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Introduction and early performance of queen bees
- some factors affecting success

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Foreword

The Australian beekeeping industry comprises a number of groups of specialists such as honey producers, queen bee breeders, honey packers, pollinators etc. Each group relies upon the expertise of one or more of the other groups for the success of their own business.

This project examined the quality of queen bees produced by a commercial queen bee breeder based in Queensland using standard production methods. The queen bees were introduced into two commercially operated honey production apiaries in different areas of NSW. Numbers of queen bees surviving the introduction process, and those surviving 15 weeks after introduction were recorded.

Same aged sister queens to those introduced into honey production hives were forwarded to the CSIRO Entomology Laboratory, Canberra where they were examined for pheromone levels, disease presence, and a number of physical characteristics considered likely to impact on survival success of the queens.

Data obtained from the laboratory examinations were used to explain queen survival data recorded from the field experiments.

This project was funded from industry revenue matched by funds provided by the Federal Government. The New South Wales Department of Agriculture provided staff and facilities.

This report, a new addition to RIRDC`s diverse range of over 900 research publications, forms part of our honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry.

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Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

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Contents

Executive Summary

Over a number of recent years commercial honey producers have reported that commercially reared queen bees often have a low survival rate when introduced into their hives, or they may have a satisfactory introduction success rate which is followed by poor performance by the established queen bee.

A three year trial beginning in 1999 investigated the introduction and early performance success rates of queen bees caught from their mating nucleus or taken from a queen bank and introduced at ages between 7 and 35 days into established commercial honey production colonies.

Sister queen bees were reared each year from one or two AI *Apis mellifera ligustica* breeder queens. Queens were grafted on the same day, reared in cell rearing colonies in the same apiary, and mated in the same queen mating apiary at the same time. Based on available information, sufficient drone mother colonies were supplied to the mating apiary.

A commercial queen bee breeder in Queensland produced the queens and mailed them to two commercial honey producers in climatically separate parts of New South Wales, as well as mailing queens to the CSIRO Entomology Laboratories, Canberra, ACT. Transport effects during mailing were recorded by enclosing dataloggers in envelopes with queens during transit and recording queen and escort mortality on arrival.

For 1999 and 2000 queen bees were caught from their mating nucleus at 7, 14, 21, 28 and 35 days of age for introduction into bee colonies and laboratory examination. For 2001, queens were caught from their mating nucleus at 17, 24 and 31 days of age or were caught at 17 days and placed in a queen bank until 24 and 31 days and introduced into colonies or examined in the laboratory.

Queen survival and performance data were based on Introduction Success (IS) – the number of queens surviving 14 days after introduction, Early Survival (ES) – the number surviving 15 weeks after introduction, and Satisfactory Performance (SP) – field evaluation for satisfactory performance by queens surviving 15 weeks after introduction.

Measurements made on each queen in the laboratory included – number of sperm present in the spermatheca, number of ovarioles in one ovary, nosema disease spore count (years 1, 2, 3). Identification and levels of pheromone components in each queen`s head gland (year1). Queen weight, spermatheca diameter and presence of semen in oviducts (years 2 and 3).

Nosema and brood disease status of all colonies used in the trial were recorded.

Results showed that Introduction Success, Early Survival and Satisfactory Performance increased with increase in queen age at introduction between 7 and 24 days of age where it began to level off with the youngest age showing high survival success being 28 days of age. A small increase was obtained for queens introduced at older than 28 days.

The data demonstrated that 7, 14 and 17 days (21%, 59% and 67% IS respectively) are not suitable ages for catching queen bees for introduction into established bee colonies immediately on arrival at their destination. Introducing queens caught at 21 days provided an IS rate of 82% which was improved to 90% for queens caught at 28 days and 92% for 35 day old.

Improved survival rates with increased age at introduction were carried through for numbers of queens surviving 15 weeks after introduction, ES, with 20% of 7 d.o. surviving, 48% of 14 d.o., 67% of 17 d.o., 70% of 21 d.o., 79% of 28 d.o., and 81% of 35 d.o. queens.

An attempt to age queens by catching them from their mating nucleus at 17 days of age and placing them in a queen bank until 24 and 31 days of age before being introduced into established bee colonies provided inconclusive results. The results suggest that catching queens at 17 days of age and holding them in a queen bank until 28 days or older may be a satisfactory alternative method for aging queens prior to introduction.

Low numbers of sperm present in the queen`s spermathecae was common for all ages of queens examined for all years. Low sperm counts did not appear to affect queen survival for Introduction Success and Early Survival Success 15 weeks after introduction.

Ten pheromone components were identified in queen bee head glands. Results were characterised by the large range of levels present within and between each component for each age group of queens examined. Levels of pheromone components varied between age groups suggesting a relationship between queen pheromone level and introduction survival success.

Other factors examined including transport effects, disease status of hives queens were introduced into, and queen physical characteristics were shown not to have a measurable effect on queen survival following introduction.

1. Introduction

A Pilot Project, DAN 164A, carried out during 1997-98 by NSW Agriculture investigated the introduction and early performance success of commercially reared queen bees introduced into commercial honey production apiaries. The Pilot Project identified two factors to be sufficiently important to warrant further investigation – the age of the queen bee when introduced into an established hive, and the wide range of sperm counts in the spermathecae of same-aged sister queen bees reared under the same conditions and mated in the same mating apiary at the same time.

The importance of these two factors was examined in detail by obtaining field data on queen bee survival (Objective 1) and explaining the field data using data obtained from laboratory examinations of sister queen bees (Objectives 2, 3 and 4), and by identifying stress situations queen bees in the field trials may have been subjected to during transport (Objective 5). Required data were obtained from three experiments on queen bee introduction and early performance success, DAN 182A, carried out over three years between November 1999 and April 2002.

2. Objectives

The project contained a number of similar objectives incorporated into Years 1, 2 and 3 –

(1) Effects of Queen Age on - Introduction, Early Performance and Satisfactory Performance Success.

For Years 1 and 2 (1999-2000) - field experiments were carried out to determine differences between the Introduction Success Rate (number of queens surviving 14 days after introduction), Early Performance Success Rate (number of queens surviving 15 weeks after introduction), and Satisfactory Performance Success (field evaluation of queen performance carried out at the 15 week inspection), of commercially reared queen bees caught from their mating nuclei and introduced into commercial honey production hives at 7, 14, 21, 28 and 35 days of age.

For Year 3 (2001) – to determine the effects of holding queen bees to various ages prior to introduction by comparing field survival data from queens either retained in a mating nucleus or held in a queen bank. Field survival data were collected for queen bees (a) caught from mating nuclei at 17, 24 and 31 days of age and introduced into commercial honey hives, and (b) caught from mating nuclei at 17 days of age, placed in a queen bank, and introduced into commercial honey production hives at 24 and 31 days of age.

(2) Number of Spermatozoa in the Spermatheca

To examine relationships between queen bee survival and the number of sperm in the spermathecae of queen bees. Sister queen bees corresponding to each of the age groups queen bees were caught for field trials for Years 1, 2 and 3, were examined in a laboratory and the number of spermatozoa in their spermatheca determined.

(3) Pheromones Present in the Queen Mandibular and Head Glands

To determine the effects of queen age on mandibular and head gland pheromone production, for Year 1 only. The number and level of pheromones present in the mandibular and head glands of queen bees caught at 7, 14, 21, 28 and 35 days of age were determined.

(4) Physical characteristics of queens and disease status of queen bees and hive bees were measured as an indicator of queen quality.

Year 1 – queen bees examined for the number of ovarioles in the left and right ovaries, and the number of Nosema (*Nosema apis)* spores present in the mid and hind gut. Honey hives were examined for the presence of brood diseases, hives displaying brood disease were not used. Years 2 and 3 – queen bees examined for the number of ovarioles in left ovary, diameter of the spermatheca, presence or absence of sperm in the oviducts, queen weight, and number of *N apis* spores present. Nosema disease and brood diseases prevalences were recorded in honey hives.

(5) To Examine Transport Effects on Queen Survival

The effects of temperature and humidities queen bees were subjected to during transport on subsequent introduction and early performance success rates were examined.

The Project contained a number of objectives carried out during Year 2 –

(6) To determine relationships between position of the queen cell on the cell bar during larval feeding and – the number of sperm in the queen`s spermatheca, diameter of the spermatheca, queen weight, queen survival following introduction of those queens, and the number of ovarioles in one ovary.

(7) To determine whether storage by freezing whole queen bees resulted in physical damage eg. fragmentation, of spermatozoa stored in the spermathecae of queens when sperm counts were carried out.

(8) To obtain data on sperm counts of a sample of mature age drones collected from drone mother hives at the time queens used in the project were being mated.

(9) To compare the number of sperm in the spermathecae of sister queen bees mated in (a) a commercial queen bee breeder`s mating apiary, and (b) a commercial honey producer`s queen mating apiary

(10) To compare sperm counts present in queen bees examined at 7 and 14 days of age with sperm counts of their sister queens introduced into honey hives at 7 or 14 days of age and caught from those hives 15 weeks after introduction.

(11) To develop a method for storing adult drone bees which would allow the number of sperm produced by each drone to be determined 7 days after storage.

3. Methodology

Year 1 (1999)

Objectives (1), (2), (3), (4) and (5) were examined.

Arrangements were made with a recognised Queensland based commercial queen bee breeder to produce 300 queen bees to be grafted on the same day and to be mated in the same mating apiary at the same time. Two sister *A m ligustica* breeder queens provided sufficient larvae.

Mating nuclei were ten frame, full depth brood chambers divided into three by three frame nuclei. Based on available information (Rhodes 1999 a, b), the mating apiary was encircled by three drone mother apiaries, each apiary contained 20 hives which had been managed to provide mature age drones at the time the test queen bees were expected to be mating. The three drone mother apiaries were placed 2.3, 1.5, and 1.0 km from the mating apiary.

