Australian Government
Rural Industries Research and Development Corporation

Honeybee R&D

A beekeepers' guide to understanding control measures for European Foulbrood

by Russell Goodman, Ben McKee and Peter Kaczynski

Publication No 04/091
RIRDC Project No DAV-157A

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May 2004

RIRDC Publication No 04/091
RIRDC Project No DAV 157A

ISBN 1 74151 000 7
ISSN 1440-6845

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Published in May 2004
Printed on environmentally friendly paper by Union Offset Printers, Canberra

Foreword

In 2002, RIRDC produced a five year research and development plan for the Honeybee Program. This plan was formulated in consultation with industry to provide the research direction necessary for the current best practice and preparation for future development in the honey industry.

The use of the antibiotic oxytetracycline hydrochloride (OTC) for the control of the honey bee brood disease, European foulbrood, occurs in all Australian states except Western Australia. The potential for oxytetracycline residues to occur in honey poses a serious threat to the future marketability of domestic and export honey.

This publication is an abridged edition of the comprehensive companion RIRDC report entitled 'European Foulbrood – investigating control measures'. This abridged edition presents outcomes of investigations concerning the application of OTC to honey bee colonies, degradation of OTC in honey extracted from treated hives and efficacy of lower doses of OTC to control European foulbrood. Studies on *Melissococcus pluton*, the causal organism of the disease are also reported.

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

This report, a new addition to RIRDC's diverse range of over 1000 research publications, forms part of our Honeybee R&D program, which aims to contribute to the productivity and profitability of the Australian beekeeping industry.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

Acknowledgements

The project was financially supported by the Rural Industries Research and Development Corporation (RIRDC), Department of Primary Industries and the National Residue Survey (NRS) of Agriculture Fisheries and Forestry Australia.

We thank Dr Heloisa Mariath**,** NRS; Phil Reeves, Australian Pesticides and Veterinary Medicines Authority; Keith McIlvride, Chairman, RIRDC Honeybee R & D Committee; Graham Kleinschmidt and Dr Jeff Davis, former and present RIRDC Honeybee Program Research Managers respectively, for their support. We thank the Australian Honey Bee Industry Council and Federal Council of Australian Apiarists' Associations for helpful advice.

Appreciation is extended to Max Cane, apiarist, Ararat, for apiary sites, honeybee colonies, honey extraction facilities and valuable time, expertise, support and advice throughout the project. We also thank Bob McDonald, apiarist, Castlemaine, who kindly supplied apiary sites, honeybee colonies, and advice for some of the oxytetracycline hydrochloride (OTC) trials. Trevor Richards, Amphitheatre; Dale Richards, Bendigo and Robert Kilpatrick, Joel Joel, provided apiary sites and advice for the management of hives. Apiarists Ian Fenselau, Bendigo; Ken Gell, Maryborough; Ray Hall, Dunolly and Ron Rich, Maryborough loaned hives for *Melissococcus pluton* and OTC dose response trials.

The authors wish to acknowledge the apiarists and honey packing companies who supplied honey for the OTC spiking experiments and hemi-nested polymerase chain reaction (PCR) studies. These included apiarists, Harold Ayton, Tasmania; Ailsa J Collins, Ravenshoe; Margaret Mikits and Richard West, Mareeba and Capilano Honey, Richlands, Queensland.

We thank Drs Michael Hornitzky and Steven Djordjevic, senior research scientists, Elizabeth Macarthur Agricultural Institute (EMAI), NSW Agriculture, and Dr Peter Taylor, The University of Melbourne, Parkville, Victoria, for helpful advice throughout the project. We thank Bruce Abaloz and David Paul, Department of Zoology, The University of Melbourne for assistance with histology sections of honey bee larvae. We thank Fiona Cooper, Brendan Rodoni, and Kerry Paice, Research and Development Division, Department of Primary Industries, Knoxfield, for counts of *Nosema apis* spores in samples of adult honey bees and assistance with PCR and culture assays for detection of *Melissococcus pluton* respectively. Dr Michael Hornitzky, EMAI, arranged for culture of honey samples for presence of *M. pluton*.

