THE INFLUENCE OF FEEDING ON *APIS MELLIFERA SCUTELLATA* QUEEN REARING AND BROOD PRODUCTION

by

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Dedicated to my Parents

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ABSTRACT

After vast numbers of *Apis mellifera scutellata* colonies had to be destroyed as a result of *Apis mellifera capensis* infestations, the so-called Capensis problem, it became clear that there was a great need for efficient queen rearing in South Africa.

A queen rearing programme in a commercial beekeeping business at Douglas was implemented to replace the large number of lost *scutellata* colonies. Within the first week of queen rearing having started, large numbers of eggs were noticed in the queen cells, indicating *capensis* laying workers. The acceptance of grafted queen cell cups was very low in general, the highest percentage being 48 %. The percentage emergence of queen cells introduced into mating nucleus colonies was high, namely 95 %. However, only 44 % of these queens mated successfully, and had a normal brood pattern. This gave an overall success rate of 20 % maximally in the presence of Cape laying workers.

A number of factors that influence queen rearing, excluding Cape laying workers, were investigated, namely different queen cup lengths, dry and wet grafting, and the interval between dequeening and grafting on acceptance on the grafted larvae. The bees preferred queen cell cups with a length of 9 mm (73,3 %) to other lengths of 7 mm (0,0 %), 8 mm (52,5 %) and 11,5 mm (33,4 %). Acceptance of queen cells was higher when larvae were grafted into a droplet of water (72 %), compared to dry grafts (57 %). Acceptance of grafted queen cells was 73,3 % after a 24 hour queenless period, compared to 7,2 % after 8,5 hours without a queen.

A 60 % sugar solution is recommended for feeding honeybees before a honeyflow or during queen rearing, because it was found not to ferment easily, thus necessitating feeding once a week only.

Different pollen supplements and substitutes were tested for preference and brood production. On dry substitutes outside the hives, most bees were counted on the mixture of sifted maize and Lotmix ® (a cattle feed), to which dry powdered sugar had been added. The other substitutes that contained no maize meal, namely yeast and mixtures of yeast, soy and powder milk, were not collected. When natural pollen became more freely available, pollen substitutes were generally ignored.

The following substitutes/supplements, in decreasing order of preference, were tested as moist patties inside hives: Beltsville substitute, fine maize meal, soy + pollen (3:1), yeast + milk + pollen (2:2:1), Pronutro ® (breakfast cereal), soy + pollen (9:1), and soy + yeast + milk (3:1:1).

The following substitutes/supplements, in decreasing order of brood production, were tested: Pronutro ® + pollen (4:1), Beltsville substitute, soy + pollen (4:1), soy + yeast (3:2), soy + yeast + milk (3:1:1), and soy + yeast + egg (2:1:1). The soy + pollen combination is recommended for the highest brood production at the lowest price.

Natural pollen was most plentiful during September, November, December and April in Bloemfontein. The most abundant pollens were from *Eucalyptus* spp., *Tribulus terrestris*, *Rhus lancea* and *Helianthus annuus*. The different pollen types and their percentages were tabled for every month. The total amount of pollen trapped for the one year period was 3580,6 g. Pollen trap efficiency was calculated to be 10 %, therefore the total amount of pollen collected by the colony was approximately 35,8 kg.

UITTREKSEL

Nadat groot getalle *Apis mellifera scutellata* kolonies vernietig moes word as gevolg van die sogenaamde Capensis probleem, het daar 'n groot behoefte in Suid-Afrika vir effektiewe koninginteelt ontstaan.

'n Koninginteelt program in 'n kommersiële byeboerdery by Douglas is geïmplementeer, om die groot getalle verlore *scutellata* kolonies te vervang. Binne die eerste week nadat daar met koninginteelt begin is, is baie eiers in die koninginselle opgemerk, 'n aanduiding van *capensis* lêende werker besmetting. Aanvaarding van geënte koninginselkelkies was oor die algemeen baie laag, met die beste aanvaarding synde 48 %. Die persentasie voltooide koninginselle wat in kernkolonies uitgebroei het, was hoog, naamlik 95 %. Slegs 44 % van hierdie koninginne het egter suksesvol gepaar en 'n normale broedpatroon gehad. In geheel was die maksimale suksessyfer dus slegs 20 % in die teenwoordigheid van Kaapse lêende werkers.

Verskeie faktore wat 'n invloed op koninginteelt het, bo en behalwe die Kaapse by, is ondersoek, naamlik verskillende lengtes van koninginkelkies, nat en droë enting, en verskillende tydperke van koninginloosheid op aanvaarding van geënte larwes. Koninginselkelkies met 'n lengte van 9 mm (73,3 %) is verkies bo die kelkies van 7 mm (0,0 %), 8 mm (52,2 %), en 11,5 mm (33,4 %). Aanvaarding van koninginselle was hoër wanneer die larwes in 'n druppeltjie water geënt is, teenoor droë enting, met 'n persentasie aanvaarding van 72 % en 57 % onderskeidelik. Na 'n 24 uur koninginlose tydperk was aanvaarding van geënte kelkies 73,3 % in vergelyking met 7,2 % na 8,5 uur sonder 'n koningin.

'n Suiker oplossing van 60 % word aanbeveel vir die voer van heuningbye voor 'n heuningvloei of tydens koninginteelt, aangesien dit nie maklik gis nie en daarom net een keer per week gevoer hoef te word.

Verskillende stuifmeel plaasvervangers en aanvullings is getoets vir hulle voorkeur en broedproduksie. Op droë plaasvervangers wat buite die korwe getoets is, is die meeste bye getel op die mengsel van gesifte mieliemeel, Lotmix ® ('n veevoer) en poeier suiker. Die ander plaasvervangers wat geen mieliemeel bevat het nie, naamlik brouersgis en mengsels van brouersgis, soja en poeiermelk, is gladnie versamel nie. Nadat stuifmeel in die natuur meer vryelik beskikbaar geraak het, is die plaasvervangers oor die algemeen geïgnoreer.

Die volgende plaasvervangers/aanvullings, in volgorde van afnemende voorkeur, is as klam stuifmeelkoekies binne die korwe getoets: Beltsville plaasvervanger, fyn mieliemeel, soja + stuifmeel (3:1), brouersgis + melkpoeier + stuifmeel (2:2:1), Pronutro ® ('n graankos), soja + stuifmeel (9:1) en soja + brouersgis + melkpoeier (3:1:1).

Die volgende plaasvervangers/aanvullings, in volgorde van afgemende broedproduksie, is getoets: Pronutro ® + stuifmeel (4:1), Beltsville plaasvervanger, soja + stuifmeel (4:1), soja + brouersgis (3:2), soja + brouersgis + melkpoeier (3:1:1), soja + brouersgis + eierpoeier (2:1:1). Die soja + stuifmeel mengsel word aanbeveel vir die beste broedprodukse teen die laagste prys.

Natuurlike stuifmeel was die volopste gedurende September, November, Desember en April by Bloemfontein. Die stuifmeel wat die meeste voorgekom het, was *Eucalyptus* spp., *Tribulus terrestris*, *Rhus lancea* en *Helianthus annuus*. Die verskillende stuifmeelsoorte en hulle persentasies is getabelleer vir elke maand. In totaal is 3580,6 g stuifmeel gedurende die jaar versamel. Die

effektiwiteit van die stuifmeelval is bereken as 10 %. Die totale hoeveelheid stuifmeel wat deur die betrokke kolonie versamel is, was dus ongeveer 35,8 kg vir die periode.

CHAPTER 1

GENERAL INTRODUCTION

More than 40 crops grown commercially in South Africa, such as deciduous fruits and hybrid sunflowers, are dependent on, or benefit from honeybee pollination. More than 20 000 colonies are currently used annually for the pollination of hybrid seed sunflower in the summer rainfall region. In the Cape, beekeepers supply approximately 18 000 colonies to apple and pear producers for the pollination of their crops. There are more than 3 000 beekeepers in South Africa, and it is estimated that these beekeepers operate 75 000 beehives (Du Toit, 2001).

Annually, the honeybee industry contributes R 2,5 billion to South Africa's GDP, of which the major portion can be ascribed to the value of bee-dependent agricultural crops. Honey, beeswax and other hive products contribute only R60 million. Beekeepers create almost 10 000 direct job opportunities. The input suppliers (manufacturers of equipment and protective clothing, transporters, etc.) and the output suppliers (processing equipment, packaging and retailing) also create many other jobs (Du Toit, 2001).

Two subspecies of honeybees are found in South Africa. The African honeybee (*Apis mellifera scutellata*), notorious for its aggressive behaviour, occurs in the greater, summer rainfall, region of South Africa. The second race, the Cape honeybee (*Apis mellifera capensis*) occurs along the southern, eastern and western Cape coasts and mountains, which roughly correspond with the distribution of fynbos vegetation (Tribe, 1983). Historically, the two subspecies have remained geographically distinct, with a hybrid zone between them.

In the early nineties, Cape bee colonies were taken by beekeepers to Gauteng province where they were introduced into commercial *scutellata* apiaries. Because of the inability of *scutellata* queens to pheromonally prevent *capensis* workers from reproducing, and because laying workers of the Cape bee produce female offspring, their presence in *scutellata* colonies results in the eventual loss of the *scutellata* queen and they take over all reproduction. Foraging from such colonies gradually diminishes as *scutellata* workers die of old age, the colonies dwindle and eventually abscond or die (Swart *et al.*, 2001). Most of the commercial African honeybee colonies have since become infested with *capensis* laying workers, and are continuously being destroyed.

The Department of Agriculture tried to eradicate the problem bee by ordering the initial killing of about 50 000 migratory colonies. This proved futile. Ten years later the problem was still acute, with only limited progress for practical solutions having been made (Johannsmeier, 2001b).

Measures taken by the Government were the legislation on bee pests and diseases. According to Government Notice R 159 of 5 February 1993, no Cape honeybees were allowed to be moved north, or African bees south of a demarcated line. This line corresponds with the probable northernmost distribution of the Cape bee. All colonies north of the line infested by Cape bees, had to be destroyed. This regulation was amended as R 1674 of 24 December 1998, and stipulated that the keeping of Cape bees north of the line was prohibited, and that all honeybee colonies that were queenless or had Cape laying workers, had to be destroyed within 72 hours (Johannsmeier, 2001a).

Commercial beekeepers in particular lost thousands of colonies annually, which forced some beekeepers out of business, and increased honey prices and the cost of pollination. The total (as well as per hive) honey production in South

Africa dropped because of the Cape bee problem, so that about 20 % of the country's honey needs had to be imported (Johannsmeier, 2001b).

Because vast numbers of *Apis mellifera scutellata* colonies had to be destroyed as a result of the Cape bee infestation, a need arose for effective queen rearing. One of the conclusions by the South African Professional Bee Farmers' Co-operative (1996), was that regular requeening with mated queens, preferably mated under controlled conditions, delayed infestation. This point of view was shared by the Plant Protection Research Institute (PPRI), which recommended regular requeening as a practical control measure in overcoming the Capensis problem (Johannsmeier, 1997).

Before 1992 it was possible to successfully split colonies, and the queenless part of the colony would rear its own queen. Most of the migratory beekeepers made use of the winter aloe flow (*Aloe greatheadii davyana*) to build up colonies and increase colony numbers, but because of the presence of the problem bee, they had to resort to special measures or take the risk of Cape bee infestation. According to Hepburn *et al.* (1991), there was considerable ovarian development among the *capensis* workers in the absence of a mated queen. They also showed that the younger the *capensis* worker bees when the queen was lost, the greater the likelihood of ovarian development.

It also became clear that the Capensis problem was enhanced by the aloe flow. When thousands of beehives were moved to the aloes, the *A.m. capensis* were given the chance to spread between and within apiaries and hives. In addition, the aloes activated the ovaries of the workers even in the presence of a queen. The mechanism behind this phenomenon is unclear, but related to the nutritious aloe pollen (Kryger *et al.*, 2000).

Kryger & Van der Schyf (1999) found, through the genetic analysis of Capensis problem bees in one apiary, that all the bees examined were genetically identical. Based on these results it seems that differences can exist between the bee families in their resistance to Capensis take-over, instead of simply being related to chance. According to them these bees are actually close to the range of being a new species with no interbreeding with the Scutellata bees, because all reproduction is asexual in the workers' ovaries. Therefore, they concluded that there is no possibility of any Scutellata x Capensis hybrids.

Some questions arise in connection with the above findings. Were there no drones present in the original colonies of Capensis bees? Was it not possible for a Capensis drone to mate with a Scutellata queen? Could, at some stage, a Capensis queen have mated with a Scutellata drone? According to Lundie (1954) and Johannsmeier (1983) there were several reports that the workers resulting from Capensis x Scutellata matings had colours in-between the typical black and yellow.

In a queenless colony, comb building stops, field activities and normal defence behaviour diminishes, as does cooperation within the colony (Ruttner, 1983). According to Morse (1985), honeybee queens can live from one to five years. This means that an average apiary will have as many four to five year old queens as one to two year old queens, if the beekeeper has no requeening program. Old, failing or weak queens mean less populous colonies, which in turn mean less honey produced. Lundie (1929) stressed the fact that, of all the factors that contribute to the prosperity of a colony of honeybees, the fecundity of the queen bee is undoubtedly one of the most important.

According to Lawrence & Cobey (1991), there are several problems when bees are allowed to rear their own queens after a colony is split. The first problem is that no selection for desirable traits is taking place. Another problem

is the poor nutritional quality of the developing larvae. This may result in small, unproductive queens. The diet of the larvae during the first 72 hours determines caste differentiation. If the diet of the worker larvae changes to queen royal jelly within the first 24 to 36 hours after egg hatching, the larvae will develop into an acceptable queen. Yet another problem is the age of a larva. If an older larva is chosen to be the queen, one and a half to three days after egg hatch, the queen will develop into an intercaste (a queen with worker characteristics). Comparatively, these queens will have fewer ovarioles, probably produce less queen substance (pheromones), and be smaller and less productive.

When a frame of eggs and larvae is given to a queenless colony, the larva the bees select to rear a queen may be very young, or may be up to three days old and already well on its way to becoming a worker. The older larva chosen will emerge before the younger, larger queens. The first queen to emerge will destroy her competition. Consequently, the result is an inferior queen in the colony (Lawrence & Cobey, 1991).

The maintenance of broodrearing in honeybee colonies is entirely dependent upon both adult nurse bees and developing larvae receiving adequate supplies of carbohydrates, proteins, vitamins, minerals and fats. In nature, these nutrients are obtained from nectar and pollen from flowering plants. Carbohydrates are provided by the sugars in nectar, while the other nutrients are obtained from pollen. In undisturbed colonies, the rate of broodrearing varies throughout the year according to the amount of pollen available.

Complete dependence on natural supplies of pollen often creates difficulties for beekeepers, because pollen is not simply a source of nutrients for honeybees, but is also the source of some important stimuli that influence the activities of nurse bees. Pollen must be available to stimulate secretory activity

in the brood food glands of nurse bees and to provide the nutrients that are required for the growth of the larvae. In bee management it is therefore useful it a beekeeper can intervene and provide the essential nutrients in the form of a pollen substitute or supplement. A pollen substitute contains no pollen in the ingredients, while a pollen supplement contains bee-collected pollen in the ingredients.

The stimulus that elicits oviposition by the queen and leads to the initiation and maintenance of broodrearing, is an intake of sugars. This stimulus is provided naturally when the bees locate a source of nectar, and is provided artificially whenever a colony is given sugar syrup. When the queen is laying eggs in response to the intake of sugars, an adequate supply of pollen must be available to feed the larvae that hatch from the eggs.

It is evident that feeding is one of the most important factors when rearing queens. The availability of natural pollen is dependent on the season as well as on weather conditions. One aim of the present study was therefore to find a suitable replacement for nectar and pollen to secure optimum brood production and to accelerate the build-up of colonies during early spring and when rearing queens. Strong colonies are needed for pollination and for preparing nucleus mating colonies used for queen rearing.

The varroa mite was discovered in the South-Western Cape in 1997 and has since spread throughout South Africa. Varroa has caused the mortality of a small percentage of honeybee colonies of both Cape and African bees in the Western Cape, Kwazulu-Natal and Gauteng. Colonies infested with large numbers of varroa mites are weakened further by other diseases and pests. A 14 % reduction in pollination efficiency was recorded in colonies that were heavily infested with varroa in the South-Western Cape (Swart *et al.*, 2001). Schehle (1996) reviewed the status of the South African beekeeping industry at

the end of the 20th century. According to him there was still no solution to the Cape bee problem and bee losses due to pesticides on crop plants were increasing. When the losses as a result of the varroa mite are added, it is clear that queen rearing and artificial feeding of honeybees is an essential part of beekeeping in the 21st century.

1.1 References

ANDERSON, R.H., BUYS, B. & JOHANNSMEIER, M.F. 1983. *Beekeeping in South Africa.* Bulletin 394, Department of Agriculture, Pretoria. ix + 207 pp.

DU TOIT, A.P. 2001. The South African Beekeeping Industry. *South African Bee Journal* 73 (3): 161 - 165.

HEPBURN, H.R., MAGNUSON, P., HERBERT, L. & WHIFFLER, L.A. 1991. The development of laying workers in field colonies of the Cape honey bee. *Journal of Apicultural Research* 30 (2): 107 – 112.

JOHANNSMEIER, M.F. 1983. Experiences with the Cape bee in the Transvaal. *South African Bee Journal* 55 (6): 130 – 138.

JOHANNSMEIER, M.F. 1997. Overcoming the Capensis problem. *South African Bee Journal* 69 (4): 63 – 70.

JOHANNSMEIER, M.F. (editor). 2001a. *Beekeeping in South Africa.* Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria. 288 pp.

JOHANNSMEIER, M.F. 2001b. Beekeeping history. pp. 1 – 8 in: Johannsmeier, M.F. (editor). *Beekeeping in South Africa.* Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria. 288 pp.

KRYGER, P., SWART, D. & JOHANNSMEIER, M.F. 2000. The Capensis problem and the aloe flow. *South African Bee Journal* 72 (1): 11 – 12.

KRYGER, P. & VAN DER SCHYF, A. 1999. The *capensis* honey bee problem: A genetic analysis using single locus DNA fingerprinting. *Final report for the South African Federation of Bee-Farmers' Associations.*

LAWRENCE, T. & COBEY, S. 1991. Queen rearing requirements. *Gleanings in Bee Culture* 119 (5): 264 – 265.

LUNDIE, A.E. 1929. *The rearing of queen-bees.* Bulletin 76, Department of Agriculture, Pretoria. 21 pp.

LUNDIE, A.E. 1954. Laying worker bees produce worker bees. *South African Bee Journal* 29: 10 – 11.

McGREGOR, L. 1990. Queen rearing with *Apis mellifera scutellata.* pp.95 – 103 in: Anderson, R.H. & Buys, B. (editors). *Proceedings of the International Beekeepers' Symposium "Bees and Beekeeping in Southern Africa", Stellenbosch.* 160 pp.

MORSE, G.D. 1985. A requeening plan. *Gleanings in Bee Culture* 113 (3): 137 – 138.

RUTTNER, F. 1983. *Queen rearing.* Apimondia Publishing House, Bucharest. 353 pp.

SCHEHLE, A. 1996. Die huidige stand van die Suid-Afrikaanse bye-industrie. *South African Bee Journal* 68 (5): 137 – 140.

SOUTH AFRICAN PROFESSIONAL BEE FARMERS' COOPERATIVE. 1996. A brief report on the control of the Cape Honeybee and the provision of financial assistance to beekeepers in the summer rainfall region. *South African Bee Journal* 68 (1): 7 – 10.

SWART, D.J., JOHANNSMEIER, M.F., TRIBE, G.D. & KRYGER, P. 2001. Diseases and pests of honeybees. pp. 198 – 222 in: Johannsmeier, M.F. (editor). *Beekeeping in South Africa*. Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria. 288 pp.

TRIBE, G.D. 1983. What is the Cape bee? *South African Bee Journal* 55(4): 77 – 87.

CHAPTER 2

QUEEN REARING WITH *APIS MELLIFERA SCUTELLATA*

"There is no one right way to rear queens; there are, however, a number of wrong ways" (Prof. Harry Laidlaw, 1979).

2.1 Introduction

In a honeybee colony, the queen is of major importance, because it is she who has the responsibility of producing sufficient eggs to maintain or increase the population size. The queen is dependent on the workers to feed her, and therefore they regulate her rate of egg production to some degree (Anderson *et al.*, 1983). A weak or failing queen will not respond to the feeding by the workers. In such case the colony will gradually become weaker because the rate of loss of adult bees will be greater than their rate of replacement. Lundie (1929) also stressed the fact that, of all the factors that contribute to the prosperity of a colony of honeybees, the fecundity of the queen bee is undoubtedly one of the most important. Poor queens head poor colonies, resulting in poor honey crops and ineffective pollination (Cobey & Lawrence, 1991).

According to Swart *et al.* (2001), in South Africa the queen may exhaust her egg-laying potential within a year where heavy demands are placed on her in migratory beekeeping. Requeening of colonies is therefore very important.

In 1978, Fletcher and Johannsmeier reported losses of colonies in commercial apiaries due to absconding, theft and the failure or loss of queens, as high as 30 % per annum, with individual commercial beekeepers having reported losses of 40 % and even 50 % of their entire stocks.

Since the early nineties, beekeepers in the summer rainfall regions of South Africa have lost thousands of colonies as a result of the infestation of African bee colonies by Cape bee workers. Some beekeepers were therefore forced to leave the business, and the price of honey and pollination increased. South Africa, formerly exporting honey, became a nett honey-importing country. Swart *et al.* (2001) recommended that queens be replaced annually in the African bee areas of South Africa to help control Cape laying workers from taking over colonies. The replacement of queens has therefore become essential in the regions where African honeybees occur.

The quality of a reared queen depends on the quality of the genes she receives from her parents, the nutrition and care she is given as a larva, and the drones she mates with (Caldeira, 1991). In this study aspects were investigated that influence the quality of reared African queen bees, namely the selection of breeder colonies, feeding of rearing colonies and using different types of nursing colonies. Additionally, the introduction of queen cells and mated queens was examined.

2.2 Material and Methods

2.2.1 Breeder colonies

The breeding colonies which supplied the larvae used for queen rearing, were subjected to strict selection. They were selected from sedentary colonies

in the Douglas region, most of them near the Orange and Vaal rivers. They were monitored over a period of one year before selection. Colour, temperament, low swarming tendency, honey production and a good brood pattern were used as criteria. Three breeder colonies were eventually selected as stock for providing material to rear queens from.

A full depth hive body insert with small frames (Figure 2.1) was used to house the breeder queen, which was confined to one half of the insert. The two long sides were covered with queen excluders, and the top with sailcloth to prevent the queen from escaping. Each half had three frames with drawn comb (Figure 2.3). The insert was constructed as described in Laidlaw (1979), and the dimensions shown in (Figure 2.2).

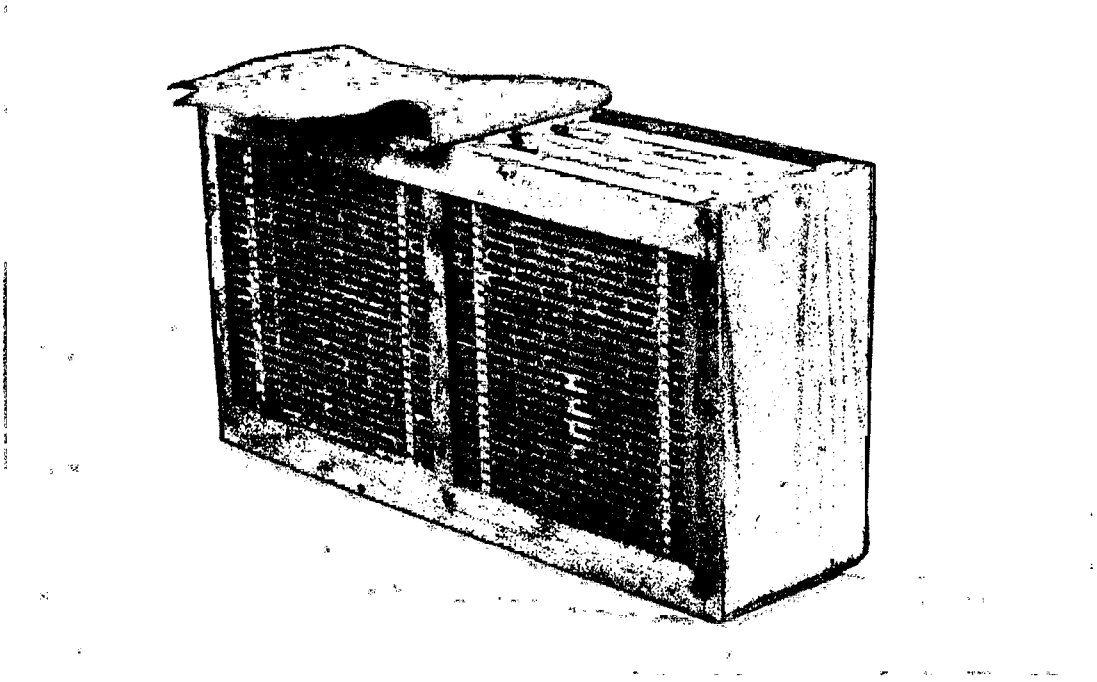

Figure 2.1 Full depth hive body insert with sailcloth cover

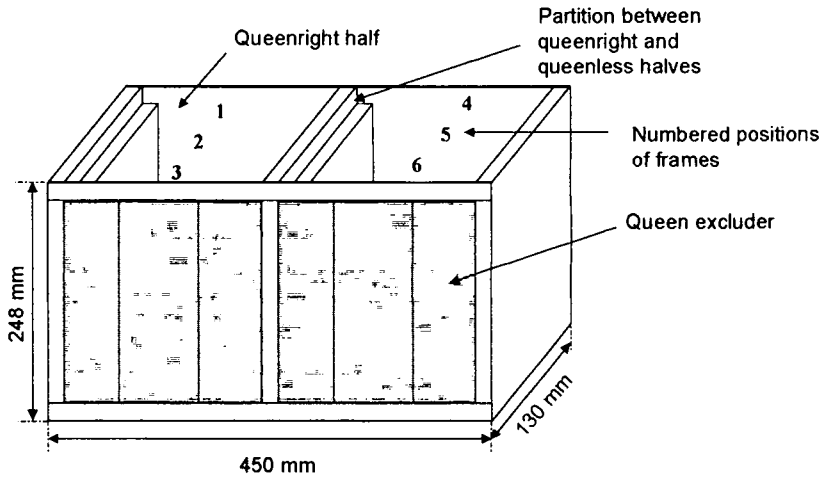

Figure 2.2 Dimensions of full depth hive body insert without sailcloth cover

The insert was placed at the side of a standard Langstroth brood chamber, the other half of the brood chamber being filled by a brood frame with pollen next to the insert, and five brood frames with sealed and emerging combs of brood. As this brood emerged, it was continuously replaced with additional sealed or emerging brood.

