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## Field Trials to Test Supplementary Feeding Strategies for Commercial Honeybees

by Doug Somerville and Damian Collins

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## Foreword

The Australian honeybee industry has historically relied on honey production as its main source of income with the need to manage extremes in floral resource availability in the form of suitable flowering events. Honeybees collect nectar and pollen both of which are vital to maintain high productivity levels in managed bee hives. Periodically there is a short fall in one or both these floral rewards which produces difficulties for beekeepers to maintain healthy populous colonies of honeybees. Supplementary feeding has been trialled by many beekeepers over many decades with mixed success.

This research provides evidence of the vagrancies of supplementary feeding honeybees over the winter. It also provides a direction for future research in this area and a number of suitable options to consider.

Beekeepers should carefully consider the economics of supplementary feeding honeybees and provide controls in any future feeding strategies they may adopt. Only by this approach of measuring production increases as compared against controls will individual beekeeping managers become confident that supplementary feeding is a paying proposition under some circumstances.

The most positive finding of the research in both the winter of 2003 and 2004 was the reinforcement of the need for 'good' autumn management of colonies if strong populous colonies are desired to harvest surplus nectar, to have a winter honey flow, or to provide a pollination service at the conclusion on the winter period before warm weather and fresh pollen sources combine to stimulate colony expansion in the early spring.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 1600 research publications, forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry

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**Peter O'Brien** Managing Director Rural Industries Research and Development Corporation

## Acknowledgements

#### 2003 trial

The 2003 project was made possible by the generous support of two commercial beekeepers— Trevor Monson, Gol Gol, NSW and Craig Scott, Echuca, Victoria. Both beekeepers provided the use of a commercial load of bee hives and donated their time and expertise to conduct the research, they also assisted with measurements and treatments as and when required. The 2003 trial was designed around a pilot study conducted in 2002 initiated by Trevor Monson at his own cost.

Each measurement period required a number of assistants to weigh, scribe, take samples, etc. Many thanks are extended to Dale Chalker, NSW DPI; Michael Cutting, technical supervisor, Kyndalyn Park Almond Farm; Ray Phillips, Laurie Fry and Kevin Emmins, commercial beekeepers.

Thank you to Susan Burrows (ANU) who assisted with the winter (June) measurements as well as conducting the protein analysis of pollen and pupae samples collected for the project. Thanks to Michael Hornitzky, NSW DPI, who coordinated the processing of the adult bee sample for nosema. Thanks to the NSW DPI Goulburn office staff, including Joanne Ottaway and Alisha Nichol for the typing and layout of the 2003 trial component of the final report.

#### 2004 trial

The 2004 project was designed around a mugga ironbark nectar flow which is regarded as pollen deficient by beekeepers. The two commercial beekeepers who generously made their bee hives available and assisted with all stages of the trial were Tony Thomas, Queanbeyan NSW, and Des Cannon, Urila, via Queanbeyan NSW.

All colonies were re-queened in the two apiaries utilised in the trial. This was conducted with the assistance from Tony Thomas and Des Cannon (beekeepers), Dale Chalker and Wayne Haigh (NSW DPI) on the 1–2 March 2004. The initial measurements for allocation of colonies for each treatment were conducted from the 27-29 April by Tony Thomas, Des Cannon and Reg Marsh (beekeepers), Mike Moncur (RIRDC committee), Dale Chalker and Wayne Haigh (NSW DPI).

The final measurement was conducted from the 23-25 August with assistance from Tony Thomas, Des Cannon, Reg Marsh, Darren and Lisa Caves (beekeepers), Bruce White, Dale Chalker, Rob Gorman (NSW DPI) and Mike Moncur (RIRDC). The task of feeding the different supplements to each colony throughout the trial period was primarily the responsibility of Dale Chalker (NSW DPI).

Thanks to Susan Burrows (ANU) for conducting the protein analysis of pupae samples. Thanks to Michael Hornitzky (NSW DPI) for coordinating the processing of the adult bee samples for nosema. The final report was typed and laid out with the assistance of Karen Webster and Vicki Saville (NSW DPI). The final report was proof read by Annette Somerville.

## **Executive Summary**

#### Background

The Australian honeybee industry has historically relied on honey production as its main source of income, with the need to manage extremes in floral resource availability in the form of suitable flowering events as the critical factor. Honeybees collect nectar and pollen, both of which are vital to maintain high productivity levels in managed bee hives. Periodically there is a short fall in one or both of these floral rewards which produces difficulties for beekeepers to maintain healthy populous colonies of honeybees. Supplementary feeding has been trialled by many beekeepers over many decades with mixed success.

Trials involving four commercial apiaries testing various supplementary feeding strategies were conducted over the winter periods of 2003 and 2004. This research provided evidence of the vagrancies of supplementary feeding honeybees over the winter. It also provided a direction for future research in this area and a number of suitable options to consider.

#### **Aims and Objectives**

The objective for the 2003 trial was to provide evidence that supplementary feeding honeybee colonies will increase bee populations through a winter period with the aim to test supplementary feeding strategies to increase the colony population in each hive prior to the onset of almond bloom in mid August. This necessitated the provision of supplements during a winter period with the colonies exposed to the prevailing climatic winter conditions.

The objective for the 2004 trial was to maintain colony populations on a pollen deficient nectar flow using pollen supplement with the aim to test the effectiveness of various pollen supplements against a control, while colonies of bees were foraging on a pollen deficient nectar flow. The floral species chosen for this trial was winter flowering mugga ironbark (*Eucalyptus sideroxylon*).

#### **Materials and Methods**

Two commercial apiaries were utilised in the 2003 trial. All colonies were re-queened in early April. The initial measurement was taken in early June, with an interim measurement in mid August and the final measurement in late October. Treatments were sugar syrup, pollen supplement and a combination of both, compared to the control. Treatments were applied either every three or six weeks. Ten colonies from each apiary were allocated to each treatment. Initial and final measurements included total weight gain, frames covered in bees, area of brood, area of pollen, frames of honey, nosema levels of adult bees and the crude protein levels of pupae.

Similar to the 2003 trial, two commercial apiaries were selected for the 2004 trial. All colonies were re-queened in early March, initial measurements were taken in late April and the final measurements taken in late August. The measurement criterion was the same as in 2003. Treatments were pollen, soyflour or a mix of soyflour (50%), pollen (25%) and yeast (25%), split into a feeding regime of every two or four weeks. These treatments were compared to a control. The apiaries were located on a mugga ironbark (*Eucalyptus sideroxylon*) nectar flow which does not provide pollen that is attractive to honeybees. Traditionally, this floral species is responsible for some excellent honey crops but managing the short fall of pollen has been a major management issue for beekeepers.

#### **Results and Discussion**

In the 2003 trial there were significant differences between the apiaries independent of the treatments, indicating strong climatic and floral reward variations between apiary locations. The strongest response was observed when one apiary had access to a flowering canola crop after almond pollination, and the other apiary did not. In this case, the frames of bees per hive were not significantly different between apiaries although the area of brood was twice as large in the colonies that had access to canola and pear blossom, than the colonies that did not.

There was no significant difference in the crude protein levels of the pupae between treatments. Also there was no significant trend from the August measurement across either apiary suggesting that any one treatment was superior to the control. In the October measurement, again there was no clear benefit by adopting any one of the feeding practices tested.

The five litres of sugar syrup and 500 gram pollen patty per application was, in many cases, excessive for the colonies' ability to remove the supplements. These treatment volumes were applied irrespective of the size of the colony. Based on the data obtained from recording unused sugar syrup and pollen supplement, it is recommended that 50 grams of patty and 500 ml of syrup per frame of bees per month should be considered as a maximum quantity for mild winter conditions as experienced in western NSW and Victoria. Pollen supplements and sugar syrup should be provided to a colony on a volume or weight formula, based on the size of the population of the colony.

There was strong evidence that any benefit from the various supplements provided to the colonies was overridden by the adult bee disease *Nosema apis*. This disease is known to reduce the longevity of adult bees and thus suppresses population increase when nectar and pollen conditions are suitable for providing stimulus for a colony to expand its population. Also, the trial provided evidence that the provision of supplements to a colony during the winter period may have increased nosema levels in adult bees.

The results from the 2004 trial provided evidence that all three different preparations of supplementary feeding had some benefit with a ranking of pollen, then the soyflour/pollen/yeast mix and then soyflour last. Even so, the control colonies produced more honey in one apiary, thus the benefit was not uniform across both apiaries. There was a significant spread in the results for each treatment, suggesting that the responses from individual colonies can be considerable given the same set of circumstances.

Nosema disease was not a significant factor in 2004. The reasons for this were unclear, although the colonies were 'interfered' with less than in 2003 and hives were not dismantled to apply the treatments as was the case in 2003.

An attempt to measure the attractiveness of three sources of soyflour provided in bulk containers to foraging bees was not conclusive. The technique used to measure the feed left over in this trial was not satisfactory due to the feeding behaviour of bees, scratching the flour out of the containers.

There was a lack of replication in this experiment. Even so, observation suggests bees will favour one source of soyflour over another, particularly when exposed in a bulk feeder. Soyflour on its own as a supplement was far more attractive to bees within the bulk containers than when placed on trays under the lids of each bee hive.

#### Implications

Essentially, the 2003 trial failed to provide a strategy for apiarists to artificially increase populations of bees over the winter period. Even so, a significant result was achieved in providing reasons why this was not accomplished, and possible future research directions.

If colonies are required to be a certain size population in late winter or early spring, then management strategies must be implemented during the autumn period prior to winter. This will give sufficient time to expand the population of the colonies to the required size with little or no management activity to the colonies during the winter period. In the event of imminent starvation, sugar supplementation in the form of dry sugar rather than syrup may be preferable.

The 2004 winter trial was primarily aimed at trialling pollen-supplement, as nectar was not a limiting factor. While pollen on its own as a supplement was the most attractive substance, the cost benefit of the exercise to a beekeeper was questionable. This conclusion was based on honey yields and subsequent financial returns. There may be a benefit of providing pollen if colonies are to be maintained for pollination services particularly for the late winter and early spring period. Essentially this trial supports autumn preparation for a winter nectar flow, as a large percentage of the colonies in both apiaries declined in number of bees in winter even with pollen and nectar available.

#### Recommendations

Beekeepers should carefully consider the economics of supplementary feeding honeybees and provide controls in any future feeding strategies that they may adopt. Only by this approach of measuring production increase, compared against a control, will individual beekeeping managers become confident that supplementary feeding, under certain circumstances, is economically beneficial.

It is also recommended that future field research on supplements should include greater numbers of colonies per treatment or use package bees of known weight on empty combs.

The main take-home message for beekeepers from the 2004 research was similar to that in 2003, autumn preparation and management is vital to ensure a populous colony of bees is maintained through winter and early spring. The results of the research did not support 'costly' supplementary feeding practices to be carried out through winter.

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2004 trial: Soyflour treatment, note discoloration of flour due to mould growth. Mould was not a problem in the hives proved with pollen only.

exposed nature of site.

Comparing the attractiveness (chapter 12) of three types/sources of soyflour in bulk feeders. Wire placed over drum to keep sheep and other larger animals

from consuming the flour.

2004 trial site north of Young, final measurement August. Note open



















Site of Tony's apiary east of Temora, May 2004 trial. Note: lack of ground cover due to very dry conditions

2004 trial – placement of feeding trays under lid. Soyflour, soyflour 50% - pollen 25% -yeast 25% or pollen.