Objective 1

At 7 days after the expected date of emergence, 60 queen bees were caught, marked on the thorax with a Posca water-based poster coloured marking pen, and placed in mailing cages for dispatch. At 14 days after emergence, all remaining test queen bees in the mating apiary were marked on the thorax with a Posca marking pen. As well, one forewing of each queen was clipped to ensure that test queens would be able to be identified during the course of the project.

Sixty queen bees were caught from the mating apiary each week when the queens were estimated to be 7, 14, 21, 28 and 35 days of age. Twenty queen bees were sent to a commercial honey producer in south eastern NSW (Apiary A), 20 to a commercial honey producer in central western NSW (Apiary B), and 20 were sent to the CSIRO Entomology Department Laboratory, Canberra.

Queen bees were subjected to the normal management practices used by each honey producer when receiving and introducing queen bees – in Apiary A queen bees were introduced in mailing cages with the escort bees present, in Apiary B the queen bees were introduced in Miller cages without escort bees. Apiaries used in the project contained 100 hives or more which, in the opinion of the owner, required requeening.

All hives containing marked test queens were inspected 14 days after introduction and the presence or absence of the marked queen recorded (Introduction Success).

All hives containing a marked queen 14 days after introduction were inspected a second time 15 weeks after the date of queen introduction and the presence or absence of the marked queen recorded (Early Performance Success). At the same time it was recorded whether the hive was “Satisfactory” or “Not Satisfactory” based on the performance of the queen bee under the conditions the apiary was being inspected (Satisfactory Performance).

The third envelope each week, containing 20 queen bees, was mailed to Dr. D. Anderson, CSIRO Entomology Department, Canberra, for laboratory examinations of the test queen bees.

On the day each envelope of queen bees arrived at the laboratory, each queen was coded for identification, narcotised with carbon dioxide, and its head was removed and placed into 200 µl of dichloromethane and passed on to Dr. M. Lacey for pheromone analysis. The remaining part of the body of each queen was then stored at -20°C until needed for determinations of ovariole and spermatozoa numbers and levels of Nosema disease.

The code used to identify each queen was used by both Drs. Anderson and Lacey so that data obtained for each queen from the two laboratories could be compared.

Objective 2

To detemine the number of spermatozoa, the spermatheca of each queen was removed at the same time the ovarioles were removed. The spermatheca was placed in 100 µl of phosphate buffered saline in a small Eppendorf tube, mashed, and then diluted 1:80 or 1:160 in distilled water. The spermatozoa were then counted using a haemocytometer at x160 magnifications with the aid of a light microscope (Anderson 1999).

Objective 3

The heads of the 20 queen bees from each of the 5 groups were examined by Dr. M. Lacey, CSIRO Entomology Department, Canberra, for pheromone presence and level. Methodology used by Dr. Lacey involved each dissected head being placed individually into dichloromethane (0.2 ml) within a screw-capped vial (Alltech, 12x32 mm). Pentadecanoic acid was added to each vial as the internal standard. The heads were extracted with agitation for 1.5 h at room temperature (22°C), after which period the heads were removed from the solvent and amalgamated for a second extraction in fresh dichloromethane (1 ml, 1.5 h). The second extract was used for validating the identity of known components by gas chromatography/mass spectrometry and for elucidating the molecular structures of unknown components.

Each of the individual first extracts in dichloromethane was gently evaporated in an updraught of nitrogen in subdued light to ca. 25 µl, a strategy that diminishes potential losses of small quantities of the more volatile solutes (Harris et al. 1987). The final stages of evaporation were carried out within a glass insert (Alltech, 100 µl).

To make the pheromone extracts from *A mellifera* more amenable to analysis by gas chromatography, the hydroxyl and carboxyl groups of the chemical components are generally derivatised at room temperature. However, silylation of alcohol functional groups with BSTFA (Slessor et al. 1990) is slow at room temperature and may be incomplete before analysis. Conversely, the conditions for esterification with diazomethane (Engels et al. 1997) may also allow partial methylation of the phenolic groups. To avoid the complication of multiple derivatives for the present analyses, a trace of pyridine was added to catalyse the persilylations. To the concentrated individual pheromone extract containing the internal standard was added BSTFA (Aldrich, 10 µl) and dry pyridine (2 µl) and the solution was left for 15 min at room temperature after vortex mixing before storage at -20°C for analysis.

Glycerides were also found in the solvent extracts and the fatty acid moieties of the glycerides in an extract were determined by transesterification. After completion of the pheromone analysis, the silyated extract was evaporated to dryness and dissolved in methanol (50 µl) containing sulphuric acid (2%). The solution was heated to 90°C for 1 hour and the resulting methyl esters (including methyl pentadecanoate) extracted with hexane (50 µl) and washed with water (20 µl) before gas chromatographic analysis.

Gas chromatography was carried out using a Varian model 3300 with a cool on-column injector, a flame-ionisation detector and a computer with data acquisition, plotting and analysis (DAPA) software. The column was a bonded phase methyl silicone (Econocap, SE-30), 30 m x 0.32 mm ID with a phase thickness of 0.25 micrometres and a helium carrier gas flow rate of 2 ml/min. The column was preceded by a retention gap (fore-column) of deactivated silica (2 m).

At the beginning of a series of analyses, the retention gap was back-flushed successively with methanol and dichloromethane in order to remove accumulated glycerides and other involatiles and to preserve the chromatographic high-resolution of the column. The temperature program used was 40°C

for 2 min followed by a temperature gradient of 20°C/min to 150°C; a temperature gradient of 5°C/min to 215°C; and finally a temperature gradient of 25°C/min to 300°C isothermally for 10 min, sufficient to elute the remaining volatile components (Lacey 1999).

Objective 4

Ovarioles - to determine the number of ovarioles, each queen was removed from -20°C, thawed, and its ovaries dissected in distilled water. The number of ovarioles in the right and left ovaries were then counted with the aid of a dissecting microscope. To assist in distinguishing and counting the ovarioles, each ovary was first placed for 30 seconds in electrophoresis gel stain (0.1% Coomassie brilliant blue R250 in 45% methanol plus 10% acetic acid), then placed in 35% ethanol and examined.

This procedure stained the linings of each ovariole blue, but left the eggs inside white. To count the ovarioles, a transverse section was cut from the middle of each ovariole and placed in a small watch glass containing 35% ethanol. The number of ovarioles were then carefully counted by teasing the ovarioles apart and moving each ovariole to the side of the watch glass as it was counted (Anderson 1999).

Nosema disease – to determine the levels of Nosema disease, *N apis,* the mid and hind gut of each queen were removed at the same time the ovaries and spermatheca were removed. The gut material was placed in 0.5 ml distilled water and examined for *N apis* spores at x400 magnifications with the aid of a light microscope (Anderson 1999).

Objective 5

The 60 queen bees caught each week were caged with escort bees in wooden queen mailing cages 8x3x2 cm with either a wire gauze or a punched plastic cover. Groups of 20 cages were selected at random and placed into plastic Australia Post Express Post Envelopes, 35x20 cm, and containing 58-60 air holes 14 mm in diameter around three sides of the envelope.

The packages mailed to each of the two honey producers contained a Tinytag Ultra Datalogger which recorded temperature and humidity within the envelope at 10 minute intervals between the period when the envelope was sealed by the queen bee breeder and it was opened by the honey producer.

Year 2 (2000)

Objectives (1), (2), (4), (5), (6), (7), (8), (9), (10) and (11) were examined.

The same Queensland based commercial queen bee breeder supplying queen bees to the Year 1 project supplied queens to the Year 2 project. Fifty mature queen cells and 370 queen bees grafted from the same *A m ligustica* breeder queen mother on the same day, reared in the same cell raising apiary, and mated in the same mating apiary at the same time were supplied. Based on available data, sufficient drone mother colonies were provided around the mating apiary as for Year 1. The same NSW based commercial honey producers providing Apiaries A and B to the Year 1 project also provided apiaries to the Year 2 project.

Objective 1

Introduction Success, Early Performance Success and Satisfactory Performance Success field experiments were carried out as for Year 1 with some modifications.

(i) Queens were marked with 5 colours corresponding to their position on the cell bar during the larval feeding period. (Objective 6). Each consignment of 20 queens comprised 4 queens of each of the 5 colours.

(ii) At 7 days of age. 60 queens were caught, marked, and 20 per envelope were dispatched to Beekeeper A, Beekeeper B, and to Dr. D. Anderson.

(iii) At 14 days of age. The remaining 310 test queens were caught and marked, 60 of these queens had both wings clipped (clipped queens) to prevent them from flying (Objective 2).
60 unclipped queens were caught, caged and dispatched as for the 7 day old queens. The remaining 250 queens were returned to their mating nucleus.

(iv) At 21 days of age. 90 queens were caught. Twenty unclipped queens were sent to Apiary A and 20 to Apiary B. Twenty clipped and 20 unclipped queens were sent to Dr. D. Anderson. An extra 10 unclipped queens were sent to Dr. D. Anderson for sperm fragmentation experiments (Objective 7).

(v) At 28 days of age 80 queens were caught. Twenty unclipped queens were sent to Apiary A and 20 to Apiary B. Twenty clipped and 20 unclipped queens were sent to Dr. D. Anderson.

(vi) At 35 days of age. 80 queens were caught. Distribution was the same for 28 day old queens.

The disease status of honey production hives used in the field experiments was recorded. For hives used on each day queen bees were introduced, the following data were recorded –

- Nosema disease — 4 adult bees collected from the top bars of middle brood frames to provide one bulk sample/apiary

- EFB, Sacbrood, Chalkbrood — Brood examined visually and disease presence recorded on the basis of –
  O – no disease observed
  L – light infection, <1% brood infected
  M – medium infection, 1-10% brood infected
  H – heavy infection, 10-25% brood infected

Objectives 2 and 4, examinations were carried out at the laboratory of Dr. D. Anderson, CSIRO Entomology, Canberra. Twenty queen bees with unclipped wings were caught at 7, 14, 21, 28 and 35 days of age (total 100 queens), and 20 queens with wings clipped at 14 days of age and caught at 21, 28 and 35 days of age (total 60 queens) from their mating nucleus.