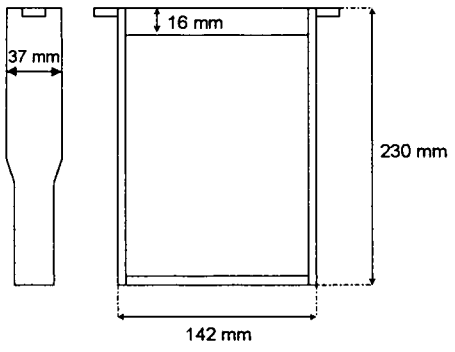

Figure 2.3 Dimensions of frame used in hive insert

The breeder colonies had to contain at least ten brood frames of bees. Each colony had a super containing five frames with unsealed honey, with the

feeder taking up the space of four super frames. The feeder was placed on the top bars of the brood frames against the side of the brood chamber.

Each day the comb in position 2 was moved to position 4. A newly drawn comb was then given to the queen in position 2. The drawn combs were produced in colonies used only for the purpose of drawing combs. For this purpose a standard Langstroth hive was used containing two feeders as described in Figure 2.9. The feeders occupied the space of eight brood frames and a standard brood frame was placed on each side of the brood chamber. The modified nucs were filled with the small frames with foundation, and removed when they were drawn. The frame in position 4 of the insert was moved to position 5, and the frame in position 5 moved to position 6. In this way, by rotating the combs each day, larvae 24 hours old and younger were obtained from day 4 onwards. Initially all the combs used in the insert were drawn combs. Combs 1 and 3 remained in the same position and were never replaced. They aided in the supply of young bees.

The breeder colony was placed as near as possible to the entrance of the room used for grafting, so that the frame containing the larvae could be transferred to the grafting room with as little delay as possible. After removal, the frame was wrapped in a damp towel to lessen the risk of desiccation.

All breeder colonies were fed continuously with a 60 % sugar solution. A plastic two-litre milk bottle, was used for syrup feeding (Figure 2.4). The bottle was placed on its side inside a wooden tray (16 x 30 x 4,5 cm). Two small holes, approximately 2 mm in diameter, were made on the lower side below the handle. These leaked syrup into the tray until they were covered by the rising level of the syrup.

Figure 2.4 Syrup feeder used in queen breeder colonies

2.2.2 Queen cell frame and queen cell cups

Artificial queen cell cups were prepared by dipping a forming stick into molten beeswax. This stick was 100 mm long and had a diameter of 7 mm. The end of the stick was rounded to give the bottom of the wax cup a concave form. A mark was made on the stick to indicate the desired depth of the cells. Cells of different depths were used, namely 7 mm, 8 mm, 9 mm and 11,5 mm (Figure 2.5).

The wax was melted in a double-jacketed container filled with water. A thermostatically controlled hot plate was used to keep the wax just above melting point at approximately 70 °C. The stick was first moistened by dipping

it into water, and any excess water shaken off. By dipping the stick four or five times with successively shallower immersions into the wax, a wax cup with a good base and tapering to a light thin edge was obtained. Each time the stick was dipped and pulled up, the wax was first allowed to harden before the next wax layer was applied.

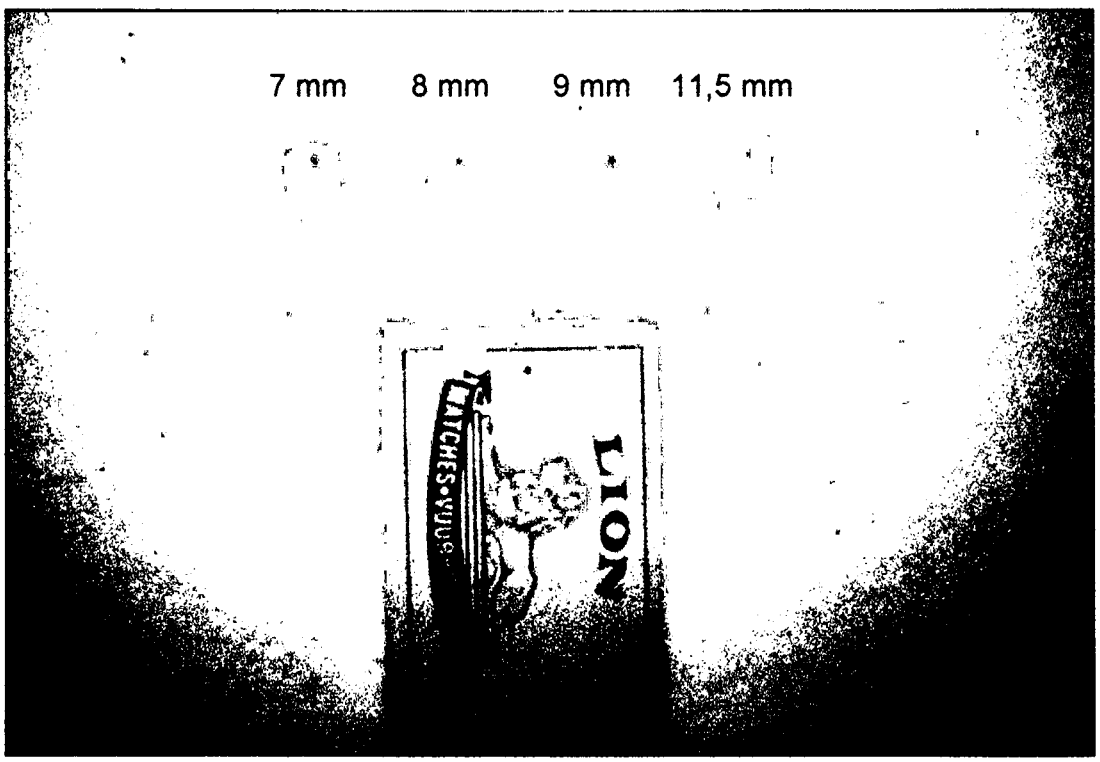

Figure 2.5 Queen cells of different lengths used in experiments

A queen cell frame was prepared by using three cell bars, which slide into the thickened side bars of a brood frame (Figure 2.6). A piece of thin aluminium was fastened over one side of the slots, to prevent the bars from falling out. The cell cups were fastened to the cell bars by pouring a drop of molten wax onto the bar, and pressing the thickened cell base into the drop of molten wax before it solidified. The cell cups were spaced 20 mm apart from centre to centre.

Twenty cell cups were fastened to each bar. The cups on the ends of a bar were 15 mm from the side bar of the frame.

The length of queens, measured just after emergence, was compared with the length of the cells from which they emerged. The cells were measured from the base to the tip while still capped. The queens were allowed to emerge from the cells into queen cages. The queens were then individually caught in a marking net and measured with callipers from the front of the head to the tip of the abdomen.

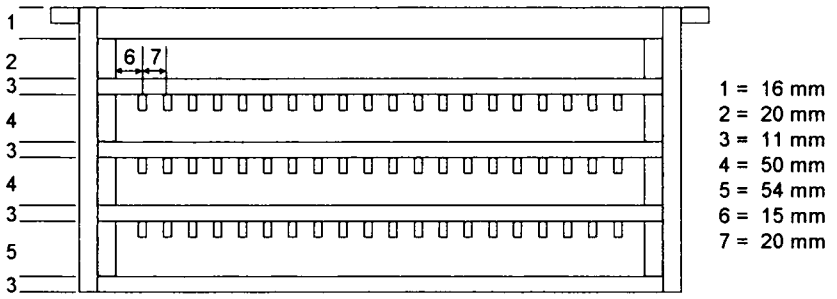

Figure 2.6 Measurements of a queen cell frame with cell cups

Thirty cups of each length were used in the acceptance test. By dividing the frame vertically, the 7 mm and 8 mm cups were tested on the same frame, and the 9 mm and 11,5 mm cups on the same frame. Three repetitions of each test were done, i.e. 3 x 30 cups of each length were tested.

2.2.3 Grafting

Frame no. 6 with grafting material of the right age was removed from the breeder colony and the bees gently brushed off with a bee brush. The frame was covered with a moist towel and taken into the nearby grafting room.

Before grafting, the room and the frames were mist-sprayed. The temperature of the room was between 25 – 28 °C. A flashlight was used as light source.

A grafting needle was used to remove the larvae floating on royal jelly, and then to deposit them in the centre of the cell cup. The home-made grafting needle was made of steel wire. If the larva was not removed with the first try, it was discarded, and another larva used.

Before this transfer, a drop of water was placed in each cell cup by dipping the grafting needle into water and shaking a droplet of water into the cell cup. This was done to facilitate the removal of the larvae from the grafting needle. Each completed bar was protected with a moist cloth while the other bars were grafted.

Larvae were also grafted into dry cells to determine if there was a difference in acceptance between wet and dry grafted larvae. Dividing the frame vertically, 30 larvae were grafted dry (10 on each bar), and 30 larvae were grafted into a drop of water. Three repetitions of each test were done.

2.2.4 Treatment of grafts

In order to obtain enough worker bees of the right age for the 4-frame starter nucleus colony, it was placed on top of the brood chamber of a colony at least 12 frames strong, separated from the latter by a queen excluder. This was done at about 08:00. A frame with young larvae, two frames with pollen and unsealed honey, as well as the grafting frame with empty artificial queen cells (3 bars with 20 cells on each bar) were placed inside the nucleus hive. The frames with pollen and honey were placed on either side of the grafting frame.

Care was taken to make sure the queen was not in the nucleus. At 17:00 the same day, the nucleus hive was removed and placed in the same position as the brood chamber, the latter being relocated about 10 metres away.

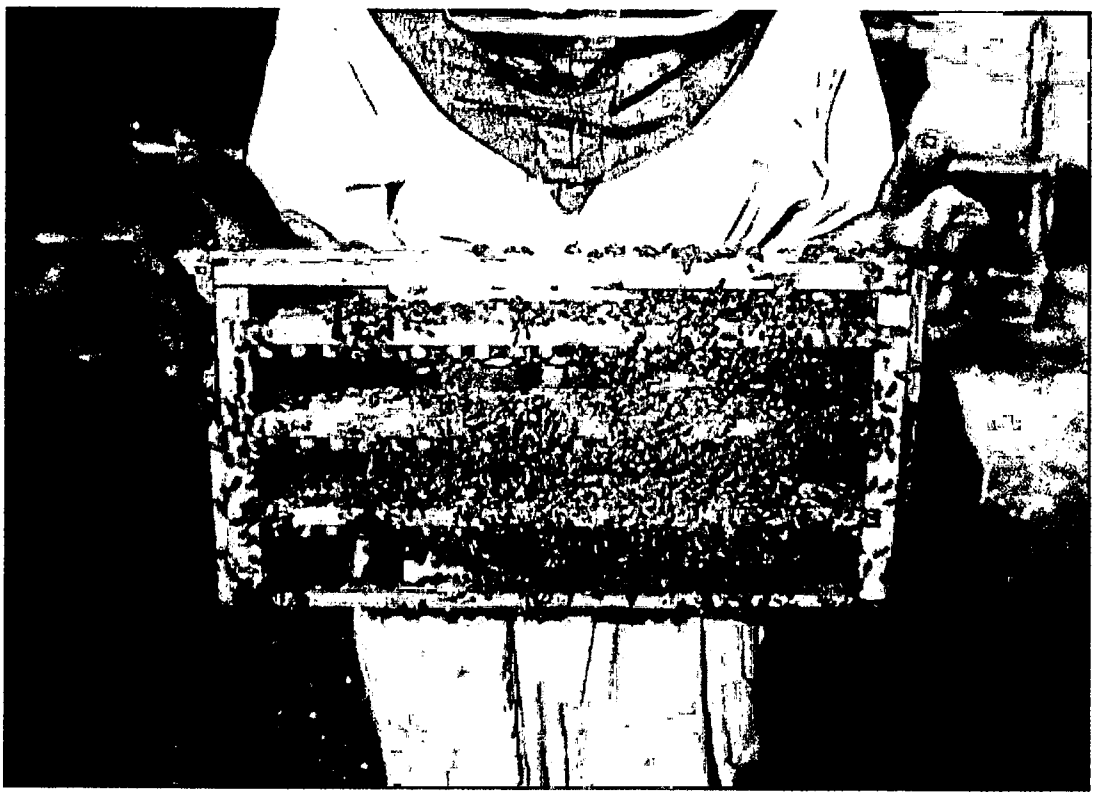

Figure 2.7 A grafting frame removed from a queenless nucleus colony just before grafting. The number of bees on the frame indicate a potentially good acceptance

The frame with larvae was removed from the starter nucleus colony before grafting. Grafting was usually done between 16:00 and 17:00 (23 – 24 hours after the nucleus colony was prepared). The number of worker bees on the familiarised grafting frame and on the cell cups (Figure 2.7) before grafting, was a good indication whether the cells would be accepted or not.

Frame no. 6 was removed from the hive body insert and one worker larva, 24 hours or less old, was transplanted into each cup of the grafting frame, which was then positioned in the centre of the starter colony. In each grafting frame, all transplanted larvae were taken from the same breeder colony. The following day the queen cells were inspected to count the number of accepted queen cells, i.e. those from which larvae had not been removed. On day 11 following grafting, the finished queen cells were removed for introduction into queenless colonies.

The influence of interval between preparation of the starter nucleus colony, and insertion of the grafted frame on the percentage cells accepted, was examined. The following intervals were evaluated: 0 minutes, 30 minutes, 4,5 hours, 8,5 hours, 16 hours, 20 hours, 22 hours and 24 hours. Six starter colonies were used for each treatment.

The influence of familiarisation of the grafting frames was also examined. The grafting frames were left in the queenless starter nucleus colony for 24 hours before grafting. Alternatively the larvae were grafted into cell cups of unfamiliarised frames. Six test frames and six control frames were used in this experiment.

The effect of the number of grafted cells per starting colony on percentage cells accepted was also investigated. Either 60 or 120 (2 cell bars next to each other, on the three different levels in the same frame) grafted cell cups were introduced into queenless colonies that were prepared in the same way as described earlier on in this section. When 120 grafted cell cups were introduced, a wider queen cell frame was used, containing 6 cell bars. Twenty replications of 60 cells and sixteen replications of 120 cells were used. The hives had to contain at least four frames densely covered with bees.

The percentage acceptance of queen cells in queenless brood chambers (n = 180) were compared to the acceptance of queen cells in queenless 4-frame nucleus hives (n = 180).

2.2.5 Queenless starting and queenright finishing of cells

The starting colony was prepared as follows: Two brood chambers on top of each other, with a queen excluder between them, were used. The queen was confined to the bottom brood chamber. Twenty four hours before inserting the grafts, the bottom brood chamber, was placed at the back of the top brood chamber, with its entrance in the opposite direction. Simultaneously, the frame with empty queen cell cups was placed inside the queenless brood chamber for familiarisation. This brood chamber contained frames with pollen and honey as well as frames with sealed and emerging brood.

The following day, i.e. 24 hours later, (day 1) larvae were grafted into the familiarised queen cell cups in the queenless colony. On day 2 the grafts were inspected to determine the percentage acceptance, and moved to a finishing colony. The two brood chambers of the starter colony were then placed in their original positions again.

The cell finishing colonies consisted of two brood chambers, separated by a shallow super, with the queen in the bottom brood chamber. A queen excluder confined the queen to the bottom brood chamber. The frame with accepted grafts was placed in the top brood chamber containing four or five frames of pollen and unsealed honey. Every ten days, the combs in the bottom and top chambers were interchanged so that the top brood chamber always contained emerging bees. All the combs were examined for queen cells each

time they were interchanged. The colony was liberally fed throughout the period that the queen cells remained in the colony.

Finished queen cells were introduced into queenless colonies on day 10 (29 replications) or 11 (19 replications) following grafting to test acceptance of capped cells differing by 24 hours in age. The frame with ripe cells was removed from the finishing colony during the afternoon, and the bees brushed off the frame, which was then carried to the grafting room, where the cells were cut from the bars with a sharpened hive tool.

2.2.6 Comparing baby and 4-frame mating nucs

In this experiment the success of the 4-frame nuclei and the baby nuclei as mating hives were compared.

The baby nucleus hive consisted of a modified 4-brood frame size nucleus (Figure 2.8). The nucleus was divided by a double wooden feeder (Figure 2.9). The feeder was dipped into molten beeswax before it was used to prevent leakage of the sugar syrup. A loose wooden block was used in each feeder to prevent the bees from drowning. Initially, the colonies were fed with a 60 % sugar solution, but too many bees drowned in the sugar syrup despite the wooden float. The sugar syrup was then replaced with dry ground sugar. Two flight holes, 9 mm in diameter were provided on both short sides of the nucleus hive. The frames in each half of the nucleus were the same size as those that were used in the hive insert in the breeder colonies.

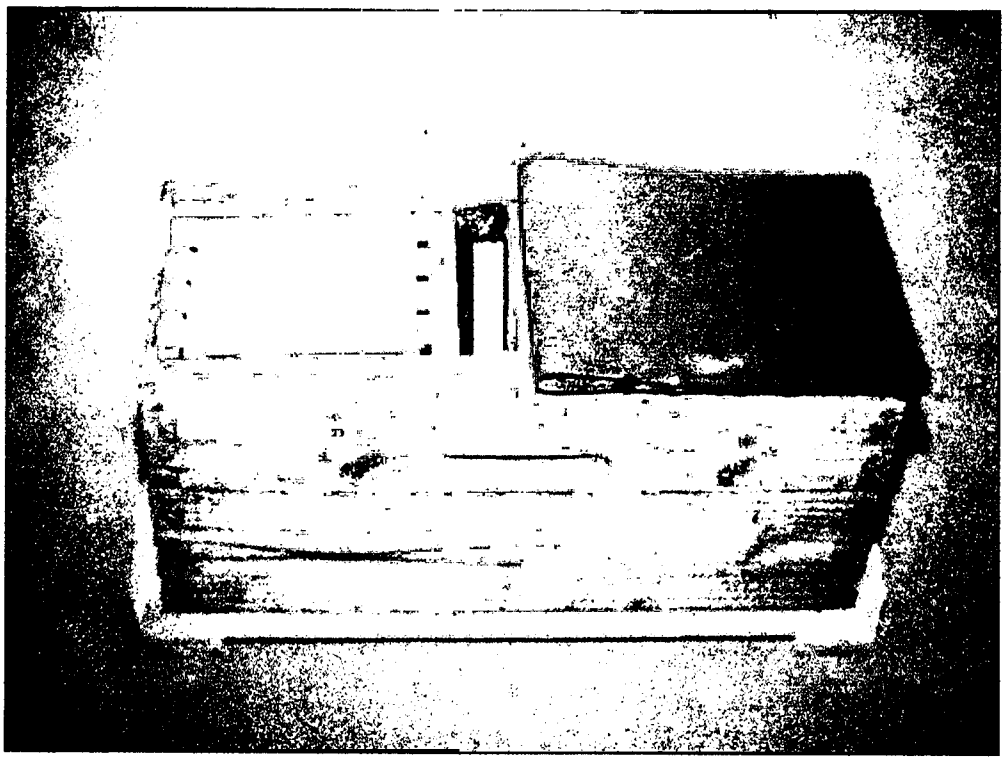

Figure 2.8 Modified 4-frame nucleus used as two baby nucs for mating

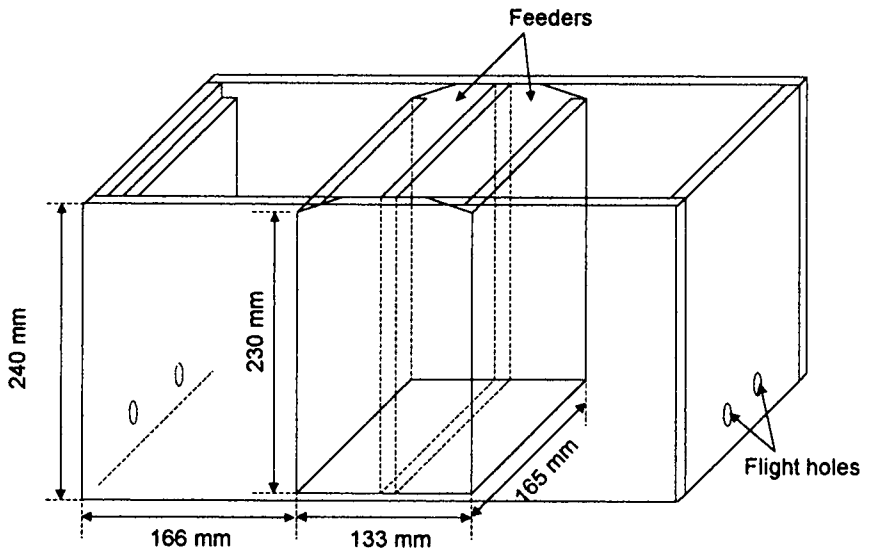

Figure 2.9 Dimensions of the modified 4-frame nucleus hive divided by feeders

Every baby mating colony had two frames with honey and pollen, one frame with wax foundation and one frame with young larvae and eggs (Figure 2.10). The frames were placed in a specific order into the baby nucleus (Figure 2.12).

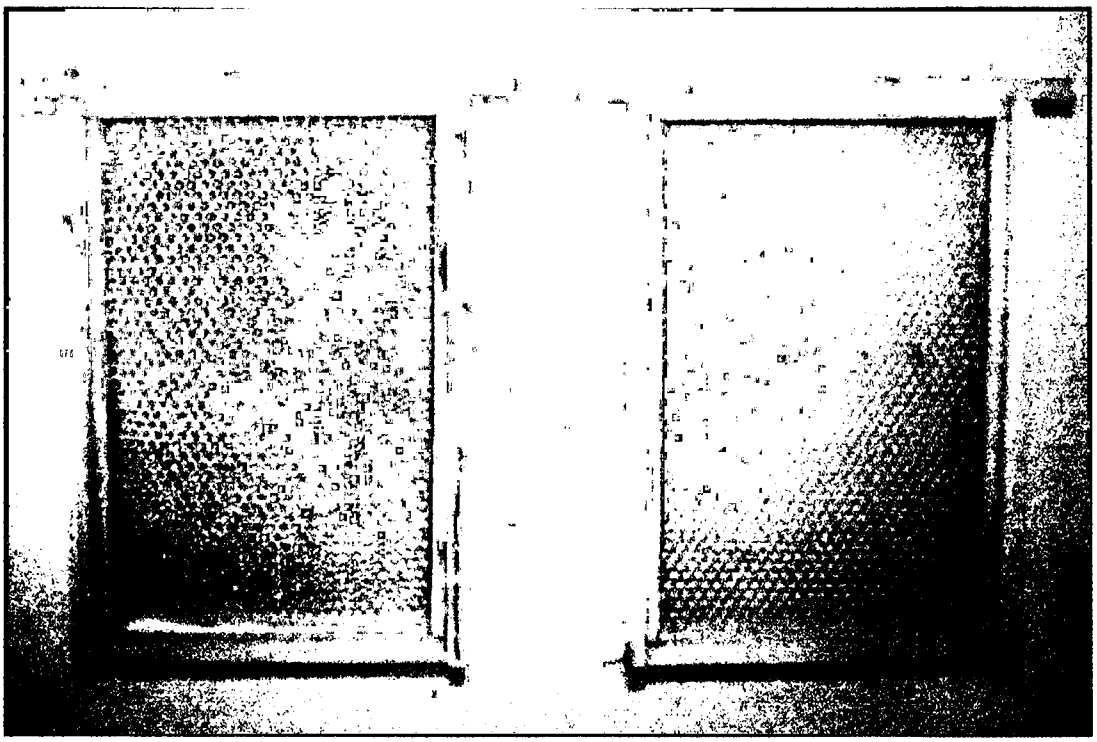

Figure 2.10 Frame with honey (left) and with wax foundation (right) used in baby mating nucleus

The ripe queen cell was pressed into the comb of the frame with young larvae and eggs (Figure 2.11). The queen cells were handled very carefully and only touched at their bases where they were attached to the cell bar. A mug full of bees (250 cm^3, containing approximately 600 bees, weighing 60g) was shaken into each baby mating nuc. This was done in the morning, when the bees were actively foraging, to reduce the number of old bees. After they were collected, they were finely sprayed with a 30 % sugar solution, in order to unite

them without stinging. The shaker box, with gauze sides, was kept in a cool, dark room (about 17°C) until needed.

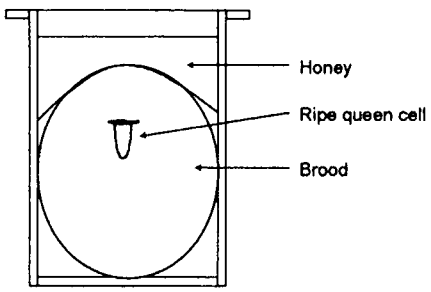

Figure 2.11 Position of ripe queen cell on the baby nuc frame

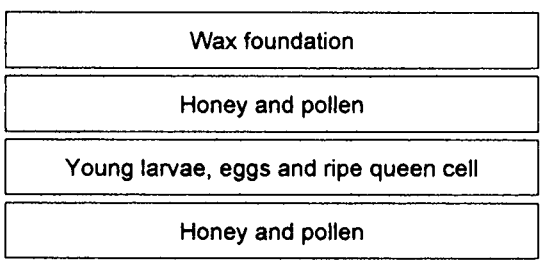

Figure 2.12 Order of frames in baby mating nucleus colony

The flight holes of the baby nucs were closed before shaking the bees into them at approximately 17:00. They were then kept in a cool, dark room (approximately 17°C) for 48 hours, after which they were taken to the mating sites, where the entrances were only opened after dark. The entrances were pointed in different directions, to minimise the loss of queens returning from their orientation and mating flights. Fifty colonies in baby nucs were used in this experiment, and their mating success compared with 20 four-frame nuclei.

The 4-frame nuclei were furnished with two frames of pollen and unsealed honey, one frame of young larvae and eggs, and one old empty comb.

These nuclei were not fed. In the morning a nuc was placed on top of the brood chamber of a strong colony, separated by a queen excluder. It was removed in the afternoon and taken to a mating site at least 3 kilometres away. Sometimes problems were experienced with too few bees entering a nuc, as it had to contain at least three frames of bees.