Comparison between the consumption of pollen on the left and soyflour on the right. Straight pollen was more palatable to bees.

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## 1. Introduction

Honeybees (*Apis mellifera*) collect nectar and pollen to obtain the necessary dietary components for survival and reproduction. The ability of honeybees to store nectar in the form of honey, and pollen within the beeswax comb structure of a colony has enabled the species to maintain breeding and populations well after the natural source of these two substances have ceased to be available in the field.

It is possible to provide dietary components as a supplement to colonies to satisfy any real or perceived deficiencies within naturally available foods. It is also possible to create a stimulus effect by mimicking a nectar flow or providing pollen supplement to create a given response within the colony.

Population increase is a direct result of the vigour of the resident queen bee, the present population of the colony and the food stimulus available either naturally or artificially. It is normal practice in Tasmania to provide sugar syrup as a supplement throughout the spring period thereby artificially stimulating colonies to expand their population more rapidly than they would do naturally (Honey Research Council 1990). The aim of this is to maximise colony populations prior to a specified nectar flow known to commence within a certain time frame. Traditionally, commercial beekeepers in mainland Australia have utilised flowering events which provided sufficient quantities of nectar and pollen to stimulate colonies to increase worker bee populations. This method of management has proven historically to be economically feasible due to the choice of flowering events over time, within the operational area.

This traditional method of managing honeybee population increase has, in recent years, been found wanting due to a number of factors. Increasingly, the availability and reliability of flowering events is diminishing due to reduced floral resources, resulting from restrictions on access to bee forage areas, pressure from urban expansion, reduced health of native vegetation, drought and fire events. Thus the ability of beekeepers to move their hives from one flowering event to another to stimulate population increase is severely reduced. The use of a nectar flow to stimulate the expansion of a colony is a dubious economic decision if artificial stimulation can be provided prior to a nectar flow to increase the population of a colony. In this scenario the nectar flow has the potential of turning into a honey crop for the beekeeper, increasing the annual harvest of honey per hive. When ever there is a significant price differential between sugar syrup and honey in favour of honey, removal of honey and replacing with sugar syrup for winter stores must be seriously considered as a viable economic option by beekeepers.

In each year, the location, and the condition of bees will be in some way different and as such any field research trial will provide data only for those given circumstances. Depending on individual circumstances the use of sugar syrup or pollen supplement or both, may provide a stimulatory response. Mitev (1970) indicated that in a Bulgarian trial the stimulating effect on brood rearing and consequently on honey production was exerted by syrup only when sufficient fresh pollen was available. Providing supplements to a colony was shown to be worthwhile for a four month period through winter in order to build up colonies for almond pollination in California, although feeding of pollen supplement was of no benefit when pollen was available in the field (Stanser and Laidlaw 1974).

Two trials in the USA (Abdellatif *et al.* 1971 and Standifer *et al.* 1973) indicated that using pollen supplements stimulated brood rearing and increased honey production but the results were not statistically significant when compared with the controls which were not provided with a supplement. Herbert *et al.* (1976) provided evidence supporting the importance of timing when feeding pollen supplements and sugar syrup. They indicated that a six week period between providing supplements to two sets of colonies produced a significantly greater harvest

of honey in the earlier fed colonies. The combination of pollen supplements and sugar syrup was found to be more effective than either pollen supplement or sugar syrup alone. Herbert and Shimanuki (1979), in a spring trial with sugar syrup and pollen supplements, produced no significant response as the conditions prevailing provided enough flowering plants for the colonies to gather naturally occurring pollen and nectar. This was also found by Skowronek (1979), a Polish author who indicated that 'good conditions' provided the necessary pollen and nectar requirements of a colony, and supplementary feeding of bee colonies did not generate a response. During poor conditions when pollen and nectar were in short supply the artificial provision of sugar syrup and pollen supplements did encourage brood development.

Eijnde and Smeeken (1982) provided evidence that sugar syrup without a pollen supplement or a natural pollen source gave no measurable response whereas in the second year of providing sugar syrup, ample pollen was available from willow and a significant response was achieved in population expansion. Imdorf *et al.* (1984) found that there was no difference in colony populations feeding sugar syrup or 'pollen patties' in spring over two consecutive years even though pollen was considered in short supply in the field. They did not trial both pollen supplement and sugar syrup to ascertain a combined response.

Feeding pollen supplement alone without sugar syrup may not stimulate brood rearing (Cook and Wilkinson 1986). A Brazilian experiment demonstrated that the combination of sugar syrup and pollen supplements was marginally more beneficial to population increase than sugar syrup alone and far more beneficial than pollen supplement alone or no treatment. The treatments were applied to the colonies 6 to 7 weeks before the nectar flow.

There is some evidence to suggest that the volume of supplements provided influences colony response, e.g. a trial in Venezuela provided evidence that one litre of sugar syrup was of equal benefit as three litres when both were fed twice a week over a six month period. The higher rate of feeding resulted in slower nest expansion and thus restricted colony development, pollen in this case was not a limiting factor (Pesante *et al.* 1992). Much of the research by Goodwin and Houten (1988) in New Zealand suggests that greater pollen collection occurs when colonies are fed one litre daily compared with colonies fed three litres every third day.

The dilemma Australian commercial beekeepers have is that the large number of colonies under management, in association with the time available and the distance needed to travel to service the colonies, will prohibit daily feeding of supplements. Even weekly feeding may prove to be too costly in time and travel. The frequency and amount of supplement provided in this research is designed to be as realistic as possible as far as Australian commercial beekeepers are likely to repeat as a regular management strategy. Some weaknesses of many of the papers cited are the low numbers of colonies utilised in the trials, the lack of clear information on the external weather and floral conditions prevailing over the trial period, the age and condition of the queen, and the disease and pest status of the colonies utilised. These factors will potentially impact on the results and thus need to be recorded and published to enable the results to be better interpreted for specific conditions.

## 2. Objectives

## 2003 Trial

"To provide evidence that supplementary feeding honeybee colonies will achieve population increases."

In the 2003 experiment, the aim was to test supplementary feeding strategies to increase colony population prior to the onset of almond bloom in mid August. This necessitated the provision of supplements during a winter period with the colonies exposed to the prevailing climatic conditions.

## 2004 Trial

"To maintain colony populations on a pollen deficient nectar flow using pollen supplement."

The aim of the 2004 experiments was to test the effectiveness of various pollen (protein) supplements against a control, while colonies of bees were working a pollen deficient nectar flow. The floral species chosen for the experiment was winter flowering mugga ironbark (*Eucalyptus sideroxylon*).

## 3. Methodology (2003)

Two commercial apiaries were re-queened from the 9<sup>th</sup>-11<sup>th</sup> April with young queens of the same age, grafted from the same queen mother and mated in the same mating yard. Both apiaries were typical commercial loads of bee hives for Australian mainland conditions with approximately 100 colonies in each apiary. Both apiaries were managed independently from each other belonging to separate beekeeping enterprises, periodically being transported to various sites as the ownership decided.

## 3.1 Trevor's apiary

April—located approx. 10 km east of Gol Gol, NSW in an open mallee landscape. White mallee (*Eucalyptus gracilis*) flowered from April through to the end of September.

August—approximately 15 km east of Robinvale, Victoria, in a commercial almond (*Amygdalus communis*) orchard in flower.

September—returned to the original site east of Gol Gol. White mallee (*E. gracilis*) flowering followed by orange (*Citrus* spp.) flowering prior to the final measurement.

## 3.2 Craig's apiary

April to the end of July—located approximately 10 km west of Rushworth Victoria, on an open grazing cropping landscape with grey box (*E. macrocarpa*) in flower along the ridges.

August—approximately 15 km east of Robinvale, Victoria in a commercial almond *(A. communis)* orchard in flower.

September—Moama region, NSW, adjacent to a commercial canola (*Brassica napus*) crop in flower.

Last two weeks of September—Cobram, Victoria, apiary placed in a commercial pear (*Pyrus communis*) (Packham) orchard in flower.

Beginning of October—for two days returned to canola crop near the end of its flowering period.

October—returned to Cobram, Victoria into a commercial pear (William) orchard in flower.

## 3.3 Measurement periods

Three measurement periods were selected to minimise physical disturbance to colonies which may have influenced the development of the colony by reducing egg laying of the queen or increasing disease occurrence.

Initial measurements and sample date:	3–5 June
Interim measurement period:	19–21 August
Final measurement period:	27–29 October

## 3.4 Treatments

Each colony was allocated a treatment based on the number of frames of bees it contained in June ensuring that each treatment was equally represented based on the number of frames of bees per colony for each apiary. Each treatment was allocated 10 colonies per apiary with a total of 20 colonies per treatment.

Treatments per colony were:

- a) 5 litres of sugar syrup at 6 week intervals.
- b) 5 litres of sugar syrup plus 500 grams of pollen supplement (patty) every 6 weeks.
- c) 500 grams of pollen supplement every 6 weeks.
- d) 5 litres of sugar syrup every 3 weeks.
- e) 5 litres of sugar syrup plus 500 grams of pollen supplement every 3 weeks.
- f) 500 grams of pollen supplement every 3 weeks.
- g) control.
- h) 5 litres of sugar syrup every 3 weeks with pollen traps attached.
- i) no syrup or pollen supplement with pollen traps attached.

## 3.5 Supplements

Sugar syrup provided to treatments a, b, d, e and h varied in concentration from 59.5% to 63% brix. The syrup was placed in a plastic tray located on the top of each hive protected by a wooden box. Each tray contained dry hay material or similar to prevent worker bees drowning while accessing the syrup.

The protein supplement was made by mixing:

- 3 x 25 kg bags of de-bittered soyflour—full fat, non-genetically modified, manufacturing code 03—May 2002, packed for HJ Langdon & Co, CAN 006 641 701, 4-8 Parker Street, Footscray, Victoria 3011.
- 2 x 15 kg bags of expeller processed soyflour manufactured November 1999 by Macquarie Mills Pty Ltd, Narromine. Australia, 33 Industry Avenue, Narromine NSW 2821.
- 25 kg of irradiated Mauri and Jarrah pollen from Western Australia. February/March 2003.
- 8 x 15 litre buckets of sugar syrup 45% brix.
- 8 x 40 grams of Solaminovit®. All Farm Animal Health, 4 Handley Crescent, Dandenong, Victoria 3175

Ingredients were thoroughly mixed in May 2003 and divided into approximately 500 gram patties. The N% of the de-bittered soyflour was 6.589 calculated to 41.2% crude protein. The N% of the final mixed pollen supplement was 4.268 calculated to 26.7% crude protein. The patties were placed above the brood under the queen excluder.

## 3.7 Laboratory

Nitrogen content of pollen supplement and soyflour was determined using a Kjeldahl nitrogen test (Mitchell 1972) multiplying the N x 6.25 to determine the crude protein levels and near infrared reflectance spectrometry (Berding 1998) was used to measure the CP% of the bee pupae. Nosema disease levels were determined by the Cantwell (1970) method.

## 3.8 Statistics

Brood area, numbered frames of bees and honey, area of pollen, pupae crude protein levels, and nosema levels were each analysed using univariate anova techniques, treating the associated initial response (June) as a covariate (where available). For the 'frames of bees' data collected in August and October, the effect of initial 'frames of bees' data differed significantly between apiaries, and so two separate coefficients were fitted. For the brood area analyses, initial 'frames of bees' data was also included as a covariate, as it was significant even after allowing for differences in initial brood area. Nosema counts were analysed on a squareroot scale.