Objective 2

Methodology as for Year 1. Sperm counts of spermathecae of queen bees were carried out to identify relationships between sperm numbers in a queen`s spermatheca and (i) increasing age of the queen, (ii) inability of the queen to continue to fly after 14 days of age.

Objective 4

(i) Diameter of spermatheca – the spermatheca was removed and measured with a gradicule using a WILD M3C dissecting microscope at x40 magnifications. Data were converted to mm using the conversion factor –
1 eyepiece unit @ x40 magnifications = 0.23809 mm. Measurement was carried out on the horizontal plane (Medveczky K. 2001, pers. com.).

(ii) Presence or absence of spermatozoa in a queen`s oviducts – oviducts were dissected out, homogenised in 100 µl water and another 100 µl water added. The sample was then examined on a haemocytometer for the presence or absence of sperm using phase contrast. When sperm were present, sperm numbers were counted, average of 10 fields, at x160 magnifications. (Medveczky K. 2001, pers.com.).

(iii) Number of ovarioles in one ovary – methodology as for Year 1.

(iv) Weight of each queen – queens were weighed on an AND HR-200 electronic analytical scale. (Medveczky K. 2001, pers.com.).

(v) Nosema disease status of each queen – methodology as for Year 1.

Objective 5

Transport effects on queen survival – methodology as for Year 1.

Objective 6

The position of the queen cell cup on the cell bar in cell rearing colonies during the critical larval feeding period was examined for effects on – (i) queen bee survival following introduction into honey hives, (ii) number of sperm in the queen`s spermatheca, (iii) spermatheca diameter, (iv) queen weight, and (v) number of ovarioles in the left ovary.

At grafting, plastic cell cups of five colours were used. The day following grafting, cell cups containing an accepted larva were arranged with 2 cell cups of the same colour together, in the following order from one end of the cell bar – red, green, dark grey, maroon, light grey, light grey, maroon, dark grey, green and red, to provide 20 cell cups on each bar.

Cell cups were placed randomly, by colour, in mating nuclei in the mating apiary. When queens were caught and marked, the marking colour used on each queen corresponded to the colour of the cell cup from which she had emerged. The colour code remained with each queen for those placed in field trials and for those examined in the laboratory.

Objective 7

An experiment was carried out by Dr. D. Anderson, CSIRO, Canberra, to establish whether storage of whole queen bodies by freezing resulted in fragmentation of spermatozoa present in the spermathecae of frozen queen bees. Methodology –

- 4 sister queen bees used

- On arrival at the laboratory (live condition), the spermatheca was removed from one queen and the number of spermatozoa in a defined area examined in the fresh state. The numbers of whole and damaged spermatozoa were recorded.

- The remaining 3 queen bees were stored by freezing at -20°C.

- Similar counts were carried out to record whole and damaged spermatozoa on one frozen queen bee at intervals of 1, 2 and 3 months after being frozen.

- Examination equipment used was a Cryo-Scanning microscope, JOEL SM 6400 fitted to a biorad cryostage at magnifications ranging between x600 and x4000.

Objective 8

An experiment was carried out to obtain data on spermatozoa counts of mature age drones caught at the estimated peak seasonal conditions to provide initial data on this subject. Methodology –

- Drone mother colonies placed around the mating apiary were examined 20 days before the test queen bees were expected to commence mating (at 7 days of age), and newly emerged drones were marked on the thorax with a Posca marking pen.

- 120 marked drones were caught at 20 days of age and transported by car to Ms. G. Wheen, Richmond, NSW. Spermatozoa counts were carried out on 100 drones over the following 4 days.

Objective 9

An experiment was carried out to compare sperm counts from the spermathecae of sister queen bees mated in a commercial queen bee breeder`s apiary and queens mated in a mating apiary used by a commercial honey producer. Methodology –

- 50 extra cells produced by the queen bee breeder supplying queen bees to this project were packaged in warm sawdust in a small polystyrene esky and forwarded by overnight bus service a distance of approx. 1000 km to the owner of Apiary B.

- The 50 cells were distributed in a queen bee mating apiary operated by the owner of Apiary B on the same day that the Queensland based commercial queen bee breeder distributed the sister queen cells in his queen mating apiary.

- Queen bees in the honey producer`s mating apiary were caught at 21 days of age and forwarded to Dr. D. Anderson`s laboratory where sperm counts were carried out.

- Data on sperm counts from the 50 cells mated in the honey producer`s mating apiary were compared to sperm counts obtained from queen bees caught in the queen bee breeder`s mating apiary at 21 days of age.

Objective 10

An experiment was carried out to compare sperm counts of queen bees caught from mating nuclei at 7 and 14 days of age with sperm counts from sister queens introduced into honey hives at 7 and 14 days of age and then caught from the honey hives 15 weeks after introduction. Methodology –

- For Apiary B. All of the queen bees introduced into honey hives at 7 and 14 days of age which were present in the hives at the 15 week inspection were caught. Sperm counts were carried out on those queens at the laboratory of Dr. D. Anderson.

- Sperm counts from the two groups of queens caught 15 weeks after introduction into honey hives were compared with sperm counts from their sister queens obtained after they had been caught and examined at 7 and 14 days of age respectively.

Objective 11

An experiment was carried out to develop a method for storing whole, dead, adult drones for a small number of days before carrying out sperm counts.

Mature, adult drones were caught and stored either (i) in an insect preservative 4% glutaraldehyde (equal volumes of 8% glutaraldehyde and phosphate buffer, ph 7.2), or (ii) by freezing at -4°C. At 7 days after treatment, attempts were made to evert drones, collect semen, and carry out sperm counts.

Year 3 (2001)

Objectives (1), (2), (4) and (5) were examined.

The Year 3 experiments carried out field and laboratory comparisons between queen bees which had been maintained either in a mating nucleus or in a queen bank prior to their introduction into honey hives.

Persons providing queen bees, apiaries and laboratory examinations for the Years 1 and 2 experiments also provided similar services for the Year 3 experiment.

The commercial queen bee breeder provided 300 queen bees which had been grafted on the same day from the same *A m ligustica* AI breeder queen, reared in the same cell rearing apiary, and mated in the same mating apiary at the same time. Based on available information, sufficient drone mother colonies were established to provide drones for the mating apiary.

Objective 1

Introduction, Early Performance and Satisfactory Performance Success.
Methodology was modified for Year 3 compared to Years 1 and 2.

Queen marking and distribution -

- At 17 days of age.
  - all queen bees were marked on the thorax with a coloured Posca pen. Two colours were used, (a) 180 queen bees marked colour A, to be supplied directly to the project from their mating nucleus, and (b) 120 queen bees marked colour B, to be banked at 17 days of age and supplied to the project from the queen bank.

  - 60 queens marked colour A caught. Twenty queens sent to Apiary A, Apiary B, and to the CSIRO laboratory (total 60 queens).

- At 24 days of age.
  - 20 queens from the mating nuclei and 20 queens from the queen bank were sent to Apiaries A and B, and to the CSIRO laboratories (total 120 queens).

- At 31 days of age.
  - numbers and distribution of queens the same as for 24 days (total 120 queens).

- Nosema disease.
  - one bulk sample was colleted from the mating apiary at the time of queen marking. The queen bank was sampled 3 times – when it was established, and 7 days and 14 days after establishment.

- Queen bank management followed recommendations in Kleinschmidt G K (undated).

Field Evaluation

- Queen bees sent to Apiaries A and B were subjected to inspections for the number of queens surviving 14 days after introduction (Introduction Success Rate), number surviving 15 weeks

after introduction (Early Performance Success Rate), and a field evaluation of performance carried out at the 15th week inspection (Satisfactory Performance Success).

- Nosema disease. At each of the three queen introduction dates at each apiary, a bulk sample of worker bees was collected from hives which had test queens introduced into them.

- Brood diseases. Hives displaying zero, or low incidence (<10 cells/colony) of EFB, Sacbrood and Chalkbrood disease were selected for use in the experiment.

Objective 2

Number of Spermatozoa in the Spermatheca. Methodology – same as for Year 1.

Objective 4

Physical Characteristics Measured to Determine Queen Quality.

Queen bees were examined for – number of ovarioles in the left ovary, spermatheca diameter, presence or absence of sperm in the oviducts, queen weight, and *N apis* levels. Methodology – same as for Year 2.

Objective 5

To Examine Transport Effects on Queen Survival. Methodology – same as for Year 1.

4. Results

Objective 1 – Effects of queen age on - introduction, early performance and satisfactory performance success

Introduction Success

Introduction Success – measured by the number of introduced queen bees present in hives 14 days after introduction into commercial honey hives. Results are shown in Table 1.

Table 1. Introduction success. The number of queen bees present in hives 14 days after introduction at each apiary for each of the age groups queen bees were caught from their mating nucleus or queen bank (n=20).

Date of introduction	Age of queen when caught (days)	Number of queen bees present 14 days after introduction			
		Apiary A	Apiary B	Total/40	% #
1999					
10.11	7 n	2*	4	6**	15.4 a
17.11	14 n	10	9	19	47.5 b
24.11	21 n	15	19	34	85.0 c
1.12	28 n	18	16	34	85.0 c
8.12	35 n	20	16	36	90.0 c
2000					
17.11	7 n	5	6*	11**	28.2 a
23.11	14 n	15	13	28	70.0 b
30.11	21 n	15	17	32	80.0 bc
7.12	28 n	18*	19	37**	94.9 c
14.12	35 n	18	20	38	95.0 c
2001					
23.11	17 n	15	11*	26**	66.7 a
29.11	24 n	18	19	37	92.5 b
29.11	24 b	13	14	27	67.5 a
6.12	31 n	17	18	35	87.5 ab
6.12	31 b	18	18	36	90.0 ab

\# groups with no letters in common are significantly different, $P<0.05$
* /19
** /39
n caught from mating nucleus
b caught from queen bank

There was no significant difference between survival rates 14 days after introduction for each age group for queen bees supplied to Apiary A and Apiary B for 1999, 2000 and 2001.