Analyses of honey to determine OTC concentrations were conducted by Hugh Mawhinney, Stephen Were and Warwick Turner, Chemical Residues Laboratory, Animal and Plant Health Service, Queensland Department of Primary Industries; Christian Saywell and Heather Lindsay, State Chemistry Laboratory, Department of Primary Industries. Paul Lawicki and Phil Zeglinski, State Chemistry Laboratory, analysed adult bees and larvae for crude protein and honey for hydroxymethylfurfural content respectively.

We thank Drs Stephen Tate, Senior Veterinary Officer; Jenny Turton, Veterinary Officer and John Galvin, Manager, Animal Health Operations, Department of Primary Industries, for prescribing OTC products. We thank Drs Graham Hepworth and Nam-ky Nguyen, former and present biometricians respectively, Department of Primary Industries, Knoxfield. Drs Jane Moran, Jo Luck and Stephen Doughty, Knoxfield, gave helpful comments and suggestions for this manuscript.

The large proportion of the work described in this report was conducted by co-author Dr Ben McKee towards his PhD thesis entitled 'A study of European foulbrood (*Melissococcus pluton*) in honey bee colonies (*Apis mellifera*) in Australia'. The thesis was submitted to the School of Agriculture and Food Systems, The University of Melbourne.

Contents

Executive summary

The endemic, bacterial honey bee brood disease, European Foulbrood (EFB) (*Melissococcus pluton*) can seriously affect honey bee colonies and reduce honey production. The disease is controlled by apiary management practices and when necessary by the antibiotic oxytetracycline hydrochloride (OTC).

The extent of OTC residues in honey extracted from hives treated with the antibiotic was determined. Groups of six colonies were treated with one of three OTC products in either sugar syrup or caster sugar at the rate of 1g active ingredient and moved to a honey flow 23 days later. Honey from the supers of the six hives of each treatment group was extracted, sampled and tested for OTC. Residues were detected in honey from all treatment groups at the first extraction but not at a second later extraction. Residues were higher in honey of the sugar syrup treatments (mean 0.34 mg/kg) than the caster sugar treatments (mean 0.065 mg/kg).

The rate of degradation of OTC in honey was determined by storing honey of the six treatment groups of the first extraction at ambient temperature and at 22ºC. Residues were detected in samples of honey of all six treatment groups stored at ambient temperature at twelve months from the date of OTC application to the hives. There was greater degradation of OTC in the same six lines of honey stored at 22ºC.

In another degradation study, residues were still present in OTC contaminated honey stored at 25ºC 18 months from the time of application to honey bee colonies. OTC was not detected at 15.5, 8.5 and 3.5 months for the same honey stored at 30ºC, 35ºC and 40ºC respectively.

Experiments were conducted to determine the concentration of OTC in larvae sampled from honey bee colonies treated with various doses of the antibiotic in caster sugar. On each of six days after treatment of colonies, mean concentrations of OTC in larvae were significantly greater in larvae of the 1.0 g OTC double-storey colonies than of the 0.5 g OTC double-storey colonies, usually by a factor of 2 to 3. In another experiment, 0.5 g OTC dissolved in 200 mL of distilled water was applied to two-storey colonies. This dose gave an equal or higher level of OTC on the first two days after treatment when compared with the same dose applied in caster sugar to two-storey colonies. All these doses of OTC in cater sugar or water, delivered sufficient antibiotic to exceed the 1-2 µg/mL minimum inhibitory concentration of OTC to *Melissococcus pluton* by a minimum of 4 times on the day immediately after treatment. However, because the results of the three above experiments were based on assay of whole honey bee larvae rather than larval guts where *M. pluton* multiplies and causes infection, it is suggested that further laboratory and field work be conducted to confirm their validity relating to the concentration of OTC in the honey bee larval guts.