## 4. Results (2003)

The results means have been plotted in graph form as well as presented in the following tables. The key for all tables and Figures is as follows, S = syrup; SP = syrup and pollen patty; P = pollen patty; A = 5 litres of syrup every 6 weeks; B = 5 litres of syrup and 500 grams of pollen supplement every 6 weeks; C = 500 grams of pollen supplement every 6 weeks; D = 5 litres of syrup and 500 grams of pollen supplement every 3 weeks; E = 5 litres of syrup and 500 grams of pollen supplement every 3 weeks; F = 500 grams of pollen supplement every 3 weeks; F = 500 grams of pollen supplement every 3 weeks; G = control; H = 5 litres of syrup every 3 weeks with pollen traps; I = pollen traps control.

Due to variability in the weight of the empty material and variations in the size of the material it was difficult to determine a base weight. Also, as the bees were not exposed to a specific nectar flow designed to harvest a honey crop, the opportunity for colonies to store large amounts of nectar in the form of honey did not occur. The colonies in both apiaries were maintained for the duration of the trial for pollination purposes and building colony strength was a higher priority than seeking a suitable nectar flow.

## 4.0 Initial measurements (3-5 June)

Colonies were allocated to treatments based on frames of bees. For Trevor's apiary the mean for the 'frames of bees' data was  $5\frac{1}{2}$ , the mean for the 'area of brood' data was  $30\frac{1}{2}$  cm<sup>2</sup> with only 17 colonies with brood, the mean for the 'frames of honey' data was  $6\frac{1}{2}$ . For Craig's apiary the mean for the 'frames of bees' data was  $6\frac{1}{2}$ , the mean for the 'area of brood' data was  $275\frac{1}{2}$  cm<sup>2</sup>, the mean for the 'frames of honey' data was  $6\frac{1}{2}$ .

### 4.1 August 2003 (mid-term measurement)

#### 4.1.1 Frames of bees

The interaction between apiary and treatment was only just significant (P<0.1), and after examination of the treatment means it was decided to consider each apiary separately. There were no significant treatment effects for Trevor's apiary. For Craig's apiary, average frames of bees were significantly lower for the supplement treated colonies (a–f) than for the control colonies (g) (P<0.05). In turn, average frames of bees were significantly lower for the colonies supplemented every 3 weeks (d–f) compared to those supplemented every 6 weeks (a–c) (P<0.05).

Table 4.1.1 The means for frames of bees for each treatment in each apiary and the average for both apiaries after 11 weeks during winter – measurement 19 August. (n= number of hives).

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	H:S3p	I:Cp	AvSED
Craig	4.9 abc	4.7 <sup>abc</sup>	5.6 <sup>bcd</sup>	4.1 <sup>ab</sup>	3.0 <sup>a</sup>	4.2 <sup>ab</sup>	6.9 <sup>d</sup>	4.2 <sup>ab</sup>	6.5 <sup>cd</sup>	1.03
n =	7	9	8	8	10	9	10	9	6	
Trevor	7.3	6.5	4.9	5.9	5.8	6.4	6.3	6.3	6.3	0.98
n =	9	9	9	10	9	10	9	9	10	
Average	6.1	5.6	5.3	5.0	4.4	5.3	6.6	5.2	6.4	0.71



Figure 4.1.1 The distribution and means for frames of bees for each treatment after 11 weeks – measurement 19 August. (S = syrup, P = pollen supplement, Cnt = control)

### 4.1.2 Frames of honey

There were significant differences in frames of honey between apiaries (P<0.01), and significant differences between treatments (P<0.001), but no significant interaction between apiary and treatment. Average honey was significantly greater (P<0.001) for colonies receiving syrup supplement (a–b, d–e, h) than colonies which did not.

Table 4.1.2The r	neans for frames	of honey for each	treatment in each	apiary and the
average for both	apiaries after 11	weeks during wi	nter – measuremen	t 19 August.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	H:S3p	I:Cp	AvSED
Craig	$6.2^{bc}$	5.8 <sup>bc</sup>	$4.0^{a}$	$6.0^{bc}$ .	5.3 <sup>abc</sup>	$4.8^{ab}$	4.2 <sup>a</sup>	6.5 <sup>c</sup>	4.1 <sup>a</sup>	0.75
Trevor	$4.6^{bc}$	4.4 <sup>abc</sup>	3.7 <sup>ab</sup>	4.3 <sup>abc</sup>	5.5 °	3.0 <sup>a</sup>	3.3 <sup>ab</sup>	$4.6^{bc}$	4.4 <sup>bc</sup>	0.72
Average	5.4 °	5.1 <sup>bc</sup>	3.8 <sup>a</sup>	5.1 <sup>bc</sup>	5.4 °	3.9 <sup>a</sup>	3.8 <sup>a</sup>	5.5 °	4.3 <sup>ab</sup>	0.52



Figure 4.1.2 The distribution and means for the frames of honey for each treatment after 11 weeks – measurement 19 August.

#### 4.1.3 Brood area

Treatment effects on brood area were similar to those on frames of bees. The interaction between treatment and apiary was almost significant (P=0.1), and after examination of the treatment means it was decided to consider each apiary separately. There were no significant treatment effects for Trevor's apiary. For Craig's apiary, average brood area was significantly lower for the supplement treated colonies (a–f) than for the control colonies (g) (P<0.05). In turn, average brood area was significantly lower for the colonies supplemented every 3 weeks (d–f) compared to those supplemented every 6 weeks (a–c) (P<0.05).

Table 4.1.3 The means for brood area  $(cm^2)$  for each treatment in each apiary and the average for both apiaries after 11 weeks during winter – measurement 19 August.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	H:S3p	I:Cp	AvSED
Craig	2349 abc	2863 <sup>bc</sup>	3063 bc	2225 <sup>ab</sup>	1711 <sup>a</sup>	2295 <sup>ab</sup>	3327 °	2529 <sup>abc</sup>	3081 <sup>bc</sup>	570
Trevor	2755	2790	2262	2888	2599	2857	2261	2723	2394	540
Average	2552	2826	2662	2556	2155	2576	2794	2626	2737	384



Figure 4.1.3 The distribution and means for the brood area  $(cm^2)$  for each treatment after 11 weeks – measurement 19 August.

### 4.1.4 Crude protein of pupae

There were no significant differences between treatments (crude protein of pupae was not measured for colonies in treatments h and i).

# Table 4.1.4 The means for crude protein (% of dry weight) of pupae for each treatment in each apiary and the average for both apiaries after 11 weeks during winter – measurement 19 August.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	AvSED
Craig	45.7	46.6	44.7	46.3	45.1	45.6	45.1	1.3
Trevor	46.0	45.7	46.3	45.8	46.4	46.1	45.1	1.3
Average	45.8	46.2	45.5	46.0	45.8	45.9	45.1	0.9



Figure 4.1.4 The distribution and means for the crude protein (% of dry weight) of pupae for each treatment in each apiary and the average for both apiaries after 11 weeks during winter – measurement 19 August.

#### 4.1.5 Stored pollen

There was a significant difference between apiaries (P<0.01), but no significant difference between treatments.

Table 4.1.5 The means for area of store pollen (cm<sup>2</sup>) for each treatment in each apiary and the average for both apiaries after 11 weeks during winter – measurement 19 August.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	H:S3p	I:Cp	AvSED
Craig	269.4	275.0	462.5	278.6	197.5	285.0	382.5	191.7	341.7	92.0
Trevor	133.3	163.9	205.6	190.0	211.1	205.0	183.3	138.9	140.0	87.5
Average	201.4	219.4	334.0	243.3	204.3	245.0	282.9	165.3	240.8	63.5



Figure 4.1.5 The distribution and means for the area of stored pollen (cm<sup>2</sup>) for each treatment after 11 weeks – measurement 19 August.

#### 4.1.6 Nosema

The treatments had significant effects on nosema levels (P<0.05). The interaction between treatment and apiary was almost significant (P=0.1). Average nosema levels in colonies with supplement treatments (a–f) were significantly higher than the control (P<0.01) across both apiaries. However, for Craig's apiary, nosema levels in treatment c (a pattie every 6 weeks) were significantly lower than the other supplement treatment trials.

Table 4.1.6 The means for nosema levels (x  $10^6$  spores) for each treatment and the average for both apiaries after 11 weeks during winter – measurement 19 August.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	H:S3p	I:Cp	AvSED
Craig	9.2 °	7.7 <sup>bc</sup>	3.1 <sup>a</sup>	5.8 <sup>abc</sup>	7.9 °	8.3 °	$4.0^{ab}$	$6.0^{\text{ abc}}$	3.6 <sup>ab</sup>	2.1
Trevor	6.7 <sup>abc</sup>	9.0 °	8.8 bc	5.6 <sup>abc</sup>	7.7 <sup>bc</sup>	4.7 <sup>ab</sup>	3.5 <sup>a</sup>	$6.5^{\text{abc}}$	5.2 <sup>abc</sup>	2.0
Average	7.9 °	8.3 °	5.6 <sup>abc</sup>	5.7 <sup>abc</sup>	7.8 °	6.4 <sup>bc</sup>	3.7 <sup>a</sup>	6.3 <sup>abc</sup>	4.4 <sup>ab</sup>	1.5



Figure 4.1.6 The distribution and means for nosema levels (x  $10^6$  spores) for each treatment after 11 weeks – measurement 19 August.

## 4.2 October 2003 (final measurement)

Colonies were removed from the trial due to a range of reasons beyond those that may be associated with the treatments. A number of queens failed during the trial period with the eventual death of the colony, brood diseases including chalkbrood (*Ascosphaera apis*) and European foulbrood (*Melissococcus pluton*) (EFB) were present in a few colonies. Some colonies had also swarmed and others were in the process of swarming, or had produced supersedure queens, making it impossible to measure the frames of bees that would have been associated with the treatments only.

Colonies culled during the August measurement period from Trevor's apiary included 4 due to queen failure. Colonies culled from Craig's apiary included 11 due to queen failure, 1 with a significant infection of EFB and another with a significant infection of chalkbrood. Colonies culled during the October measurement period from Trevor's apiary included 12 due to queen failure and swarming, 6 with EFB and 1 with chalkbrood. In the same period 15 colonies were culled from Craig's apiary due to swarming and queen failure.

### 4.2.1 Frames of bees

The number of frames of bees were significantly lower in the supplement treatments on average than in the control (P<0.05). This was more pronounced for Trevor's apiary but not significantly so. There were no other significant treatment effects on frames of bees for either apiary.

Table 4.2.1 The means for frames of bees for each treatment and the average for both apiaries after 21 weeks over winter and early spring – measurement 28 October. (n = number of hives).

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	AvSED
Craig	12.8 a	13.1 a	14.3 a	13.2 a	12.9 a	12.4 a	14.4 a	2.2
n =	4	7	7	4	8	9	9	
Trevor	17.9 bc	14.3 ab	13.4 a	15.0 ab	18.6 bc	16.0 abc	19.2 c	2.2
n =	5	7	7	7	5	8	8	
Average	15.4 ab	13.7 a	13.8 a	14.1 ab	15.7 ab	14.2 ab	16.8 b	1.6



Figure 4.2.1 The distribution and means for the frames of bees 21 weeks over winter and early spring – measurement 28 October.