For 1999 there was a significant difference, $P<0.05$, between survival rates of queen bees introduced at 7 and 14 days compared to 21, 28 and 35 days.

For 2000, survival rates were improved for all ages compared to 1999 data. There was a significant difference, $P<0.05$, for queens introduced at 28 and 35 days old.

For 2001, there was a significant difference, $P<0.05$, for 24 day old queens caught from mating nuclei and for 31 day old queens caught from mating nuclei and from a queen bank compared to 17 day old queens caught from mating nuclei and 24 day old queens caught from a queen bank.

Early Performance Success

Early Performance Success – measured by the number of queen bees present in hives 15 weeks after introduction into commercial honey hives. Results are shown in Table 2.

Table 2. Early performance success. The number of queen bees present in hives 15 weeks after introduction at each apiary for each of the age groups queen bees were caught from their mating nucleus or queen bank (n=20).

Date of introduction	Age of queen when caught (days)	Number of queen bees present 15 weeks after introduction			
		Apiary A	Apiary B	Total/40	% #
1999					
10.11	7 n	1*	3	4**	10.3 a
17.11	14 n	3	3	6	15.0 a
24.11	21 n	9	16	25	62.5 b
1.12	28 n	11	13	24	60.0 b
8.12	35 n	14	15	29	72.5 b
2000					
17.11	7 n	4	6*	10**	25.6 a
23.11	14 n	15	13	28	70.0 b
30.11	21 n	14	15	29	72.5 b
7.12	28 n	18*	16	34**	87.2 b
14.12	35 n	16	19	35	87.5 b
2001					
23.11	17 n	15	11*	26**	66.7 a
29.11	24 n	16	19	35	87.5 a
29.11	24 b	13	14	27	67.5 a
6.12	31 n	15	17	32	80.0 a
6.12	31 b	17	16	33	82.5 a

\# groups with no letters in common are significantly different, $P<0.05$
* /19
** /39
n caught from mating nucleus
b caught from queen bank

There was no significant difference for queen bee survival rates 15 weeks after introduction for each age group of queens supplied to Apiary A and Apiary B for 1999, 2000 and 2001.

For 1999, there was a significant difference, $P<0.05$, for queen bees introduced at 7 and 14 days of age compared to 21, 28 and 35 day old queens.

For 2000, there was a significant difference, $P<0.05$, in queen survival rates for queens introduced at 14, 21, 28 and 35 days of age compared to queens introduced at 7 days.

For 2001, there was no significant difference between survival rates for queen bees introduced at 17, 24 or 31 days of age from mating nuclei or a queen bank..

Satisfactory Performance Success

Satisfactory Performance Success – was measured by the number of introduced queen bees present at the 15 week inspection displaying satisfactory performance based on the performance of the queen bee under the conditions the apiary was being inspected. Results are shown in Table 3.

Table 3. Satisfactory performance success. The number of queen bees present at the 15 week inspection displaying satisfactory performance for each of the age groups queens were caught from their mating nucleus or queen bank (n=20).

Date of introduction	Age of queen when caught (days)	Number of queen bees present 15 weeks after introduction and displaying satisfactory performance			
		Apiary A	Apiary B	Total/40	% #
1999					
10.11	7 n	1*	3	4**	10.3 a
17.11	14 n	2	3	5	12.5 a
24.11	21 n	7	15	22	55.0 b
1.12	28 n	11	12	23	57.5 b
8.12	35 n	14	12	26	65.0 b
2000					
17.11	7 n	1	2*	3**	7.7 a
23.11	14 n	14	10	24	60.0 b
30.11	21 n	14	13	27	67.5 bc
7.12	28 n	18*	16	34**	87.2 c
14.12	35 n	16	19	35	87.5 c
2001					
23.11	17 n	15	10*	25**	64.1 a
29.11	24 n	16	19	35	87.5 a
29.11	24 b	13	13	26	65.0 a
6.12	31 n	15	17	32	80.0 a
6.12	31 b	17	15	32	80.0 a

\# groups with no letters in common are significantly different, $P<0.05$
* /19
** /39
n caught from mating nucleus
b caught from queen bank

There was no significant difference between queen bee performance success 15 weeks after introduction for each age group for queens supplied to Apiary A and Apiary B for 1999, 2000 and 2001.

For 1999, there was a significant difference, $P<0.05$, for satisfactory performance by queen bees introduced at 21, 28 and 35 days of age compared to queens introduced at 7 and 14 days of age.

For 2000, there was a significant difference, $P<0.05$, between the number of queen bees displaying satisfactory performance for queens introduced at 7 days and those introduced at 14, 21, 28 and 35 days of age, and no significant difference between queens introduced at 14 and 21 days of age.

For 2001, there was no significant difference in the number of queen bees displaying satisfactory performance for those introduced at 17, 24 or 31 days of age and introduced from mating nuclei or a queen bank.

Objective 2 – Number of spermatozoa in the spermatheca of queen bees

Sister queen bees corresponding to each of the age groups queen bees were caught for field trials for Years 1, 2 and 3 were examined in a laboratory and the number of spermatozoa in their spermathecae determined. Results are shown in Table 4.

Table 4. The average number and range of spermatozoa present in the spermathecae of queen bees for each of the age groups queen bees were caught from their mating nucleus or queen bank (20 queen bees/sample).

Age of queen when caught (days)	**1999**	**2000**	**2001**
	Number of spermatozoa 10^6 av.(range)	**Number of spermatozoa 10^6 av.(range)**	**Number of spermatozoa 10^6 av.(range)**
7 n	0.34 (0.00-1.31)	0.06 (0.00-0.53)	
14 n	2.33 (0.78-3.56)	1.60 (0.31-7.25)	
17 n			3.21 (2.07-4.16)
21 n	3.51 (1.84-5.78)	1.94 (0.26-3.10)	
24 n			2.80 (1.40-5.07)
24 b			4.11 (2.03-6.75)
28 n	5.21 (1.49-9.32)	1.44 (0.39-2.84)	
31 n			3.20 (0.53-7.28)
31 b			3.10 (0.90-6.20)
35 n	3.96 (1.82-6.02)	1.52 (0.25-3.63)	

n caught from mating nucleus
b caught from queen bank

For 1999, there was a significant difference, $P<0.05$, between the number of sperm in the spermathecae of 7 day old queens compared to 14, 21, 28 and 35 day old queens; 14 day old compared to 21, 28 and 35 day old queens; and no significant difference between 28 and 35 day old queens.

For 2000, there was a significant difference, P<0.05, between the number of sperm in the spermathecae of 7 day old queens compared to 14, 21, 28 and 35 day old queens.

For 2001, there was a significant difference, P<0.05, between the number of sperm in the spermathecae of 24(queen bank) day old queens and 17(nucleus), 24(nucleus), 31(nucleus), 31(queen bank) day old queens.

For Year 2000, for each age group examined, 20 queen bees had their wings clipped at 14 days of age and 20 did not have their wings clipped. Results of sperm numbers in the spermathecae of clipped and unclipped queen bees for the age groups sperm counts were carried out are shown in Table 5.

Table 5. The average number and range of spermatozoa present in the spermathecae of queen bees caught from their mating nucleus for queens with their wings clipped at 14 days of age and queens with unclipped wings.

Age of queen *when caught* **(days)**	**2000**	
	Number of spermatozoa 10^6 **av. (range)#**	
	Wings not clipped	*Wings clipped*
21	1.94 (0.26-3.10)	2.14 (0.04-4.73)
28	1.44 (0.39-2.84)	2.16 (0.15-4.20)
35	1.52 (0.25-3.63)	2.45 (1.13-3.83)

a difference of >0.56 is needed for a significant difference between pairs of results, P<0.05

The percentages of queen bees aged between 14 and 35 days which contained –

(i) <3 million sperm/queen were – 42.5% (1999); 81.4% (2000); and 46% (2001).
(ii) 3-4.5 million sperm/queen were – 23.8% (1999); 16.4% (2000); and 33% (2001).
(iii) >4.5 million sperm/queen were – 33.7 (1999); 2.2% (2000); and 21% (2001).

the age queen bees commence to mate. Queen bees caught from their mating nuclei at 7 days of age and examined for sperm in their spermathecae resulted in 40% (1999) and 15% (2000) of queen bees with sperm. For 1999 and 2000, 100% of 14 day old queen bees examined contained sperm in their spermathecae. This indicates that queen bees commenced mating at an age just prior to 7 days old with the majority commencing at an age older than 7 days.

Objective 3 – Pheromones present in the mandibular and head glands of queen bees

The effects of queen age on mandibular and head gland pheromone production of queen bees caught at 7, 14, 21, 28 and 35 days of age were determined for the 1999 project. Ten pheromones identified in amounts >0.05µgm/queen are shown in Table 6.

Table 6. Pheromones identified from the mandibular and head glands of queen honey bees aged 7-35 days of age.

MHB	methyl 4 - hydroxybenzoate
7-HO	7- hydroxyoctanoic acid
8-HO	8- hydroxyoctanoic acid
9-ODA	9-oxo-2 (E) - decenoic acid
HVA	4- hydroxy - 3- methoxyphenethanol
9-HDAA	(R, S) - 9- hydroxydecanoic acid
9-HDA	(R, S) - 9- hydroxy - 2(E) - decenoic acid
10-HDAA	10 - hydroxydecanoic acid
10-HDA	10- hydroxy - 2- (E) - decenoic acid
HFA	4- hydroxy - 3 methoxybenzene - propanoic acid

The level of each pheromone produced by each queen varied between queen bees in the same age group. The average level of each pheromone produced by each age group varied between the five age groups of queen bees examined. The means ± s.d. of pheromone levels recorded for each pheromone for each age group are shown in Table 7.