Field trials were conducted to determine effect of doses lower than those stipulated on OTC product labels on EFB. Single-storey colonies with advanced EFB were treated with a standard 0.5 g dose or a reduced dose of 0.3 g or 0.4 g active ingredient. All doses resulted in significantly less diseased larvae than the controls at 12 and 22 day post-treatment inspections, but not at the 41 day post-treatment inspection. No dose enabled all colonies to totally clean up the disease. It was concluded that until the efficacy of low doses of OTC are further investigated using laboratory reared honey bee larvae and subsequent field trials, the current 0.5 g and 1.0 g doses for single and double-storey colonies respectively were appropriate for control of EFB in Australia.

During 2000 and 2001, commercially managed honey bee colonies were fed either 1.0g OTC, protein dietary supplement or left as untreated controls to determine their effect on the incidence of EFB. The application of OTC resulted in total protection against EFB for these colonies in 2000 and for 12 of 13 treated colonies in 2001. Over all the observations during 2001, the mean incidence of EFB in colonies fed protein supplement was 9% lower than the controls, but this result was not statistically significant.

The mean number of combs of adult bees and brood in colonies of the three treatment groups did not differ significantly at any of the eleven observation dates over the two years of the trial. This suggests that regardless of antibiotic treatment or supplementary feeding, other factors such as nectar and pollen resources may have influenced the number of combs of brood and adult bees. There was a significant statistical relationship between the number of days with rain per month and incidence of EFB in colonies fed supplement. There was no significant statistical difference in spring honey production between the three treatment groups. There was no significant difference in the mean crude protein of adult bees sampled from the 3 treatments at the completion of the trial in 2001.

Honey bee larvae, approximately 4-5 days old, were sampled from randomly selected colonies at five commercially managed apiaries in central and western Victoria. The mean pH varied significantly between apiary locations (range 6.258 to 6.506). This may be a result of bees foraging on different pollen and nectar plants at these locations. The pH of larval guts sampled from colonies approximately 100 km apart but foraging on the same plant species was similar at 6.277 and 6.258 respectively. Laboratory studies also indicated that honey bee larvae have a capacity to buffer their diet and consequently may not be adversely affected by changes in pH of larval food as previously suggested by other reports.

An existing hemi-nested polymerase chain reaction (PCR) assay was modified to obtain increased sensitivity for the detection of *M. pluton* in bees and their products. The modified PCR confirmed *M. pluton* in the following items sampled from infected colonies: larvae, individual body components of adult bees, pollen, washes with water of brood comb cells that contained a freshly laid egg, and broodnest honey. *M. pluton* was found in larvae, adult bee digestive tracts and broodnest honey of healthy colonies, confirming the existence of the bacterium even though disease signs were not evident in the brood.

Honey bee larvae were successfully reared in the laboratory from an age of less than 24 hours to the stage of defecation and commencement of pupation. Successful inoculation of laboratory reared larvae with suspensions of *M. pluton* proved to be very effective in causing disease. These procedures will enable fast, relatively inexpensive, fully controlled laboratory studies to be conducted on the effect of nutrition and other potential treatments for the control of all honey bee larval diseases. Such studies could replace costly field and cage trials that are invariably affected by uncontrollable factors such as variable pollen and nectar resources, weather conditions and bee disease.

A survey of selected Victorian and Tasmanian apiarists was conducted in 1999 to determine OTC use patterns and apiary management practices used for the control of EFB. Sixty-two percent of Victorian respondents treated colonies with OTC only when EFB symptoms were present in the brood, while the remaining respondents treated colonies before symptoms were evident. Forty-nine percent of respondents chose to spot treat only infected colonies rather than blanket treat all colonies in an apiary.