#### 4.2.2 Frames of honey

Most combs were only partly filled with ripening nectar, with the remainder of the comb containing stored pollen and brood, all contributing to a degree of difficulty in measuring frames of actual honey. It was deemed that the data presented too much field error thus it was not analysed for the October period.

#### 4.2.3 Brood area

Brood area was significantly lower for Trevor's apiary than for Craig's (P<0.001). There were no significant treatment effects.

# Table 4.2.2 The means for brood area (cm<sup>2</sup>) for each treatment and the average for both apiaries after 21 weeks during winter and early spring – measurement 28 October.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	AvSED
Craig	7241	7673	8421	7864	6687	7097	7694	659
Trevor	4919	4083	4172	4583	4032	5035	4774	631
Average	6080	5878	6297	6223	5360	6066	6234	452



Figure 4.2.2 The distribution and means for brood area (cm<sup>2</sup>) for each treatment after 21 weeks – measurement 28 October.

#### 4.2.4 Crude protein of pupae

There were no significant differences in crude protein of pupae between treatments for Craig's apiary. For Trevor's apiary, pupae crude protein was significantly higher on average for the pollen supplement only treatments (c and f) than the syrup only treatments (a and d) and the control (g) (P<0.05).

Table 4.2.4 The means for crude protein (% of dry weight) for each treatment and the average for both apiaries after 21 weeks during winter and early spring – measurement 28 October.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	AvSED
Craig	45.1	45.1	45.0	44.9	45.5	46.3	45.4	2.0
Trevor	42.6 ab	44.3 ab	46.0 b	43.9 ab	41.8 a	46.0 b	41.3 a	2.0
Average	43.9 ab	44.7 ab	45.5 ab	44.4 ab	43.6 ab	46.1 b	43.4 a	1.4



Figure 4.2.4 The distribution and means for crude protein (% of dry weight) for each treatment after 21 weeks – measurement period 3 June to 28 October.

#### 4.2.5 Stored pollen

As the amount of pollen stored was quite extensive by the October measurement period and very scattered through the combs, it was not seen as a reliable measure of any differentiation between treatments. The area of stored pollen was also consistent for colonies in all treatments for both apiaries, thus this data was not analysed.

#### 4.2.6 Nosema

Samples of bees collected 3-5 June provided data indicating a significant variation between colonies with nosema infection. For Trevor's apiary 36 samples had no detectable spores (51%), the mean infection was  $0.385 \times 10^6$  spores. There were 9 samples with spore counts a million or greater up to 7.3 x  $10^6$  spores. For Craig's apiary 31 samples had no detectable spores (44%), the mean infection was  $0.379 \times 10^6$  spores. There were 5 samples with spore counts a million or greater up to 6.6 x  $10^6$  spores.

After treatments had been applied to the colonies the nosema count was significantly higher, on average, for the syrup and pollen supplement (b, e) treatments than for treatments with syrup or pollen supplement alone (a, c, d and f) (P < 0.05). There were no other significant differences.

Table 4.2.6 The means for nosema levels (x  $10^6$  spores) for each treatment and the average for both apiaries after 21 weeks during winter and early spring – measurement 28 October.

	A:S6	B:SP6	C:P6	D:S3	E:SP3	F:P3	G:Cnt	AvSED
Craig	0.14 ab	0.20 ab	0.07 a	0.34 ab	0.36 b	0.14 ab	0.18 ab	0.16
Trevor	0.14 a	0.28 a	0.14 a	0.17 a	0.39 a	0.10 a	0.12 a	0.15
Average	0.14 ab	0.24 ab	0.10 a	0.24 ab	0.37 b	0.12 a	0.15 ab	0.10





Figure 4.2.6 The distribution and means for nosema levels (millions spores) for each treatment after 21 weeks – measurement 28 October.

#### 4.3 Associations between nosema infection and colony size

The poorer performance of the syrup supplement treatments in comparison to the control, in particular the ones supplemented at 3 week intervals, might be related to the increase in nosema infection.

## 4.3.1 Association between nosema infection and frames of bees in June

In June, almost half the colonies in each apiary had no detectable nosema (refer 4.2.6). It was decided to examine the probability of detecting nosema against the size of the colony using a binary logistic model. Across both apiaries, there was a significant (P<0.01) effect of frames of bees on the probability of detecting nosema infection. For Trevor's apiary, nosema was detected in more than half (58%) of the 62 colonies with <6 frames, but only a third (34%) of the 47 colonies with >= 6 frames. For Craig's apiary, nosema was detected in  $\frac{34}{75\%}$  of 20 colonies with <6 frames, but only in half (49%) of the 70 colonies with >=6 frames.

## 4.3.2 Association between nosema infection and frames of bees in August

Initial number of frames of bees was not a significant covariate in the model for nosema in August, as noted above. Therefore, there is no evidence that a smaller colony at the start of winter has a higher rate of nosema infection during the winter. When nosema (on squareroot scale) is plotted against frames of bees, there appears to be a similar negative relation for the two apiaries (Figure 4.3.2a). However, this relation could be an artefact of the treatment effects on each of these variables, as noted in the previous section.



Figure 4.3.2a Nosema count (squareroot scale) versus frames of bees for each apiary in August.

To examine this, nosema was fitted against both treatment and frames of bees for both apiary and then each apiary in turn. After adjusting for differences between treatments, there is no significant relation between nosema infection and frames of bees for Craig's apiary, but there is a significant relation for Trevor's apiary (P < 0.05). This relation predicts that average nosema concentration will decrease by more than 50% across the range of frames of bees (from 8.1E6 at 2 frames of bees to 3.4E6 at 12 frames of bees).



Figure 4.3.2b Nosema versus frames of bees for Trevor's apiary. The linear relation, adjusted for treatment differences, is superimposed, August.

The inclusion of frames of bees as a covariate had little effect on the magnitude of the treatment differences at either apiary – specifically, the significant difference between supplement treatments and control. Therefore, there is no evidence to indicate that treatment effects on late winter nosema are explained by treatment effects on frames of bees.

#### 4.3.3 Nosema in June versus nosema in August

The inclusion of the June nosema data was not significant when included in this model or when fitted against the August data alone. (Nor was the inclusion of June nosema reading at all significant when it was dichotomised according to whether is was detected or not). This lack of association may suggest a real biological effect – that the level of nosema infection during winter is not related to the level at the start of winter. But it also may be just caused by high measurement error in both readings (i.e. only 25 bees were sampled from each colony to measure nosema infection).

#### 4.3.4 Nosema infection in October

After allowing for any treatment effects on nosema in October, there was no significant association between October nosema infection and either colony size (frames of bees in June, August or October) or previous nosema levels (June and August).

#### 4.4 Leftover supplement in August

Another reason for the poor performance of the supplementary treatments could be that the supplement was not consumed. Weaker colonies with fewer frames of bees would be more likely not to consume all the supplement. Both the leftover syrup and pollen supplement were examined against colony size where they were applied (for the sake of simplicity, a simple linear fit was used in all cases, despite the number of zeros and the skewed distributions of the left over amounts).

The amount of syrup left over was related to colony size (Figure 4.4a), but much more strongly for Craig's apiary. The amount of pollen supplement left over was also negatively related to the colony size for both apiaries (Figure 4.4b).



Figure 4.4a Syrup left over versus colony size.



Figure 4.4b The amount of pollen supplement left over as a function of colony size.

## 5. Discussion (2003)

## 5.1 August measurement

The lower number of frames of bees in Craig's apiary may have been a factor of the location of the apiary prior to the August measurement which was in a more exposed position, experiencing windier and cooler conditions with broken shade, as compared to Trevor's apiary located in a warmer position located in the Mallee region.

Hives fed sugar syrup consumed far less stored honey than hives not fed syrup. The implications for this response by a colony is to only feed sugar syrup if they are low in stored honey or there is a desire to deter colonies eating stored honey to enable the honey to be removed and extracted at a later date.

The area of brood closely relates to the frames of bees in both apiaries for the August measurement. Therefore, if a quick field estimate was to be taken of the strength of individual colonies, counting the frames covered with adult bees would be sufficient without the need to remove combs and measure brood area as well. Why there was no significant treatment effect on the brood area with Trevor's apiary is not apparent. In Craig's apiary it is interesting to note that brood area was less for all three week treatment intervals, compared with six week treatment intervals. As brood area eventually equates to the future population of a colony, the results did not generally support the management practice of providing supplements to colonies on a three week interval, compared with six week intervals. This response was not experienced across both apiaries thus this view is inconclusive.

The August measurements for crude protein content of 12 to 14 day old pupae provided no evidence to suggest supplementary feeding had a negative or a positive impact. There was no indication from the data and observations that the area of pollen stored by colonies was influenced by any treatment. There is certainly a strong location variation indicating Craig's apiary possibly had access to greater volumes of pollen in the field than Trevor's. There is no immediate benefit apparent to Craig's apiary from this larger volume of pollen available to his apiary; however, there may be a longer term benefit that was not recorded by this experiment.

Given that almost half the colonies in early June had no detectable nosema and the levels in the remaining colonies were low, it can be assumed that nosema levels increased from this point until the August measurement period. There was evidence in both apiaries that nosema levels were proportionally lower in larger sized colonies, providing further evidence that building colony strength prior to the winter period assisted in reducing the impact of nosema on weaker colonies.

The impact of nosema on the trial was the most significant result. Nosema counts were consistent across both apiaries. The control colonies in both apiaries experienced lower nosema counts than the colonies provided with pollen supplement and sugar syrup except in Craig's apiary for colonies provided pollen supplement every six weeks. The results provided evidence that the action of opening a hive and delivering syrup or pollen supplement may increase the incidence of nosema. Increased nosema could also be a cause of the stimulation to the colony from the supply of supplements. By reducing manipulation of the hive and providing supplement by other means may reduce nosema incidence.

The population of the colony was proportional to nosema incidence, indicating that smaller colonies are more likely to suffer from nosema infection than larger colonies. The implications of this possibility are quite serious as nosema will reduce the lifespan of infected adult bees and reduce the colony's ability to expand in population.

In conclusion, there was no major benefit in population increase by any of the supplementary feeding strategies applied to colonies between June and August. In fact, there was a detrimental impact from increased nosema levels as a result of the treatments. The provision of sugar syrup would be beneficial to colonies with little or no honey stored to avoid starvation or where it was desirable to deter colonies from consuming the stored honey.

## 5.2 October measurement

Soon after the August measurement the treatments associated with the pollen traps ceased as no meaningful data was being provided. There was also some question about the efficiency of the traps, particularly where small pellets of pollen were being brought back to the colony which would, in most cases, pass through the trapping screen without being removed. Generally there were fewer frames of bees in the hives provided supplements as compared to the control. In October, this was more so in Trevor's apiary, whereas in the August measurement, the trend was more prominent in Craig's apiary.

The brood area for Craig's apiary was significantly greater than Trevor's apiary independent of the treatments, providing strong evidence of a location effect. The probable reason for this was the movement of Craig's apiary onto a flowering canola crop in September. The area of pollen in each colony was not measured in either apiary as pollen was randomly stored in ample quantities throughout all brood combs which made it difficult to assess accurately.