Table 7. Means ± s.d. levels for the ten pheromones at each of the five age groups of queen bees examined (20 queen bees/sample).

Pheromone	**Pheromone level mean ± s.d. µgm/queen**				
	Age of queen (days)				
	7	**14**	**21**	**28**	**35**
MHB	0.04 ± 0.05	0.20 ± 0.16	3.21 ± 2.15	2.38 ±1.99	1.52 ± 1.40
7-HO	0.15 ± 0.11	0.08 ± 0.07	0.52 ± 0.21	0.91 ± 0.37	0.58 ± 0.26
8-HO	1.99 ± 0.86	0.94 ± 0.72	6.52 ± 3.02	10.56 ± 4.10	7.84 ± 3.24
9-ODA	40.48 ± 15.13	12.63 ± 8.16	72.20 ± 26.19	57.43 ± 27.25	32.57 ± 13.32
HVA	0.03 ± 0.06	1.08 ± 1.07	7.01 ± 2.55	8.10 ± 4.28	7.40 ± 3.70
9-HDAA	0.43 ± 0.14	0.25 ± 0.13	0.80 ± 0.39	1.07 ± 0.33	0.63 ± 0.22
9-HDA	8.81 ± 2.46	2.90 ± 2.04	25.85 ± 12.78	42.27 ± 14.49	17.07 ± 7.12
10-HDAA	0.34 ± 0.26	0.27 ± 0.36	2.28 ± 0.95	2.64 ± 1.28	0.97 ± 0.42
10-HDA	9.14 ± 3.95	1.93 ± 2.40	15.84 ± 8.52	13.22 ± 6.54	4.05 ± 2.03
HFA	0.13 ±0.13	0.49 ± 0.66	3.75 ± 2.35	2.64 ± 1.78	1.55 ± 0.89

With the exceptions of HVA, HFA and MHB, mean pheromone levels for queen bees from each age group were at a low level for 7 day old queens, reduced to a minimum level at 14 days, increased to peak at either 21 or 28 days, and began to reduce for 35 day old queens. HVA and HFA increased

between days 7 and 14 with HVA continuing to increase up to 28 days with a slight reduction at 35 days, HFA and MHB peaked at day 21.

9-ODA was the dominant pheromone being present in larger quantities than any other pheromone. 9-HDA and 10-HDA were present in quantities larger than the remaining 7 pheromones.

A correlation was found between HVA and sperm counts of 0.53 which was the largest correlation for pheromones and sperm counts. The highest positive correlation between a pheromone and queen survival was between HVA and queen survival.

Objective 4 – Physical characteristics and disease status of queen bees and disease status of hive bees

(i) Number of ovarioles

The number of ovarioles was counted from queen bees from each age group queens were caught. For the 1999 project, the number of ovarioles present in both the left and the right ovaries were counted, there was no significant difference between the number of ovarioles in the left ovary compared to the number in the right ovary (Table 8). For the 2000 and 2001 projects, the number of ovarioles in the left ovary only was counted. Results are shown in Table 8.

Table 8. The range and average number of ovarioles present in queen bees from each of the age groups queen bees were examined for the 1999, 2000 and 2001 projects. (20 queen bees/sample)

Age of queen when examined (days) *	Number of ovarioles/ovary av. (range) #				
	1999			2000	2001
	Left ovary	Right ovary	Av. left and right	Left ovary	Left ovary
7 n	158.3 (139-178)	153.0 (126-173)	155.6 a (126-178)	163.5 (147-188)	
14 n	176.5 (155-194)	172.0 (154-189)	174.2 b (154-194)	175.0 (153-197)	
17 n					171.7 (153-194)
21 n	172.1 (157-194)	171.1 (158-183)	171.6 bc (157-194)	173.4 (138-213)	
24 n					171.0 (120-207)
24 b					154.7 (120-175)
28 n	179.9 (161-202)	179.2 (159-201)	179.7 bd (159-202)	169.2 (145-194)	
31 n					143.2 (120-172)
31 b					149.9 (125-177)
35 n	168.5 (151-192)	167.5 (143-180)	168.0 be (143-180)	171.0 (147-192)	

for letters different from each other, $P<0.05$

* n caught from mating nucleus b caught from queen bank

(ii) Queen weight, spermatheca diameter, and presence or absence of semen in the oviducts

For Years 2000 and 2001 projects, for each age group queen bees were caught, data were collected for each queen bee on queen weight, diameter of the spermatheca, and oviducts examined for the presence or absence of semen. Results are shown in Table 9.

Table 9. The means±s.e for queen weight, spermatheca diameter, and the presence or absence of semen in the oviducts of queen bees for each of the ages queen bees were caught for the 2000 and 2001 projects. (number of queen bees/sample = 20).

Queen age (days) *	**Queen weight g mean±s.e.#**		**Spermatheca diameter mm mean±s.e.#**		**Number of queen bees with semen present in oviducts**	
	2000	**2001**	**2000**	**2001**	**2000**	**2001**
7n	0.173a ±0.003		1.377b ±0.024		2/20	
14n	0.214b ±0.003		1.364b ±0.024		2/20	
17n		0.220b ±0.003		1.235a ±0.017		8/20
21n	0.226b ±0.002		1.374b ±0.013		4/40	
24n		0.212b ±0.003		1.285b ±0.017		3/20
24b		0.197a ±0.003		1.299b ±0.017		1/20
28n	0.221b ±0.002		1.323a ±0.017		4/40	
31n		0.214b ±0.003		1.262ab ±0.017		2/20
31b		0.200a ±0.003		1.238a ±0.017		4/20
35n	0.218b ±0.002		1.380b ±0.017		0/40	

for each year, groups with no letters in common are significantly different, $P<0.05$
* n caught from mating nucleus b caught from queen bank

Queen weight – queen age was significant for 7 day old queens, banked queens were significantly lighter in weight than queens caught from mating nuclei.
Spermatheca diameter – significant differences ,$P<0.05$, occurred between groups of queens examined at different ages.

(iii) Nosema disease

Queen bees – all queen bees forwarded for laboratory examination were examined for the presence of *N apis* spores. Test queen bees were caught from their mating nucleus or queen bank at each of the ages queens were caught for the 1999, 2000 and 2001 projects.

1999 project – 0/100 queen bees identified with *N apis* spores,
2000 project – 3/160 queen bees contained *N apis* spores, all were 28 day old queens caught from their mating nuclei, spore numbers averaged 1.66 x10^6, range 1.16-3.82 x10^6 .
2001 project – 0/100 queen bees identified with *N apis* spores.

Honey and Queen Rearing Hives

Data on Nosema prevalence in honey hives queen bees were introduced into and queen bee production hives for the years data were recorded are shown in Table 10.

Table 10. Nosema disease *N apis* spore counts from honey hives at queen introduction and queen rearing hives at time of queen production for the years shown.

Honey production hives	**Queen bee production hives**	***Nosema apis* spores/bee x10^6 average(range)**	
		2000	**2001**
Apiary A		0.39(0.05-0.90)	<0.05
Apiary B		0.42(0.10-1.35)	0.13(0.10-0.15)
	Mating nuclei		0.88(0.55-1.20)
	Queen bank – at establishment		<0.05
	after 7 days		1.70
	after 14 days		0.15

(iv) Brood diseases

Honey hives

The disease status of commercial honey hives receiving test queen bees was assessed at the date queen bees were being introduced. Based on visual inspection, in 1999 hives used were free from or contained <5 cells of EFB, Chalkbrood and Sacbrood. For the 2000 and 2001 projects, disease prevalence was recorded. A summary of the brood disease status of hives used in 2000 is shown in Table 11. For the 2001 project, Chalkbrood was recorded in two colonies (1Light infection and 1Medium infection in Apiary A) during the introduction of 17 day old queens.

Table 11. Brood disease status of honey production hives used for queen bee introductions in the Year 2000 project

Queen age (days)	**Apiary A No. hives with infection /20**			**Apiary B No. hives with infection /20**		
	EFB	**C`brood**	**Sacbrood**	**EFB**	**C`brood**	**Sacbrood**
7	0	0	0	3L	0	0
14	0	3L	1L	3L	2L	0
21	0	1L, 1M	2L	0	3L	0
28	0	1M	0	0	0	0
35	0	6L	1L	0	0	0

0 No infection observed
L Light, <1% brood infected
M Medium, 1-10% brood infected

(v) Other factors

Climate, foraging and general hive conditions during the production of test queens and for both commercial honey production apiaries for the duration of the 1999, 2000 and 2001 projects were average to good. 1999 provided the best overall foraging conditions.

Objective 5 – Transport effects on queen bee survival and performance

Factors related to the transport of queen bees which were able to be recorded and which would be expected to express their effects in terms of damage to the queen as a result of the transport process were the time in transit and exposure to a range of temperatures and humidities.

Major damage was determined from the number of dead queens and escorts in cages on arrival at their destination, and minor damage by the number of queens and escorts arriving alive but not surviving 14 days after introduction into honey hives. Results for the 1999, 2000 and 2001 experiments are shown in Tables 12, 13 and 14 respectively.

Table 12. Queen transport information for each age group of queens for shipments mailed November-December 1999. Time spent in transport, temperatures and humidities in envelopes, queen and escort survival on arrival, and queen survival 14 days after arrival.

		Honey producer	Age of queen (days) 7	14	21	28	35
Time (hours)		A	33	20	20	22	26
		B	31	29	18	18	22
Temperature °C	Min	A	18.9	-2.5	18.3	20.2	22.1
	Max	A	28.3	28.3	29.1	31.0	30.6
	Min	B	19.2	19.2	22.1	19.5	19.5
	Max	B	28.3	31.0	29.1	32.2	31.4
Relative Humidity %	Min	A	51.6	26.7	56.3	43.4	58.2
	Max	A	73.5	49.6	87.2	63.9	77.6
	Min	B	51.9	62.7	78.3	60.3	56.4
	Max	B	69.3	73.3	87.7	69.9	72.3
Number * of bees dead on arrival	Q	A	0	1	0	0	0
	E	A	0	0	1	1	0
	Q	B	0	0	0	0	0
	E	B	0	2	4	0	0
Queen survival**		A	2	10	15	18	20
		B	4	9	19	16	16

* Q=queen (/20), E=escorts (/120)
** number of queen bees alive 14 days after introduction into honey hives, /20

There were no significant differences for queen bee survival between Honey Producer A and Honey producer B for each of the age groups 14 days after queen bees were introduced into honey hives.