There was a strong indication that ‘good conditions’ (provided by good nectar and pollen flows) were very important as a means of reducing or preventing the incidence of EFB. In contrast, the feeding of protein supplement to improve honey bee nutrition did not appear to prevent EFB. Locating colonies on sunny, warm, sheltered wintering and spring apiary sites was very important. Many respondents thought that regular requeening of colonies reduced the potential for EFB. Most practiced regular replacement of aging broodnest combs in an attempt to lower the population of *M. pluton* in the hive.

1. Introduction

European foulbrood (EFB) is a serious economic bacterial disease of honey bee brood. In Australia, the disease was first confirmed in honey bee colonies in South Australia and Victoria in 1977 and has spread to all Australian States except Western Australia.

When honey bee larvae die as a result of EFB infection there is a reduction of adult bees in the colony and a subsequent loss of honey production depending on the degree of infection. In severe cases where many larvae die, the colony may decline and eventually die. However, many colonies although seriously weakened by the disease can slowly recover when good nectar and pollen flows occur.

In Australia EFB primarily occurs in spring. The incidence and intensity of EFB infections varies from year to year, but appears greatest in districts that have inclement, changeable spring weather that interrupts honey bee foraging and the collection of nectar and pollen. In Victoria, where such weather is typical, the incidence of EFB appears greatest in the southern, cooler parts of the State.

Apiarists use a number of apiary management techniques to lessen the impact of EFB in their colonies. These may include:

- regular requeening of colonies to ensure a queen capable of prolific egg laying
- keeping colonies warm and compact
- placing colonies on apiary sites with good nectar and pollen yielding flora
- feeding of dietary supplement to colonies to ensure optimum honey bee nutrition.

The antibiotic oxytetracycline hydrochloride (OTC) may be used by some apiarists to control the disease. A number of OTC products are registered by the Australian Pesticides and Veterinary Medicines Authority for application to beehives for the control of EFB.

Goodman and Azuolas (1994) alluded to potential repercussions in the Australian honey industry including the possible loss of export and domestic honey markets if OTC residues were detected in extracted honey.

The project described in this report was developed in 1998 following detection by the Australian National Residue Survey of OTC residues in commercially produced honey. Several experiments were conducted to provide comprehensive information on the occurrence of OTC residues in honey. In 1999, the project was modified and enlarged to include a range of studies on EFB and *M. pluton* in addition to further investigations about the usage of OTC.

Introductory notes:

- All OTC treatments mentioned in the following experiments were prescribed by registered veterinary surgeons and some were applied to hives as an “off-label” use. Off-label use of an OTC product in these experiments does not imply endorsement by the authors, Department of Primary Industries or the Rural Industries Research and Development Corporation. OTC is an S4 drug and must be prescribed and used in accordance with the relevant regulations of the particular State or Territory.
- The application of low doses of OTC in dose-response experiments, where the amount of OTC applied to hives was lower than that stipulated by label directions, does not imply endorsement by the authors, Department of Primary Industries or the Rural Industries Research and Development Corporation. The authors advise full adherence to OTC product label directions.
- Full details of methodology used in the following investigations, together with detailed discussion and conclusions, are presented in RIRDC’s research report ‘European foulbrood – investigating control measures’.

2. Oxytetracycline hydrochloride in honey

Residues in honey extracted from hives treated with OTC

Experiment 1

Aim
To determine the level of residues in honey extracted from hives treated with OTC according to doses stipulated on labels of three OTC products and according to apiary industry practice current at 1998.

Methodology
Hives were located at Ararat on a yellow box (*Eucalyptus melliodora*) nectar flow that provided enough nectar for the colonies to only maintain themselves. These conditions mimicked those commonly found at the time of the year when apiarists would normally treat hives with OTC. Before treatment was applied, a sample of honey was obtained from each hive to determine if any residual OTC was present.