The large difference between the area of brood in Craig's apiary and Trevor's apiary provides evidence of a significant impact of one or more variables that were not equal between apiaries. Sister queens were used in both apiaries and hive materials were standard Langstroth. The three variables were location, climate and nutrient intake. The variable most important in this case appeared to be the supply of available nutrients from the field. Craig's apiary had access to canola and pear blossom, both provided fresh nectar and pollen, compared to Trevor's apiary which was deprived of these flowering events.

The results in this trial indicate that any population increase is unlikely to occur during the winter period, with or without supplementary feeding. Any target population required for almond pollination in August should be achieved before colonies enter the winter period in early June and any colony manipulation should be carried out in autumn. Supplements should be provided to colonies in autumn to build bees up to the desirable population prior to the negative influence of cooler weather and nosema disease.

## 5.3 Leftover supplement

Ideally, colonies should be provided supplement on a "needs" basis, but unfortunately this is not always practical. The leftover syrup in both Craig's apiary and Trevor's apiary was related to the number of bees available to consume the syrup. The leftover syrup in Craig's apiary indicated that 500 ml per frame of bees would have been a suitable volume when the majority of syrup was to be consumed in 2–3 weeks, whereas 300 ml of syrup per frame of bees would be sufficient for Trevor's apiary. Why there should be a difference between apiaries was not apparent, although the exposure of the apiary sites to different climatic variables may have influenced consumption of the syrup. Even so, any volume of syrup leftover after a three week period may introduce problems such as fermentation and dysentery. If a stimulation response was required, then a much lower volume than 300 ml per frame of bees could be considered with each application.

The consumption by colonies of pollen supplement was proportional to the syrup consumed based on colony strength. Colonies with 8–10 frames of bees consumed most of, if not all the

500 grams of pollen supplement. As the population decreased, as measured by the frames of bees, so did the volume of pollen supplement consumed. Based on the results most colonies with less than 10 frames of bees would be able to consume 50 grams of pollen supplement per frame of bees, thus a colony with 4 frames of bees should be able to consume a 200 grams pollen supplement within a three week period.

The consumption of both pollen supplement and sugar syrup would almost certainly be a factor of in-hive and atmospheric temperatures, thus these assumptions are made in the context of a winter period and consumption of both sugar syrup and pollen supplement may increase with warmer conditions.

## 6. Recommendations (2003)

- Supplementary Feeding—The differences in brood area between Craig's apiary and Trevor's apiary in the October measurement was likely to be associated with the floral conditions available to each apiary. In this case naturally available nutrients provided the stimulus required for the brood area to rapidly expand in Craig's apiary. Therefore, it could be possible to emulate this natural response to nutrient availability in an artificial environment by providing pollen and sugar supplements if nosema infections could be controlled and a suitable pollen supplement provided. The mechanism, time of year, location and impact of disease on the colonies requires further investigation to fine tune supplementary feeding strategies for commercial apiaries within the Australian context.
- Providing sugar syrup to colonies low on stored honey still has merit in preventing starvation over winter. Dry sugar or candy feeding was not tested in this trial, this may be a suitable alternative to sugar syrup during winter months although further research is required before it can be a recommendation.
- The influence of nosema disease on adult bee longevity and thus honeybee populations must be of major concern to apiarists contemplating supplementary feeding strategies for commercial apiaries within the southern half of Australia over a winter period. Given the techniques used in this trial it cannot be recommended that supplementary feeding is a worthwhile exercise over a winter period with the aim of population increase.
- Further studies should be considered into the incidence and prevalence of nosema disease in commercial apiaries from various geographical and climatic areas of Australia. From the results of this study and tests conducted on apiaries from various regions of Victoria and NSW during the 2002 pilot trial, nosema disease was widely spread and prevalent. General advice/extension strategies should be considered to assist beekeepers in the management of this significant disease.
- Advice on the volume and quantity of supplement to feed to colonies should be based on the strength of the colony which can quickly be measured by estimating frames covered by adult bees. The trial data suggested that 50 grams per frame of bees of pollen supplement and half a litre per frame of bees of sugar syrup would be adequate for a colony to take up in a 3 week period. Pollen supplement requirements would be a factor of the area of brood, stored pollen and fresh pollen available to foraging bees. Pollen supplement uptake would also be linked to the attractiveness of the supplement to honeybees.
- Placement of the supplement within a hive remains an issue. Practice suggests that patties placed in the proximity of the brood area will be consumed in preference to supplement placed further away from the brood. In this experiment common practice was followed, yet it is still not known if bees remove supplement close to the brood due to the nutrient value and attractiveness of the supplement or remove the supplement due to a hygienic behaviour response.

## 7. Introduction (2004)

There are many flowering events worked by Australian beekeepers that produce ample quantities of nectar but are not supported by adequate volumes of pollen. These are usually referred to as pollen deficient honey flows where a beekeeper will experience a decline in bee populations. At first the area of brood will diminish, followed by a declining adult bee population. The demise of a colony can be relatively rapid due to the heavy work load the adult bees are exposed to due to the strong nectar flow.

Beekeepers have traditionally reacted to these events with one of two responses. The apiary is transported away from the nectar flow onto floral conditions providing 'good' breeding opportunities. Good breeding conditions are usually defined by ample quantities of pollen combined with a stimulating nectar flow. Collecting and harvesting surplus nectar in the form of honey is not generally the aim of such a move.

An alternative to moving the apiary is to provide pollen supplement to each colony to overcome the protein shortfall. A variety of different recipes and formulas have been trialled over many decades (Somerville 2005) with various degrees of success. Honeybee collected pollen, trapped and fed back to colonies at a later date has been the most successful, but unfortunately this is one of the most expensive. Thus beekeepers have trialled many other substitutes to pollen in an effort to be more cost effective.

Soyflour has been demonstrated to be attractive to honeybees and there is ample evidence and experience that bees will actively gather this material and continue to produce brood. How effective soyflour is on its own is debatable, but if a colony has access to some stored pollen or there were small amounts of fresh pollen being collected by field bees, then soyflour may extend the value of any limited supplies of pollen.

Soyflour is not always attractive to bees and often an amount of pollen is added to various recipes to increase the consumption of the supplement. Yeast is also a common additive to soyflour based supplement to balance any vitamin or amino acid deficiency the soyflour may have.

Mugga ironbark (*Eucalyptus sideroxylon*) is one of the most reliable honey-producing trees in Australia and is a major source of honey in NSW (Clemson 1985). This species unfortunately is regarded as a very poor source of pollen for foraging honeybees. Unless colonies have access to alternate sources of pollen during a mugga ironbark flowering event, it is possible for a colony to die.

The colony will continue to breed due to the nectar stimulus but with a declining area of brood. Eventually the colony will go broodless but the worker population will continue to harvest mugga ironbark nectar. Without careful management of the colonies before, during and after a mugga ironbark nectar flow, the population of a colony will be seriously compromised.

Mugga ironbark flowers every 2 to 3 years from April through to September and the average honey crop harvested has been stated as 35kg/colony (Somerville 1999). There have been many attempts to provide protein in the form of pollen supplement with various degrees of success. A more popular pollen supplement has been to provide soyflour to an apiary, either on its own in a dry powder form or combined with pollen, yeast and other ingredients in the form of a patty.

It has been suggested that the ingredients added to 'patties' e.g. either sugar syrup or gamma irradiated honey, are in fact what stimulates a colony to consume the supplement (Somerville 2005). For this reason this trial was established to provide soyflour, pollen and a mixture of soyflour, yeast and pollen in a dry form without the stimulus of sugar or honey in the supplement as provided to the colony.

Dry soyflour has been increasingly provided by commercial beekeepers to bees on mugga ironbark flowering events, but in bulk open containers. It is not possible under these feeding circumstances to restrict the intake of soyflour by any single set of colonies within an apiary. Providing the treatments (soyflour, pollen, soyflour + pollen + yeast and control) to separate colonies within the same apiary was deemed the most appropriate design under the circumstances.

## 8. Methodology (2004)

Two commercial apiaries were re-queened on the 1-2 March with young queens of the same age, grafted from the same queen mother and mated in the same mating yard. Both apiaries were commercial apiaries typical of southern NSW with approximately 100 colonies in each apiary. Both apiaries were managed independently belonging to separate beekeeping enterprises.

## 8.1 Apiary management prior to trial

The two apiaries, although managed by separate beekeeping enterprises, experienced similar floral conditions from December 2003. Both apiaries were located on the Monaro region of NSW for Vipers' bugloss (*Echium vulgare*) nectar flow. At the time of requeening at the end of February both apiaries were experiencing a significant deterioration in the floral conditions with very little nectar and pollen being collected. Of the two apiaries (Tony and Des), the floral conditions were marginally better for Tony's apiary.

Both apiaries were moved to floral conditions providing some pollen and light nectar closer to Queanbeyan (Captains Flat region). During March and April both apiaries were supplied with sugar syrup to stimulate brood rearing and pollen foraging activities prior to being moved onto the mugga ironbark sites in late April.

Tony's apiary was fed with a bulk feeder placed in the apiary where field bees were required to fly to the sugar syrup. The amount of syrup provided equated to 2 litres per colony although in this circumstance the uptake was based on the number of field bees from each colony collecting the syrup. All colonies were observed to actively forage for sugar syrup at the bulk feeder. No robbing or other problems were encountered with this method of syrup feeding. Tony's apiary was fed sugar syrup twice. Des's apiary was fitted with individual in hive tray feeders. Approximately 2 litres per colony was provided to Des's apiary on three occasions, 24 March, 8 and 15 April. The syrup in both cases was a 50:50 sugar water mix. Field bees in both apiaries were observed to be actively collecting pollen from a range of floral species during this March/April period.

Both apiaries were moved onto winter flowering mugga ironbark in late April. Tony's apiary was located east of Temora in NSW and Des's apiary was located north of Young. The primary target species had some blossom but most trees were not in flower. The conditions on both sites were dry with no weeds or herbaceous plants providing any prospects for bee forage.

## 8.2 Measurement periods

The initial measurement of all colonies in both apiaries took place on the 28-29 April. Data was collected on 'area of brood', 'area of pollen' and 'frames of bees'. The queen and disease status were recorded for each colony. The principle criteria for allocating colonies to treatments were 'frames of bees'. The area of brood was fortunately well correlated to the frames covered in bees. The mean 'frames of bees' for Tony's apiary was 13 ½ and the area of brood was 2125 cm<sup>2</sup>. The mean 'frames of bees' for Des's apiary was 14 and the area of brood was 2375 cm<sup>2</sup>. Measuring the area of pollen stored was very difficult as there was ample evidence that the colonies had in many cases covered the stored pollen with honey.

All hives were weighed and treatments allocated on the 3-4 May which marks the beginning of the experiment. The trial concluded on the 24-25 August. The final measurements were 'frames of bees', 'area of brood', 'area of pollen' and hive weight. Adult bee samples and pupae samples were collected for Nosema examination and CP% determination. During the trial, supplement was provided every 2 and 4 weeks, supplement not consumed was collected and weighed throughout the trial.

A second experiment was conducted from 3-30 June with the purpose of measuring the relative attractiveness of soyflour from different manufacturers. This involved two separate apiaries not related to the principle experiment. These apiaries were located east of Temora on the same floral conditions. The hives involved were estimated for colony strength and allocated a treatment.