The nuclei were placed in the mating station as widely spaced as possible, at least three metres apart, with entrances facing in different directions. Care was taken to site hives in such a way that vegetation provided landmarks to prevent drifting and the possibility of queens returning to the wrong hive.

Queen cells were only introduced after a 24 hour queenless period. A ripe queen cell was also gently pushed into the surface of the comb with the brood, near young larvae. After a week, the colony was inspected to determine if the queen had emerged and whether she was present. The sites were visited once a week for feeding purposes, therefore combining inspections with feeding.

2.2.7 Introducing mated queens

Mated, laying queens were introduced into 4-frame nucleus colonies 24 hours or less after removing the old queens. These colonies were made up "artificially" (splits), and were not fed. A total of 50 queens were introduced into 50 different colonies during October and November of the same year. The success rate of the 50 queen introductions was determined.

A new queen was caged without attendants or food, in a plastic hair curler, which was inserted between two combs of unsealed brood near the centre of the hive. A paper clip was partially opened, and the straight end used to suspend the curler from the brood frame. A piece of newspaper, held in

position by a thin elastic band, covered one end of the curler tube, allowing the worker bees gradual access to the queen by chewing away the newspaper. The other end of the curler was closed with a cork stopper. The newspaper end of the curler pointed downwards.

2.3 Results and Discussion

2.3.1 Breeder colonies

The breeder colonies were selected for a yellow colour to try and avoid the risk of *capensis* infestation. It was attempted to select more docile colonies to facilitate the management of colonies. The criterium of low swarming tendency is important for maximum honey production, whereas a good brood pattern ensures optimum bee production, which is important for good pollination and a better honey crop.

The breeder colony system was very successful in supplying abundant well-fed larvae younger than 24 hours. No time was wasted in selecting the right size larvae, or larvae with abundant royal jelly. A disadvantage of this system is that the inserts and their frames were not standard Langstroth equipment, and had to be specially manufactured.

The use of larvae of the correct age is very important. According to Alber (1965), the first worker-type development in larvae takes place on the first day in the spermatheca and head, and on the second day in the legs and hairs. Experiments showed that the average number of ovarioles was significantly greater in queens reared from eggs, than in queens reared from grafted larvae. It can therefore be expected that the younger the larvae used for grafting, the

more ovarioles the resulting queen will have. This view is also shared by Sarling (1992).

Ruttner (1983) used larvae of different ages for grafting. According to him, the percentage acceptances of older and younger larvae were approximately the same. The bees showed a preference for older larvae, but not for larvae older than 24 hours. He also found that queens reared from larvae 36 hours old, weighed less than those reared from younger larvae. Lundie (1929) recommended larvae 12 hours old.

The feeding method with the 2 litre milk bottle was very successful when used with the breeder colonies, because the level of the sugar syrup could be monitored daily when the frames were moved. No problems were experienced with robbing and with ants. It was a very easy and cheap method to use.

The colonies used in the experiments were not fed any pollen substitutes or supplements, because there was enough natural pollen available during the time when the queen rearing was done. If no pollen or pollen supplement is available, the colonies should not be fed sugar syrup, because the nurse bees would deplete their own body protein, and would then only be able to rear queens of good quality for about 4 weeks (Kleinschmidt & Kondos, 1979). Queen rearing would therefore not be successful if pollen, or a pollen supplement, is not available. Very little natural pollen was available during August, while pollen was abundant during November and December. The queen cells produced during August 1993, were much smaller than those produced during November and December 1993 (Figure 2.13).

Food is the critical element in the determination of the queen caste and in the development of this caste to its full potential. It is very important that all

larvae that are to become queens are fed abundantly from the time they hatch from the egg, until they stop feeding in the sealed cell.

According to Jay (1963), dwarf adults can be reared from undernourished worker, drone and queen larvae. The effect of underfeeding is more marked the sooner feeding is stopped. The effect is not significant unless the queen larva is removed from its food when its mass is 60 – 65 % or less of the mass of the well-nourished larva.

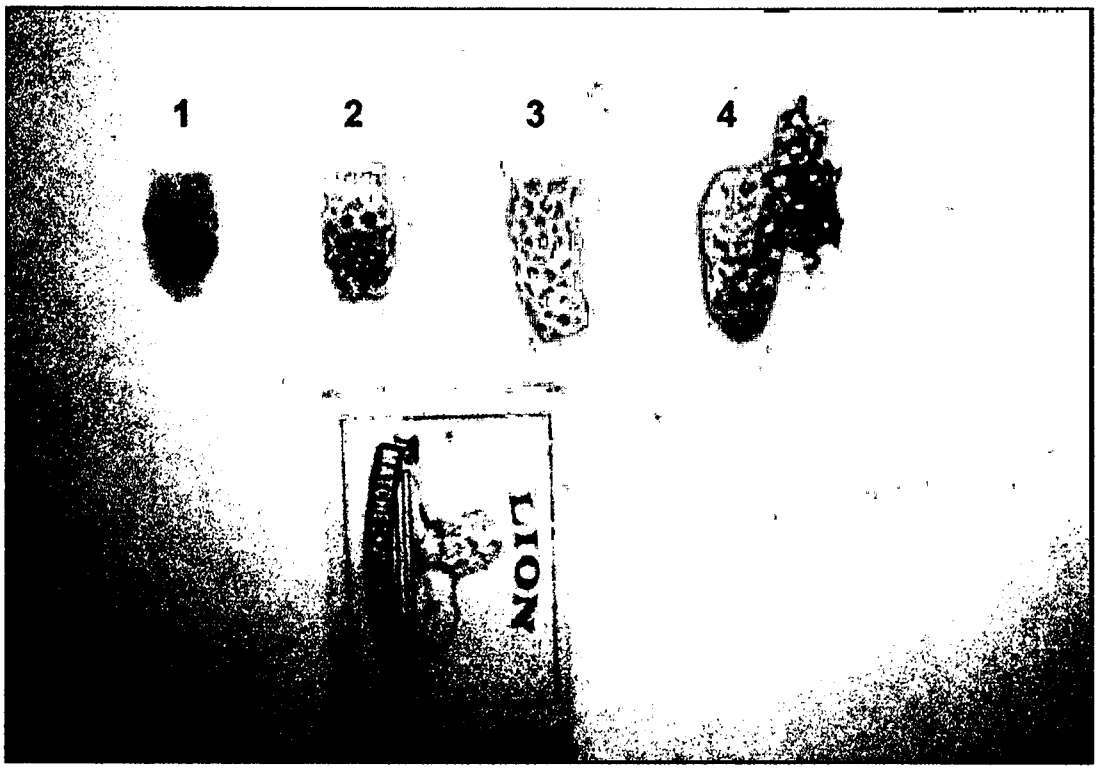

Figure 2.13 Finished queen cells produced during August (No. 1 & 2) and November 1993 (No. 3), with a natural queen cell (4) for comparison

Beyleveld (1939), using African bees, recommended that the breeder colonies should be fed liberally for two days prior to grafting. According to him,

it ensures well-fed larvae that can easily be lifted out of the cells, reducing the risk of injuring the larvae. Ebadi & Gary (1980) fed their cell builder colonies continuously with 50 % sucrose syrup in Boardman entrance feeders. Lundie (1929) recommended that the colony containing the breeder queen should be fed liberally with thin sugar syrup to ensure a generous feeding of the larvae from their earliest development.

Beyleveld (1939) reported that if bees were fed excessively, especially when there was a honey flow, the bees would build brace comb from one queen cell to the other, and in some cases right over the cells. Lundie (1929) also proposed that feeding of queen rearing colonies must be liberal, not only in quantity of syrup given, but also in its rate of flow or availability to the bees.

2.3.2 Results with queen cell cups of variable dimensions

The queen cells made from beeswax had the advantage that they were relatively cheap to make, and that they did not have to be removed after the finished queen cells were introduced, like plastic queen cells. A disadvantage of the beeswax queen cells was that their preparation was time-consuming.

The bees preferred queen cells cups of specific lengths as indicated in Table 2.1. The 9 mm cell cups were preferred, followed by the 8 mm cell cups. Cell cups of 7 mm were not accepted at all. According to Kither & Pickard (1983), the design of queen cups can significantly affect acceptance of larvae, and the characteristics of the queens that are subsequently produced. They also reported that a high level of acceptance was usually obtained with cups having a rounded internal cell base, circular transverse section and a diameter of 8 – 9 mm and length of 7 – 15 mm. According to Lundie (1929) queen cups should be 7,8 - 11 mm deep and 7,8 - 9,4 mm in diameter. He also reported that

the bees did not accept cell cups made from overheated wax. For the same reason, cells should be kept free from dust. Ebadi & Gary (1980) found that artificial queen cups made of new beeswax and of beeswax from old combs, were equally acceptable.

Table 2.1 Preference for queen cell cups of different lengths

Cell cup length (mm)	Percentage acceptance (%)
7,0	0,0
8,0	52,5
9,0	73,3
11,5	33,4

According to Ruttner (1983), natural queen cells are 7,8 mm in diameter, and 8 - 10 mm deep. He had better results with queen cells 9 mm in diameter than with cells 8 mm in diameter. Beyleveld (1939) used a forming stick with a diameter of 9,38 mm and spaced the cell cups 20 mm apart. He reported that the bees tended to build brace comb from one cell to the other if they were placed any closer. He used three bars in each frame, spaced 25 mm, 80 mm and 140 mm respectively below the top bar of the frame. Ebadi and Gary (1980) spaced queen cups 19 mm apart from centre to centre on each bar.

The finished queen cells produced during August were smaller than those produced during November and December in the present study. The queen cells produced during August had a mean length of 17,3 mm, compared to 23,8 mm of these produced during November and December. Alber (1965) found that the heaviest queens did not emerge from the largest cells. Comparable results were found in the present study when the length of queens,

measured just after emergence, was compared with the length of the cells from which they emerged (Table 2.2). A beekeeper can therefore use smaller queen cells as well, without fear of compromising on the quality of the resulting queens.

Table 2.2 Length of queens upon emergence from cells

Length of cell (mm)	Length of queen (mm)
21,0	15,0
21,5	16,5
22,0	15,5
25,0	14,0

Abdellatif *et al.* (1970) in Ruttner (1983) gave the rates of acceptance for queen cells in Egypt for March as 46 %, for May as 60 % and for July as 72 %. The reason for the low acceptance is given as high external temperatures, but the authors also mention that nectar and pollen supplies were influential. He also found that the acceptance of queen cells in Egypt was better in spring and summer than in autumn, and was worst in winter.

2.3.3 Wet and dry grafting

Cell priming is the placement of a drop of royal jelly, or dilute honey or a droplet of water into a cell cup before a larva is grafted into it. This simplifies grafting and reduces dehydration and injury to larvae. According to Delaplane (1988), priming of cell cups before grafting into them did not improve weight of queens, but it did improve cell acceptance in nurse colonies. Free & Spencer-

Booth (1961), using European bees, thought that priming cell cups with royal jelly was not necessary.

In the present study, the percentage acceptance was higher when larvae were grafted into a droplet of water (72 %), compared to the dry grafted treatment (57 %). According to Free and Spencer-Booth (1961), it did not make any difference to percentage acceptance, when small quantities of worker jelly were put in the queen cups with the larvae.

Beyleveld (1939) recommended the grafting method with a grafting needle, pointing out that the temperature and humidity of the grafting room was important. The temperature should be kept at about 30 – 32 °C, and the floor sprinkled with water, in order to make the atmosphere humid. Care should also be taken to brush the bees from the frame, and not to shake it. Enough light was also essential. A bright fluorescent lamp was most satisfactory, though other lamps or sunlight could also be used, but care should be taken that the larvae were not exposed to excessive heat. The grafting frames had to be covered with a moist towel as the larvae desiccate easily. According to McGregor (1990), successful grafting using the African bee, was best achieved in a warm moist environment. Temperature was less important than humidity.

2.3.4 Treatment of grafted queen cell cups

When a frame of young larvae was placed in a nucleus colony being prepared for queen rearing, more bees moved into the nucleus compared to nucs without larvae. Before the frame with grafted cells was placed into the starter nuc, the frame with young larvae was removed. Ruttner (1983) found that the presence of open brood decreased the percentage acceptance of queen cells. The arrangement of the frames in the starter nucleus hive is important.

Bees do not relocate pollen in the hive as they do honey (Laidlaw, 1979). The pollen supply should therefore be as close as possible to the larvae that have to be fed.

The frame with young larvae in the nucleus hive during the preparation stage before grafting, is important to assure a large preponderance of young bees (about seven days old). It is believed that it is at this stage that the glands secreting the larval food are most active (Lundie, 1929). Nurse bees are young bees (3 – 13 days old) that are not yet foragers. Soon after emergence, worker bees eat stored pollen, which develops their fat bodies and wax glands, and causes the hypopharyngeal glands and mandibular glands to secrete the main components of royal jelly (Herbert, 1992). They must continue to eat pollen as long as they have to secrete royal jelly and feed the queen larvae.

When checking the cells, they should be handled carefully, particularly after the 5th day when the cell is sealed (probably between 17:00 on day 4 and 09:00 on day 5 following grafting). The young developing queens appear to be vulnerable to chilling, tilting and any jarring, especially on day 8, when wing development may be impaired through handling. Because they are delicate, it is advisable to leave the cells until day 11 (they hatch on day 12) before placing them in mating nuclei. By day 11 the queen is fully developed and is less susceptible to damage. Although mature cells may generally be safely handled, they should still be treated with care (McGregor, 1990).

Queen rearing with a queenless colony used both as a starter as well as a finisher, is very successful if only a few queens have to be produced. The percentage acceptance of the queen cells in these colonies was 73 % (using a 24 hour queenless period). Free (1987) was able to induce construction of queen cell cups by experimentally crowding colonies, and so disrupting pheromone distribution, both inside and outside the normal swarming season.

Above a certain threshold (2,3 workers/mL hive space), the number of queen cell cups increased with colony density.

Based on the rate of contact and dispersal of queen pheromone by court bees, Free (1987) calculated that, as the nurse bee population increased, there was a sudden disproportionate decrease in the percentage of bees that were pheromonally inhibited from queen rearing. This helped to account for the sudden onset of queen rearing following colony expansion in spring. When the acceptance of queen cells is low, a possible cause may therefore be too few nurse bees or the accidental inclusion of a queen in the starter.

It is evident that the influence of the interval between dequeening and introduction of the grafted cells has an influence on the percentage cells accepted and finished (Figure 2.14). The best results were obtained with a 24 hour interval when 73 % of the cells were accepted and 68 % of the cells were finished. The results of 20 and 22 hour intervals were almost the same with 45 % cell acceptance and 41 % finished cells. An interval of more than 24 hours was not used, to try and prevent *capensis* infestation.

The data was statistically analysed with a One-Way Analysis of Variance test and with the Tukey-Kramer Multiple Comparisons Test. The most significant difference was between 30 minutes and 24 hours, and between 4,5 hours and 24 hours ($P < 0,001$). According to the statistics, 8,5 and 16 hours were also significantly different from 24 hours ($P < 0,01$ and $P < 0,05$ respectively). Significantly more cells were accepted after a 24 hour queenless period (73,3 %) than after 8,5, 16, 20 or 22 hours (7,2, 30,6, 45,6 and 44,8 % respectively).

According to Free (1987) it is possible but not proven, that the number of queen cells a colony builds generally increases with its population and is an

approximate measure of its queen pheromone deficiency. According to Ruttner (1983), a period of 2 - 24 hours is needed for a colony after dequeening, to become aware of the fact that they are queenless.

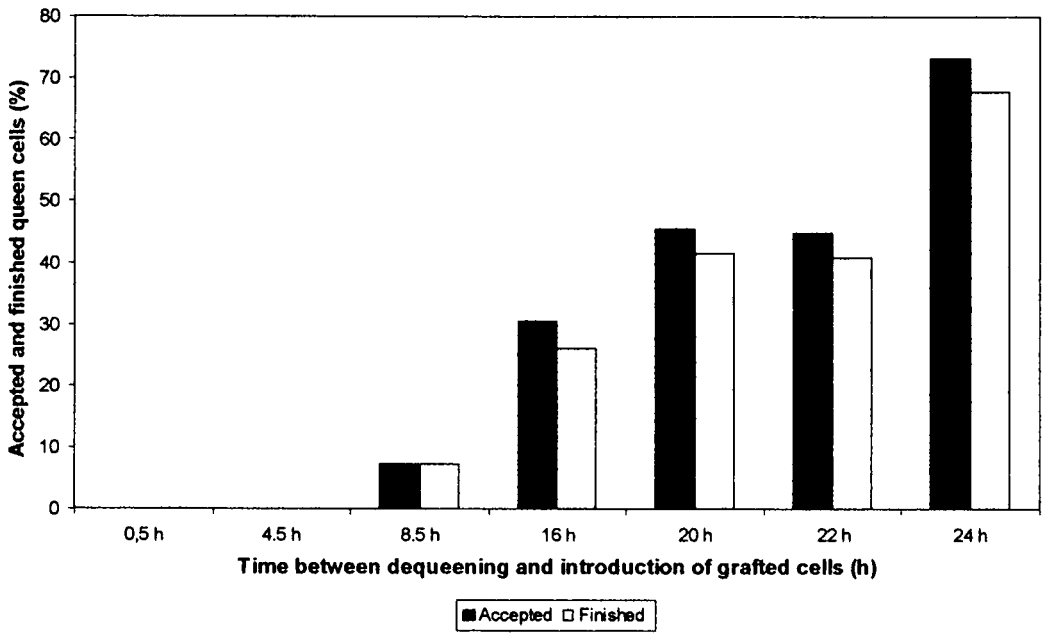

Figure 2.14 Influence of interval between dequeening and introduction of grafts on the percentage cells accepted and finished

If the grafts were given too early, the bees would remove some or all larvae from their cups. Ebadi & Gary (1980) also reported that queen pheromone could inhibit the acceptance or feeding of larvae grafted into artificial queen cups. According to Free (1987), a cage occupied by a queen does not lose its attractiveness completely until 90 minutes after the queen had been removed. He also monitored the attractiveness of a cage, the mesh of which was covered with beeswax, suspended in the brood chamber of a colony. The number of bees clustering on the cage diminished to about half, 20 minutes after the queen had been removed, and to about one-fifth after a further 20 minutes. Hence, queen pheromone deposited on the wax was sufficiently persistent to suggest that the widespread trails made by the queen on the surfaces of wax

combs, form an additional means by which the queen's presence is communicated within the hive.

The difference between the percentage cells accepted and finished at 24 hours queenlessness (73,3 and 67,8 % respectively), were statistically significant at a 95 % level of certainty. Beyleveld (1939) also found that there was usually a big difference between the number of cells accepted initially, and the number of finished cells procured at the end of the tenth day. At one time it was thought that the bees neglected a large number of cells if they were left for the full ten-day period in a queenless colony. Hence the cells were usually transferred after 24 hours to a special cell-finishing colony to be completed under a supersedure impulse. He then found that there were other more important factors that contribute to successful cell building. Of these, the judicious feeding of the colony is of paramount importance, i.e. an ample pollen supply and continuous sugar syrup feeding.

Butler (1954) found that one or more emergency queen cells would be started within a few hours after a colony had lost its queen. McGregor (1990), using African bees, observed that a reduction in the time that elapsed between dequeening and introducing grafts or between dequeening and the introduction of mature cells or mated queens, gave a consistently higher acceptance rate. She introduced grafted queen cells approximately three hours after she dequeened the nursing colony.

In the present study, very few or no grafted cells were accepted if they were not familiarised. When the cells were familiarised, the percentage acceptance was 73,3 % after a 24 hour queenless period, and only 2,0 % if they were not familiarised. In a similar study done by Kither & Pickard (1983), the acceptance of larvae in 12 mm cups that had been drawn over periods of 7, 14, 21 or 26 hours in the same queenless colony, was determined. The percentage

acceptance associated with each of the four times was 50 %, 70 %, 75 % and 87,5 % respectively. They concluded that some factor promoting the acceptability of the artificial queen cups was being continuously acquired during the first 26 hours of the drawing process.

Kither & Pickard (1983) found that a transplanted larva was more likely to be accepted in an artificial queen cup that had been familiarised over a two hour period, than in an undrawn one of the same initial length. Within a group of drawn or undrawn cups, larvae in the longer cups were more likely to be accepted than those in the shorter ones, and larvae in long undrawn cells were more likely to be accepted than those in short drawn cells. They also showed that the percentage acceptance of queen cups made of wax from cappings and old comb was increased in both cases after a 24 hour period in a colony prior to their use. They proposed that the new cups had acquired an acceptance-promoting substance from worker bees which had an opposite effect to any acceptance-inhibiting substance derived from queens. Ruttner (1983) found no significant difference between the percentage acceptance in familiarised and unfamiliarised queen cells.

There was no significant difference, in the present study, between the percentage acceptance when 60 or 120 cells were grafted and placed into nucleus starter colonies. It was not possible to compare the mass and performance of the queens that emerged from these grafts, but since nutrition is so important, the grafting of 120 cells into a colony is not recommended. Snelgrove (1949) found that the greater the number of queen cells reared at one time, the lighter in weight the queens, the smaller the ovaries, and the lower the number of ovarioles.

Significantly more queens (ANOVA) emerged and were accepted from queen cells introduced on day 11 compared to day 10 following grafting. The

percentage acceptance on day 10 was 77,8 % and on day 11 it was 85,8 %. A possible reason for the higher percentage acceptance on day 11, could have been that the queen was very near to emergence and not that delicate any more. In another study, when ripe queen cells (9-10 days after grafting) were introduced into queenless colonies, 90 % of the colonies were successfully requeened using Italian bees (Boch & Avitable, 1979). These colonies were queenless for only a few hours. Free & Spencer-Booth (1961) proposed that dequeening some days before virgin queen or queen cell introduction, increased success.

In the present study it was found that if a cell cup was presented to the starter colony without a larva, a rim was added at right angles to the cup wall and the cell opening was eccentric. If a cup was presented with a larva and accepted, the rim was added as a downward-facing cone with a centrally positioned cell opening. If a cup was presented with a larva but rejected, the rim was added at right angles to the cup wall, but the cell opening was centrally positioned.

Kither & Pickard (1983) observed that queenright colonies drew artificial queen cups in a similar fashion to those in queenless colonies, although the process tended to be slower and many cells were not modified for several days. The rate of drawing was also directly proportional to the size of a colony. These authors also found that the most successful undrawn cell had an opening diameter of 8,87 ± 0,027 mm and a mean length of 11,97 ± 0,029 mm (n = 50)

When rearing queen cells in a queenright colony, the batch size is already limited by the low rate of acceptance in such a stock. Many breeders who use this queenright method, find a batch size of 15 is best. When rearing queens in a queenless colony, it is not recommended to introduce more cells than the number of swarm cells that such a colony would raise under natural

conditions. Ruttner (1983) believed that 50 - 60 cells could be used in European races if open brood was removed before grafting. Boch (1979) reported that a dequeened colony may rear an increasing number of queen larvae and pupae until, as preliminary tests have shown, the pooled output of pheromone of several cells eventually reached the same level of inhibitory activity as the pheromone output of an adult queen. No more queen cells were subsequently built.

2.3.5 Queenless starting and queenright finishing of cells

The percentage acceptance of the queen cells was higher in the queenless brood chambers, than in the queenless 4-frame nucleus hives (78,6 % and 73,3 % respectively). The higher percentage acceptance in the brood chamber could be attributed to more nurse bees available. It is important that the starter colonies have a large population of nurse bees of appropriate age. Ruttner (1988) recommended that the minimum strength for a high quality nursing colony be at least eight frames, densely covered with bees. The crowding of bees into one chamber when preparing a colony for nursing was recommended, since the ratio of bees to space was of utmost importance for nursing. Additionally, the nursing colony has the most significant effect on the development of queens.

The chances of acceptance of grafted cells are certainly greater in queenless stock. In the present study, the percentage queen cells completed were also lower in queenright colonies than in the queenless colonies (62,4 % and 67,8 %). The construction of queen cells is in part inhibited by pheromones from mated laying queens, virgin queens and immature queens, the first being the more effective (Free, 1987). The pheromones produced by immature and adult queens suppress only the initial phase of queen cell construction, but they

do not inhibit the completion of the queen rearing process after it has been initiated (Boch, 1979).

The arrangement of the frames in the nucleus hive is important, because bees will not move pollen over any distance. The frame with young larvae (that was removed prior to the insertion of the frame with grafted larvae) is also important to assure a large preponderance of young bees (about seven days old) in this colony.

According to Butler (1954), the queen is able to inhibit the construction of emergency queen cells even if she is caged, provided it is a small colony. It is necessary for any given number of bees to acquire a certain minimum amount of queen substance if they are to remain inhibited from building emergency queen cells. He also showed that failing queens were able to prevent the rearing of supersedure queens, when the number of worker bees in their colonies was greatly reduced.

Queens can be reared continuously using queenless starters and queenright finishers, which is an advantage where queens are regularly needed. The fact that a queen is present in the queenright finishers, ensures that enough nurse bees are available.

2.3.6 Comparing baby and 4-frame mating nucs

The introduction of the finished queen cells into baby nucs had a success rate of 81,3 %, i.e. queens that emerged. However the percentage of mated queens obtained was considerably lower (67,4 %). One reason for this lower percentage was the method of feeding. Initially the baby nucleus colonies were fed with sugar syrup, but many bees drowned, and sometimes the queens as

well. Instead of sugar syrup, ground sugar was then used. More success was achieved with this method of feeding.

Some of the disadvantages when feeding sugar syrup is that moisture condenses at night on smooth surfaces (metal, plastic), and bees slide into the syrup and drown. Therefore the feeder must have a roughened interior with a float. Syrup can spill during transport. Thin syrup may ferment more quickly: rather use two parts sugar to one part water. Liquid feeding also promotes robbing.

An important factor in introducing queen cells is to keep the cells warm, but to avoid direct sunlight. The cells were covered in a towel after removal and introduced as soon as possible into the prepared queenless colonies. The successful introduction of queen cells to queenless colonies increases (within limits) with the length of time the colonies have been queenless (Baribeau, 1976). This author proposed that colonies stay closed in a cool dark room for at least 48 hours before taking them to the mating sites. Ruttner (1983) recommended that nucs with bees should be stored for three to five days after the introduction of the queen cells, in a moderately cool but dark and quiet room. Colonies placed out too soon, easily absconded.

Some of the advantages of using baby nuclei were that the small colonies required relatively few bees and little food to establish. The small hives were also easy to handle and the queen could be spotted and caught without difficulty. In Table 2.3, baby mating nuclei and 4-frame mating nuclei are compared to show the problems and advantages experienced with the different mating nuclei.