There were six groups of six colonies. On 16 December 1998, each group of colonies was given one of the following OTC treatments:

1. OTC from product No. 1 - wet treatment (P1-W)
   1 g equivalent active OTC in 500 ml of sugar syrup (1:1 sugar:water by volume)
2. OTC from product No. 1 - dry treatment (P1-D)
   1 g equivalent active OTC in caster sugar to make a total mixture of 100 g
3. OTC from product No. 2 - wet treatment (P2-W)
   1 g equivalent active OTC in 500 ml of sugar syrup (1:1 sugar:water by volume)
4. OTC from product No. 2 dry treatment (P2-D)
   1 g equivalent active OTC in caster sugar to make a total mixture of 100 g
5. OTC from product No. 3 - wet treatment (P3-W)
   1 g equivalent active OTC in 500 ml of sugar syrup (1:1 sugar:water by volume)
6. OTC from product No. 3 - dry treatment (P3-D)
   1 g equivalent active OTC in caster sugar to make a total mixture of 100 g

OTC was applied to the brood nest after the queen excluder and supers were temporarily removed.

On 9 January 1999, the colonies were moved to Moyston at the base of the Grampians in western Victoria where river tea-tree (*Leptospermum obovatum*) and messmate (*E. obliqua*) were flowering.

On 28 January 1999, the supers of honey from each of the six hives belonging to a treatment group were extracted as one batch. The honey from the six supers was pooled and sampled for later analysis for OTC residues.

The extractor, uncapping knife, honey tank, bucket and pipe beneath the extractor were thoroughly cleaned before extracting the honey of the next treatment group. After cleaning, honey from untreated (control) hives was extracted and sampled. These samples were analysed to demonstrate effective cleaning of the extraction equipment and absence of cross-contamination of OTC between the treatment groups.

On 10 February 1999, the hives were robbed a second time and these supers were extracted on the 15 February 1999 using the above method.

Results
The results of the analyses of the first and second extraction samples are presented in Table 1.

Table 1. Results of analysis of the first and second extract samples of honey derived from the six hives in each treatment group.

OTC treatment group	OTC residue (mg/kg)	
	First extract (27/1/99)	Second extract (15/2/99)
P1 - Wet	0.34	0*
P1 - Dry	0.053	0
P2 - Wet	0.41	0
P2 - Dry	0.093	0
P3 - Wet	0.28	0
P3 - Dry	0.050	0

** less than the level of reporting of 0.05 mg/kg.*

Residues were not found in any of the pre-treatment and non-treated control honey samples. This demonstrated effective cleaning of the extraction equipment and absence of cross-contamination between treatments.

Conclusions

- Application of 1gram of OTC according to label directions resulted in residues in extracted honey from all treatment groups.
- The application of OTC in caster sugar resulted in less residues in extracted honey than when the antibiotic was applied in sugar syrup.
- In this experiment, OTC residues were not detected in the second extraction honey of any treatment group. If this were true for every scenario, apiarists would have confidence that honey of the second extraction would be free of OTC residues provided no further medication had been applied.

Experiment 2

Aim

To determine if the application of OTC to hives early in the beekeeping season (late winter) when bees usually consume much of their honey stores might reduce the occurrence of residues in extracted honey.

Methodology

On 13 August 2001, two-storey colonies located at Boundary Bend, northern Victoria, were treated during early almond bloom with OTC as detailed in Table 2. Due to cold weather pre-treatment honey samples were not collected.

Table 2. OTC treatment and number of hives per treatment.

Treatment	No of hives
0.3 g OTC active ingredient in 30g caster sugar	5
0.5 g OTC active ingredient in 45g caster sugar	5
1.0 g OTC active ingredient in 90g caster sugar	5
Control – no OTC treatment	6

All hives were moved to the following districts for nectar supplies:

- 7 September 2001, Corowa, NSW, - canola
- 17 September 2001, Shepparton, Victoria, - nashi fruit pollination
- 28 September 2001, Corowa, NSW, - canola.

On 3 October 2001 the super of honey was removed from each hive and extracted on the following day using the method described in Experiment 1.