### 8.3 Treatments

Each colony was allocated a treatment based on the number of frames of bees it contained on the 3-4 May. Each treatment was allocated 10 colonies per apiary with a total of 20 colonies per treatment.

Treatments per colony were:

- a. 250 grams of pollen 2 week intervals
- b. 500 grams of pollen 4 week intervals
- c. 250 grams of soyflour 2 week intervals
- d. 500 grams of soyflour 4 week intervals
- e. 250 grams of soyflour, pollen and yeast 2 week intervals
- f. 500 grams of soyflour, pollen and yeast 4 week intervals
- g. control

The supplement was provided on the:

3-4	May	a, b, c, d, e, f
18-19	May	a, c, e
2-3	June	a, b, c, d, e, f
15-16	June	a, c, e
30-1	June - July	a, b, c, d, e, f
15	July	a, c, e
29-30	July	a, b, c, d, e, f
11	August	a, c, e

## 8.4 Supplement

The supplement was placed in styrofoam trays under the lids of the hives. The various supplements were all in a dry powder form and were not mixed with sugar syrup or any other ingredient. The pollen was purchased from Western Australia and was a mixture of floral species including various pasture 'weeds', acacias, white gum (*Eucalyptus wandoo*), jarrah (*E. marginata*) and 50% red gum (*Corymbia calophylla*). The pollen was transported to Sydney where it was gamma irradiated prior to its inclusion in the trial. The pollen pellets were dried and crushed in Western Australia.

The soyflour was purchased from Hyfeed in Toowoomba, Queensland. This product was chosen based on the popularity of the product by beekeepers for use as a bee feed. No specifications for the flour were provided by the manufacturer. The mix (treatment e and f) was a combination of 50% soyflour, 25% pollen and 25% yeast. The yeast was described by the manufacturer, Bakels-Lesaffre Yeast Pty Ltd, as 'inactive dried yeast powder'.

## 8.5 Measurements

Due to the desire to disturb colonies as infrequently as possible, there was no interim measurement. The initial measurement of 'frames of bees', 'weight of hives', 'area of brood', 'area of pollen' was conducted on 28-29 April. The final measurement was conducted 24-25 August, which included the above measurements. The final measurement also included samples of 25 adult bees which were collected from the top of the cluster from each colony and placed in jars containing methylated spirits for nosema spore counts. Ten pupae were collected from each colony and stored at -20°C prior to examination for nitrogen content expressed as crude protein percentage.

## 8.6 Laboratory

Crude protein content (Nitrogen) of the bee pupae was determined using near infrared reflectance spectrometry (Berding 1998). Nosema spore levels were determined by the Cantwell (1970) method.

## 8.7 Statistics

Each of the post-trial response variables – 'frames of bees', 'brood area', 'pollen area', 'hive weight' percentage protein of the brood and nosema – were analysed using univariate ANOVA. The ANOVA model consisted of the effects of treatments, apiaries and their interactions, and the respective pre-trial response if available. The effects of apiaries were considered as a fixed effect since preliminary inspection suggested strong differences in the treatment effects between apiaries. For the analysis of nosema, a binary logistic model was used to analyse the presence/absence of nosema – the objective was to determine whether the probability of detecting nosema varied between apiaries and treatments.

The following six treatment contrasts were formally tested:

- 1. average supplement effect: control vs treatment (g vs a,b,c,d,e,f)
- 2. comparison of supplements:
  - a. pollen vs soyflour/mixed treatments (a,b vs c,d,e,f)
  - b. soyflour vs mixed (c,d vs e,f)
- 3. timing of supplement application: fortnightly vs monthly (a,c,e vs b,d,f)
- 4. interactions between comparison of supplements and timing of application:
  - a. interaction between (pollen vs soyflour) and timing of application
  - b. interaction between (soyflour vs mix) and timing of application

The interactions of apiary with each of these six treatment contrasts were also tested.

## 9. Results (2004)

### 9.1 Initial measurements compared to final measurements

The 'frames of bees' was the primary variable on which colonies were allocated to a treatment. (A = pollen every 2 weeks, B = pollen every 4 weeks, C = soyflour every 2 weeks, D = soyflour every 4 weeks, E = mix every 2 weeks, F = mix every 4 weeks, G = control).



Figure 9.1.1 Initial measurement for frames of bees for apiary 1, 27-29 April.



Figure 9.1.2 Final measurement for frames of bees for apiary 1, 23-24 August.



Figure 9.1.3 Initial measurement for frames of bees for apiary 2, 27-29 April.



Figure 9.1.4 Final measurement for frames of bees for apiary 2, 23-24 August.

The area of brood was closely aligned with frames of bees.



Figure 9.1.5 Initial measurement for area of brood for apiary 1, (cm2) 27-29 April.



Figure 9.1.6 Final measurement for area of brood for apiary 1 (cm2) 23-24 August.



Figure 9.1.7 Initial measurement for area of brood for apiary 2 (cm2) 27-29 April.



Figure 9.1.8 Final measurement for area of brood for apiary 2 (cm2 ) 23-24 August.

### 9.2 Brood area

Table 9.2 Adjusted treatment means (±SE) and contrasts for the analysis of brood area (cm<sup>2</sup>). Treatments which are significantly different from the control are asterisked (\*:P<0.05, \*\*:P<0.01, \*\*\*:P<0.001).

	Apiary 1	Apiary 2	Average
	Means		
A: pollen, fortnightly ± 219***	3642 ± 282***	2232 ± 337*	2937
<b>B: pollen, monthly</b> ± 210***	3654 ± 298***	$1863\pm297$	2758
C: soyflour, fortnightly ± 199	$2210\pm282$	$1936\pm283$	2073
D: soyflour, monthly ± 205	$1682\pm282$	$1342\pm297$	1512
E: mix, fortnightly ± 210***	3332 ± 297***	$1849\pm297$	2591
F: mix, monthly + 230	$2688 \pm 283 *$	$1321\pm364$	2005
<b>G: control</b> + 211	$1803\pm282$	$1292\pm316$	1547
supplements vs control	Contrasts $1065 \pm 305$	$466 \pm 340$	765 ±
228			
pollen vs soyflour/mix 185	$1170\pm250$	436 ± 273	$803~\pm$
soyflour vs mix ± 211	$-1065 \pm 285$	54 ± 311	-505
fortnightly vs monthly 174	$387 \pm 234$	$497\pm256$	$442 \pm$
(fortnightly v month) x (pollen v soyflour/mix) ± 185	$-299 \pm 249$	-96 ± 273	-197
(fortnightly v month) x (soyflour v mix) 211	$58 \pm 285$	$-33 \pm 311$	13 ±

There was no significant interaction between apiary and average supplement effect. Across both apiaries, the average brood area was higher for supplemented treatments than for the control (P<0.01). There were no significant interactions between timing of application and the comparison of supplements. There were significant interactions between apiary and comparison of supplements. For apiary 1, average brood area was significantly higher for pollen treatments than for soyflour/mix treatments (P<0.001), and significantly lower for soyflour than mix treatments (P<0.01). For apiary 2, there was no significant comparison of supplements and no significant interaction between apiary and timing of application. Across both apiaries, the average brood area was significantly higher for the fortnightly treatments than for the monthly treatments (P<0.05).

## 9.3 Pollen area

There were no significant treatment comparisons.

Table 9.3 Adjusted treatment means (±SE) and contrasts for the analysis of pollen (cm <sup>2</sup> ).
Treatments which are significantly different from the control are asterisked (*:P<0.05,
**:P<0.01, ***:P<0.001).

	Apiary 1	Apiary 2	Average
	Means		
A: pollen, fortnightly	$204 \pm 63$	$432\pm74$	$318 \pm$
49			
B: pollen, monthly	$140 \pm 66$	$365 \pm 67$	$252 \pm$
46			
C: soyflour, fortnightly	$183 \pm 62$	$405 \pm 64$	294 ±
	200 + 62	$201 \pm 66$	261
<i>D</i> : soynour, monuny 45	$200 \pm 62$	$521\pm00$	201 ±
T: mix, fortnightly	$170 \pm 66$	$392 \pm 67$	281 +
46	170 - 00	572 = 07	201 -
F: mix, monthly	$156 \pm 63$	$263 \pm 80$	$210 \pm$
51			
G: control	$159 \pm 63$	$343\pm69$	$251 \pm$
47			
	Contrasts		
supplements vs control	$17 \pm 67$	$20\pm75$	$18 \pm$
50 <b>m G ( ·</b>		$52 \pm 60$	24
pollen vs soyllour/mix	$-5 \pm 55$	$53\pm60$	24 ±
41 sovflour vs miv	$28 \pm 63$	35 + 69	32 +
47	$20\pm05$	55 ± 07	52 ±
fortnightly vs monthly	$20 \pm 52$	$94 \pm 56$	57 ±
38		, . <u> </u>	
(fortnight v month) x (pollen v soyflour/mix)	$33 \pm 55$	$-20 \pm 62$	$7 \pm 42$
(fortnightly v month) x (soyflour v mix)	$16 \pm 63$	$22 \pm 69$	$19 \pm$
47			

### 9.4 Frames of bees

	Apiary 1	Apiary 2	Average
	Means		
A: pollen, fortnightly 0.7***	14.4 ± 0.9***	$11.8 \pm 1.0$	13.1±
<b>B: pollen, monthly</b> 0.6	$11.6\pm0.9$	$95\pm0.9$	10.5 ±
<b>C: soyflour, fortnightly</b> 0.6	$10.9\pm0.9$	$10.4\pm0.9$	10.6 ±
<b>D: soyflour, monthly</b> 0.6	$9.7\pm0.9$	$10.2\pm0.9$	9.9 ±
E: mixed, fortnightly 0.6	13.3 ± 0.9**	$10.1\pm0.9$	11.7 ±
<b>F: mixed, monthly</b> 0.7	$10.1\pm0.9$	$7.5 \pm 1.1$	$8.9 \pm$
G: control			
	Contrasts		
<b>supplements vs control</b> 0.7	$1.8 \pm 0.9$	$-0.3 \pm 1.0$	$0.8 \pm$
pollen vs soyflour/mix 0.6	$2.0 \pm 0.8$	$1.1 \pm 0.8$	1.5 ±
soyflour vs mix 0.6	$-1.4 \pm 0.9$	$1.4\pm0.9$	$0.0 \pm$
fortnightly vs monthly 0.5	$2.4\pm0.7$	$1.7\pm0.8$	2.1 ±
(fortnightly v month) x (pollen v soyflour/mix) 0.6	$0.3 \pm 0.8$	$0.5\pm0.8$	$0.4 \pm$
(fortnightly v month) x (soyflour v mix) 0.6	$1.0\pm0.9$	$1.1\pm0.9$	$1.1 \pm$

Table 9.4 Adjusted treatment means  $(\pm SE)$  and contrasts for the analysis of frames of bees. Treatments which are significantly different from the control are asterisked (\*:P<0.05, \*\*:P<0.01, \*\*\*:P<0.001).

There was no significant average supplement effect, and no significant interaction between apiary and average supplement effect. There were no significant interactions between timing of application and the comparison of supplements. There were significant interactions between apiary and the comparison of supplements. For apiary 1, average frames of bees was higher for pollen treatments than for soyflour/mix treatments (P<0.01) and lower for soyflour than mix treatments (P<0.05). There were no significant comparisons of supplements for apiary 2. There was no significant interaction between apiary and timing of application. Across both apiaries, the average frames of bees was significantly higher for fortnightly treatments than for monthly treatments (P<0.001).