The introduction of the finished queen cells into 4-frame nuclei had a success rate of 85,8 % for emerged queens. The percentage mated queens obtained was 79,5 %.

Table 2.3 Comparison between baby mating nuclei and 4-frame mating nuclei

Baby mating nuclei	4-Frame mating nuclei
1. Require little food for maintaining the colony.	1. Require more food for maintaining the colony.
2. Equipment is not standard.	2. Standard equipment is used.
3. Few bees needed for establishing nuclei, therefore fewer colonies are needed to provide bees.	3. More bees needed for establishing nuclei, therefore more colonies are needed to provide bees.
4. Queen can easily be found.	4. Queen less easily found.
5. Difficult to maintain. Without brood, they sometimes abscond a few days after the young queen begins to lay.	5. Easier to maintain because of the larger number of bees, greater quantity of brood, and larger frames.
6. More care needed to maintain them in proper condition, i.e. enough bees and enough brood.	6. Almost self supporting during average conditions.
7. More preparation needed to ensure enough frames with honey, pollen and brood.	7. Because standard equipment is used, frames with honey, pollen and brood are obtained easily.

The slightly higher emergence rate in the 4-frame nuclei compared to the baby nucs, may be ascribed to the greater number of bees that could maintain the correct temperature of the brood, and therefore of the introduced queen cell. The number of queens lost after emergence in 4-frame nuclei was small, partly because no queens drowned in syrup, since the nuclei were not fed. The frames

with pollen and honey were adequate feed during the time of the experiment (November).

According to Beyleveld (1939) many virgin queens get lost on their mating flights, either by getting caught by insectivorous birds or bee pirates, or for various unknown reasons. In some localities the losses may be so great that queen rearing operations are seriously hampered: as many as 40 % of the virgin queens never reaching the laying stage. Sarling (1992) reported that a mating average of 70 % was acceptable in the USA. According to Allsopp *et al.* (1997), 85 % of the queens of *A. m. capensis* queens mated successfully.

2.3.7 Introducing mated queens

In this study the success rate of the introduction of mated queens was 75 % (n = 50). The colony into which the queen was introduced was queenless for a day or less. If this period exceeded 24 hours, it was necessary to check for queen cells and to remove them. The danger of Cape laying worker infestation is also greater the longer the colony remains queenless. As with the introduction of mature cells or virgin queens, the time elapsing between dequeening and requeening seems to be critical in *scutellata* for mated queens.

The introduction of a queen in a hair curler, enable the workers of the recipient colony to contact the queen and to obtain queen pheromones from her, thus inhibiting the changes which take place in a queenless colony. There are three mechanisms of queen pheromone transfer, by antennal contact, in transferred food, and by pheromone trails (Free, 1987). Since the presence of foreign workers alert the members of a colony, making successful queen introduction difficult, no attendants were introduced with a queen. When no food is given in a cage, the queen is compelled to solicit food from the workers of the

recipient colony. She would thus receive food having the same odour as that circulating in the colony, and the bees would be able to take queen substance from the queen's body (Butler & Simpson, 1956). The queens in the hair curlers were therefore not fed. The method is also economical in time and money, and the cage can be left in a hive indefinitely.

Because the amount of inhibitory pheromone produced depends on the age of the queen, requeening colonies at frequent intervals helps ensure that there is an adequate supply of natural pheromones in the colony (Free, 1987). The mesh of the cage containing the new queen should be large enough for the bees to make antennal contact with their queen's body. The cage should be introduced into the brood area of the colony so bees which normally provide the queen's court, are well placed to receive the queen pheromones (Free, 1987).

According to Morse (1985), honeybee queens live from one to five years. This means that an average apiary will have as many four to five year old queens as one to two year olds if the beekeeper has no requeening program. Older queens are more likely to leave with swarms than one-year old queens are. A colony that swarms seldom produces surplus honey for the beekeeper. In addition, colonies headed by older queens have smaller populations. A colony with a one-year old queen will produce on average as much as 30 pounds of honey more than a colony headed by an older queen, particularly where the queen is more than two years old (Morse, 1985).

According the Morse (1991), young queens lay more eggs, have greater populations, lay later in autumn and earlier in spring, head colonies that are less likely to swarm, and in general do all of those things that lead to greater honey production. He also points out the importance of young queens producing greater quantities of pheromones.

If there is no nectar flow when requeening is done, it is advisable to feed the colonies. Lundie (1929) observed that a strange queen or queen cell, is more readily accepted by a colony of bees during a honey flow than during a dearth of nectar, presumably because the colonies are less alert to intruders. He suggested that the queen should be caged in the hive for 24 – 48 hours or longer before she was liberated, in order to acquire the hive odour of the colony into which she was being introduced.

According to Free (1987), there are two reasons why it is difficult to introduce queens. The first reason is that the new queen will have an alien odour. She will therefore be readily recognised and rejected by the bees. It is for this reason that it is necessary to cage the new queen within the recipient colony for several hours so her body surface has the opportunity to absorb the odour of the recipient colony. The second reason is that the quality and composition of the pheromone the new queen produces is different from that of her predecessor.

The success obtained in introducing queen cells, virgin queens, or mated laying queens is greatest when the queen of the recipient colony is either at the same stage of development or the one immediately preceding that of the type of the introduced queen (Free & Spencer-Booth, 1961). Szabo (1977) showed that workers did not attack queens that were similar in age and condition, but that they were hostile to queens that were not similar. Usually no difficulty is experienced in replacing an immature queen with a virgin queen, or a virgin queen with a mated laying queen (i.e. a sequence that occurs naturally) (Free, 1987).

The successful introduction of queen cells into queenless colonies increases (within limits) with the length of time the colonies have been queenless (Free & Spencer-Booth, 1961; Baribeau, 1976). Under such conditions, the

introduced queen would maintain or increase the amount of queen pheromone in the colony.

A failing queen should be replaced as soon as possible irrespective of the season. In some parts of South Africa, where the queen has to maintain her egg-laying activities throughout the year, or where she undergoes only a very short period of rest when little or no brood is reared, annual requeening is recommended to help control Cape laying workers from taking over colonies. In non-migrating colonies, biennial queen replacement could be more than sufficient (Swart *et al.*, 2001).

Requeening became very important during the past ten years in the South African beekeeping industry. Before the Cape Bee problem became acute in 1992, and Varroa was discovered in 1997, queen rearing was generally not essential. It is increasingly becoming more difficult for beekeepers to maintain their colony numbers through splitting, and trapping swarms. Queen rearing has therefore become an essential part of beekeeping in South Africa.

2.4 References

ALBER, M.A. 1965. A study of queen-rearing methods. *Bee World* 46(1): 25 - 31.

ALLSOPP, M.H., KRYGER, P., MESKE, U. & MORITZ, R.F.A. 1997. Do Cape honeybee queens struggle in the wind? p. 147 in: Robertson, H.G. (editor). *Proceedings of the Joint Congress of the Entomological Society of Southern Africa (11 th congress) and the African Association of Insect Scientists (12 th congress), Stellenbosch.* 255 pp.

ANDERSON, R.H., BUYS, B. & JOHANNSMEIER, M.F. 1983. *Beekeeping in South Africa.* Bulletin 394, Department of Agriculture, Pretoria. ix + 207 pp.

BARIBEAU, M. 1976. Timing on introducing queen cells when requeening established colonies. *American Bee Journal* 116(3): 109.

BEYLEVELD, G.P. 1939. *A method of queen rearing for the commercial beekeeper.* Bulletin 193, Department of Agriculture and Forestry, Pretoria. 15 pp.

BOCH, R. 1979. Queen substance pheromone produced by immature queen honeybees. *Journal of Apicultural Research* 18(1): 12 – 15.

BOCH, R. & AVITABLE, A. 1979. Requeening honeybee colonies without dequeening. *Journal of Apicultural Research* 18(1): 47 – 51.

BUTLER, C.G. 1954. The importance of 'Queen Substance' in the life of a honeybee colony. *Bee World* 35(9): 169 – 176.

BUTLER, C.G. & SIMPSON, J. 1956. The introduction of virgin and mated queens, directly and in a simple cage. *Bee World* 37(6): 105 – 114.

CALDEIRA, J. 1991. Jenter queen rearing. *Gleanings in Bee Culture* 119(1): 23 – 25.

COBEY, S. & LAWRENCE, T. 1991. Queen rearing requirements. *Gleanings in Bee Culture* 119(5): 264 – 265.

DELAPLANE, K.S. 1988. A re-examination of double grafting. *American Bee Journal* 128(6): 439 – 440.

EBADI, R. & GARY, N.E. 1980. Acceptance by honeybee colonies of larvae in artificial queen cells. *Journal of Apicultural Research* 19(2): 127 – 132.

FLETCHER, D.J.C. & JOHANNSMEIER, M.F. 1978. The status of beekeeping in South Africa. *South African Bee Journal* 50(4): 5 – 20.

FREE, J.B. 1987. *Pheromones of social bees*. Chapman and Hall, London. 218 pp.

FREE, J.B. & SPENCER-BOOTH, Y. 1961. Analysis of honey farmers' records on queen rearing and queen introduction. *Journal of Agricultural Science* 56: 325 – 331.

HERBERT, E.W. 1992. Honey bee nutrition. pp. 197 – 233 in: Graham, J. (editor). *The hive and the honey bee.* Dadant & Sons, Hamilton, Illinois. 1324 pp.

JAY, S.C. 1963. The development of honeybees in their cells. *Journal of Apicultural Research* 2 (2): 117 – 134.

KITHER, G.Y. & PICKARD, R.S. 1983. Increasing the acceptance of transplanted honeybee worker larvae by queen-cell starter colonies with the use of partially drawn artificial queen cups. *Journal of Apicultural Research* 22(3): 175 – 183.

KLEINSCHMIDT, G.J. & KONDOS, A.C. 1979. Colony management for low quality pollens. *Australasian Beekeeper* 81(1): 5-6.

LAIDLAW, H.H. 1979. *Contemporary queen rearing.* Dadant & Sons, Hamilton, Illinois. 199 pp.

LUNDIE, A.E. 1929. *The rearing of queen-bees.* Bulletin No. 76, Department of Agriculture, Pretoria. 23 pp.

McGREGOR, L. 1990. Queen rearing with *Apis mellifera scutellata.* pp. 95 – 103 in: Anderson R.H. & Buys, B. (editors). *Proceedings of the International Beekeepers' Symposium "Bees and Beekeeping in Southern Africa", Stellenbosch.* 160 pp.

MORSE, G.D. 1985. A requeening plan. *Gleanings in Bee Culture* 113(3): 137 – 138.

MORSE, R.A. 1991. Are two queens best? *Gleanings in Bee Culture* 119(5): 342.

RUTTNER, F. 1956. The mating of the honeybee. *Bee World* 37(1): 2 – 15.

RUTTNER, F. (editor). 1983. *Queen rearing*. Apimondia Publishing House, Bucharest. 358 pp.

RUTTNER, F. 1988. *Breeding techniques and selection for breeding of the honeybee*. British Isles Bee Breeders Association, by arrangement with Ehrenwirth, Munich. 151 pp.

SARLING, A.J. 1992. The lofty heights of queen rearing. *Gleanings in Bee Culture* 120(5): 290 – 293.

SNELGROVE, L.E. 1949. *Queen Rearing*. Publisher: Miss I. Snelgrove, Bleadon, Somerset, UK. 344 pp.

SZABO, T.I. 1977. Overwintering of honeybee queens. 2. Maintenance of caged queens in queenless colonies. *Journal of Apicultural Research* 16(1): 41 – 46.

SWART, D.J., KRYGER, P. & JOHANNSMEIER, M.F. 2001. Queen rearing. pp. 95 – 110 in: Johannsmeier, M.F. (editor). *Beekeeping in South Africa*. Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria. 288 pp.

CHAPTER 3

ARTIFICIAL FEEDING

3.1 Introduction

Honeybees, like most animals, require proteins, carbohydrates, minerals, fats (lipids), vitamins, and water for normal growth and development. These nutritional needs are satisfied by the collection of nectar, pollen and water. Nectar, which is collected by honeybees from either floral or extra-floral nectaries, satisfies the carbohydrate requirement of the bees. Pollen, which is collected from a wide range of flowering plants, normally satisfies the dietary requirements for proteins, minerals, lipids and vitamins (Herbert, 1992).

Nectar ranges in sugar content from 4 % to 60 % or higher depending upon the floral source and weather conditions. Nectar and sugar solutions containing 30 – 50 % sugar seem to elicit the maximum collection response by honeybees. This is the reason why nectar containing small amounts of sugar, such as in pears, may not be attractive to bees (Herbert, 1992).

Sugar syrup feeding is beneficial to any colonies short of food reserves, for newly formed small colonies or nuclei, or when an unseasonable cold spell occurs after foraging and substantial brood rearing have commenced (Crane, 1990). Sugar syrup feeding is also used when a colony is subjected to certain manipulations, such as queen rearing.

Although honeybees can utilise many sugars in their diets, there are also several sugars that are either toxic or useless to honeybees because the bees lack the proper enzymes for digestion. Sugars which are either toxic to honeybees or reduce their longevity include mannose, lactose, galactose and raffinose. Mannose is especially toxic and will kill honeybees within a few minutes of feeding. Both lactose and raffinose reduce the longevity of bees when offered to caged honeybees. Lactose is found in milk and milk products, and raffinose occurs naturally in soybeans (Herbert, 1992).

According to Herbert (1992) the development of body tissue, muscles and glands, depends upon adequate amounts of protein in the honeybee's diet. During the early adult life of worker bees all nitrogen is derived from pollen protein. Consequently, young bees must consume a large quantity of pollen in the first two weeks of their adult life. Some worker bees begin consuming pollen within one to two hours after emergence. Within twelve hours after emergence, 50 % or more of the workers have already consumed pollen in small amounts. Mass consumption begins when the bees are 42 to 52 hours old, and reaches a maximum when they are five days old (Herbert, 1992).

According to Nabors (1996), large quantities of pollen are carried in the form of pollen loads into every honeybee colony during a single season. Reliable estimates suggest that from 20 to 32 kg per year are required by an average colony.

The honeybee colony is completely dependent on factors enabling the collection of pollen, such as season, ambient temperature, distance to the pollen source and amounts of pollen available. Therefore, the pollen supply is a limiting factor in beekeeping practice (De Groot, 1953).

Since pollen is often not present in adequate quantities in the field, beekeepers frequently supplement fresh pollen supplies by feeding bees with pollen substitutes or supplements or commercial trapped pollen. Colonies are normally fed supplemental foods to produce strong colonies for package production, to develop colonies with optimum populations for pollination of crops, to build up colony population for autumn and spring division, for queen production, to help overcome pesticide damage, or to assist colonies to overcome diseases which may be associated with nutritional deficiencies (Winston *et al.*, 1983).

A pollen substitute is any material which, when fed to colonies of honeybees, replaces the pollen requirement of that colony for a short period of time. The same pollen substitute or any other proteins becomes a pollen supplement when pollen is added to the diet as an attractant or to increase its nutritive value. Pollen substitutes can be fed outside the hive in trays where the substitute can be collected by foraging bees, or inside the hive as a moist patty over the brood frames.

In many areas in South Africa, honeybee colonies are able to collect little or no natural food during certain times of the year, especially during winter. These are periods of resource expenditure where colonies steadily consume their honey and pollen stores and their hive weights constantly fall. Artificial sugar feeding is relatively easy to apply but the feeding of pollen supplements and substitutes pose problems. According to literature, there have been many attempts to develop pollen substitutes suitable for rearing honeybee brood in times of pollen scarcity. Acceptability of such substitutes was usually a problem. In South Africa artificial feeding of pollen supplements and substitutes has been tried by commercial and small-scale beekeepers, with mostly negative results.

In this artificial feeds study, the following was investigated:

1. Different methods of sugar syrup feeding.
2. The preference of honeybees for:
   - 2.1 dry and moist substitutes outside hives.
   - 2.2 dry substitutes and supplements outside hives.
   - 2.3 moist substitute and supplement patties inside hives.
3. Brood production with moist substitutes and supplements inside hives.

3.2 Material and Methods

3.2.1 Sugar syrup

3.2.1.1 Testing of different sugar syrup concentrations

The experiment was conducted in Douglas, Northern Cape Province during October 1993 and the temperature during this period ranged from 19,6 °C to 36,0 °C.

Different concentrations of sugar syrup, namely 40 %, 50 %, 60 %, 70 %, 80 % and 90 %, were prepared and left at room temperature in the laboratory. Observations were made for changes in colour, fungal growth on the surface, odour, crystallisation in the form of a surface layer, and crystals or other changes in the syrup.

In this experiment sterilised plastic honey jars were used as containers. The concentrations were made up with tap water, boiled or unboiled. In the

experiment with the unboiled water, the sugar was dissolved in cold water, and in the experiment with the boiling water, the sugar was dissolved in hot water.

3.2.1.2 Testing of two different methods of sugar syrup feeding

The experiments were conducted during March and April 1995 on an agricultural holding in Bainsvlei (29° 03' S, 26° 07' E, 1372 m above sea level), 12 km west of Bloemfontein. Two different methods of feeding sugar syrup were used.

In the first, a plastic two-litre bottle with two holes (approximately 2 mm in diameter) at the opposite side of the handle, was used (Figure 3.1). Four super frames had to be removed so that the bottle could fit into the super. The bottle was placed on its side inside a wooden tray (16 x 30 x 4,5 cm). The wooden trays were manufactured especially for feeding purposes and dipped into molten beeswax before they were used, to prevent leakage of the sugar syrup. Four colonies containing approximately 8 brood frames of bees were used in this experiment.

In the second method, a Park feeder (Laidlaw, 1979) was located on the outside, at the back, of the hive (Figure 3.2). The feeder was fitted to a thin frame, open at the back, that is held between the brood chamber and the super. The feeders were constructed of metal. Three colonies were used for this experiment, because only three feeders were available. The bees could reach the sugar syrup through the opening at the back of the feeder frame. A small compartment of expanded metal inside the feeder aided in preventing the bees from drowning (Figure 3.3). The feeders had a volume of 2,8 litre, but only two litre sugar syrup were fed at a time.

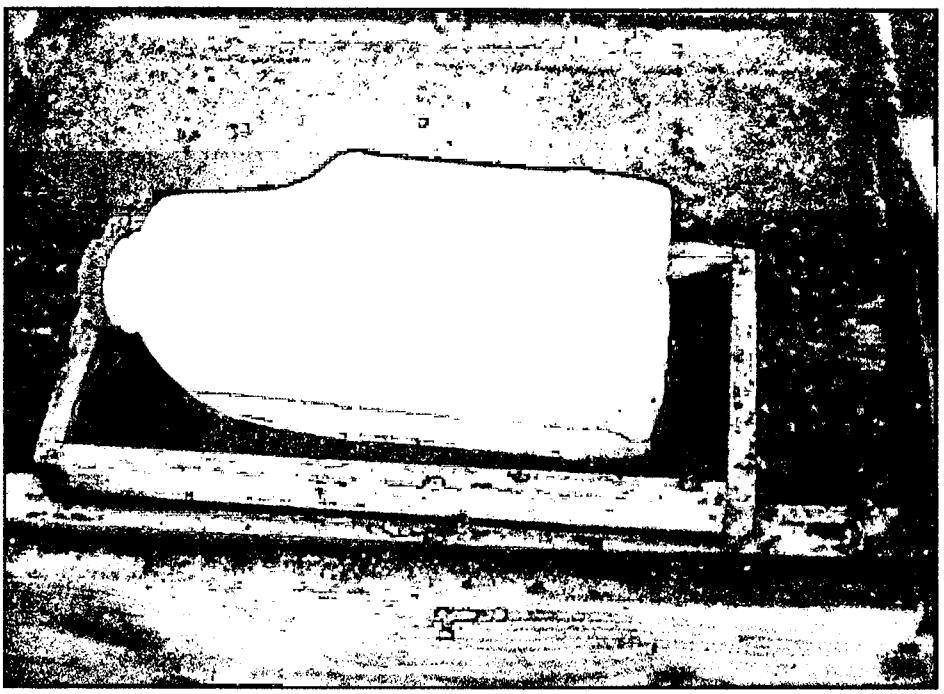

Figure 3.1 Feeding sugar syrup inside the hive

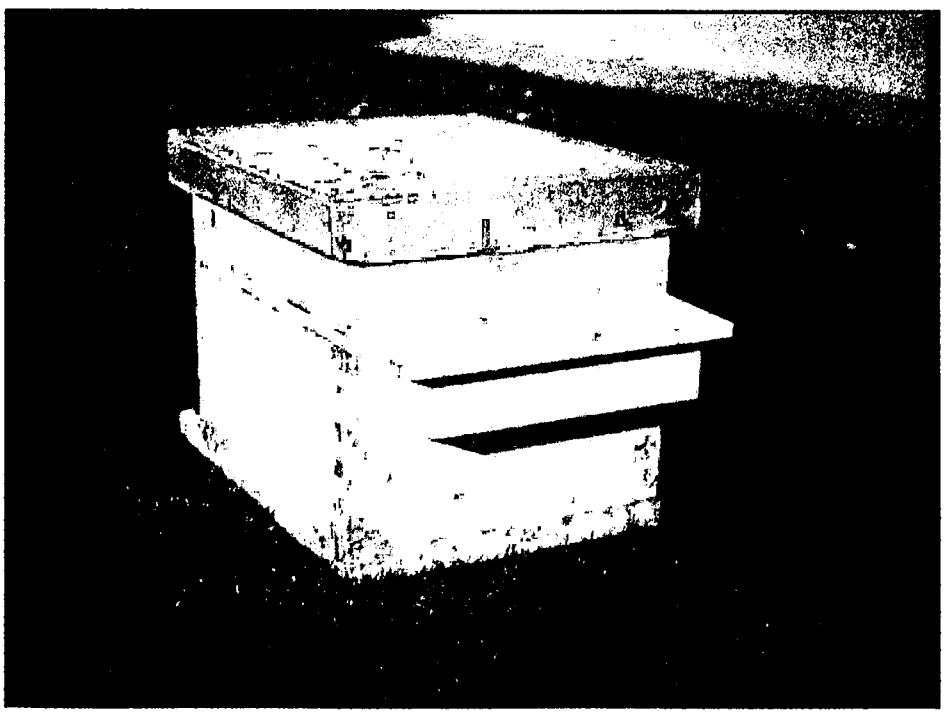

Figure 3.2 Feeding sugar syrup with a Park feeder on the outside of the hive

The feeders were compared in terms of the bees' preference for the feeder, the speed with which the bees consumed the sugar syrup and the efficacy of the feeders.

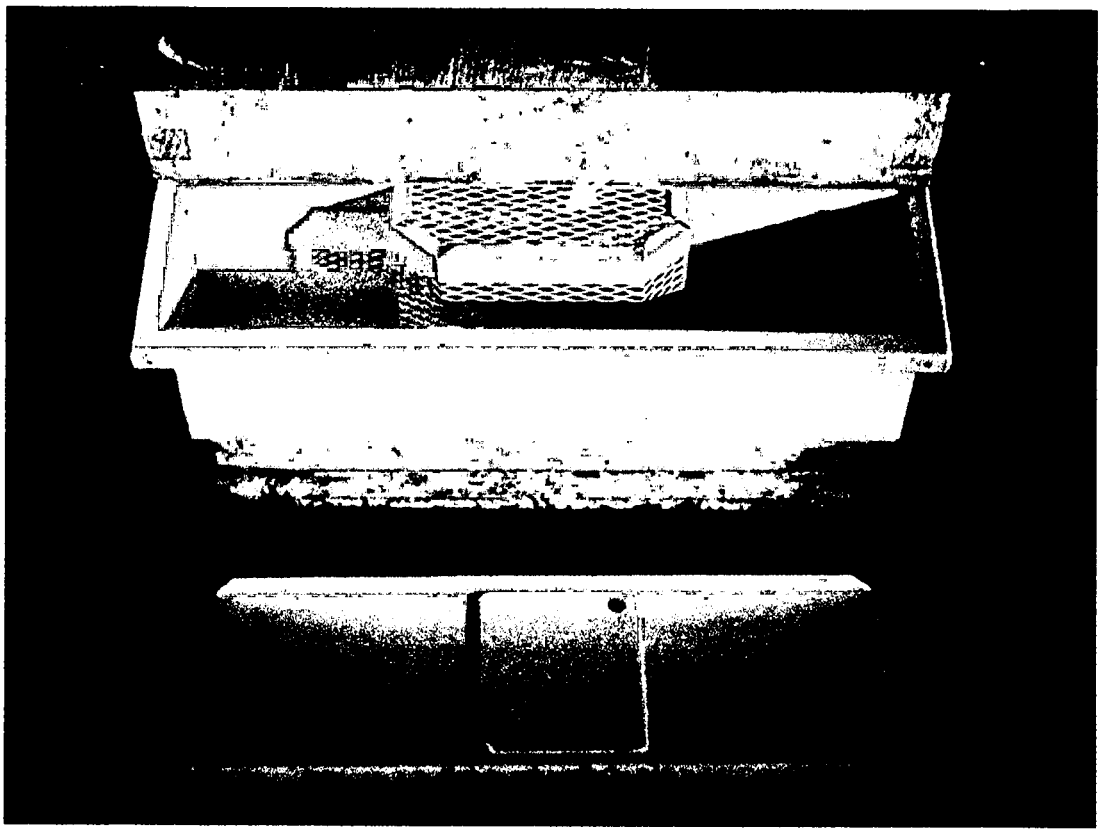

Figure 3.3 Compartment of expanded metal inside the Park feeder.

3.2.2 Pollen substitutes/supplements

3.2.2.1 Preference for dry and moist substitutes outside hives

The experiments were conducted from May 1994 to April 1995 on a smallholding 12 km west of Bloemfontein. Six different pollen substitutes were placed outside the hives (Figure 3.4) once a month, for eleven consecutive

months. The substitutes were placed in petri dishes on a table approximately 25 m north of the colonies. The number of bees foraging in the petri dishes was counted every hour during daylight to determine which substitute was preferred. The substitutes were placed outside before dawn and were removed at dusk, when the bees ceased to work.