Results

- OTC residues were not found in any of the control honeys, demonstrating effective cleaning of the extraction equipment and absence of cross-contamination of OTC between treatments.
- Each of the four samples collected from the treatment of 1.0 g OTC had 0.07 mg/kg residues.
- Residues were not found in any of the samples derived from the 0.3 g and 0.5 g treatment groups.

Conclusion

- In this experiment, the treatment of colonies with 1.0 g OTC early in the beekeeping season failed to prevent residues of OTC in the extracted honey. Readers should consider the warning in the following chapters in relation to the reduction of the standard 1 g (active ingredient) of OTC dose.

Experiment 3

Aim

To determine the concentration of residues in extracted honey following the application of 0.5 g and 1.0 g OTC in caster sugar and water to hives in late spring.

Methodology

Forty-two hives with no history of OTC treatment in the two previous seasons were selected. A pre-treatment sample of honey was obtained from each hive by scraping at least two combs.

The hives were moved in the early morning of 22 November 2001 to an apiary site for maintenance nectar and pollen supplies. The colonies were treated as per Table 3 at midday 22 November 2001 when the bees were foraging well and bringing pollen to the hive.

Table 3. OTC treatment and number of hives per treatment group.

OTC treatment	No of hives
1.0 g OTC active ingredient in caster sugar	5
1.0 g OTC active ingredient in caster sugar	5
1.0 g OTC active ingredient in caster sugar	5
1.0 g OTC active ingredient in distilled water	5
0.5 g OTC active ingredient in caster sugar	5
0.5 g OTC active ingredient in distilled water	5
Control – no OTC treatment	12

All colonies were moved to a yellow box nectar flow on 4 December 2001. On 2 January 2002, a super of empty combs and a honey bee clearer (escape) board, both with no history of OTC, were inserted beneath each super of honey to allow the bees to clear from the combs before extraction of the honey. The full super of honey was removed on 7 January 2002 and extracted the same day using the method of Experiment 1. The honey was then sampled and frozen for later analysis.

Results

No OTC was detected in any of the pre-treatment samples and control samples.

Table 4. Treatment, concentration of OTC in four replicate honey samples and mean OTC residue per treatment group by HPLC.

Treatment	Concentration of OTC residue in 4 replicate samples (mg/kg)	Concentration of OTC residue Mean of 4 samples (mg/kg)
1.0 g OTC (ai) caster sugar	0.25; 0.22; 0.22; 0.23	0.23
1.0 g OTC (ai) in caster sugar	0.13; 0.13; 0.14; 0.12	0.13
1.0 g OTC (ai) in caster sugar	0.14; 0.16; 0.15; 0.13	0.145
1.0 g OTC (ai) in distilled water	0.28; 0.24; 0.23; 0.26	0.253
0.5 g OTC (ai) in caster sugar	0.07; 0.07; 0.08; <0.05	0.073*
0.5 g OTC (ai) in distilled water	0.05; 0.05; 0.05; 0.05	0.05

(ai) = active ingredient. ** Mean of three samples*

Conclusion and recommendation

- The mean concentration of OTC was lower in honey derived from hives treated with 0.5 g OTC in distilled water than in honey derived from hives treated with the same dose in caster sugar.
- In contrast, the mean concentration of OTC was higher in honey derived from hives treated with 1.0 g OTC in distilled water than in honey derived from hives treated with the same dose in caster sugar.
- We suggest that additional trials be conducted to provide more data to confirm the appropriateness of applying OTC in water to hives.

Degradation of OTC in honey extracted from treated hives

Experiment 4

Aim

To determine the rate of degradation of OTC in extracted honey stored at ambient temperature and 22˚Celsius.

Methodology

Honey derived from the first extraction of each of the six groups of hives treated with 1 g OTC in Experiment 1 was placed in 30 kg labelled plastic pails. The honey to be stored at ambient temperature was placed in a shed to mimic customary industry storage practices. Honey to be stored at 22^{o}C was placed in a laboratory constant temperature room. The honeys were sampled as per Table 5 and 6.