### 9.5 Hive weight gain

Table 9.5	Adjusted treatment means (±SE) and contrasts for the analysis of hive weight
gain (kg).	Treatments which are significantly different from the control are asterisked
(*:P<0.05,	**:P<0.01, ***:P<0.001).

	Apiary 1	Apiary 2	Average
	Means		
A: pollen, fortnightly	$14.2 \pm 2.2^{**}$	$15.9\pm2.6$	$15.1 \pm$
1.7			
B: pollen, monthly	$10.8 \pm 2.3$	$12.9 \pm 2.4 **$	11.9 ±
C: soyflour, fortnightly	$6.3 \pm 2.2$	$17.7 \pm 2.2$	$12.0 \pm$
	0.2 + 2.2	15 6 1 0 0*	10.0
D: soyflour, monthly	$8.3 \pm 2.2$	$15.6 \pm 2.3^*$	$12.0 \pm$
1.0 E. mir. fortnightly	$11.0 \pm 0.2$	$126 \pm 2.4 **$	124+
1 7	$11.2 \pm 2.3$	$15.0 \pm 2.4$	12.4 ±
F: mix monthly	97+22	182+31	139+
1.9	<i>y</i> . <i>r</i> <u>→</u> <u>2</u> . <u></u>	$10.2 \pm 5.1$	15.7 ±
G: control	$6.0 \pm 2.2$	$22.6 \pm 2.4$	$14.3 \pm$
1.6			
	Contrasts		
supplements vs control	$4.0 \pm 2.4$	$-6.9\pm2.6$	-1.4 ±
1.8			
pollen vs soyflour/mix	$3.6 \pm 1.9$	$-1.9 \pm 2.2$	$0.9 \pm$
1.5			
soyflour vs mix	$-3.1 \pm 2.2$	$0.8 \pm 2.5$	$-1.2 \pm$
1.7			
fortnightly vs monthly	$1.0 \pm 1.8$	$0.2 \pm 2.1$	$0.6 \pm$
	10 + 10		2 0 1
(fortnightly v month) x (pollen v soyflour/mix)	$1.8 \pm 1.9$	$2.2 \pm 2.2$	$2.0 \pm$
	10100	24+25	
(Iortnightly v month) x (soyflour v mix)	$1.8 \pm 2.2$	$-3.4 \pm 2.5$	-0.8 ±
1./			

There was a significant interaction between apiary and average supplement effect. For apiary 1, average hive weight gain was significantly higher for supplemented treatments than for controls (P<0.05), but for apiary 2, this was significantly lower for supplemented treatments (P<0.05). There were no significant interactions between timing of application and the comparison of supplements.

There were significant interactions between apiary and comparison of supplements. For apiary 1, average hive weight gain was higher for pollen treatments than for soyflour/mix treatments (P<0.05). There was no significant difference between pollen and soyflour/mix treatments for apiary 2 and no significant difference between soyflour and mix treatments for either apiary. There were no significant effects of timing of application.



Figure 9.5.1 Total weight (kg) gain for apiary 1.



Figure 9.5.2 Total weight (kg) gain for apiary 2.

### 9.6 Crude protein

	Apiary 1	Apiary 2	Average
	Means		
A: pollen, fortnightly	$40.2\pm1.3$	$40.6\pm1.7$	$40.4 \pm$
<b>B: pollen, monthly</b>	$40.0\pm1.4$	$42.5\pm1.4$	$41.2 \pm$
C: soyflour, fortnightly	$37.0 \pm 1.3$	39.1 ± 1.3	$38.0 \pm$
<b>D: soyflour, monthly</b>	33.8 ± 1.4*	$38.3 \pm 1.6$	$36.0 \pm$
E: mix, fortnightly	$38.6 \pm 1.4$	$39.9 \pm 1.4$	39.2 ±
<b>F: mix, monthly</b>	39.6 ± 1.3	39.7 ± 1.9	39.7 ±
G: control	$38.5 \pm 1.3$	$38.5\pm1.6$	38.5 ±
	Contrasts	1 5 1 1 5	
supplements vs control       1.1	$-0.3 \pm 1.5$	$1.5 \pm 1.7$	$0.6 \pm$
pollen vs soyflour/mix 0.9	$2.9 \pm 1.2$	$2.3 \pm 1.4$	2.6 ±
soyflour vs mix	$-3.7 \pm 1.4$	-1.1 ± 1.6	-2.4 ±
fortnightly vs monthly 0.9	$0.8 \pm 1.1$	$-0.3 \pm 1.3$	0.3 ±
(fortnightly v month) x (pollen v soyflour/mix) 0.9	$-0.4 \pm 1.2$	$-1.2 \pm 1.4$	-0.8 $\pm$
(fortnightly v month) x (soyflour v mix) 1.1	$-2.1 \pm 1.4$	$-0.4 \pm 1.6$	-1.2 ±

Table 9.6 Adjusted treatment means (±SE) and contrasts for the analysis of crude protein (%) of pupae. Treatments which are significantly different from the control are asterisked (\*:P<0.05, \*\*:P<0.01, \*\*\*:P<0.001).

There was no significant average supplement effect. There were no significant interactions between timing of application and the comparison of supplements. There were no significant differences in the comparison of supplements between apiaries. Across both apiaries, average crude protein was higher for pollen treatments than for soyflour/mix treatments (P<0.01) and lower for soyflour treatments than mix treatments (P<0.05). There were no significant effects of timing of application.

## 9.7 Nosema

			•
	Aplary I	Aplary 2	Average
	Means		
A: pollen, fortnightly	-1.4 ± 0.8 (20%)	$-17.6 \pm 1495.3 \ 0\%)$	-9.5 ±
747.6 (0%)			
B: pollen, monthly	$0.2 \pm 0.7 \ (56\%)$	$-0.2 \pm 0.7 (44\%)$	-0.0 $\pm$
0.5 (50%)			
C: soyflour, fortnightly	$-0.4 \pm 0.6$ (40%)	$-1.4 \pm 0.8 (20\%)$	$-0.9 \pm$
0.5 (29%)			
D: soyflour, monthly	$-0.8 \pm 0.7 (30\%)$	$-0.2 \pm 0.7$ (44%)	-0.5 $\pm$
0.5 (37%)			
E: mix, fortnightly	$-0.7 \pm 07 (33\%)$	0.7 ± 0.7 (67%)*	$-0.0 \pm$
0.5 (50%)*			
F: mix, monthly	$-2.2 \pm 1.1 (10\%)$	$-1.6 \pm 1.1 (17\%)$	-1.9 ±
0.8 (13%)	× /	× ,	
G: control	$-1.4 \pm 0.8$ (20%)	$-1.9 \pm 1.1 (12\%)$	-1.7 ±
0.7 (16%)	× /	× ,	
	Contrasts		
supplements vs control	$0.5 \pm 0.9$	$-1.4 \pm 249.2$	-0.5 ±
124.6			
pollen vs sovflour/mix	$0.5 \pm 0.7$	$-8.3 \pm 747.6$	-3.9 ±
373.8			
sovflour vs mix	$0.8 \pm 0.8$	$-0.3 \pm 0.8$	$0.2 \pm 0.6$
fortnightly vs monthly	$0.1 \pm 0.6$	$-5.4 \pm 498.4$	-2.6+
249.2			
(fortnightly y month) x	$-1.3 \pm 0.7$	$-9.0 \pm 747.6$	-5.1 ±
373.8			
(pollen v sovflour/mix)			
(fortnightly v month) x (soyflour v mix)	$0.5 \pm 0.8$	$1.7 \pm 0.8$	$1.1 \pm 0.6$

Table 9.7 Adjusted treatment means ( $\pm$ SE) and contrasts for the analysis of nosema detection (million spores). Treatments which are significantly different from the control are asterisked (\*:P<0.05, \*\*:P<0.01, \*\*\*:P<0.001).

There were no significant treatment comparisons.

#### 9.8 Leftover feed

Consumption of pollen (A and B) was significantly higher than the mixture (E and F) of soyflour 50%, pollen 25% and yeast 25%, (P<0.001), which was in turn significantly higher than the soyflour (C and D). There were no significant differences between the 250g/fortnight and 500 grams per 4 week application for any of the three supplements (pollen, soyflour or mix).



Figure 9.8.1 Leftover feed (supplement for apiary 1 in grams over the experimental period.



Figure 9.8.2 Leftover feed (supplement) for apiary 2 in grams, over the experimental period.

## **10. Discussion (2004)**

In the 2004 trial, there was a significant difference in the results between apiaries. This could be as a result of the local climate experienced at each geographically distinct location. Generally, apiary 1 experienced better weather with slightly warmer conditions and less wind than apiary 2. Apiary 1 was also placed in the protection of a line of trees whereas apiary 2 was placed in the middle of a grazing paddock without any vegetation acting as a windbreak. Even so, this should indicate a result in favour of apiary 1, not apiary 2. In fact the total weight gain was substantially greater for apiary 2 than apiary 1.

## **10.1** Timing of supplement feeding (2 or 4 weeks)

The response to providing supplement either every 2 weeks (250 grams) or every 4 weeks (500 grams) varied. Although there was a slight advantage in providing supplement every 2 weeks rather than every 4 weeks. This may not be a sufficiently significant difference to justify the expense of visiting apiaries fortnightly to feed supplements. Thus, if pollen supplement is to be provided during a winter nectar flow, then feeding periods of not less than 4 weeks should be sufficient, given that the colonies are provisioned with adequate supplement on each visit.

## 10.2 Leftover feed

The data for leftover feed throughout the trial period clearly supports the high palatability of bee-collected pollen as a supplement as compared to soyflour or a contribution of soyflour, pollen and yeast. An addition of (25%) pollen to the mixture of soyflour (50%) and yeast (25%) increased the attractiveness of this supplement to the bees. Even with the very high humidity levels within the hive in the location of the feed trays, the resident bees were frequently able to completely remove all the pollen provided, whereas it was observed in a number of hives the bees had not attempted to remove the soyflour.

## 10.3 Brood area

The area of brood was significantly greater for the pollen fed bees in apiary 1, although this was not the case in the data for apiary 2. Even so, in apiary 2 (Figure 9.1.8) the area of brood was favourable for the pollen treatments compared to the other treatments, particularly in comparison to the control.

A comparison between the area of brood for soyflour and mix treatments suggests a difference in favour of the mix for apiary 1 but not significant in apiary 2. In both apiaries the brood area for the controls was the smallest in size compared to colonies fed supplement.

### 10.4 Pollen area

The area of pollen measured initially and at the end of the trial did not provide evidence for any treatment effect. This could be due to the difficulty of measuring pollen in the combs as a result of the haphazard storage in the cells and the difficulty of initially measuring the pollen in combs. At the initial measurement the colonies were 'closing down' by reducing the brood area and filling up brood comb cells with honey. In this process, cells with pollen were covered with honey thus masking its presence.

## 10.5 Frames of bees

The results do not provide any strong evidence that any of the treatments across both apiaries increased the total population of adult bees. In fact both apiaries demonstrate a loss of bees over the trial period. In the initial measurements no colonies were allocated to any treatments with frames of bees below 10. In the final measurement in apiary 1, 25% of all colonies that were measured were less than the lowest initial measurement for frames of bees. In apiary 2 this Figure rose to 48% of colonies with less than 10 frames of bees representing a net loss of bees over the winter period for both apiaries. The colonies in apiary 1, fed pollen every fortnight, were the only group to remain with the same number of frames covered in bees from the initial measurement.