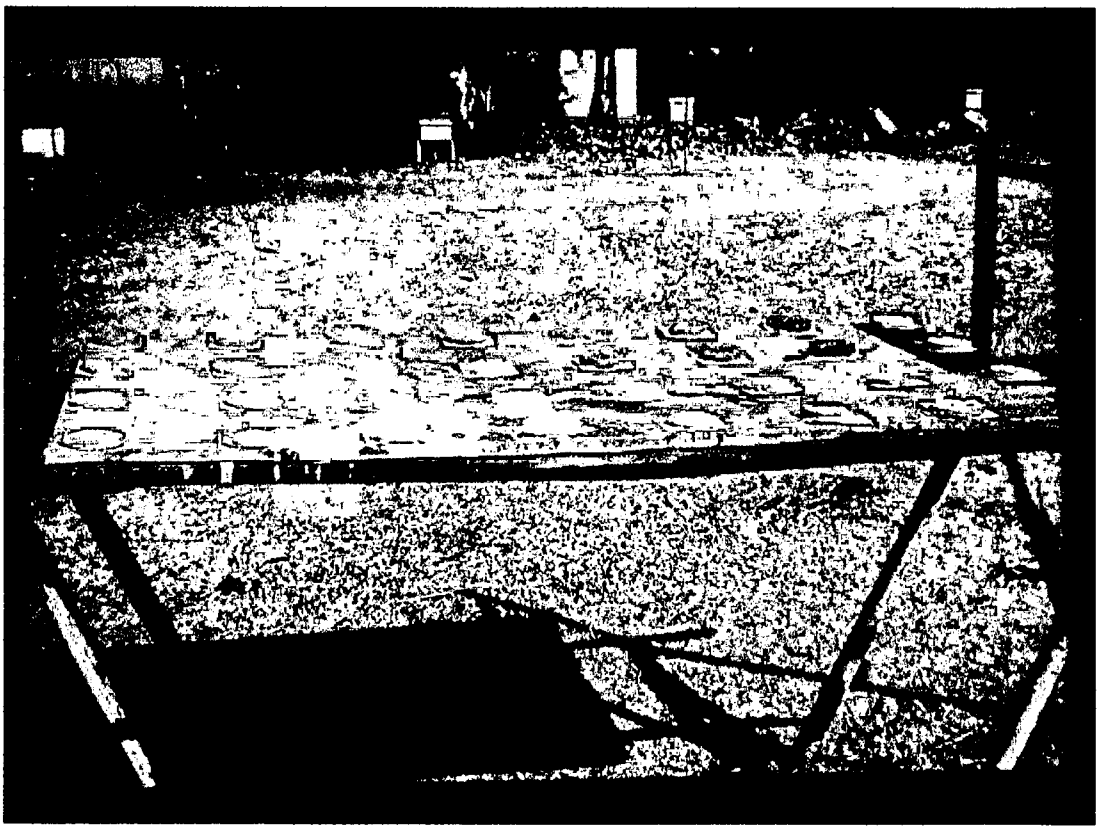

Figure 3.4 Testing preferences for pollen substitutes outside hives

Each of the following substitutes were mixed with sugar, or sugar syrup, or honey, or without anything added, i.e. 24 different treatments. The substitutes were:

1. Maize meal (unsifted)
2. Maize meal (sifted)

3. Maize meal (sifted) + Lotmix ® (19:1 w/w) (Lotmix ® is a high protein concentrate for ruminants, used in feedlots for cattle fattening)
4. Soy flour (defatted, heated) + commercial brewer's yeast + skimmed milk powder (household use) (3:1:1)
5. Soy flour + brewer's yeast (3:1)
6. Brewer's yeast

In conjunction with the experiments on pollen substitutes, natural pollen was collected with a pollen trap on one colony, to determine when pollen was available. Pollen was removed once a week from the pollen trap and weighed. Pollen pellets were sorted, counted and identified. The pollen trap was installed on 30 June 1994 and used until 3 August 1995. The pollen trap remained on the same hive for the entire year.

3.2.2.2 Preference for dry substitutes and supplements outside hives

A second test for the preference of dry substitutes and supplements was conducted outside hives in Pretoria on 21 – 22 May 1997.

The substitutes/supplements were placed in petri dishes on variously coloured paper disks (12 x 20 cm) on a table (20 m south of the colonies) in a 12-hive apiary, one hive having been fitted with a pollen trap and four others with sugar syrup feeders. Two petri dishes for each of the substitute treatments were used. The number of bees foraging in the petri dishes were counted at three different times on two days namely 11:30 and 13:00 on 21 May and 10:45 on 22 May 1997. The positions of the petri dishes with their paper disks were changed on the table at least two hours prior to counting.

The following dry substitutes and supplements was tested:

1. Soy flour (defatted, heated) + maize meal (household) (3:1 w/w)
2. Soy flour + maize meal + sugar (castor) (6:2:1)
3. Soy flour + pollen (eucalypt, ground) (3:1)
4. Soy flour + brewer's yeast + skimmed powder milk (3:1:1)
5. Maize meal
6. Chicken mash (commercial brand)
7. Maize meal + sugar (2:1)
8. Pronutro ® (commercial breakfast food, ground, containing maize, soy flour, milk solids, minerals, vitamins) + sugar (2:1).

3.2.2.3 Preference for moist substitute and supplement patties inside hives

Experiment 1 - Bloemfontein

The preference for moist substitute patties was tested in Bloemfontein during June 1995. Six colonies were used in this experiment.

The preference for the following substances was tested inside the hives:

1. Maize meal
2. Soy flour (defatted, heated) + commercial brewer's yeast + skimmed milk powder (household use) (3:1:1)

Patties of the pollen substitutes were prepared, by mixing them with honey and 60 % sugar syrup respectively until a non-runny, doughy consistency was attained. The patties were weighed, covered with wax paper and placed on

the top bars of the brood frames (Figure 3.5). Three hives were used to test the maize meal patties, and three hives were used to test the soy flour patties. The sugar syrup and honey patties were tested in the same hive. The patties were removed once a week and replaced with fresh patties.

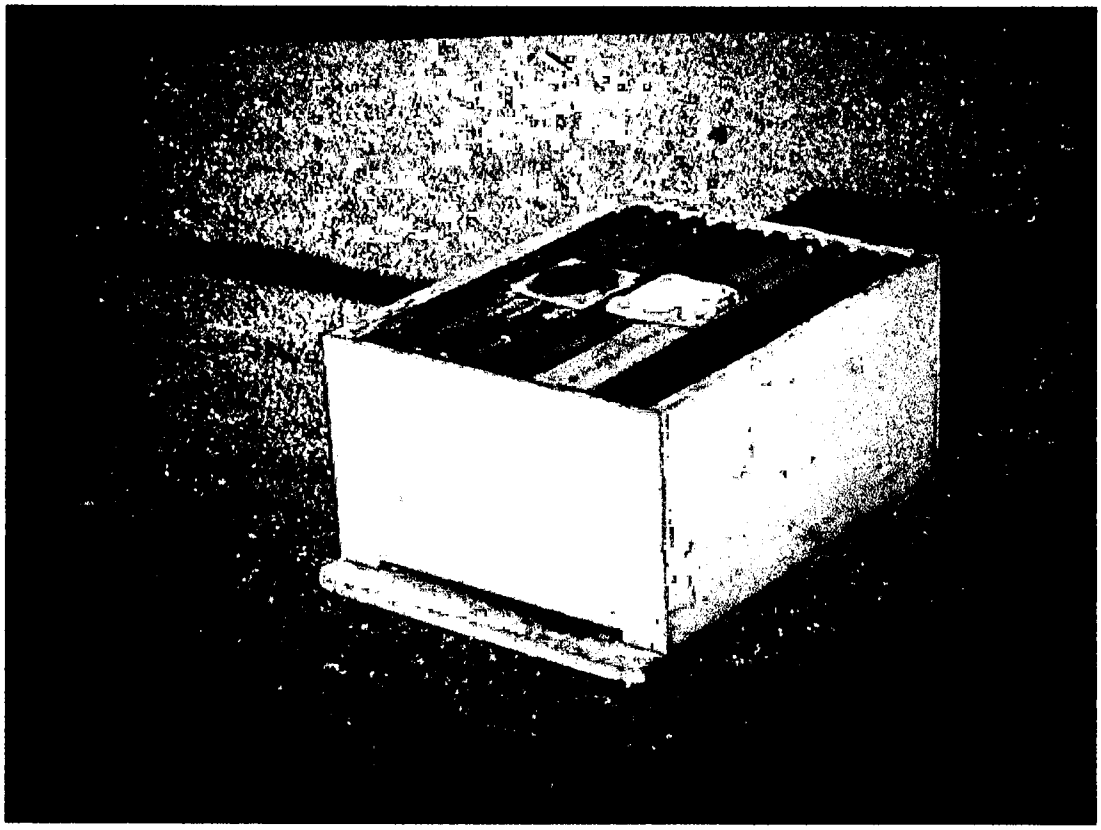

Figure 3.5 Feeding substitute patties inside the hive

Experiment 2 - Pretoria

The preference for moist substitute and supplement patties were tested in Pretoria from 20 May 1997 and terminated on 10 June 1997.

The various compounds were mixed with 40 % sucrose syrup until a non-runny, doughy consistency was attained. This was then pressed into petri dishes, which were weighed and placed open-side up on the top of the brood frames of four different colonies that were fed with sugar syrup. Once in between, the petri dishes were weighed, filled and returned upside down. The petri dishes were again weighed at the end of the test.

Preference for the following patties was tested inside hives:

1. Beltsville substitute(a commercial USA bee feed containing torula yeast, lactalbumin, vitamins and minerals)
2. Maize meal
3. Soy flour + pollen (3:1 w/w) (pollen trapped from *Eucalyptus tereticornis*)
4. Yeast + milk + pollen (2:2:1)
5. Pronutro ®
6. Soy + pollen (9:1)
7. Soy + yeast + milk (3:1:1)

3.2.2.4 Brood production with moist substitutes and supplements inside hives

Brood production with moist substitutes and supplements were tested from 3 June 1997 to 18 July 1997 at Roodeplaat north of Pretoria. The colonies were fed sugar syrup throughout this period.

Brood production with the following substitutes/supplements was tested:

1. Pronutro ® + pollen (4:1 w/w)
2. Beltsville substitute
3. Soy and pollen (4:1)
4. Soy + yeast + milk (3:1:1)
5. Soy + yeast (3:2)
6. Soy + yeast + egg (commercial whole egg powder) (2:1:2)

The compounds were mixed with 40 % sugar syrup to a doughy consistency. This was then pressed into flat styrofoam punnets as patties that were weighed and then placed upside down on the top bars of brood frames.

Plastic 2-litre milk bottles were used as syrup containers. They were provided with two 3 mm holes at the top of one side, the side which was laid flat into a shallow rectangular plastic tray with a 15 mm rim, used for flowerpots. This feeder occupied the space of three super frames.

Feeding and inspection was done weekly and colony evaluations fortnightly, namely amount of substitute/supplement consumed, brood area, colony size, and amounts of pollen and honey. The substitutes/supplements were replaced and weighed when they were almost consumed. Only three replications for each treatment were possible and no controls were employed, because of colony losses due to honey-badger damage just prior to the test. One hive was fitted with a pollen trap.

3.3 Results and Discussion

3.3.1 Sugar syrup

3.3.1.1 Testing of different sugar syrup concentrations

In the solutions mixed with unboiled water, fermentation began on Day 4 in the 40 % solution. Fermentation of the 50, 60 and 70 % solutions began on day 7, 8 and 9 respectively (Table 3.1).

Fermentation in the boiled water solutions, started on day 6 (40 % solution). None of the 50 to 90 % solutions fermented during the experiment.

Crystallisation in the form of a surface layer developed on both the 90 % solutions on day 6 i.e. mixed with unboiled and with hot water (Table 3.1).

A 60 % sugar solution in boiled water is recommended for feeding honeybees before a honeyflow or during queen rearing. This concentration does not ferment easily, fermentation started on day 8 (Table 3.1) and permits feeding once a week. Labour can therefore be reduced, as it allows the provision of enough syrup to last a week.

3.3.1.2 Testing of two different methods of sugar syrup feeding

The sugar syrup in the bottle feeder was consumed faster, presumably because the temperature of the syrup was higher than that of the Park feeder. Both types of feeders were very efficient, however, requiring refilling once a week only.

Table 3.1 Changes in sugar syrup of different concentrations

DAY	TYPE OF CHANGE	UNBOILED WATER						BOILING WATER					
		40%	50%	60%	70%	80%	90%	40%	50%	60%	70%	80%	90%
1	1*												
	2*												
	3*												
	4*												
	5*												
2	1*												
	2*												
	3*												
	4*												
	5*												
3	1*												
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	5*												
4	1*												
	2*	X											
	3*	X											
	4*												
	5*												
5	1*												
	2*	X											
	3*	X											
	4*												
	5*												
6	1*												
	2*	X						X					
	3*	X						X					
	4*						X						X
	5*												
7	1*												
	2*	X	X					X					
	3*	X	X					X					
	4*						X					X	X
	5*												
8	1*												
	2*	X	X	X				X					
	3*	X	X	X				X					
	4*						X					X	X
	5*												
9	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*						X					X	X
	5*												
10	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*						X					X	X
	5*												
11	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*						X				X	X	X
	5*												
12	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*					X	X				X	X	X
	5*												
13	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*					X	X				X	X	X
	5*												
14	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*					X	X				X	X	X
	5*												
15	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*					X	X				X	X	X
	5*												
16	1*												
	2*	X	X	X	X			X					
	3*	X	X	X	X			X					
	4*					X	X				X	X	X
	5*												

1* Colour, 2* Fungal growth on the surface, 3* Odour, 4* Crystallisation in the form of a surface layer,

5* Crystals or changes in the syrup

An advantage of the feeding method inside the hive is that the temperature of the sugar syrup remains more constant than that of the feeder on the outside of the hive. However, if the colony is very small, the bees are not able to regulate the temperature of the extra hive space. No problems with robbing or with ants were experienced with the bottle feeder. A disadvantage of the bottle feeder is that the hive has to be opened every time sugar syrup has to be replenished, resulting in a drop of the temperature of the hive, particularly in winter. It is important to avoid a drop in temperature, because at 31 °C there can be damage to the open brood (Table 3.4) and at 26 °C there can be damage to emerging brood. This method is also more labour intensive.

Feeding sugar syrup from outside the hive has the advantage that the bees are not disturbed. In this type of feeder, the ease of refilling may be counter-balanced by the lack of heat from the colony to keep the syrup warm. According to Laidlaw (1979), the Park feeder is one of the most convenient feeders. The lids of the feeders used in the present test did not close properly at the beginning, and robber bees managed to get into the feeders, but when the lids were repaired, no further problems were experienced.

When sugar syrup is fed to bees, it is important that a sufficient volume that can last for at least a week is provided, because feeding bees is time-consuming and expensive.

3.3.2 Pollen substitutes/supplements

3.3.2.1 Preference for dry and moist substitutes outside hives

The results of the Bloemfontein substitute preference test are presented in Table 3.2. Yeast and mixtures of yeast, soy and powder milk did not attract

any bees, whether dry or moist. Many bees were attracted to dry maize meal and most to the Lotmix + maize mixture. Dry mixtures were far more attractive to bees than moist mixtures containing honey and sugar syrup.

Table 3.2 Preference for dry and moist pollen substitutes outside hives

Substitute		Mean number of foraging bees			
Substitute ⇓	Additive ⇒	Nothing	Dry Sugar	Sugar syrup	Honey
Unsifted Maize		26,5 ± 17,7	32,5 ± 21,7	0,8 ± 0,5	2,3 ± 1,7
Sifted Maize		28,3 ± 20,5	34,3 ± 29,2	1,5 ± 1,3	0,8 ± 0,5
Sifted maize + Lotmix (14:1)		39,0 ± 26,2	61,5 ± 41,2	3,0 ± 2,0	2,0 ± 2,0
Soy + Yeast + Milk (3:1:1)		0	0	0	0
Soy + Yeast (3:1)		0	0	0	0
Yeast		0	0	0	0

The acceptance of the different pollen substitutes was correlated with the amount of natural pollen available. Only during the winter months, May to August, when very little pollen was available, did the bees collect the substitutes. The mean number of bees collecting pollen substitutes were the highest during June and July, when hardly any natural pollen was available in comparison with the 400 - 500 g/month during September to December and again in March and April (Table 3.3). No bees collected any of the substitutes during February 1995, despite the low availability of natural pollen. Presumably there was still enough stored pollen available in the colonies. Figures for the amount of pollen collected for May and June 1994 were not available, because the pollen trap was only installed on 30 June 1994.

The bees preferred the substitutes fed dry, and collected very little of the substitutes mixed with honey or sugar syrup. When natural pollen became abundant, all the pollen substitutes were ignored.

The highest number of bees collecting pollen substitute was counted on the mixture of sifted maize meal and Lotmix® with dry sugar added (Table 3.2). It is not known why this mixture was more attractive, but a possible reason may be the smell or physical property of the mixture.

The substitutes that contained no maize meal were not collected. Mixtures containing fine maize meal were preferred to the mixtures containing brewer's yeast, probably because of the physical nature of the maize meal which made gathering of loads in the corbiculae easier. According to Johannsmeier (2001) the texture of the substitute is very important: the finer, the better.

The dry substitutes were collected in the following descending order of preference: sifted maize meal + Lotmix®, sifted maize meal, unsifted maize meal. The substitutes with ground sugar added, were preferred to the other substitutes. These results correspond to those of Jones (1995), who found that the addition of powdered sugar increased the attractiveness of substitutes. A possible reason for the preference of added sugar, the results of which are opposite to those under 3.3.2.2, may be that in this experiment, fresh substitute was used at each replication, but in 3.3.2.2 the substitutes remained outside overnight and a crust formed.

Foraging commenced at about 11:30, and continued until 17:30 (Figure 3.6). A possible explanation is that the temperature during May to July was very low early in the morning and late in the afternoon. According to Crane (1990), the lowest temperature for flight in winter is 8 °C - 10 °C and the lowest temperature for foraging is 13 °C - 14 °C (Table 3.4).

Table 3.3 Amount of natural pollen collected on a monthly basis by one colony over a period of one year, compared to the mean number of bees collecting pollen substitutes

Month	Amount of pollen (g)	Number of substitute collectors
May 1994	?	270
June	?	314
July	11,71	340
August	115,08	5
September	441,33	0
October	345,80	0
November	527,09	0
December	557,61	0
January 1995	378,68	0
February	107,73	0
March	384,08	0
April	542,22	0
May	102,34	-
June	36,21	-
July	30,69	-

Feeding dry substitutes to honeybees outside the hives, presents several problems. The strongest colonies take the major share of the food, whereas the weaker colonies, which may need it most, collect the least amount (Herbert, 1992). If there are neighbouring colonies of bees within a radius of a few kilometres, they may also collect this food.

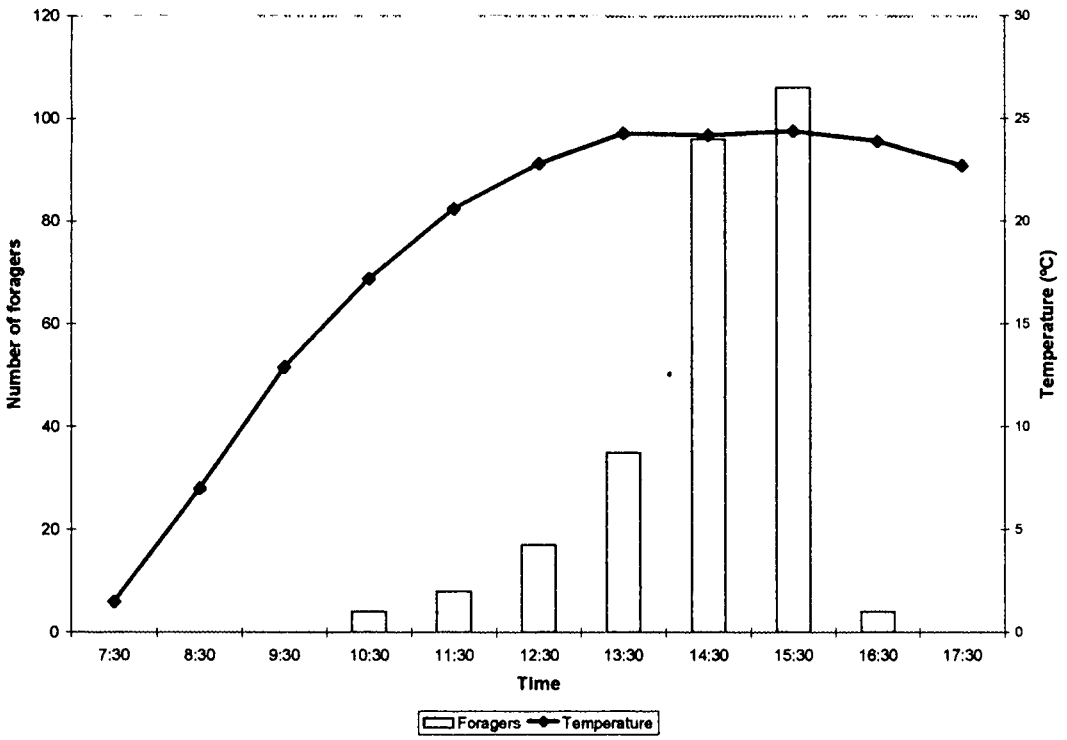

Figure 3.6 Number of pollen substitute foragers counted hourly on 14 May 1994 at Bloemfontein

Table 3.4 Critical temperatures for honeybees*

Normal temperatures		Damaging temperatures	
14 °C	Hive temperature at which colony forms winter cluster	31 °C	Damage to open brood
13 °C to 14 °C	Lowest temperature for foraging	26 °C	Damage to emerging brood
8 °C to 10 °C	Lowest temperature for flight in winter	-2 °C to -6 °C	Workers die (depending on period of exposure)

*(after Crane, 1990)

3.3.2.2 Preference for dry substitutes and supplements outside hives

Honeybees are often a nuisance, collecting cattle and chicken feeds that contain crushed maize. Van den Heever (1997) found maize meal (dry as well as moist) preferred to several other pollen substitutes. Maize meal was therefore included in this test on its own and in combination (w/w) with soy powder and castor sugar (Table 3.5). Alone it ranks 5th in the eight tested substitute combinations, but with soy, it ranks 1st and 2nd (Table 3.5). The soy:maize combinations produced slightly higher collecting activity than the soy:pollen combination, also indicating the attractiveness of maize meal which unfortunately has a very low nutritional value for bees (Johannsmeier, 2001). Soy combinations occupy all first four positions, evidently denoting a high acceptability in this dried form (Johannsmeier, 1998).

According to international literature, e.g. Jones (1995), the impression was gained that the addition of powdered sugar increased the attractiveness of powdered substitutes, and it was therefore included in this test. The results, however, indicated the opposite to be true, one reason possibly being that a thin crust forms with high moisture at night.

The medium attractiveness of maize meal and commercial chicken mash would corroborate the reports of bees causing problems on chicken and stock farms, stinging cattle when they collect these feeds.

Very little pollen was trapped, about 50 pellets a day, on the two test days, indicating a relative shortage of natural pollen.

Table 3.5 Preference test of dry pollen substitutes and supplements outside hives

Substitute/Supplement	Number of foraging bees
Soy + Maize (3:1)	70
Soy + Maize + Sugar (6:2:1)	60
Soy + Pollen (3:1)	58
Soy + Yeast + Milk (3:1:1)	56
Maize	48
Chicken mash	44
Maize + Sugar (2:1)	19
Pronutro + Sugar (2:1)	14

3.3.2.3 Preference for moist substitute and supplement patties inside hives

Experiment 1 - Bloemfontein

None of the patties fed inside the hives, were accepted and collected by the bees. Very little natural pollen was available during June (36,21 g, Table 3.3). One possible reason for the results may be that the wax paper used to cover the patties was not punctured before the patties were placed on the top bars. The wax paper was used to cover the patties to prevent them from drying out. The colonies used in this experiment were also not fed with sugar syrup. Another reason why none of the patties were accepted, is because there was no honeyflow, i.e. sugar syrup or nectar at the time of the experiment.

Experiment 2 - Pretoria

Acceptability of substitute patties is the foremost problem worldwide with artificial bee feeds. Since pollen substitutes are used as patties inside hives almost without exception, and because no published information on substitute tests exist, using the African bee, preference and brood production tests were carried out near Pretoria during winter months.

The Beltsville substitute (not tested in the dry form) and maize meal surpassed the other compounds by far in acceptability (Table 3.6). The results of the two soy + pollen supplements confirmed overseas conclusions that attractiveness increases with an increase in the pollen quota (here 10 % versus 20 % versus 25 %). The "classic" combination of soy + yeast + milk was the least attractive of the seven treatments. Torula yeast seems more attractive to bees than brewer's yeast (M. Allsopp, Stellenbosch, personal communication).

Table 3.6 In-hive preference tests of pollen substitute and supplement patties

Substitute/Supplement	Total amount consumed (g)
Beltsville	127,7
Maize	109,4
Soy + Pollen (3:1)	68,6
Yeast + Milk + Pollen (2:2:1)	68,2
Pronutro	62,7
Soy + Pollen (9:1)	43,8
Soy + Yeast + Milk (3:1:1)	27,0

The results in Table 3.7 verify observations by beekeepers and bee scientists that individual colonies in the same apiary may have preferences for different pollens or substitutes if they have a choice (Johannsmeier, 1998).

Table 3.7 Preference of individual colonies for pollen substitutes and supplement patties

Colony Number	First Choice	Amount consumed (g)	Second Choice	Amount consumed (g)
1	Maize	38,3	Soy + Pollen	28,7
2	Beltsville	51,5	Yeast + milk	28,7
3	Beltsville	54,1	Soy + Pollen	43,2
4	Pronutro	35,4	Maize	27,4

3.3.2.4 Brood production with moist substitutes and supplements inside hives

The results in Table 3.8 are difficult to interpret, more so because only three replications were used, and no control colonies were included (shortage of colonies due to honey-badger damage). Some general conclusions can nevertheless be made.

The amounts of substitute/supplement consumed are an indication of their attractiveness and broadly agree with the results in Table 3.6 – the Beltsville and Pronutro + pollen patties had to be replenished frequently. The Beltsville substitute has a drawback, however, in that it tends to set hard, making its removal by bees difficult.

Table 3.8 Brood production, consumption and crude protein content of six pollen substitutes/supplements

Pollen substitute/supplement	Amount consumed (g)	Total brood increase (dm^2)	Crude protein (%)
Pronutro + Pollen (4:1)	961	106	21,4
Beltsville	784	106	14,2
Soy + Pollen (4:1)	623	100	35,6
Soy + Yeast + Milk (3:1:1)	353	188	39.2
Soy + Yeast (3:2)	111	97	41,3
Soy + Yeast + Egg (2:1:1)	50	86	43,9

The high brood production of the last three soy substitutes, compared to the small amounts consumed strongly points to an extraneous protein source. Very little pollen was trapped, initially from *Asparagus*, later from *Rhus lancea*. Because of the low attractiveness of said substitutes (yeast and egg), scouts and foragers at the start of the experiment probably ventured further afield to find the sources of identified pollens.