Table 13. Queen transport information for each age group of queens for shipments mailed November-December 2000. Time spent in transport, temperatures and humidities in envelopes, queen and escort survival on arrival, and queen survival 14 days after arrival.

		Honey producer	Age of queen (days) 7	14	21	28	35
Time (hours)		A	22	22	20	25	22
		B	31	33	31	32	31
Temperature °C	Min	A	19.6	16.5	23.7	22.3	23.0
	Max	A	26.5	28.4	31.5	31.0	33.1
	Min	B	20.2	20.7	24.8	22.7	23.3
	Max	B	26.1	29.1	33.1	29.1	32.3
Relative Humidity %	Min	A	64.1	66.7	59.9	67.4	51.9
	Max	A	76.4	81.1	81.1	76.9	65.5
	Min	B	56.2	65.1	58.9	70.2	49.0
	Max	B	78.4	88.0	76.2	88.4	66.6
Number * of bees dead on arrival	Q	A	0	0	0	1	0
	E	A	0	1	0	1	0
	Q	B	0	0	0	0	0
	E	B	0	4	0	0	0
Queen survival**		A	5	15	15	18	18
		B	6	13	17	19	20

* Q=queen (/20), E=escorts (/120)

** number of queen bees alive 14 days after introduction into honey hives, /20

There were no significant differences for queen bee survival between Honey Producer A and Honey Producer B for each of the age groups 14 days after queen bees were introduced into honey hives.

Table 14. Queen transport information for each age group of queens for shipments mailed November-December 2001. Time spent in transport, temperatures and humidities in envelopes, queen and escort survival on arrival, and queen survival 14 days after arrival.

		Honey producer	Age of queen (days) 17	24	31
Time (hours)		A	18	21	20
		B	31	33	32
Temperature °C	Min	A	20.2	20.8	18.9
	Max	A	31.4	29.8	32.2
	Min	B	17.4	22.7	26.5
	Max	B	31.0	31.0	32.6
Relative Humidity %	Min	A	51.2	54.0	42.6
	Max	A	68.9	78.7	71.0
	Min	B	47.4	58.3	47.8
	Max	B	56.9	69.3	64.9
Number * of bees dead on arrival	Q	A	0	0	0
	E	A	0	5	1
	Q	B	0	0	0
	E	B	0	4	2
Queen survival**		A	15n	18n 13b	17n 18b
		B	11n	19n 14b	18n 18b

* Q=queen (/20 for 17 d.o. and /40 for 24 and 31 d.o).,
E=escorts (/120 for 17 d.o. and /240 for 24 and 31 d.o).

** number of queen bees alive 14 days after introduction into honey hives, (/20)
n caught from mating nucleus, b caught from queen bank

There were no significant differences for queen survival between Honey Producer A and Honey producer B for each of the age groups 14 days after queen bees were introduced into honey hives.

Objective 6 – Position of the queen cell cup on the cell bar – effects on queen survival and physical characteristics

For the Year 2000 project, queen cells were positioned in pairs of the same colour using five colours from the middle of the cell bar to the end of the cell bar, a total of 20 cells/bar – 10 positions x 2 cells/position. The middle pairs of cells were numbered Position 1, and the pairs at the end of the cell bar numbered Position 5.

Queen bee survival data for the number of queen bees surviving 15 weeks after introduction, and laboratory data for each queen examined for the number of sperm in the spermatheca, spermatheca diameter, queen weight, and the number of ovarioles in one ovary were collated according to the position of the cell cup on the cell bar which produced each queen. Results are shown in Tables 15 and 16 respectively.

Table 15. The percentages of queen bees developed from cell cups at Positions 1 to 5 on the cell bar and which survived to 15 weeks after introduction of the queen bee into a honey hive.

Cell * position	Apiary A %	Apiary B %	Average ** %
1	68.2	76.2	72.1
2	68.2	68.2	68.2
3	60.0	66.7	63.4
4	61.1	70.6	65.7
5	77.8	63.2	70.3

* 1 = middle of cell bar, 5 = end of cell bar
** data from queen bees 7-35 days of age at introduction, n = 40

Cell cup position on the cell bar had no significant effect on survival rates for each age group at 15 weeks after introduction.

Table 16. Data recorded for adult queen bees developed in queen cell cups for the five positions on the cell bar for the number of sperm in the spermatheca, spermatheca diameter, queen weight, and the number of ovarioles in one ovary.

Cell * position	Number of sperm in spermatheca 10^6 av.(range) **	Spermatheca diameter mm av.(range) #	Queen weight g av.(range) #	Number of ovarioles in one ovary av.(range)#
1	1.627 (0.788-3.633)	1.377 (1.167-1.738)	0.2118 (0.1542-0.2536)	164.9 (140-195)
2	1.608 (0.363-2.838)	1.354 (1.214-1.524)	0.2123 (0.1399-0.2456)	171.1 (153-197)
3	1.404 (0.250-3.167)	1.386 (1.214-1.595)	0.2139 (0.1523-0.2435)	173.9 (151-213)
4	1.330 (0.263-3.450)	1.387 (1.119-1.595)	0.2087 (0.1609-0.2492)	172.1 (149-199)
5	1.959 (0.390-7.250)	1.321 (1.190-1.571)	0.2124 (0.1663-0.2469)	170.7 (154-188)

* 1 middle of cell bar, 5 end of cell bar
** data from queen bees 14-35 days of age at introduction, n = 16
# data from queen bees 7-35 days of age at introduction, n = 20

The position of the queen cell on the cell bar during the larval stage of the queen bee`s development was not significant for number of sperm in the spermatheca, spermatheca diameter, queen weight and number of ovarioles in one ovary of queen bees developing from those cells.

Objective 7 – Freezing whole queen bee bodies – effects on sperm in the spermathecae

No physical damage was observed to individual sperm.

Objective 8 – Sperm counts from 20-24 day old November drones

To obtain data on sperm counts of 20-24 day old drones at the time of mating of test queen bees for the 2000 project

42% of drones contained $<0.5 \times 10^6$, 16% contained $0.5\text{-}5 \times 10^6$ sperm, and 42% contained $>5 \times 10^6$ sperm. For the 58% of drones which contained sperm, average number of sperm/drone was 6.45×10^6, range $0.5\text{-}10.7 \times 10^6$.

Objective 9 – Sperm counts from sister queen bees mater at two different mating apiaries

To compare sperm counts between sister queens mated in mating apiaries of (i) a commercial queen bee breeder, and (ii) a commercial honey producer. Results are shown in Table 17.

Table 17. number of sperm in the spermathecae of sister queen bees mated in one of the mating apiaries and caught at 21 days of age.

Number of sperm 106 mean (range)	
Queen bee breeder	Honey producer B
1.791 * (0.263-3.100) ** n = 20	2.057 * (0.375-5.400) ** n = 27

* no significant difference, $P<0.05$
** number of queen bees examined

Objective 10 – Sperm counts of sister queen bees examined at different ages

A comparison of sperm counts of queen bees examined at either 7 or 14 days of age and sister queen bees introduced into honey production colonies at 7 or 14 days of age and removed and examined 15 weeks after introduction for the 2000 project, results are shown in Table 18.

Table 18. Comparison of the number of sperm in the spermathecae of queen bees examined either at 7 or 14 days of age and sister queens examined 15 weeks after being introduced into honey hives at either 7 or 14 days of age.

Age of queen when caught from mating nucleus (days)	Age of queen when examined for number of sperm in the spermatheca	Number of queens examined	Number of sperm 106 range (average)
7	7 days	20	0.06 (0.00-0.53) *
7	16 weeks	5	2.21 (1.13-4.43) *
14	14 days	20	1.60 (0.31-7.25) **
14	17 weeks	11	3.18 (1.04-4.40) **

* significantly different from each other, $P<0.05$
** significantly different from each other, $P<0.05$

Objective 11 – Short-term storage of whole drone bodies - methodology

Development of a short-term storage method for adult drone bodies which would allow sperm counts to be carried out.

The two methods attempted (i) 4% glutaraldehyde, and (ii) freezing at -4°C resulted in a failure to evert drones with abdominal contents softening and becoming unsuitable for the purpose required.

5. Discussion

Objective 1 – Effects of queen age on – Introduction, Early Performance and Satisfactory Performance success rates.

There were no significant differences between survival and performance rates between queen bees introduced into either Apiary A or Apiary B for the three years data were collected (Tables 1, 2 and 3). This suggests that where differences were found within or between groups of queens, then those differences resulted from the effects of the factors being measured and not from indirect factors associated with apiary management practices, climate effects, nutritional conditions, apiary disease status etc. which can be expected to have impacted on the data obtained.

The age queen bees are introduced into established honey production hives to provide maximum survival and performance from those queen bees varies according to the criteria used in determining that age -

(i) when survival 14 days after introduction is the criteria used,(Table 1), an age at introduction between 21 and 28 days, about 24 days of age, provides the highest success rate.

(ii) when survival 15 weeks after introduction, (Table 2), and queen performance 15 weeks after introduction,(Table 3) are used, an age at introduction between 24 and 35 days, about 30 days of age, provides the highest success rate.

A comparison of survival/performance rates between queen bees held for a number of days in either their mating nucleus or in a queen bank prior to introduction into established honey production hives did not provide clear results. Queen bees caught from mating nuclei at 17 days of age were not satisfactory either for survival or performance. Queens caught from mating nuclei at 24 days of age provided superior survival and performance results compared to queens held in a queen bank between the ages of 17 and 24 days. There was no difference in survival or performance results between queen bees either caught from mating nuclei at 31 days of age or queens caught at 17 days of age and held in a queen bank until 31 days of age.