## 10.6 Hive weight gain

Overall there was a weight gain across both apiaries as a result of the bees collecting and ripening honey derived from mugga ironbark. The average weight gain across all the hives in the trial was greater for apiary 2 than apiary 1. Although the hives in the control treatment in apiary 1 faired much better for weight gain than all the supplement treatments, apiary 1 provided the opposite result to apiary 2 with weight gains for all the treatments ahead of the control. There is no clear reason why there should be such a distinction between the controls in apiary 1 and apiary 2.

## 10.7 Crude protein of pupae

The data provides evidence of a marginally higher CP% in pupae from colonies provided pollen. The CP% levels of pupae were also higher in the mixed diet than the levels in the larvae offered a soyflour diet only. Given that every colony probably had access to stored pollen over the winter period collected the previous autumn, the results have probably been compromised as regards any treatment effect.

Crude protein levels have been reported to be correlated with longevity (Somerville 2005). Thus an increase in CP% of pupae may indicate increased longevity of the emerged adult bee. An adult bee that has a longer life span is then able to conduct greater numbers of foraging trips and collect an increased volume of nectar.

## 10.8 Nosema

There were no significant differences between apiaries or treatments. In apiary 1, 71% of the colonies returned a zero spore count, compared to only 6% of the colonies with over a million spores. In apiary 2 the Figures were similar with 69% of the colonies returning a zero spore count compared to only 2% of the colonies with over a million spores. Clearly nosema disease was not a significant factor through the winter of 2004 for both apiaries involved in the trial.

## 10.9 Economic analysis

There was a sliding scale of honey produced when the means of each of the 2 and 4 week treatments are combined for the same supplement in apiary 1. The average honey yield for all pollen treatments was 12 kg, the mix of soyflour (50%), pollen (25%) and yeast (25%) was 10.45 kg, the soyflour treatment was 7.3 kg and the control was 6 kg.

The difference between the control and soyflour treatments was not significant enough to

warrant discussion on any economic benefits by its use in bee hive management. The use of pollen as a supplement was substantially beneficial to the yield of honey, doubling the crop harvested from 6 kg for the control to 12.5 kg for the pollen treated hives. Even so, there was no economic advantage by feeding pollen during the time the trial was conducted. Bulk irradiated pollen was valued at \$20/kg and the wholesale bulk price of honey was between \$2 and \$4 per kg.

Each colony was provided with 2 kg of supplement over the trial period, which equates to \$40 of pollen provided to each colony without consideration for the cost of labour and travel by the beekeeper. A weight advantage of 6.5 kg would only amount to \$13 to \$26 increase in gross income per hive, well short of the costs of providing the pollen supplement.

The honey crop harvested was not a long term industry average for the floral species in question. As previously stated, 35 kg/colony is considered an average yield for mugga ironbark. The reasons for the below average honey crop experienced in this trial could be due to adverse weather conditions preventing field bees from flying and/or drought conditions affecting nectar secretion.

At \$20/kg for irradiated pollen as a supplement for colonies deficient in stored or available field pollen, a beekeeper would need to harvest from 10 kg (at \$4/kg) to 20 kg (at \$2/kg) of honey more than untreated colonies. This may be a possibility in some years when conditions are more favourable for a heavier honey harvest from mugga ironbark.

The data indicates that in apiary 1 the area of brood and number of bees was greater for the pollen treatments compared to the control which would mean that the treated bees would be in a much better position to gather greater quantities of nectar if it was available. It would also suggest that the larger colonies would be in a better position to harvest any follow-on nectar flow following the completion of the mugga ironbark flowering in September and October. If this was evident then the economics of providing pollen would improve.

The weight gain data for apiary 2 does not provide any trends for any of the treatments with the control experiencing the greatest weight advantage. One very plausible explanation for this could be the amounts of stored pollen each colony had available as they expanded their brood area and uncovered previously stored pollen.

During the autumn period, when colonies were provided good breeding conditions, they were able to collect substantial quantities of pollen. Much of this pollen was stored and covered over with capped honey, making the task of accurately determining the amount of stored pollen per hive impossible in the initial measurements. As the winter progressed, the stored pollen would become available to the colonies making them less reliant on supplements and external sources of pollen.

## 11. Recommendations (2004)

During the 2003 winter supplementary feeding trial nosema was very prevalent within both apiaries. This was not the case during 2004. The difference in the circumstances may be due to the different floral conditions available to the bees in autumn, the geographic locations, possible different management of the colonies, reduced manipulation of the colonies during winter (2003 compared to 2004) or some other influence. It could also be a result of a combination of factors mentioned.

- Honeybee-collected pollen is far more attractive as a winter pollen supplement on its own than soyflour or a mixture with pollen as an ingredient. The strong attraction of pollen over other supplements provides cause for investigation to determine what the primary olfactory attractants are in pollen that makes it a highly attractive food for bees.
- The provision of supplements in all three forms tested did not substantially hinder the colonies in comparison to providing no supplements. The only measurement that is in disagreement with this conclusion was the total weight gain for the control hives in apiary 2 which was greater than all the treatments.
- The method of provision of supplements was designed to allow each colony to be fed with the dry supplement without the colony being unduly disturbed, particularly during cool weather. The high humidity levels in the hive created major mould problems with the supplements, adding to the weight of the left over supplement and seriously reducing the attractiveness of the supplement.

Bulk feeding in the open was successful as a means of providing bees with soyflour but the amount consumed was difficult to measure due to the activity of foraging bees kicking flour out of the feeder. The observations during the bulk feeding experiment did not provide an ideal alternative for testing supplements on an individual colony basis with controls in place. Providing supplements in bulk within an apiary would provide data on the relative attractiveness of a range of supplements.

- Bulk feeding soyflour or dry pollen supplement could have some management advantages over feeding individual colonies. This should reduce the possibility of nosema disease, reduce labour costs of opening individual hives, avoid the problems with mouldy feed left in the hive if the bees do not remove it and the beekeeper should be in a better position to monitor the consumption of the supplement.
- Unfortunately, the results do not provide clear evidence that providing supplement equates to a major economic benefit to the beekeeper. This could have been due to seasonal effects and bees going into the trial with substantial quantities of stored pollen. The final measurement was conducted in August, whereas there may have been a follow-on benefit well into spring from the provision of supplements in July and August. The results did not discount the concept of providing pollen supplement while bees are working a winter flowering, pollen deficient nectar flow but they did not demonstrate a clear benefit in the circumstances experienced during the winter of 2004.
- The economics of supplementary feeding is always going to be difficult to accurately quantify before a flowering event, as it is not known what the final yield will be. A beekeeper can only make decisions based on historical production data, current bulk honey prices and the estimated costs of providing supplements.

## 12. Soyflour comparison

## 12.1 Introduction

It was apparent by mid May 2004 that the soyflour treatment used in the main trial was not as attractive as the pollen treatment. There was a possibility that the soyflour used may not be as attractive as other sources of soyflour and, as such, a means of measuring its relative attractiveness against other sources of soyflour was devised.

### 12.2 Methods and materials

Two other soyflour products were obtained equating to a comparison between 3 sources of soyflour. The three sources of soyflour and descriptions stated by the manufacturer were as follows:

- a. soyflour, Hyfeed, Toowoomba
- b. full fat soyflour, Ben Furney Flour mills, Dubbo
- c. defatted soyflour, Ben Furney Flour mills, Dubbo

Two apiaries were selected for the soyflour comparison trial separate to those used in the trial comparing pollen, mix and soyflour. In one apiary 20 colonies were selected on their strength based on the frames of bees being greater than 10. Two styrofoam trays were placed under the lids. One tray in all cases contained the Hyfeed soyflour and the other tray in 10 hives contained the full fat soyflour and the remaining 10 hives contained the defatted soyflour from Ben Furney Flour mills. The trays were left in the hive from 3-30 June, when the remaining soyflour was removed and weighed.

During the same time in another apiary, a third experiment was established involving the bulk external feeding of soyflour. Six barrels were screened to prevent the access of livestock and provisioned with 2.5kg of soyflour, two from each soyflour a, b or c. These barrels were placed in a tent to assist in protecting the flour from the weather but without impeding the flight of field bees. The barrels were placed approximately 20 metres from the apiary and the bees were allowed to fly freely to collect the soyflour.

For the open feeding experiment with only 2 replicas it was not possible to analyse the data as there was only 2 degrees of freedom left for error. For the experiment whereby the different soyflour sources were compared within each hive, a paired t-test for each set of 7 colonies (a versus b, a versus c) was conducted.

#### 12.3 Results

Six colonies were culled as a result of unexplained population decline or queenlessness, of the remaining colonies the following Table 12.3.1 provides the left over soyflour still remaining in the tray.

	Hive number													
Treatment	301	302	303	304	305	306	308	311	314	315	317	318	319	320
а	281	269	279	259	42	90	277	276	240	270	250	281	235	245
b	-	192	242	205	-	123	215	-	-	214	-	-	-	230
с	215	-	-	-	202	-	-	206	178	-	89	81	104	-

Table 12.3.1 Left over soyflour (grams)

 Table 12.3.2 The remaining flour in the bulk drum soyflour feeding experiment (grams)

Treatment	Barrel 1	Barrel 2
a	889	992
b	180	582
с	762	1895

In the first set of 7 colonies (a versus b), there was a significant preference for soyflour b over a (P=0.034). In the second set of 7 colonies (a versus c) there was no significant preference for either source of soyflour.



Figure 12.3 Comparison of the consumption of soyflour a, b and c per hive in grams of flour left over.

## 12.4 Discussion

The results do not conclusively provide evidence that one soyflour source is superior in attractiveness to bees than another. The bulk feeding experiment demonstrated greater activity by bees for soyflour 'b'. For the in-hive experiment soyflour 'b' was more attractive than soyflour 'a', but the analysis does not show this to be the case for soyflour 'c' when compared to soyflour 'a'.

In some of the hives, the leftover soyflour gained weight above what was initially provided. This is likely to be caused by the very high moisture levels in the proximity of the soyflour. Mould was observed on the soyflour residue of at least two trays in hives 301 and 303. This may explain why there was a weight gain in the flour provided to at least eight of the hives. Even so, it is assumed that all colonies were exposed to high humidity levels within the hive and, as such, any error would be uniform across the experiment.

The foraging activity of bees while collecting soyflour can be described as very busy with bees burrowing into the soyflour. It was not uncommon to observe, at the mouth of the barrels and within hives, soyflour that had been scattered out of the container in the process. This characteristic of their foraging activity provided an unknown margin for error in determining left over ingredients.

Thus, at best the results provided an insight into the amount of foraging activity each soyflour source attracted. Even so the results do not clearly support one source of soyflour in preference to another based on the likely error in the data due to the loss of flour during the foraging activity by the worker bees.

In-hive provision of soyflour during winter is not considered a useful practice due to the very high humidity levels experienced as a result of condensation. If the provision of dry soyflour is a considered management practice this should be conducted outside of the hive in an environment where the atmospheric moisture conditions and temperature are conducive to bee flight. Any further comparisons of bulk feeding dry supplements should provide more than two replicas and also a means of collecting the feed tossed about by the foraging bees.

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