In Table 3.8, the crude protein levels of the compounds have little relevance, since the amounts of the compounds consumed determined whether adequate quantities of nutrients were consumed. The Beltsville feed contains appropriate amounts of different minerals, vitamins and specific amino acids, hence its low crude protein level, but nevertheless strong brood production capacity (Table 3.8).

The patties containing egg powder became mouldy, probably because of their composition and the fact that they were hardly consumed.

According to Standifer *et al.* (1977), none of the protein supplemental foods fed to honeybees is a complete replacement for natural pollen, nor can they be regarded as more than adequate supplements for natural pollens. However, beekeepers can use protein supplemental foods to improve the nutrition of their bees when natural pollen is scarce. A good protein supplement food for bees is one that they will readily consume and has the quality and quantity of proteins, lipids, vitamins and minerals required for growth and development of individuals and reproduction of the colony. No pollen type or substitute would furnish a complete diet for the bees on its own. In order to have a complete diet for the bees, they need a mixture of pollens so that where one kind is deficient, this deficiency would be made up by another kind (Taber, 1996).

Kleinschmidt & Kondos (1979) found that colonies with adequate brood areas and populations, but averaging low in body protein, dwindle rapidly when exposed to a heavy workload. Such colonies become too weak and respond slowly when nutritional conditions improve.

According to Kleinschmidt & Kondos (1978), bees in colonies with a rapid decrease in body protein, lived 20 – 26 days under heavy honeyflow conditions, whereas bees in colonies which maintained body protein above 40 %, had a lifespan of 46 – 50 days. Colony reproduction did not completely compensate for deaths when longevity was short, whereas increased longevity allowed colonies to maintain large populations during a twelve week honey flow.

According to Standifer *et al.* (1977), pollen supplements are usually more acceptable to bees than are pollen substitutes. Bee-collected pollen releases biostimulant chemicals in the artificial protein food supplement that are attractive to bees and contain other constituents that aid in keeping the supplement moist, soft and palatable. Pollen intended for use in protein supplemental diets should

be stored in a freezer or dried and stored in airtight containers for no more than two years. Pollen supplement diets containing 20 % or more of either soybean flour or brewer's yeast are highly palatable to bees and have the nutritive requirements for their growth and reproduction. The colony should be provided with a new supplement cake before all the previous cake is consumed. Herbert (1992), reported that honeybees fed natural pollen reared significantly more brood than those fed the pollen substitute.

Pollen stored in a freezer gradually loses its attractiveness and nutritive value for honeybees. This deterioration may be due, in part, to the loss of essential amino acids. Herbert (1992), showed that the nutritional effects of stored pollen (3 years old) could be restored to the biological value level of fresh pollen by the addition of two amino acids, namely lysin and arginine. However, after 13 years of storage, the pollen was shown to have deteriorated to such an extent that the addition of amino acids could not restore the original nutritional value (Herbert, 1992).

Disadvantages of the outdoor feeding are that weak colonies often get less of the substitute than they need, that it must be protected from inclement weather, and that during bad weather bees are often unable to collect it. A moist patty placed inside the hive is, therefore, preferred, since its availability to the bees is not influenced by the weather. However, pollen substitute patties should preferably maintain their moist consistency and high nutritional value over long periods.

Most pollen substitutes offered to bees are nutritionally adequate, and some apparently surpass pollen in nutritive value, but when bees have a choice, they usually eat considerably more natural pollen than pollen substitute (Herbert, 1992).

In the summer rainfall areas of South Africa, winter feeding of dry substitutes should be encouraged and further tested. This form of feeding elicits greater collecting activity in honeybees compared to in-hive patties, and it is easier and cheaper to apply (Johannsmeier, 1998).

Table 3.9 Approximate wholesale prices of substitutes as in 1997

Substitute	Price
Beltsville	R55-00/kg
Egg powder	R28-00/kg (1st grade); R8-00/kg (2nd grade)
Milk powder	R20-00/kg
Pronutro ®	R17-00/kg
Soy meal	R 9-00/kg
Brewer's yeast	R 3-00/kg (large quantities only); R24-00/kg retail

When the results in Table 3.6 and Table 3.8 are taken into account as well as the wholesale prices of the different substitutes (Table 3.9), the feeding of soy and pollen would be recommended.

3.4 References

CRANE, E. 1990. *Bees and beekeeping. Science, practice and world resources.* Heinemann Newnes, London. 615 pp.

DE GROOT, A.P. 1953. *Protein and amino acid requirements of the honeybee* (*Apis mellifica* L.). W. Junk, Den Haag. 90 pp.

HERBERT, E.W. 1992. Honey bee nutrition. pp. 197 – 233 in: Graham, J. (editor). *The hive and the honey bee.* Dadant & Sons, Hamilton, Illinois. 1324 pp.

JOHANNSMEIER, M.F. 1998. *Pollen substitutes and supplements for honeybees.* Unpublished PPRI report to the National Department of Agriculture, Pretoria. 9 pp.

JOHANNSMEIER, M.F. (editor). 2001. *Beekeeping in South Africa.* Plant Protection Research Institute Handbook No. 14, Agricultural Research Council, Pretoria. 288 pp.

JONES, W. 1995. Supplementary feeding. *The Australasian Beekeeper* 97(3): 119 – 120.

KLEINSCHMIDT, G.J. & KONDOS, A.C. 1978. The effect of dietary protein on colony performance. *The Australasian Beekeeper* 79(12): 251 – 257.

KLEINSCHMIDT, G.J. & KONDOS, A.C. 1979. Colony management for low quality pollens. *The Australasian Beekeeper* 81(1): 5 – 6.

LAIDLAW, H.H. 1979. *Contemporary queen rearing.* Dadant & Sons, Hamilton, Illinois. 199 pp.

NABORS, R.A. 1996. Using mixtures of different sugars to feed bees. *American Bee Journal* 136(11): 785 – 786.

STANDIFER, L.N., MOELLER, F.E., KAUFFELD, N.M., HERBERT, E.W. JR. & SHIMANUKI, H. 1977. *Supplemental feeding of honey bee colonies.* Bulletin Number 413, United States Department of Agriculture, Washington D.C. 8 pp.

TABER, S. 1996. Pollen and bee nutrition. *American Bee Journal* 136(11): 787 – 788.

VAN DEN HEEVER, C.H. 1997. African honeybees' (*Apis mellifera scutellata* Latreille) preference for six pollen substitutes. *Proceedings of the 11 th Congress of the Entomological Society of South Africa, Stellenbosch.* pp. 152 – 153.

WINSTON, M.L., CHALMERS, W.T. & LEE, P.C. 1983. Effects of two pollen substitutes on brood mortality and length of adult life in the honeybee. *Journal of Apicultural Research* 22(1): 49 – 52.

CHAPTER 4

THE INFLUENCE OF CAPE HONEYBEE (*APIS MELLIFERA CAPENSIS*) LAYING WORKERS ON QUEEN REARING AND COLONY DEVELOPMENT OF THE AFRICAN HONEYBEE (*APIS MELLIFERA SCUTELLATA*)

4.1 Introduction

Beekeeping is a very important part of agriculture in South Africa. Insects are responsible for approximately 85 % of pollination, and bees are responsible for about 80 % of pollination by insects. South Africa produces large quantities of deciduous fruits, and honeybees are also important pollinators of several other crops. The more important crops that benefit from honeybee pollination are apples, pears, plums, avocado, litchi, mango, seed sunflower, seed lucerne, seed onion and cucurbits. The value of these crops amount to R 2,2 billion per annum (Wiese *et al.*, 1992).

Regular requeening of colonies is very important. Poor queens head poor colonies, resulting in poor crops and ineffective pollinating units. Older queens do not lay as many eggs as younger queens and the colonies headed by older queens are more prone to swarming (Taber, 1985). Young queens also lay later in autumn and earlier in the spring (Morse, 1991). More eggs laid at the proper time prior to the honey flow, furnish more bees in the hive, with a greater production of honey. According to Cobey & Lawrence

(1991), there is no doubt that a young, properly reared queen from a proven genetic line of bees will be more efficient and out-produce, on average, "naturally" reared queens.

Two subspecies of honeybees are found in South Africa. The African honeybee (*Apis mellifera scutellata*), notorious for its aggressive behaviour, occurs in the greater, summer rainfall region of South Africa. The second race, the Cape honeybee (*Apis mellifera capensis*) is a coastal bee, which occurs along the southern, eastern and western Cape coasts, which roughly corresponds with the distribution of fynbos vegetation (Du Toit, 2001). Historically, the two subspecies have remained geographically distinct, with a hybrid zone between them (Hepburn & Crewe, 1990).

According to Johannsmeier (1997), by virtue of certain characteristics of the Cape honeybees, the two races of bees are incompatible when kept in close proximity to each other, and it is this incompatibility that has resulted in the *capensis* problem. These characteristics are:

1. the inability of *scutellata* queens to pheromonally control *capensis* workers, or to prevent them from reproducing;
2. the ability of *capensis* workers to become rapidly reproductively active, and their inhibition of reproductive activity in *scutellata* workers;
3. the production of female (worker) offspring by *capensis* workers, whilst *scutellata* produce only male or drone offspring.

In apiaries with *scutellata* as well as *capensis* colonies, Cape bees drift into *scutellata* colonies where they are insufficiently controlled by the *scutellata* queen. This results in the eventual loss of the *scutellata* queen, and the *capensis* workers taking over all reproduction in the colony. Further

capensis workers are produced, with the final outcome being a complete *capensis* laying worker colony. These colonies tend not to be productive, gradually dwindle and eventually abscond or die (Johannsmeier, 1997).

The first indication of a *capensis* problem in South Africa was detected in January 1992, when *scutellata* colonies were found to be losing their queens and succumbing to *capensis* laying workers. Some 80 000 *scutellata* colonies have since died out as a result.

A queen rearing programme in a commercial beekeeping business was implemented in an attempt to replace the large number of *scutellata* colonies that were lost as a result of the invasion by *capensis* laying workers.

4.2 Material and Methods

4.2.1 Queen rearing

Queen rearing was conducted from 1 December 1992 to 30 January 1993 near Douglas in the Northern Cape Province. In all stages colonies were fed with a 50 % sugar syrup solution with a 2-litre plastic bottle inside the hive (Figure 3.1, Chapter 3).

Four frame nucleus hives were used for queen cell rearing and finishing. Each nucleus hive contained two frames with pollen and unsealed honey, as well as one frame with young larvae. If the honey was sealed, the cappings were removed before the frame was placed into the hive.

Artificial queen cell cups were prepared by dipping a forming stick into molten beeswax. This stick was 100 mm long and had a diameter of 7 mm at

the dipping end. The end of the stick was rounded to give the bottom of the wax cup a concave form. A mark was made on the stick to indicate the desired depth of the cells. Cups with a depth of 9 mm were used.

A frame of young larvae for grafting was removed from a colony with the most suitable characteristics. Some of the selection criteria used were colour, temperament, low swarming tendency, honey production and a good brood pattern. The frame was covered with a moist towel after it was removed from the colony to help prevent desiccation of the larvae.

Young bees for queen cell rearing were removed from colonies with large populations in brood chambers, and sufficient amounts of sealed and unsealed brood. At 09:00 in the morning, the nucleus hive (including the frame of young larvae) was placed on top of a brood chamber, with a queen excluder between them. The queen cell rearing colonies were prepared early in the morning to allow enough nurse bees to move into the nucleus colony to attend to the larvae. The same evening, before the nucleus hive was removed, the brood chamber was first smoked. By smoking the entrance of the brood chamber, more bees moved into the nucleus hive. The nucleus hives were moved to an apiary at least 5 kilometres from the original apiary.

The following morning, the frame containing the larvae was replaced with the artificial queen cells. Eleven artificial queen cups, attached to cork stoppers, were placed in a wooden strip (Figure 4.1). The wooden strip with the queen cells was placed between the two frames containing the pollen and honey. Only eleven queen cups were used in every nucleus colony.

The cork stoppers were tapered (Figure 4.1) and an aluminium strip was attached with a drop of molten beeswax at the base of the cork stopper

between the cork and the wax cup. The aluminium strip was later used to attach the finished queen cells to a comb. Elastic bands were used to keep the cork stoppers from falling through the holes in the wooden strip.

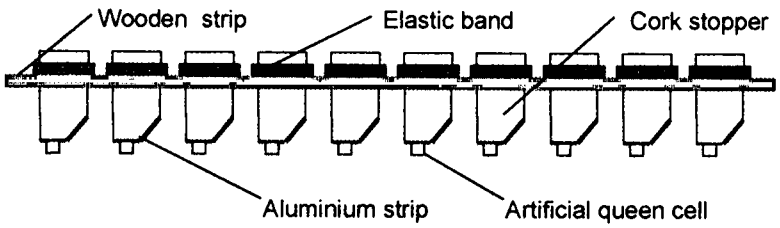

Figure 4.1 Artificial queen cells used for queen rearing

After 20 - 24 hours, larvae younger than 48 hours were grafted into the queen cells. A grafting needle was used to remove the larvae from the selected comb. Care was taken to select the smallest possible larvae floating on royal jelly. The following day the grafts were inspected, and if some larvae were rejected, they were regrafted. When the bees did not accept a larva, they removed it and cleaned the queen cup.

4.2.2 Introduction of finished queen cells

Ten days after the first grafting, the finished queen cells were removed for introduction into queenless nucleus hives. It is important to remove the queen cells at this stage, because the age of the larvae used for grafting was not known, and they were grafted on account of their size. If the queen cells are removed on day 11, it is possible that some of the queens may already have hatched.

The mating nucleus colonies were prepared with two frames of pollen and unsealed honey, one frame of brood, and one frame of young larvae. In

the morning, a nucleus hive was placed on top of a strong queenright colony in a brood chamber with a queen excluder between them, and removed in the evening to an apiary at least 5 kilometres from the original apiary.

The following morning, after the queen cells were removed from the queen cell rearing colonies, the queen cells were transported in their frames and kept warm. The queen cells were gently removed from the cork stopper. Using the aluminium base, one queen cell was pressed into the comb of the frame with young larvae approximately in the middle of the comb. Nucleus colonies were used for the introduction and mating of queens in order to avoid the loss in honey production which would have resulted if strong dequeened colonies were used. Queen cells were only introduced after a 24 hour queenless period.

When nucleus colonies have no brood, they sometimes abscond a few days after the young queens begin to lay. It was therefore advisable to give some brood to the colony.

The colonies were placed in the mating site as widely and randomly spaced as possible, with entrances facing in different directions. Care was taken to place colonies in a way that vegetation provided landmarks to prevent drifting and the possibility of queens returning to the wrong hive. After two weeks, the colonies were inspected to determine if the queens had emerged.

4.2.3 Introduction of mated queens

Mated queens were introduced 24 hours after removing the old queens or after queenless nucleus hives were prepared.

A new queen was caged without attendants or food, in a plastic hair curler, which was inserted between two combs of brood (preferably unsealed) near the centre of the brood chamber. A paper clip was used to attach the curler vertically to the comb. A single piece of newspaper, held in position by an elastic band, covered the bottom end of the curler tube, allowing the bees gradual access to the queen when chewing away the paper. The top of the curler was closed with a cork stopper. The queen was released after the bees had chewed away a large enough opening.

After a week, the colonies were inspected to determine if the queens were laying.

4.3 Results and Discussion

4.3.1 Queen rearing

Within the first week of queen rearing having started, large numbers of eggs were noticed in the queen cells, which was an indication that the colonies used for queen rearing were infected with *capensis* laying workers. The percentage acceptance of the queen cells during December 1992 was initially very low, although the cells accepted during this period, were finished to the sealed stage (Figure 4.2).

The acceptance of the queen cells was low in general, with the highest percentage acceptance on 5/1/93 in the second graft (47,95 %). During December 1992, almost all the cells accepted were completed, but during January 1993, the percentage cells completed decreased until no cells were completed on 6-7/1/93. The larvae were fed for two days after grafting, but were then rejected, and the queen cups were chewed away by the workers.

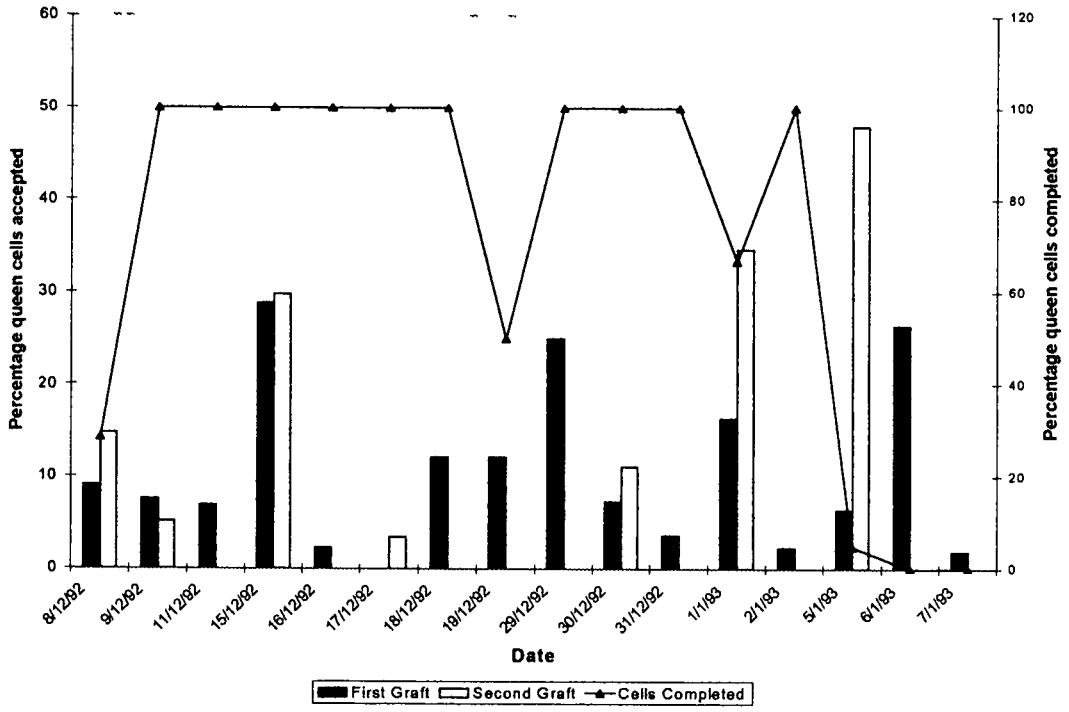

Figure 4.2 Difference between percentage queen cells accepted and percentage queen cells completed

Most of the finished cells were destroyed on day 8. In such colonies about 0,5 - 1 % black bees were present. These were a little larger than normal worker bees, and their behaviour resembled that of a *scutellata* queen. Many eggs were also noticed in worker cells, and in some of the queen cups. A possible reason for the low percentage queen cells completed on 19/12/92, 1/1/93 and from 5/1/93 onwards, was that the colonies from which the nurse bees were removed, could have been infested with *capensis* workers. Once the bees became queenless, the ovaries of the workers developed and they became laying workers.

The low percentage acceptance at the beginning of December 1992 was ascribed to too few bees in the queen rearing hives. Another factor that could have influenced the percentage acceptance was the fact that it was the

beginning of the experiment and more larvae could have been injured due to inexperienced grafting.

The temperature during December was very high, with a mean maximum temperature of 36,4 °C (highest maximum temperature was 44,7 °C), and a very low mean humidity of 56 %. The larvae could have died as a result of dessication, because grafting was done in the back of a minibus between 16:00 and 17:00.

According to Hepburn & Radloff (1998) the specific latency period between dequeening and the onset of oviposition is about 6 days for *capensis* and about 10 days for *scutellata*. Latency is related to the rate of ovariole development. The number of eggs laid by workers within 48 hours of the onset of oviposition, are significantly higher in the African races than in the European races. Likewise, significantly more eggs are laid by *capensis* than *scutellata* worker bees (Hepburn & Radloff, 1998).

Lundie (1929) reported on the laying workers of *Apis unicolor,* now known as *A. m. capensis*. According to him, these bees will fly to queenright *scutellata* colonies, and although many will be stung to death, some will survive the "balling" ordeal, and become established in the colony, and may be seen laying in the same brood combs as the queen. In the course of time the queen disappears. The brood loses its regular appearance, instead showing an irregular pattern so characteristic of these laying workers, in that the developmental stages of the brood varies a great deal in age within a small area of the comb. Furthermore, when a queen is separated from her brood for cell-building purposes, these laying workers continue to lay in the brood combs, and when the queen is allowed to return to her brood, she does not seem to be able to restore normal conditions, and finally the colony

becomes queenless. The laying workers are unable to maintain the population of the colony.

Scutellata queens seem unable to control reproduction by the *capensis* workers. When *capensis* workers enter the colony, they eventually produce a sufficient quantity of queen chemical signal to result in the loss of the *scutellata* queen. She may be killed by the *capensis* workers, or by her own workers.

The ovaries of the *capensis* workers develop because of the absence of suppressive queen pheromones and/or the availability of a high protein pollen source (Kryger *et al.*, 2000). The workers are then responsible for all subsequent reproduction. They increase as the *scutellata* workers die out. This decline in productivity is due to either the competition amongst the workers for false queen status, or more likely, the non-foraging characteristic of the clone-like *capensis* "invader" bee (Kryger & Van der Schyf, 1999). Foraging decreases and eventually ceases, the colony consumes all stored reserves, and slowly starves to death.

This unfortunate situation in South Africa has arisen as a result of the action of beekeepers. *A. m. capensis* workers have only naturally "invaded" *scutellata* colonies when beekeepers have placed their hives alongside each other. The uncontrolled migration and splitting of colonies, stress due to pollination, and generally poor management of colonies are all factors contributing to infestations. Under stressful conditions, the queen neither readily releases pheromones nor distributes it over her abdomen – usually her movements over the comb deposit a pheromone trail over its wax surface and encourage frequent changes in the composition of her court (Free, 1987). The ability of *A.m. capensis* workers to release queen-like signals

could explain the rapidity with which they establish themselves as false queens.

4.3.2 Introduction of finished queen cells

The percentage emergence of a total of 52 queen cells introduced into mating nucleus colonies, was 95 %. Only 44 % of these queens mated successfully, and had a normal brood pattern. The remaining 56 % of these colonies were queenless, with a distinct Cape honeybee laying worker presence. This gave an overall success rate of 20 % maximally (graft → laying queen) in the presence of Cape laying workers. For example on 09/01/93, queen cells were introduced into nine queenless nucleus hives. When they were inspected two weeks later, four of the colonies died (possibly absconded), three of the colonies were infested with *capensis* and the two remaining colonies were queenright with no obvious sign of *capensis* infestation.

Of 43 queen cells introduced on another occasion (24/12/92 – 18 cells, 25/12/92 – 14 cells, and 27/12/92 – 11 cells), only two queens did not emerge. When the colonies were inspected two weeks later, 18 of the colonies were queenright. The remaining 23 colonies were queenless and multiple eggs were scattered in the combs, in worker and drone cells, as well as in cells containing pollen. A distinct Cape bee presence was noted. The source of the *capensis* infestation in the nucleus colonies could possibly have been the brood that was introduced into the nucleus colonies.

According to Allsopp (1995), the time it takes for the queen to be lost following *capensis* infestation, is highly variable, and probably depends on the number of *capensis* bees drifting into the colony. The time for queen loss

was also very markedly influenced by the time of year. It is also important to note that once the queen was lost, it was impossible to requeen the colony. Whether a *scutellata* virgin or mated queen is introduced, or a *scutellata* queen cell, or a *capensis* virgin or mated queen or a queen cell, these will all be killed.

4.3.3 Introduction of mated queens

Almost no difficulty was experienced introducing mated queens to dequeened colonies. Eighteen mated queens were introduced and the percentage acceptance was 89 %. No *capensis* infestation was noted in the colonies into which the mated queens were introduced.

A colony into which a queen is to be introduced, must be queenless. The colony should not be queenless for more than a day, otherwise it may be necessary to check for queen cells and remove them. The danger of *capensis* infestation is greater the longer the colony remains queenless. If there is no nectar flow when requeening is done, it is advisable to feed the colony.

The large percentage acceptance of the mated queens, can possibly be as a result of the higher percentage of (E)-9-oxo-2-decenoic acid (9-ODA), the major chemical component of the mated queen's mandibular glands (Table 4.1). This could have suppressed ovariole development of *capensis* workers possibly present. Two other possible reasons may be the short queenless and broodless period, and the possible absence or low incidence of *capensis* workers.

Table 4.1 Mean percentage of (E)-9-oxo-2-decenoic acid present in queens and laying workers*

	Mean % 9-ODA
A. mellifera virgin queens (1 day old)	26
A. mellifera mated queens	36
A. mellifera workers in queenright colony	0
A. mellifera laying workers	0
A. m. capensis workers with partially developed ovaries	34
A. m. capensis laying workers	76

*(after Free, 1987)

The introduced queens began laying shortly after the workers released them. This brood probably also contributed to suppressing ovariole development of *capensis* worker bees. Kropáčová & Haslbachová (1971) concluded that both the absence of the queen and unsealed brood, favoured ovary development in worker bees. There was some indication that unsealed brood had a stronger inhibiting effect than the presence of the queen.

According to Free (1987), there are two reasons why it is difficult to successfully replace the old queen with a new one. The new queen will have an alien odour, and so will be readily recognised, and may be rejected by workers of the recipient colony. The other reason is because the quality and composition of pheromone a new queen produces, is different from that of her predecessor.

It is possible to successfully rear and mate queens at Douglas, if no *capensis* is present. Possible measures that can be taken to prevent apiaries from becoming *capensis* infested are to try and keep more

permanent sites and not to mix colonies from different apiaries. The interchanging of frames with brood must be avoided as this can lead to the spread of *capensis*.

4.4 References

ALLSOPP, M.H. 1995. In: Magnuson, P. (editor). *Proceedings of the First International Electronic Conference on the Cape Bee Problem in South Africa, 5 – 30 June 1995.* Hosted by the Agricultural Research Council, Pretoria, and the University of the Witwatersrand, Johannesburg. 105 pp.

COBEY, S. & LAWRENCE, T. 1991. Young, healthy queens are essential to good beekeeping. *Gleanings in Bee Culture* 119(1): 16 – 17.

DU TOIT, A.P. 2001. The South African Beekeeping Industry. *South African Bee Journal* 73 (3): 161 - 165.

FREE, J.B. 1987. *Pheromones of social bees.* Chapman and Hall, London. 218 pp.