The effects of holding queen bees in queen banks on survival and performance prior to introduction into established hives requires further investigation.

Beekeepers can expect an economic benefit, based on the percentages of queen bees surviving for each age group, from introducing queen bees at an older age, this was shown in field results for all three years of the project.

From Table 2, the percentage differences between numbers of queen bees which survived to 15 weeks after introduction –

For 1999 – about 45% more queens survived from introductions at 21 and 28 days of age compared with queens introduced at 14 days of age, and 10% more queens survived when introduced at 35 days of age than those introduced at 21 days of age.

For 2000 – about 16% more queens survived when introduced at 28 or 35 days of age compared with queens introduced at 14 and 21 days of age.

For 2001 – an average of 16% more queens survived when caught from their mating nucleus at 24 or 31 days of age compared with queens caught at 17 days of age.

The purchase price of commercially reared queen bees is low compared to the benefits provided by a young queen bee and is probably no more than the cost to the beekeeper in time and expenses in carrying out the requeening activity in the apiary.

Costs to the beekeeper when requeening increase substantially when an introduced queen bee fails to be accepted or is superseded a short time after being accepted. Financial losses arise from the beekeeper having to purchase a replacement queen and provide time and expenses associated with introducing a new queen into a colony which is queenless or in the process of raising its own queen.

Social cohesion breaks down in colonies which attempt to rear queen cells or remain queenless. Returning these colonies back into working order is generally at a cost of lost bee population numbers and lost production over an extended time period.

Benefits from introducing queen bees at an older age should be sufficient to encourage beekeepers to incorporate using queen bees of about 30 days of age at introduction in their requeening management programs.

Objective 2 – Number of spermatozoa in the spermathecae of queen bees

Mackensen (quoted in Jay and Dixon 1984) stated that queen bees with one million sperm should lay fertilised eggs for "a time" and Taber (quoted in Jay and Dixon 1984) stated that queens with sperm counts of less than 3 million are unable to head commercial honey production colonies for one season. Over a 6 year period, Jay and Dixon (1984) found that 11% of queen bees had <3 million sperm and 45-64% contained >5 million sperm.

Severson and Erickson (1989) recorded sperm counts from naturally mated queen bees over 3 years finding 41% of queens had sperm counts of at least 4.5 million and considered these queens to be adequately inseminated for utilization by beekeepers. Harizanis and Gary (1984) considered drone populations adequate which provided commercially reared queen bees with sperm counts of 4.3-4.7 million sperm. Woyke (1971) found naturally mated queen bees (n=54) produced from larvae grafted at one or two days of age contained between 5.026-5.737 million sperm in their spermathecae. Van Eaton (1986) found the mean sperm count for New Zealand queen bees was 4.72 million sperm.

The1999 experiment showed a positive correlation between increased queen survival and increased sperm counts with data suggesting that sperm counts of <2 million sperm/queen was associated with low survival of queens following introduction into honey production colonies. This result was not supported by data from the 2000 and 2001 experiments.

Data from the 2000 experiment showing high queen survival (Tables 1 and 2), and low sperm counts (Table 4), suggest that the number of sperm in a queen`s spermatheca is not an important factor for queen bee introduction success and survival during 15 weeks following introduction.

For the three years of results for this project, the percentages of queen bees examined between 14 and 35 days of age which contained less than either 3 million or 4.5 million sperm/queen are unacceptably high when compared with data and statements contained in Jay and Dixon (1984), Harizanis and Gary (1984), and Severson and Erickson (1989).

Combined with the low overall average sperm counts are the wide range of sperm counts associated with each age group of queen bees for the three years queens were examined for this project (Table 4).

The two factors of overall low sperm counts/queen and a wide range of sperm counts within each similar group of queens suggests a problem during queen bee mating, eg. insufficient numbers or inadequate quality of drones present in the mating area.

A comparison of sperm numbers present in the spermathecae of queen bees which had their wings either clipped at 14 days of age, or not clipped (Table 5) resulted in clipped queens having higher average sperm counts than non-clipped queens. Only laying queens had their wings clipped, the result may indicate that slightly older, or more mature queens which flew and mated earlier than their sisters were selected to have their wings clipped.

Data from Tables 4 and 5 indicate that queen bees are able to stop mating and commence egg laying with low sperm numbers in their spermathecae. This raises the question of whether the ability of queen bees to do this is associated with factors from within the queen or from external factors eg. insufficient drone numbers/quality to complete mating and provide queen bees with high average sperm counts. Results of physical and disease prevalence characteristics measured, Objective 4 results including Tables 8 and 9, suggest that the physical qualities of queen bees used in the project for Years 1-3 were of a high standard. The problem appears to be related to drone factors rather than queen factors and requires further attention to identify the correct source of the problem.

Objective 3 – Pheromones present in the mandibular and head glands of queen bees.

The wide range of pheromone levels between individual queen bees in each age group, and between age groups, does not allow patterns of pheromone levels to be developed for most of the pheromones.

Table 7 shows a positive relationship between increasing levels of HVA and increasing age up to day 28. Improved introduction, survival and performance success were also associated with increased age of the queen at introduction (Tables 1-3). Combined, these data suggest a positive relationship between increasing levels of HVA and increasing introduction, survival and performance success of queen bees which is able to be measured through the common factor of queen age.

HFA and MHB also showed a positive increase each week to age 21 days before decreasing.

HVA, HFA and MHB were the only pheromones displaying an increase in level with increase in queen age. This suggests that some pheromones and their levels may be involved in the introduction success of queen bees less than 28 days of age into new colonies with HVA being the important pheromone for increasing queen survival.

Objective 4 – Physical characteristics of queen bees and disease status of queen bees and hive bees.

(i) Number of ovarioles

From Table 8, the lower number of ovarioles for 7 day old queens may be explained by their incomplete development and difficulty in counting at that age. The low numbers for 31 day old queens for the 2001 experiment are not able to be explained.

Orosi-Pal (quoted in Ruttner 1983) found that the number of queens in which the number of ovarioles exceeded a certain level increased the younger the larvae had been when they were grafted. He found that 80% of queens reared from eggs, 51% of queens reared from worker larvae 18-20 hours old, and 12% of queens reared from larvae grafted at 66-78 hours old exceeded 150 ovarioles/ovary.

Woyke (1971) also found that ovariole number/ovary decreased with increase age of the grafted larva with one day old larvae averaging 154 ovarioles/ovary, 146 for two day old larvae and 136 for three day old larvae.

Casagrande-Ialoretto et. al. (1984) found an average of 175 ovarioles/ovary in *A m ligustica* queen bees (n=30), and Van Eaton (1986) examined commercial strains of queen bees in New Zealand resulting in a mean number of 148 ovarioles/ovary , range 100-182 (n=65).

Data from Ruttner (1983) and Woyke (1971) suggest that the number of ovarioles/ovary is closely related to the age of the larva when grafted allowing this data to be used as a measure of the quality of queen bees being produced by the queen bee breeder.

From Table 8, in general, data on the number of ovarioles/ovary in queen bees used in these experiments equalled or were better than similar data accepted as being of a suitable standard from a number of overseas sources.

These results are indicative of the queen bee breeder producing queen bees of high quality for this project for Years 1-3, and suggests that unsatisfactory results on queen bee survival rates and the number of sperm in queen bees` spermathecae are likely to have resulted from factors other than queen bee production factors.

(ii) Queen weight, spermatheca diameter, and presence or absence of semen in the oviducts

Queen weight – Seven day old queen bee samples comprised mated and unmated queens and the sample average could be expected to be lower than a sample comprising all mated queen bees which occurred for all samples of queens older than 7 days of age.

For the 2001 experiment, (Table 9), queens removed from queen banks after 7 and 14 days were significantly, $P<0.05$, lighter in weight than queen bees removed from mating nuclei. Temporary weight loss is expected for queen bees stored in queen banks.

Woyke (1971) reported that queen bees grafted from one and two day old larvae and weighed at emergence ranged in weight between 0.156-0.201 g; Van Eaton (1986) found a mean queen weight for mated queen bees of 0.214 g, and Nelson and Gary (quoted in Van Eaton 1986) found an average weight of 0.208 g.

Queen weights for 14-35 day old queen bees in this experiment ranged between 0.197-0.226 g, which compare well with the overseas data.

Spermatheca diameter – Significant differences, $P<0.05$, were identified between queen bees examined at different ages for the 2000 and 2001 experiments (Table 9).

Woyke (1971) identified differences in spermatheca diameter between queens grafted from larvae of different ages, one day old larvae produced newly emerged queens with spermathecae diameters between 1.225-1.375 mm, and two day old larvae produced queens ranging between 1.150-1.300 mm. A difference in the ages of larvae when grafted may explain the differences found between groups of adult queen bees from the 2000 and 2001 experiments. Van Eaton (1986) reported the mean diameter of the spermatheca of New Zealand queen bees as 1.217 mm.

Spermatheca diameter from queen bees 7-35 days of age used in this experiment ranged between 1.235-1.380 mm which compared well with overseas data.

Semen presence in oviducts – Vesely (1970) observed that infecundity and incidental death of artificially inseminated queen bees was often caused by the retention of semen in lateral oviducts. Vesely observed semen retention in - queens inseminated after they had commenced laying, queens reared in unsuitable populated mating hives, queens inseminated out of the rearing season and those inseminated with preserved semen.

Woyke and Jasinski (1978) found that as the age of drones used to inseminate queen bees increased, the percentage of queens with semen residues in their oviducts increased, a seasonal effect was also found. Queen bees which could not clear their oviducts died.

The opportunity was taken during the 2000 and 2001 experiments to examine the oviducts of queen bees for semen residue to provide data on its prevalence in naturally mated queen bees and as a measure of queen quality ie. semen residue may be a factor contributing to reduced survival of young queen bees.

