / Honigbienenkönigin / künstliche Besamung/ Leistungsfähigkeit des Bienenvolkes**

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