HEPBURN, H.R. & RADLOFF, S.E. 1998. *Honeybees of Africa.* Springer-Verlag, Berlin. 370 pp.

HEPBURN, H.R. & CREWE, R.M. 1990. Defining the Cape Honeybee: reproductive traits of queenless workers. *South African Journal of Science* 86: 524 – 527.

JOHANNSMEIER, M.F. 1997. Overcoming the Capensis problem. *South African Bee Journal* 69 (4): 63 – 70.

KROPÁČOVÁ, S. & HASLBACHOVÁ, H. 1971. The influence of queenlessness and of unsealed brood on the development of ovaries in worker honeybees. *Journal of Apicultural Research* 10(2): 57 – 61.

KRYGER, P. SWART, D. & JOHANNSMEIER, M. 2000. The Capensis Problem and the aloe flow. *South African Bee Journal* 72 (1): 11 - 12.

KRYGER, P. & VAN DER SCHYF, A. 1999. *The capensis honeybee problem: A genetic analysis using single locus DNA-fingerprinting.* ARC-Plant Protection Research Institute report for the South African Federation of Bee Farmers' Associations, Pretoria. 7 pp.

LUNDIE, A.E. 1929. The rearing of queen-bees. *Bulletin 76, Department of Agriculture, Pretoria.* 21 pp.

MORSE, R.A. 1991. Are two queens best? *Gleanings in Bee Culture* 119(5): 324.

TABER, S. 1985. Reasons for replacing queens that are in your colonies. *Gleanings in Bee Culture* 113: 206.

WIESE, I.H., SCHEHLE, J.A., ENGELBRECHT, T., KEETCH, D.P., CORNELIUS, P.J. & VON LA CHEVALLERIE, M. 1992. Bylae A: Die waarde van bye as bestuiwers van landbougewasse in die RSA. In: *Die Kaapse indringerby en hulp aan byeboere.* Taakspanverslag, Departement Landbou-Ontwikkeling, Pretoria. 22 pp. & Bylae A – I.

CHAPTER 5

SEASONAL VARIATION IN HONEYBEE COLONY SIZE, BROOD, POLLEN AND HONEY AT BLOEMFONTEIN, FREE STATE

5.1 Introduction

The honeybee colony is completely dependent on factors enabling the collection of pollen, such as season, environmental temperature, flight distance and amounts and quality available. Therefore, the pollen supply is a limiting factor in beekeeping practice (De Groot, 1953).

According to Standifer *et al.* (1983), the natural diet of the honeybee, *Apis mellifera* L., namely pollen and nectar, does not remain the same throughout the year, it changes with the season both in quality and quantity and in availability in the hive and in the field. Seasonal changes and unavailability of pollen sources for bees usually have an adverse effect on brood production by the colony. As a result, during certain times of the year, brood-rearing activity can be suspended, or brood-rearing rates can change greatly. Seasonal differences in behaviour, physiology and length of life of honeybees are known to occur. Among the chief findings in the studies of Standifer *et al.* (1983) were that winter bees differ from summer bees in the greater development of their hypopharyngeal glands and their fat bodies, in body protein reserves and in metabolism. There are also seasonal differences in the weight of the queen and in the exchange of food type from the worker bee to the queen bee. There are

annual rhythms in brood rearing, pollen supply, colony weight, egg laying, foraging activity and temperature within the hive. The trend in brood rearing is parallel to the seasonal availability of pollen and the amount of pollen collected by bees. The egg-laying peak in spring coincides with the peak of pollen flow in honeybees of temperate regions.

Adult bees can survive on carbohydrates and water. However, proteins, lipids or fats, minerals and vitamins are necessary for growth and development of young bees, and for them to rear larvae. If nurse bees do not get pollen or some other appropriate protein source, their brood food gland secretions are not adequate for support of normal growth and development of the larvae and egg production of the queen.

Estimates of the amount of pollen required by a single colony in a year in temperate climates were quoted as being 15 – 18 kg and 50 – 55 kg in North America and 15 – 28 kg and 50 – 55 kg in Europe. In Arizona it was determined from seven yearly records of pollen trapping, that colonies annually collected 43,8 ± 13,6 kg of pollen (Johannsmeier, 2001).

Factors such as soil moisture, pH, and fertility affect the nutritive value of pollen. The crude protein levels of pollen collected from different plants range from 8 % to 40 %. Pollen has been classified into four groups based upon the influence on bee longevity and development of the hypopharyngeal glands, ovaries and fat bodies (Maurizio, 1960 in Herbert, 1992). The first group of highly nutritious pollen included fruit trees, willow, and white clover. The second group of less nutritious pollen included maize, poplar, and dandelion. The third group of pollen with only a fair nutritional value, came from alder, date palm and hazelnut. The fourth group of pollen, with the poorest nutritive value, included various species of cedar and pine trees. This grouping seems to follow the crude protein levels of the pollens.

The present study was undertaken to determine the seasonal variation in size, brood, pollen and honey of honeybee colonies at Bloemfontein, Free State. Stationary and migrated hives in this province are used for honey production, pollination of different crops, and for queen rearing. Knowledge of natural food sources and colony development would indicate if and when supplemental feeding became necessary for specific colony manipulations such as queen rearing, making increase and brood production.

5.2 Material and Methods

The study site was an agricultural holding in Bainsvlei (29° 03' S, 26° 07' E, 1372 m above sea level), near Bloemfontein. The rainfall for this region ranges from 250 – 510 mm per annum (from July 1994 to July 1995 the recorded rainfall was 293,2 mm). It is a summer rainfall area and the main rainfall occurs from November to February. Frost occurs from May to August. The mean maximum and minimum temperature in midsummer is 32,5 °C and 13,3 °C respectively, and the mean maximum and minimum temperature in midwinter 16,0 °C and –3,8 °C.

The test apiary with 15 hives was located beneath eucalypt trees, mainly for the purpose of shade. The surrounding vegetation was primarily cultivated farms and smallholdings with lucerne, sunflowers, deciduous fruit trees, various weeds, ornamental trees and shrubs such as karee, poplars, wattle and ash, and natural grazing (grasses and karoo shrubs). The lucerne was irrigated, but the sunflowers not.

5.2.1 Collection and identification of pollen pellets

Pollen pellets were collected with a pollen trap (Fig. 5.1). Only one pollen trap was used. The number of pollen collecting bees returning to the hive and the number of pollen pellets collected were counted for a certain period. Trap efficiency was then calculated to be 10 %. The trap was emptied fortnightly.

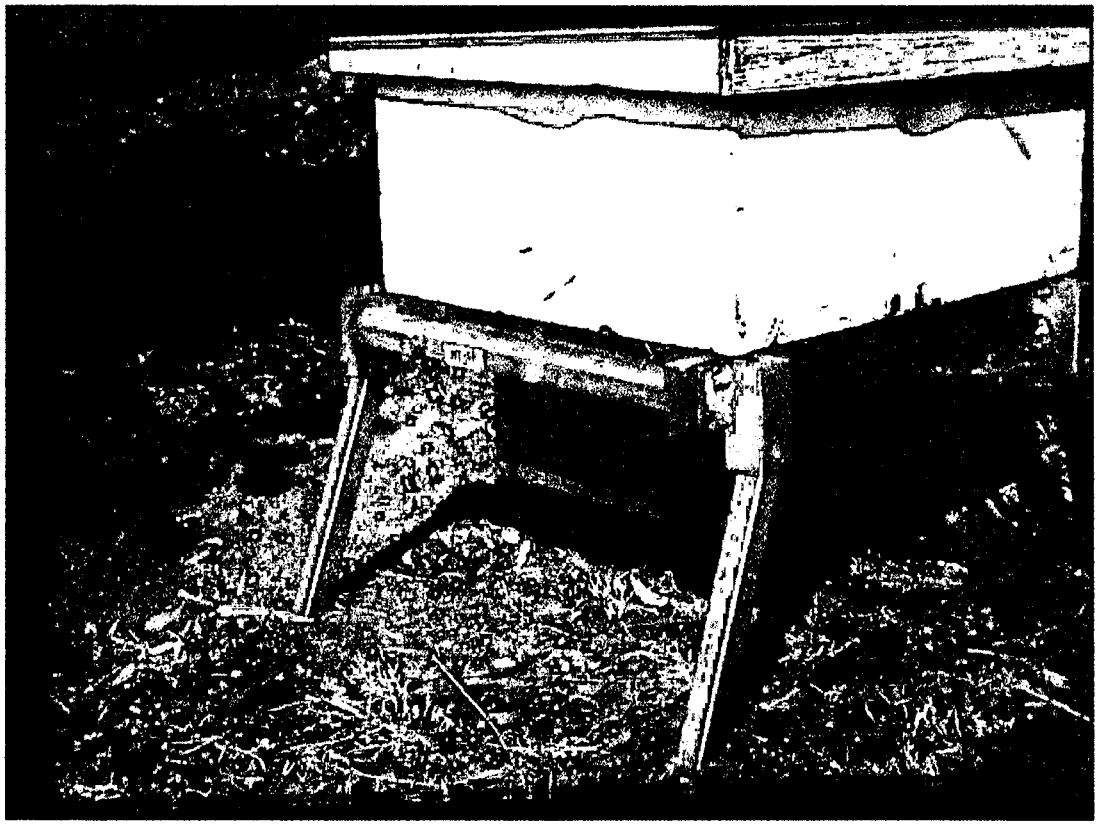

Figure 5.1 Pollen trap used for sampling pollen pellets

Pollen was trapped between 30 June 1994 and 3 August 1995 on the same colony. The trap was emptied 30 times at about fortnightly intervals. In the laboratory, ca. 3 g of each pollen sample was sorted by colour and the number of pellets per colour counted. The total number of sampled pellets for the entire year was 18 433. Several representative pellets of each colour were

individually softened in water and mixed with glycerine jelly on a microscope slide. Basic fuchsin stain in 50 % ethyl alcohol was applied on one side only of the pollen/glycerine jelly mixture, and stirred. This was done to obtain variable intensities of staining to assist in the identification of the pollen grains. The slide was then warmed to remove the alcohol and excess moisture. A glass cover slip was used to contain the sample.

The identification of pollens was accomplished mainly with the aid of the permanent pollen slide collection of the Plant Protection Research Institute. The following two publications also proved useful in this regard: Erdtman (1952) and Bonnefille & Riollet (1980). (The identification of the pollen pellets was done by M.F. Johannsmeier, Pretoria).

The pellets were identified during November 1997. The pellets were then already old and the colours, particularly of the Asteraceae, had changed in most cases compared to their fresh condition.

5.2.2 Determining colony size and amount of brood and stored food

Seven newly established colonies on the same location as the one employed for trapping pollen, were used for determining colony size and the amount of brood and stored food. The results of the seven hives were combined and the average of the values used.

A record card was used for each colony (Table 5.1). The hives were inspected fortnightly when the pollen trap was emptied.

Table 5.1 Example of record card used for determining colony size, amount of brood and stored food

Observation or Manipulation	Date	Date	Date
Number of supers			
Colony size (brood frames of bees)			
Brood: Eggs (dm^2)			
Larvae (dm^2)			
Sealed brood (dm^2)			
Pollen (dm^2)			
Honey (dm^2)			
Honey removed			

The number of supers:

- Number of supers before colony inspection.

Colony size:

- Number of brood frames, well covered with bees on both sides.

Brood:

- Visual estimate in dm^2, of the area covered with eggs, larvae and sealed brood.

Pollen:

- Visual estimate of the amount of pollen, in dm^2.

Honey:

- Visual estimate of the amount of honey, in dm^2 (in brood chamber as well as in supers).

Honey removed:

- Frames of honey removed from the supers.

5.3 Results and Discussion

5.3.1 Types and amounts of different pollens

Pollen grains from different plant species have their own distinctive shape and sculpturing which can be used in the identification of both pollen and nectar sources. Grasses produce pollen which is light and dry to facilitate wind pollination. The pollen of other plants is usually sticky and must be moved by a honeybee or some other pollinator (Dietz, 1979).

In Figure 5.2, the mass of pollen collected on the 30 dates between July 1994 and August 1995 is shown. The mass of the pollen on the different dates was grouped as shown below for every month. The reason for the above grouping of dates is because the dates, on which the pollen trap was emptied, did not correspond with the start or the end of a particular month. By grouping the dates, it was attempted to get a better total image of the pollen flow during this period and to compare it with the size of the colonies and the amount of brood and stored food.

Dates on which the pollen trap was emptied:

1. July 1994: 17/07/94; 31/07/94
2. August 1994: 14/08/94; 30/08/94
3. September 1994: 06/09/94; 13/09/94; 22/09/94; 29/09/94
4. October 1994: 13/10/94; 27/10/94
5. November 1994: 10/11/94; 24/11/94
6. December 1994: 08/12/94; 22/12/94
7. January 1995: 05/01/95; 19/01/95; 02/02/95
8. February 1995: 16/02/95; 02/03/95

9.	March 1995:	16/03/95; 30/03/95
10.	April 1995:	13/04/95; 27/04/95
11.	May 1995:	11/05/95; 25/05/95
12.	June 1995:	08/06/95; 22/06/95
13.	July 1995:	06/07/95; 20/07/95; 03/08/95

As shown in Figure 5.3, pollen was most plentiful during September, November, December and April. The most abundant pollen during September, November and December was *Eucalyptus* and during April, *Helianthus* (Table 5.3). Very little pollen was collected during the winter months of June and July. The main source of pollen during July 1994 was Asteraceae cf. *Senecio* type (42,5 %) and *Acacia* cf. *baileyana* (41 %).

The 8,4 % starch or flour particles that were collected during July 1994 could be an indication of a scarcity of pollen. According to Anderson *et al.* (1983), it is a common occurrence to find bees collecting animal feeds and different kinds of recently-milled grains during periods of pollen shortages. These substances may have some food value, but do not sustain broodrearing. They found that in times of pollen scarcity, pollen gatherers collected useless substances like sawdust, fungus spores and even coal dust. During July 1995, the main source of pollen was *Felicia/Senecio* type (49,27 %) and *Rhus* cf. *lancea* (40,6 %), with *Acacia* cf. *baileyana* only 6,23 %.

The total amount of pollen collected for the period from 30 June 1994 to 3 August 1995, was 3580,6 g. When taken into account that the trap collected only 10 % of all bee-gathered pellets, the total amount of pollen collected by the colony for the particular period was approximately 35,8 kg of pollen. No such previous data for *scutellata* seem to exist.

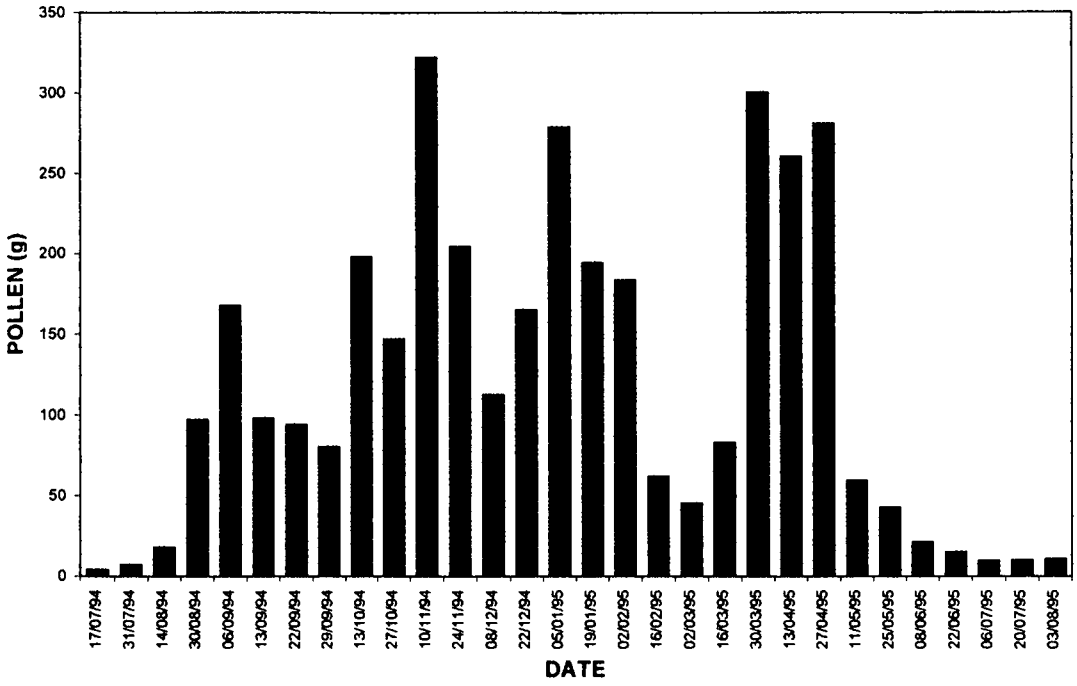

Figure 5.2 Mass of pollen collected with a pollen trap between July 1994 and August 1995

Haydak (1935) reported that honeybee colonies require 44 to 66 lbs. (20 – 30 kg) of pollen annually. Todd (1940 in Herbert, 1992), estimated the annual colony requirement to be 88 lbs. (40 kg). On an individual bee basis each larva requires more than 100 mg of pollen to complete its development (Haydak, 1935). Schmidt & Buchmann (1985 in Herbert, 1992), reported that the average weight of nitrogen consumed per individual over a 28 day period was 3,07 mg. Rashad & Parker (1958), reported that 66,5 mg of fresh pollen was needed to rear one larva. They further calculated that one cell contained 183 mg of beebread which they stated was sufficient to rear 1,2 bees. Based on the above estimates, one pound (454 g) of pollen would support the rearing of over 4 000 bees. Since a strong colony rears about 200 000 bees a year, a minimum of 44 pounds (20 kg) of pollen would be required (Herbert, 1992).

According to Seeley (1985), the total mass of food eaten by a colony in temperate zones during the winter period, is approximately 25 kg, of which 1 kg is pollen, and the rest is honey. Drops in hive weight during inclement weather range from 1 to 4 kg/week, averaging about 2,5 kg/week. Given a season of 22 weeks (late April to late September), the total mass of resources consumed over the summer is about 55 kg.

The pollen portion of this total can be estimated by noting that it requires about 130 mg of pollen to produce a bee (Seeley, 1985), and that the average colony population across the summer is about 30 000. As an average bee lives about one month (Seeley, 1985), this implies that a colony rears about 150 000 bees each summer over a five month season in temperate regions. At about 130 mg of pollen per bee reared, a colony would then require about 20 kg of pollen each summer for brood rearing. Hence the yearly food consumption of unmanaged colonies in Connecticut, U.S.A., is approximately 20 kg of pollen and 60 kg of honey (25 kg in winter, plus 35 kg in summer).

From unpublished scale hive records obtained in five of South Africa's provinces, it has been conservatively estimated that the non-migrated honeybee colonies in these localities consumed on average 50 kg of honey per year (range 34 – 65 kg) (Johannsmeier, 2001). These are only general estimates, the precise values will vary depending on colony size, climate, and forage abundance.

Table 5.2 shows the percentages of different pollens collected over one year. *Eucalyptus* was the most abundant pollen, in eight of the months, compared to the other extreme, namely *Sorghum* pollen, which was trapped only once during June 1995. Nearly all the eucalypts are highly regarded as producers of nectar and pollen, and as a group the eucalypts are the most

important bee plants in South Africa (Anderson *et al.*, 1983). The peak flowering time of *Eucalyptus* in this area was from November to December 1994.

Tribulus terrestris and *Rhus lancea* pollen played a significant role during the autumn and winter of 1995. The colonies used were able to maintain six to eight frames of bees, and although broodrearing decreased, there was brood present on every inspection date. The occurrence of *Tribulus terrestris* pollen corresponds with the rainfall during this period. The presence of cultivated fields that surrounded the agricultural holding contributed to the occurrence of the *Tribulus terrestris*, which is a weed on cultivated fields and flourishes after even a little rain. *Tribulus terrestris* is a minor to good pollen source and a minor to medium nectar source. *Rhus lancea* was an important source of pollen during May to July 1995. It is a minor to medium nectar source and a medium to good pollen source, with a flowering time from May to September and a peak flowering time from June to July.

The sand particles (0,09 %) and sand and pollen (0,013 %), is a very rare occurrence. Sand particles were collected during July 1994 (0,2 %) and during April (0,35 %) and May (0,8 %) of 1995. The sand and pollen was only collected once during April 1995 (0,2 %).

The fact that only one pollen trap was used, could have a limiting effect on the amount of pollen types collected. According to Johannsmeier (1981), flower constancy is very highly developed in honeybees, a trait that makes them eminently suitable for crop pollination. When he compared the pollen collected by three colonies fitted with pollen traps, he found that each colony collected the majority of its pollen from three quite different plants. If more than one pollen trap were used at the study site at Bainsvlei, it would be possible that a greater variety of pollen types could have been collected.

Table 5.2 Identity and percentages of corbicular loads collected during one year at Bloemfontein, Free State

PELLET SOURCE	COMMON NAME	PERCENTAGE (%) OF TOTAL (n = 18 433)
Eucalyptus	Gum, Eucalypt	26,260
Tribulus terrestris	Common dubbeltjie	14,103
Rhus cf. *lancea*	Karee	12,163
Helianthus annuus	Sunflower	7,137
Felicia / *Senecio* type	Blue aster / Senecio weed	5,600
Poaceae	Grass family	4,697
Populus	Poplar	4,533
Acacia cf. *baileyana*	Bailey's wattle	4,437
Senecio type	Senecio weed	3,613
Fabaceae	Legume family	2,907
Osteospermum / *Arctotheca* type	Free State daisy / Cape weed	2,287
Fraxinus	Ash	2,110
Leucas / *Teucrium* type	Labiate herbs	1,947
Celtis type	White Stinkwood	1,270
Morus	Mulberry	0,997
Unknown pollen		0,960
Zea mays	Maize	0,867
Prunus type	Peach / plum type	0,593
Ulmus parvifolius	Chinese elm	0,563
Starch / flour particles		0,560
Othonna / *Helichrysum* type	? / Everlasting	0,400
Type between *Senecio* and *Carduus*	Senecio weed / Thistle	0,360
Rhus type	Karee type	0,360
Manna, from *Eucalyptus viminalis*?		0,313
Plant particles		0,173
Papilionaceae	Bean / Pea family	0,157
Sand particles		0,090
Eucalyptus plus fine unidentified particles		0,077
Cucurbita	Pumpkin, Marrow	0,073
Brassicaceae	Cabbage / Mustard family	0,067
Felicia type	Blue aster	0,067
Cupressus	Cypress	0,053
Oenothera	Evening primrose	0,047
Stoebe type similarities	Bankrotbos	0,037
Pinus	Pine	0,033
Propolis and pollen		0,027
Propolis		0,017
Campanulaceae	Blue bell family	0,013
Sand and pollen mixture		0,013
Argemone	Mexican poppy	0,007
Rosaceae	Rose family	0,007
Sorghum	Sorghum	0,007

In temperate regions, nectar and pollen flows are primarily dependent on temperature. Deciduous trees at the study site that flower in spring, e.g. *Fraxinus* and *Populus,* and evergreen *Eucalyptus,* are mainly dependent on the rainfall of the previous season (summer and autumn) for their nectar and pollen flow. The Karoo shrubs and the annual weeds, as well as cultivated crops, are dependent on the rainfall of the previous one to three months, e.g. Asteraceae, *Tribulus* and *Helianthus.* In Figure 5.3, the dependence of the above-mentioned pollen sources on rainfall, both previous and current, is indicated. Figure 5.3 should be studied in conjunction with the type of pollen source and the time of its availability as shown in Table 5.3. There is no correlation between temperature and pollen flow according to the data in Figure 5.3, except that the combined effect of drought and cold during the winter months results in very few plants flowering.

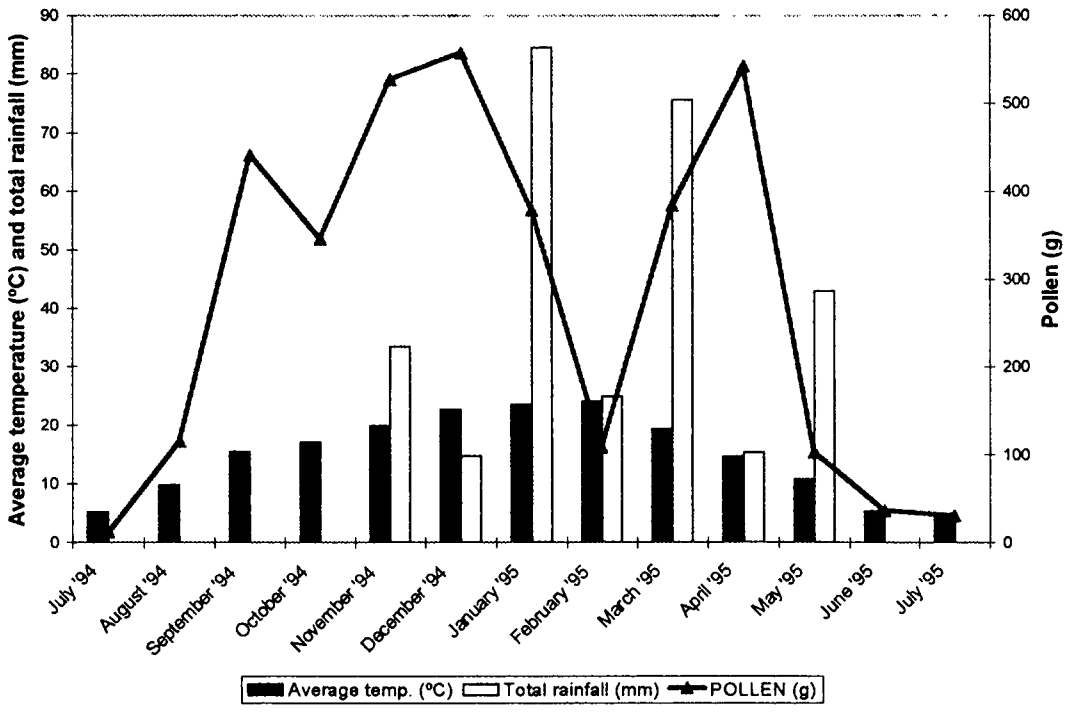

Figure 5.3 Seasonal variation in weight of trapped pollen, and fluctuation in temperature and rainfall from July 1994 to July 1995

5.3.2 Relationship between pollen flow and colony development

The amount of stored pollen does not necessarily correspond with the amount of incoming pollen, e.g. September, January and March had large amounts of incoming pollen, but this is not reflected in the beebread curve of Figure 5.4. Presumably larvae and young worker bees consumed this pollen. After pollen intake increased in September (Figure 5.3), the amount of brood (different stages) also increased (Figure 5.4). However, there is an increase in beebread and brood during December and April that corresponds to peaks in pollen collection during these months. The increased colony size in April, although it was preceded by a decrease in brood, could possible be ascribed to greater longevity of well-fed worker bees. Three to four frames of honey were removed from each colony only once at the end of December, which explains the steep downward curve of stored honey in Figure 5.4. The drop in honey after April is due to increased use of stores during winter. The decrease in colony size after April can be the result of the sharp decrease in the amount of incoming pollen, and presumably nectar. The queen therefore did not maintain her egg production as earlier in the season, because of a lack of stimulative nectar and pollen.