For queen bees aged between 7-17 days, semen residue in oviducts could be expected to result from queen bees mating in the 24 hours previous to when they were examined. For queen bees older than 17 days when examined, semen residue in oviducts may indicate a drone problem eg. age effect or seasonal effect on the drone.

Data obtained in this experiment is of interest, Table 9, as it shows that semen residue in oviducts was observed in queen bees up to 31 days of age and its occurrence in 11.5% of queens examined is not uncommon.

(iii) Nosema disease

Nosema disease *Nosema apis* present in colonies used for queen bee production or in colonies into which young queen bees are introduced is often considered as a possible cause for poor queen bee introduction or performance success.

Jay and Dixon (1984) surveyed Nosema spore presence in queen bees imported into Canada from the USA over a 6 year period. Nosema was found in 7.55 (0.5-18%) of queens examined (n=777), and a mean of 48.3% of escort bees had the disease. Jay and Dixon concluded that Nosema disease was partly responsible for queen supersedure problems.

Van Eaton (1986) found for New Zealand queen bees, 18% of the total examined contained Nosema spores at a mean level of 3.06 million spores/queen.

Czekonska (2000) introduced Nosema innoculated and non-innoculated queen bees into mating nuclei. All queens were accepted, worker bees did not supersede innoculated queen bees significantly more often than healthy ones. Significantly more worker bees were infected in the mating nuclei with innoculated queens (61%) than those with non-innoculated queens (5.3%).

For the 1999, 2000 and 2001 experiments, very low numbers (3/360) of queen bees supplied to the project by the queen bee breeder contained measurable levels of Nosema spores. Samples of worker bees from mating nuclei and the queen bank used in 2001 resulted in relatively low numbers of Nosema spores present (Table 10).

Commercial honey production hives which received the test queen bees during the 2000 and 2001 seasons also recorded relatively low Nosema spore counts (Table 10).

This data suggests that Nosema disease did not have a measurable effect on the introduction success and early performance of queen bees used in these experiments.

(iv) Brood diseases

Brood diseases, either light or heavy infections, in colonies young queen bees are being introduced into are considered by some beekeepers to be a contributing factor towards low introduction success rates and poor early performance of young, healthy queen bees.

The relatively low brood disease levels in honey production colonies at the dates of queen introduction, (Table 11), combined with data from Tables 1-3 showing there was no significant difference between introduction and early performance success rates between queen bees introduced into either Apiary A or Apiary B suggests that there was no measurable effect from brood diseases on survival and performance data obtained in these experiments.

(v) Other factors

Indirect factors such as climate, foraging conditions and beekeeper management at the time of queen introduction are considered to affect introduction and early performance success rates of young queen bees.

Records maintained at the times of queen introductions for the three years experiments were carried out did not indicate extremes or problem areas which may have impacted on the results.

Non significant differences between survival and performance rates for queen bees introduced into Apiary A and Apiary B for each of the 3 years of the experiments, with each apiary being managed by a different commercial beekeeper in different parts of NSW indicate that climate, foraging and beekeeper management practices did not have a measurable effect on the introduction and performance results obtained.

Objective 5 – Transport effects on queen bee survival and performance.

Damage to queen bees during transit between apiaries of the queen bee breeder and the honey producer were examined for each of the three years of the experiment.

Queens may be damaged directly and arrive dead or visually injured, or suffer indirect damage and arrive in an apparent healthy condition with the effect of the damage not becoming apparent until a later time.

Work on transport effects on queen bees by Forster (1971) who compared local, airmailed and surface mailed queens, concluded that there was no significant difference in honey production between the three groups examined however queen supersedure was significantly less for local queens (5.2%), than for airmailed (27.7%), and surface mailed (27.7%) queens. Forster suggested that the higher supersedure rates resulted from damage to the queens during transport.

Finley et. al. (1999) placed temperature monitoring devices in queen shipments moved across the USA. Free (quoted in Finley 1999) found that groups of 10 bees died after 5 hours at 10°C. Finley et. al. found that bees without water die at about 46°C, however bees may survive these temperature extremes for short periods of time. Small numbers of bees arrived dead in Finleys` experiment and there was no statement regarding long term effects of transport.

Over the three years data was recorded for this project (Tables 12-14) extremes of temperatures were – 2.5 to 33.1°C, and relative humidities 26.7 to 88.4%.

Small numbers of queen bees (2/300) and escort worker bees (26/800) arrived dead following transport (Tables 12-14), combined with a non significant difference between survival rates 14 days after introduction for queen bees received at Apiary A and at Apiary B (Table 1) indicate that the conditions experienced by queen bees during transport had little to no effect on their survival and performance success rates following introduction into productive honey hives.

Objective 6 – Position of the queen cell cup on the cell bar during the larval feeding stage and effects on queen survival and physical characteristics.

The position of the cell cup on the cell bar during the larval feeding stage in the cell raising colony was shown to not have a significant effect for queen survival rates 15 weeks after introduction, (Table 15), number of sperm in the spermatheca, spermatheca diameter, queen weight, or the number of ovarioles in one ovary (Table 16), of adult queen bees developed from cells at different positions on the cell bar for the 2000 experiment.

This data suggests careful management by the queen bee breeder by providing well fed, strongly populated cell raising colonies. Different results may be expected to occur from queen bees raised under conditions of nutritional stress and reduced bee population numbers in the cell raising colonies.

Objective 7 – effect of freezing whole queen bee bodies at -20°C on the physical characteristics of sperm stored in the queen`s spermatheca.

No fragmentation or other form of physical damage which would have affected the accuracy of sperm counts was observed.

Objective 8 – Sperm counts of 20-24 day old drones.

This was carried out to provide basic data on sperm production from drones of mature age, 20-24 days old, reared during the expected peak seasonal period, October-November in Queensland.

The results of 42% of drones containing less than 0.5 million sperm/drone and a further 16% containing less than 5 million sperm/drone is of concern due to – (i) the drones sampled were expected to represent drones at their peak condition ie. mature age and reared under good climate and nutritional conditions, (ii) the high number of drones with low sperm counts not being physically distinguishable externally from drones containing suitable numbers of sperm.

Management of drone mother colonies including means of determining numbers of viable drones present in drone production colonies requires further attention.

Objective 9 – Comparison of sperm counts between same aged sister queen bees mated at two different mating apiaries.

No significant difference was found between sperm counts in the spermathecae of same aged sister queen bees mated at either the queen bee breeder`s mating apiary or a queen mating apiary established by the beekeeper owner of Apiary B (Table 17).

A lower sperm count number would have been expected from the queen bee breeder`s mating apiary if the larger number of queen bees being mated in the queen breeder`s apiary, compared to the smaller number being mated in the honey producer`s mating apiary, was affecting sperm count numbers.

The mean sperm counts from the two mating apiaries, 1.791(queen breeder) and 2.057 (honey producer) million sperm/queen emphasises the problem of queen bees stopping the mating process and commencing laying with relatively low numbers of sperm in their spermathecae. These results also imply that low sperm counts in queen bees may be due to a queen factor rather than a drone factor.

Objective 10 –Comparison of sperm counts of sister queen bees examined at different time periods.

The opportunity was taken to examine queen bees 15 weeks after being successfully introduced into honey production colonies and compare sperm counts in their spermathecae with sperm counts recorded from their sister queens when caught from their mating nuclei.

Jungwirth (1972) observed two instances where he concluded that a queen can start egg laying after mating and can mate again subsequentially.

Data from the 2000 experiment showed there were significant differences, $P<0.05$, between sperm counts from spermathecae of queen bees examined at 7 and 14 days of age, and sperm counts from spermathecae of their sister queens examined 15 weeks after introduction into honey hives (Table 18).

An explanation of the significant increase in sperm counts between sister queens examined 15 weeks apart is that queen bees caught as laying queens from mating nuclei may lose weight during transit.

Once successfully introduced into a new bee colony the queen bee may recommence flying and mating. This has a practical application since queen bees mating in a honey production apiary are more likely to return to a different hive from their own and be damaged. This data also identifies a potential mating problem with queen bees caught from their mating nucleus at 14 days of age.

Objective 11 –Short term storage method for whole drone bees.

An attempt was made to develop a method for storing complete drone bodies which would allow sperm counts to be carried out over a number of following days.

Two methods using readily available materials/equipment were attempted. In both cases, drone body tissue softened and became unsuitable for allowing accurate sperm counts to be carried out on them.

Short term drone storage methodology requires further attention to allow large numbers of drones to be caught in a short period of time and then allow the relatively slow process of sperm counts to be carried out over a longer period of time.

6. Implications

This project primarily investigated the age at which queen bees were introduced into established productive honey hives and the effect of age on introduction success and short term survival. A number of objectives were examined to obtain data to assist in interpreting data on age effects. Areas of interest arising from the project are –

(i) Mating nucleus v. queen bank for holding queen bees – results from this experiment were not clear and were unsatisfactory. It would be beneficial to repeat this experiment under improved controlled conditions allowing detailed data to be collected.

(ii) Sperm numbers in queen bee spermathecae – the overall low average sperm counts recorded during this project combined with a wide range of sperm counts present for each group of queen bees examined indicate a major problem with queen mating.

Data from this project has suggested that the problem may be due to drone factors (high physical standards found for queen bees examined for this project; low number of spring reared drones 20-24 days of age producing sufficient numbers of sperm, increase in sperm numbers from sister queen bees examined at different ages), and also queen factors may be involved (sister queens mated at different mating apiaries returning similar sperm counts).

This subject requires further investigation.

(iii) The positive correlation between the queen mandibular pheromone HVA and increase in queen age, and increase in queen survival, found in the 1999 experiment suggests that a technique may be able to be developed using HVA as a management tool for improving queen bee introduction success.

(iv) Semen in queen oviducts – the implications of this characteristic and its effect on queen bee survival and performance require further investigation.

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