The population of adult bees in the colony largely determines the number of bees available for foraging. The annual cycle of the adult population is governed by the rate of brood rearing, and by the length of life of the adult workers which varies from a few weeks in summer to several months in winter. A worker that survives to adulthood emerges from its cell 3 weeks after the eggs are laid, so the maximum adult population of a colony lags somewhat behind the maximum rate of brood rearing (Crane, 1990). The same trend can be seen in Figure 5.4 where the amount of eggs, larvae and sealed brood increased during December, but the colony size peaked in January.

The reason why the amount of brood present during July 1994 was considerably lower than that of the brood during July 1995, could be ascribed to the fact that all the colonies used were newly-established, with little brood and only a few frames of bees. According to Al-Tikrity *et al.* (1972), investigations by several research workers have indicated a relationship between the amount of brood and the amount of pollen collected by colonies of honeybees. They noted that foraging activity, and the proportion of pollen gatherers to nectar gatherers, increased with an increase in amount of brood. They also showed that colony size influenced the amount of pollen stored, and that both colony size and the amount of pollen independently influenced brood rearing. A newly-established colony without brood would bring in pollen, but not as much as an established colony with brood.

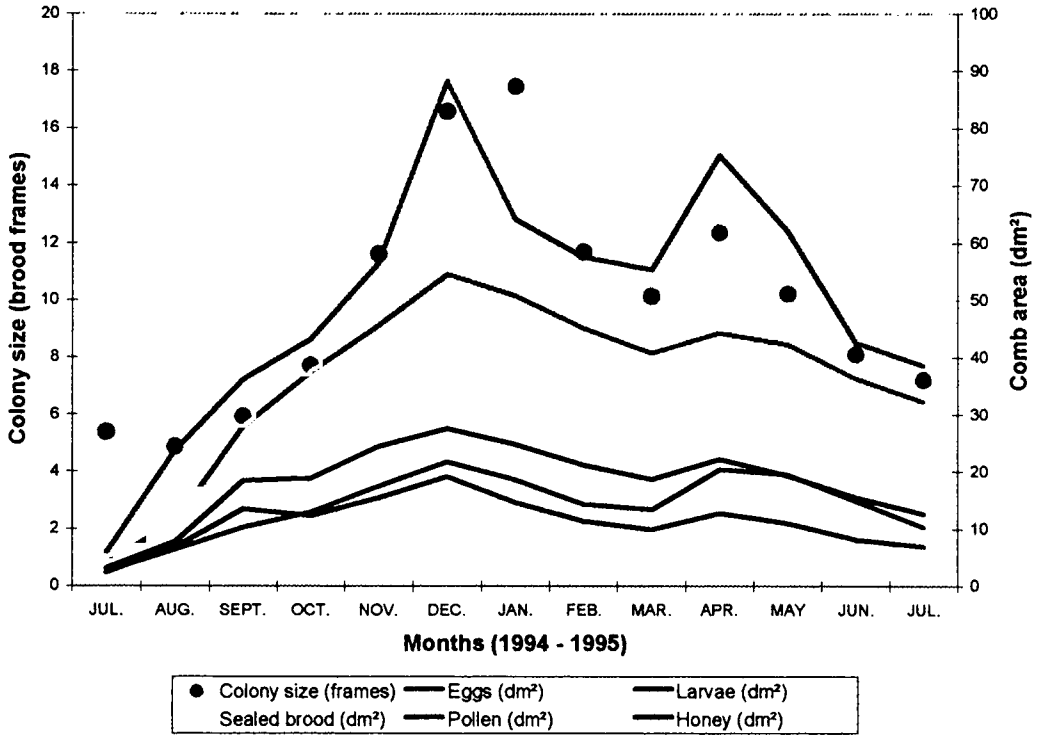

Figure 5.4 Seasonal variation in honeybee colony size and of brood, pollen and honey at Bainsvlei, Free State

Free (1967), and Todd & Reed (1970 in Al-Tikrity *et al.*, (1972) as well as Doull (1973), established a direct relationship between the amount of pollen collected by a colony and the amount of brood in it (capped and uncapped). Uncapped brood is a very effective factor in stimulating pollen collection because of brood pheromone (Herbert, 1992).

In any colony in which the queen has been laying consistently for 21 days, eggs, larvae and pupae will be present in the proportions of 3:5:13 – the three stages of the bee occupying 3, 5 and 13 days respectively. If the queen is varying her rate of egg laying, the ratios will change (Wedmore, 1932 in Doull, 1973).

Pollen is not simply a source of essential nutrients for honeybees. It appears to play other important roles in feeding behaviour, and in the process of elaboration and secretion of larval food and royal jelly (Doull, 1973).

Brood production is dependent on the availability of pollen. As soon as pollen becomes available in nature, the amount of pollen in the hives increases, as well as the production of brood. The degree of pollen collecting activity is related to the amount of unsealed brood present in a hive, and the stimulant for egg laying is derived from nectar or honey (Dietz, 1979). Another important factor which affects pollen collection, is the presence or absence of a stimulatory nectar flow. In South Africa it has been observed on several occasions that the absence of a stimulatory nectar flow or sugar feeding, eventually stops pollen collection, despite the presence of honey stores and brood in the hive (Johannsmeier, 2001). Population density and brood rearing patterns in colonies of honeybees are broadly predictable according to the seasons, but cannot be precisely predicted because they are often altered by quality and quantity of diet, climate, geographic location and age of queens.

Table 5.3 Percentages of different corbicular loads collected monthly during one year at Bloemfontein, Free State

MONTH	PELLET SOURCE	PERCENTAGE PELLETS OF MONTHLY TOTAL
July	*Senecio* type	42,55
	Acacia cf. *baileyana*	41,00
	Starch / flour particles	8,40
	Plant particles	2,60
	Eucalyptus	2,15
	Populus	2,05
	Cupressus	0,80
	Sand particles	0,20
	Propolis	0,15
	Type between *Senecio* and *Carduus*	0,10
August	*Populus*	44,50
	Eucalyptus	35,90
	Fraxinus	9,35
	Acacia cf. *baileyana*	6,90
	Senecio type	2,35
	Prunus type	1,00
September	*Eucalyptus*	29,93
	Fraxinus	11,15
	Populus	10,73
	Celtis type	9,53
	Morus	7,48
	Leucas / Teucrium type	628
	Senecio type	4,65
	Acacia cf. *baileyana*	4,60
	Prunus type	3,95
	Othonna / Helichrysum type	3,00
	Unknown	2,75
	Type between *Senecio* and *Carduus*	2,65
	Osteospermum / Arctotheca type	2,25
	Eucalyptus plus fine particles	0,58
	Brassicaceae	0,50

MONTH	PELLET SOURCE	PERCENTAGE PELLETS OF MONTHLY TOTAL
October	*Eucalyptus*	44,45
	Fabaceae	33,30
	Leucas / Teucrium type	11,50
	Osteospermum/Arctotheca type	5,70
	Unknown	5,05
November	*Eucalyptus*	81,80
	Fabaceae	10,30
	Rhus type	4,85
	Unknown	3,05
December	*Eucalyptus*	90,70
	Osteospermum / Arctotheca type	4,07
	Leucas /Teucrium type	3,43
	Tribulus terrestris	0,70
	Oenothera	0,47
	Propolis and pollen	0,27
	Rhus type	0,23
	Poaceae	0,07
	Rosaceae	0,07
January	*Tribulus terrestris*	51,60
	Eucalyptus	31,70
	Osteospermum / Arctotheca type	12,90
	Cucurbita	1,10
	Papilionaceae	1,10
	Poaceae	0,70
	Felicia type	0,45
	Zea	0,35
	Campanulaceae	0,10

MONTH	PELLET SOURCE	PERCENTAGE PELLETS OF MONTHLY TOTAL
February	*Tribulus terrestris*	82,95
	Poaceae	12,05
	Osteospermum / Arctotheca type	1,55
	Eucalyptus	1,45
	Papilionaceae	1,25
	Felicia type	0,55
	Argemone	0,10
	Campanulaceae	0,10
March	*Tribulus terrestris*	43,20
	Helianthus	24,85
	Poaceae	18,25
	Ulmus parvifolius	8,45
	Zea	3,70
	Stoebe type similarities	0,55
	Osteospermum / Arctotheca type	0,45
	Unknown	0,40
	Eucalyptus	0,15
April	*Helianthus*	46,70
	Tribulus terrestris	32,75
	Poaceae	9,65
	Zea	8,65
	Felicia / Senecio type	0,90
	Osteospermum / Arctotheca type	0,60
	Sand particles	0,35
	Sand and pollen	0,20
	Unknown	0,20
May	*Helianthus*	35,15
	Poaceae	28,90
	Rhus cf. *lancea*	25,45
	Felicia / Senecio type	9,20
	Sand particles	0,80
	Zea	0,30
	Unknown	0,20

MONTH	PELLET SOURCE	PERCENTAGE PELLETS OF MONTHLY TOTAL
June	*Rhus* cf. *lancea*	96,10
	Osteospermum / Arctotheca type	1,35
	Manna	0,85
	Poaceae	0,80
	Helianthus	0,30
	Rhus type	0,20
	Acacia cf. *baileyana*	0,10
	Eucalyptus	0,10
	Propolis	0,10
	Sorghum	0,10
July	*Felicia / Senecio* type	49,27
	Rhus cf. *lancea*	40,60
	Acacia cf. *baileyana*	6,23
	Manna	2,57
	Osteospermum / Arctotheca type	0,77
	Pinus	0,33
	Eucalyptus	0,20
	Helianthus	0,03

In summary, it was seen that food collection by honeybee colonies is an enormous undertaking. Each colony can be thought of as an organism which weighs 1 to 5 kg, rears 150 000 bees and consumes 20 kg of pollen and 60 kg of honey each year. To collect this food, which comes as tiny, widely scattered packets inside flowers, a colony must dispatch its workers on several million foraging trips, with these foragers flying 20 million kilometres overall.

According to Johannsmeier (2001), South African climatic conditions on the whole are such that beekeepers get by with little or no feeding. Nevertheless, there are regular reports of colonies that have weakened, absconded, or even died out, often as a result of honey shortages.

With the information in Tables 5.3 and 5.4, and Figures 5.3 and 5.4, beekeepers could prepare themselves in advance for possible nectar and pollen flows which could help them in selecting apiary sites according to the time of the year, the previous rainfall and the vegetation at or near the apiary site. Rainfall records can be useful indicators of the time and magnitude of flows to be expected in both the long and short term.

Table 5.4 Pollen and nectar values of, and remarks on Bainsvlei beeplants*

Botanical Name	Nectar & Pollen [1]		Flowering time [2]	Remarks
Eucalyptus camaldulensis (River Red Gum)	N3	P2	8 - 4 (10 - 1)	A species widely planted in the drier parts of the country. Flowers throughout the summer, but a short peak of about three weeks usually falls between the months October and January. In good seasons it can be regarded as a good honey and pollen source.
Eucalyptus sideroxylon (Black Ironbark)	N4	P0	1 - 12 (4 - 9)	One of the best known drought and frost-hardy eucalypts found all over South Africa. Flowering occurs anytime of the year, but mainly from April to September, varying in different stands and even in the same stand from season to season. The period from flower bud initiation to flowering is short in this species, only about four months.
Eucalyptus tereticornis (Forest Red Gum)	N4	P4	7 - 12 (8 - 10)	This drought-hardy eucalypt is widely distributed in South Africa, but nowhere abundant. It is an excellent source of both pollen and nectar. Flowering may be from July to December, but the peak falls between August and October. The Forest Red Gum is valuable for colony build-up.

Botanical Name	Nectar & Pollen [1]		Flowering time [2]	Remarks
Eucalyptus viminalis (Manna Gum)	N2	P3	10 - 3 (12 - 1)	This is a cold-hardy, quick growing species. The honey crop varies much from year to year, but is usually small. The honey is dark, almost black with a musty aroma and low density. It has good quality pollen which promotes brood-rearing.
Tribulus terrestris (Common Dubbeltjie)	N2	P3	10 - 5 (3 - 4)	This prostrate annual weed with its spiny fruit is more common in semi-arid western regions, particularly in over-grazed veld. Profuse flowering in autumn is preceded by heavy rainfalls. Dubbeltjies are good sources of pollen, and together with the stimulative amounts of nectar, promote brood production.
Rhus lancea (Karee)	N2	P3	5 - 9 (6 - 7)	In some regions the only winter pollen source. Value as nectar plant variable. Light, mild honey rarely obtained. Dioecious.
Helianthus annuus (Sunflower)	N3	P3	12 - 7 (2 - 3)	Sunflowers provide sufficient nutritious pollen on which honeybee colonies build up. In pure form a light yellow-coloured honey of fair density and mild flavour is produced. It granulates rapidly with fine to medium grain. As a general rule no surplus honey is procured from April onwards on the Highveld because nectar secretion is inhibited by the lower temperatures prevailing.
Felicia / Senecio type	N1-3	P1-3	1 - 12	Annual or perennial herbs with blue and yellow daisy type flower heads respectively. Natural vegetation or weeds. Dark strong honey sometimes obtained.
Poaceae (Grasses)	N0	P1-3	10 - 5	Minor to good sources of pollen following adequate rain.

Botanical Name	Nectar & Pollen [1]		Flowering time [2]	Remarks
Populus (Poplar)	N0	P3	8 - 9	A popular pollen, but nutritive value reported not very high. Good source of propolis.
Acacia baileyana (Bailey's wattle)	N0	P2-3	7 - 8	Other pollens are preferred if available. Relatively hardy ornamental. Yellow flower puffs. Small fern-leaves. Pollen light greyish-khaki.
Fabaceae (Crop plants and indigenous plants of the legume family)	N2-4	P1-2	5 - 8 (6 - 7)	"Heuningbossie" – Small thorny bushes. Copious nectar, particularly after good autumn rains. Honey light lemon-coloured.
	N3	P1	11 - 3	Lucerne – Although planted extensively throughout South Africa as a fodder crop, honey surpluses from lucerne are mainly reported from low rainfall areas with lime-rich soil. Conditions for nectar production are hot and dry weather, together with lime-rich or high pH soils. Pure honey is of very light colour, with a mild flavour and aroma. It has a good density and granulates slowly. Pollen gatherers prefer other sources if available, because the lucerne flowers have to be tripped to obtain pollen. In tripping, the staminal column strikes the ventral side of the bee with considerable force, at times trapping the forager.
Osteospermum / Arctotheca type (Bietou)	N1	P1	1 - 12 (10 - 1)	Yellow-flowered Karoo bushes. Good grazing.
Fraxinus (Ashes)	N0	P1-3	8 - 9	Dioecious trees. Pellets cream-coloured to beige to orange in different species.
Leucas / Teucrium type	N0-2	P0-3	9 - 10	Indigenous herbs; sage family.
Celtis type	N0	P1	9	Offer a limited supply of pollen to bees. Wind-pollinated.

Botanical Name	Nectar & Pollen [1]		Flowering time [2]	Remarks
Morus (Mulberry)	N0	P1-2	8 - 9	Pollen yellowish-grey or dark greenish-beige. Some trees predominantly male.
Zea mays (Maize)	H2	P4	12 - 3	Maize tassels worked freely during forenoon for the nutritive pollen if a nectar source is available at the same time. Aphid honeydew occasionally.
Prunus type	N2	P2-3	8 - 9	Fruit trees and ornamentals.
Ulmus parvifolius (Chinese elm)	N0	P2	10 1 - 3	Offers limited supply of pollen to bees. Dry area street tree. Sometimes second flowering.
Othonna / Helichrysum type	N2?	P2-3	9	Karoo everlasting types.
Cucurbita (Pumpkins, squashes & marrows)	N2	P2	1 - 12 (11 - 3)	Occasional surpluses of a light coloured, bland honey. Large spiny pollen grains are not collected, unless there is nothing else.
Brassicaceae	N2-4	P2-4	8 - 10 (9)	Cabbage family. Crop plants and weeds.
Cupressus (Cypress)	N0	P1	7	Pollen of very low nutritive value.
Oenothera (Evening primrose)	N1	P2-3	12	Pellets cream-coloured.
Stoebe type similarities ("Slangbosse", "bankrotbosse")	N0	P1	2 - 5	Shrublets with ericoid leaves. Small brownish flowers, not typical of the daisy family, to which they belong.
Pinus (Pines)	H1	P1	6 - 8	Although the pollen is of poor quality, it is sometimes eagerly gathered if a nectar source, such as *Eucalyptus sideroxylon*, is available simultaneously.
Campanulaceae	N1	P2	1 - 2	Probably indigenous *Wahlenbergia*. Blue bell family.

Botanical Name	Nectar & Pollen [1]		Flowering time [2]	Remarks
Argemone (Mexican poppy)	N0	P2	2	Widespread weed.
Sorghum	N0	P2	2 - 6	Fodder crop.

*(after Johannsmeier, 1995 and Johannsmeier & Mostert, 2001)

[1] Nectar and pollen sources

N = Nectar
P = Pollen
H = Honeydew
0 = no nectar/pollen is available to honeybees
1 = poor or minor source
2 = minor to medium source
3 = medium to good source
4 = very good or major source

[2] Flowering time

8 = August. The number denotes the month
8 – 12 = May be found any time from August to December
(9 – 10) = The main flowering period is September - October

5.4 References

AL-TIKRITY, W.S., BENTON, A.W., HILLMAN, R.C. & CLARKE, W.W. Jr. 1972. The relationship between the amount of unsealed brood in honeybee colonies and their pollen collection. *Journal of Apicultural Research* 11(1): 9 – 12.

ANDERSON, R.H., BUYS, B. & JOHANNSMEIER, M.F. 1983. *Beekeeping in South Africa*. Bulletin 394, Department of Agriculture, Pretoria. 207 pp.

BONNEFILLE, R. & RIOLLET, G. 1980. *Pollens des savanes d'Afrique orientale*. Centre Nat. Recherche Scientifique, Paris. 253 pp.

CRANE, EVA. 1990. *Bees and beekeeping: Science, practice and world resources*. Heinemann Newnes, London. 615 pp.

DE GROOT, A.P. 1953. *Protein and amino acid requirements of the honeybee* (*Apis mellifica* L.). Junk, Den Haag. 90 pp.

DIETZ, A. 1979. Pollen and honeybee nutrition. *Gleanings in Bee Culture* 107: 366 – 368.

DOULL, K.M. 1973. Relationships between pollen, broodrearing and consumption of pollen supplements by honeybees. *Apidologie* 4(4): 285 – 293.

ERDTMAN, G. 1952. *Pollen morphology and plant taxonomy. Angiosperms*. Chronica Botanica, Waltham, Massachusetts. 539 pp.

HAYDAK, M.H. 1935. Brood rearing by honeybees confined to a pure carbohydrate diet. *Journal of Economic Entomology* 28(4): 657 – 660.

HERBERT, E.W. 1992. Honey bee nutrition. pp. 197 – 233 in: Graham, J.M. (editor). *The hive and the honey bee.* Dadant & Sons, Hamilton, Illinois. 1324 pp.

JOHANNSMEIER, M.F. 1981. Corbicular loads of the African honeybee. *South African Bee Journal* 53 (5): 3 – 6.

JOHANNSMEIER, M.F. 1995. *Beeplants of the South-Western Cape.* Department of Agriculture, Pretoria. 59 pp.

JOHANNSMEIER, M.F. 2001. Bee nutrition and supplemental feeding. pp. 111 – 126 in: Johannsmeier, M.F. (editor). *Beekeeping in South Africa.* Plant Protection Handbook No. 14, Agricultural Research Council, Pretoria. 288 pp.

RASHAD, S.E. & PARKER, R.L. 1958. Pollen as a limiting factor in brood rearing and honey production. *Transactions of the Kansas Academy of Science* 61: 237 – 248.

SEELEY, T.D. 1985. *Honeybee ecology. A study of adaptation in social life.* Princeton University Press, Princeton, New Jersey. 201 pp.

STANDIFER, L.N., CORNELISSEN, G. & HALBERG, F. 1983. *Chronobiological appraisal of dietary quality for free-flying honey bee colonies.* Technical Bulletin Number 1674, United States Department of Agriculture, Washington DC. 39 pp.

CHAPTER 6

SUMMARY

1. The use of breeder colonies for supplying abundant well-fed larvae for queen rearing with African bees, was successfully employed. Use was made of a full depth hive body insert, with six small frames, which occupied the space of three standard Langstroth brood frames.

2. The queen cells produced during times when very little natural pollen was available, were about 30 – 50 % smaller than those produced when pollen was abundant during November and December at Douglas.

3. Queen cell cups made from beeswax were cheaper than purchased ones. It was also not necessary to remove them later after finished queen cells were introduced. Their preparation was, however, time-consuming.

4. Queen cell cups of 9 mm length were preferred by the bees (73,3 %), followed by 8 mm long cups (52,5 %) and 11,5 mm cell cups (33,4 %). No cell cups of 7 mm length were accepted.

5. The acceptance of larvae grafted into a droplet of water was 72 %, compared to the 57 % of dry grafts. To prevent desiccation, the grafting frames were covered with a damp towel.

6. The acceptance of queen cells in a queenless 4-frame nucleus colony was 73,3 % after a 24-hour queenless period, and after the queen cell cups were familiarised for a 24-hour period. In a queenless 10-frame

brood chamber, the comparable figure was 78,6 %. When the grafted cells were not familiarised, the acceptance was only 2 %. The queenless period was not extended beyond 24 hours, to minimize infestation with Cape bee laying workers. Only 7,2 % of grafted cell cups were accepted after an 8,5-hour queenless period.

7. The numbers of queen cells completed in queenright colonies were slightly lower than in queenless colonies (62,4 % and 67,8 % respectively).

8. There was no significant difference in acceptance when 60 or 120 cells were grafted and placed into a nucleus starter colony. However, other authors found that with greater numbers, the queens became lighter in weight and the ovaries smaller.

9. Statistically significant more queens emerged and were accepted from queen cells introduced on Day 11 compared to Day 10 following grafting (85,8 % and 77,8 % respectively).

10. The introduction of finished queen cells into baby mating nucs had a success rate of 81,3 %, i.e. queens that emerged, however, the percentage queens that subsequently mated successfully, was considerably lower (67,4 %). With the larger 4-brood frame mating nuclei the success rate was 85,8 % for emerged queens and the percentage mated queens obtained was 79,5 %. The better results were ascribed to a better maintenance of the correct brood temperature.

11. The success rate of the introduction of mated queens into 4-frame nuclei was 75 % after a queenless period of 24-hours or less. The queens were introduced in plastic hair curlers with a newspaper stopper.

12. A 60 % sugar solution is recommended for feeding honeybees before a honeyflow or during queen rearing. This concentration was found not to ferment easily and permitted feeding only once a week.

13. Different pollen substitutes and supplements were tested for preference and brood production. For dry substitutes outside hives, the highest number of bees was counted on the mixture of sifted maize meal and Lotmix ® (a cattle feed), to which sugar had been added. Yeast and mixtures of yeast, soy and powder milk, containing no maize meal, attracted few, if any bees. The degree of collecting activity of the substitutes was also correlated with the amount of natural pollen available. When natural pollen became more freely available, substitutes were generally ignored.

    In another trial with dry substitutes and supplements outside hives, the attractiveness of maize was specifically tested. Alone it ranked 5th in the eight tested substitute combinations, but with soy it ranked 1st and 2nd, even effecting a slightly higher collecting activity than the soy:pollen combinations. The feed value of maize meal, however is very low. Unlike overseas results, the addition of powdered sugar to the dry substitutes did not increase their attractiveness.

    The following substitutes/supplements, in decreasing order of preference, were tested as moist patties inside hives: Beltsville substitute, fine maize meal, soy + pollen (3:1), yeast + milk + pollen (2:2:1), Pronutro ®, soy + pollen (9:1), and soy + yeast + milk (3:1:1).

14. The following substitutes/supplements, in decreasing order of brood production, were tested: Pronutro ® + pollen (4:1), Beltsville substitute, soy + pollen (4:1), soy + yeast (3:2), soy + yeast + milk (3:1:1), and soy

+ yeast + egg (2:1:1). The feeding of combinations of soy + pollen is recommended for the highest brood production at the lowest price.

15. A queen rearing programme in a commercial beekeeping business at Douglas was implemented to replace the large number of lost *scutellata* colonies. Within the first week of queen rearing having started, large numbers of eggs were noticed in the queen cells, indicating *capensis* laying workers. The acceptance of grafted queen cell cups was very low in general, the highest percentage being 48 %. The percentage emergence of queen cells introduced into mating nucleus colonies was high, namely 95 %. However, only 44 % of these queens mated successfully, and had a normal brood pattern. This gave an overall success rate of 20 % maximally in the presence of Cape laying workers.

No *capensis* infestation was noted in the colonies into which mated queens were introduced, and 89 % of the introduced queens were accepted.

16. The study on the seasonal variation in colony size, brood, pollen and honey of honeybee colonies at Bloemfontein, Free State, provided knowledge of natural food sources, and indicated when supplemental feeding could be applied if need be. Natural pollen was most plentiful during September, November, December and April. The most abundant pollens were *Eucalyptus* spp., *Tribulus terrestris*, *Rhus lancea* and *Helianthus annuus*. The different pollens and their percentages were tabled for every month. Very little pollen was collected during June and July winter months. The collection of dry stock feeds during July corroborated this scarcity of natural pollen.

17. The total amount of pollen trapped for the one year period was 3580,6 g. Pollen trap efficiency was calculated to be 10 %, therefore the total amount of pollen collected by the colony was approximately 35,8 kg.

18. The amount of stored pollen did not necessarily correspond with the amount of incoming pollen. The latter showed peaks in September, November, December, March and April, while the amount of stored pollen gradually increased from July, and peaked in December and again in April.

19. During this study it became evident that it is possible to easily produce hundreds of quality cells with *A.m. scutellata*. However, the time and labour involved in the preparation and maintenance of the mating nucleus colonies is considerable. Another problem experienced is obtaining Scutellata colonies not infested with Capensis problem bees.