Results

Table 5. OTC concentration in honey derived from the first extraction from hives treated with OTC on 16 December 1998 and stored at ambient temperature.

OTC Treatment group	OTC concentration (mg/kg) Date of sampling and approximate weeks from day of treatment of honey bee colonies								
	28/1/99#	10/2/99	24/2/99	7/3/99	31/3/99	21/4/99	7/6/99	16/12/99	23/6/2000
	6	8	10	12	15	18	24	52	79
P1-W	0.34	0.38	0.32	0.28	0.36	0.36	0.28	0.17	0*
P1-D	0.053	0.058	0.056	0.05	0.068	0.058	0.041	0.035	0
P2-W	0.41	0.42	0.47	0.35	0.41	0.4	0.3	0.21	0
P2-D	0.093	0.085	0.11	0.085	0.11	0.1	0.066	0.031	0
P3-W	0.28	0.33	0.38	0.32	0.41	0.37	0.23	0.19	0
P3-D	0.05	0.065	0.072	0.05	0.068	0.068	0.049	0.024	0

 # date of extraction.

** less than the level of reporting of 0.02 mg/kg.*

Table 6. OTC concentration in honey derived from the first extraction from hives treated with OTC on 16 December 1998 (Experiment 1) and stored at 22ºC.

OTC treatment	OTC concentration (mg/kg)				
	Date of sampling and approximate weeks from day of treatment of honey bee colonies				
	28/1/99[#]	7/6/99	16/12/99	16/3/2000	23/6/2000
	6	24	52	65	79
P1-W	0.34	0.16	0.12	0.05	0[*]
P1-D	0.053	<0.02	-	-	-
P2-W	0.41	0.17	0.095	0.04	0.04
P2-D	0.093	0.045	0.021	0.02	0.01
P3-W	0.28	0.16	0.14	0.05	0.05
P3-D	0.05	0.03	<0.02	-	-

[#] *Date of extraction.* * *less than the level of reporting of 0.02 mg/kg.*

Key:

P1-W	OTC from product No. 1 applied in sugar syrup	P2-D	OTC from product No. 2 applied in caster sugar
P1-D	OTC from product No. 1 applied in caster sugar	P3-W	OTC from product No. 3 applied in sugar syrup
P2-W	OTC from product No. 2 applied in sugar syrup	P3-D	OTC from product No. 3 applied in caster sugar

Conclusion

- The fact that OTC residues were still present in first extraction honey sampled 12 months after the date of OTC treatment indicates that the antibiotic degrades only slowly in honey held at ambient temperature.
- There was a higher rate of degradation of OTC in the honey stored at 22°C when compared to the same honey stored at ambient temperature.

Experiment 5

Aim

To determine the degradation of OTC in extracted honey stored at 25°C, 30°C, 35°C and 40°C.

Methodology

Two, 2-storey hives were each treated with 1 g of OTC (ai) in 500 mL of sugar syrup on 19 October 2000. Honey from the super of each hive was extracted on 1 November 2000, thoroughly mixed and decanted into vials. These were kept at room temperature until they were placed in 25°C, 30°C, 35°C and 40°C incubators in equal numbers on 14 November 2000. Two vials were removed from each incubator at 14 day intervals for analysis.

Results

- 25ºC honey – OTC was still detectable at almost 18 months after treatment of the colonies
- 30ºC honey – residues were not detected at approximately 15.5 months after treatment
- 35ºC honey – residues were not detected at approximately 8.5 months after treatment
- 40ºC honey – residues were not detectable 3.5 months after treatment.

Conclusion

- The data from this experiment confirms the slow rate of degradation of OTC at 25°C and a faster rate of degradation at higher temperatures.

3. Oxytetracycline hydrochloride in larvae of treated honey bee colonies

Following detection of OTC residues in honey in 1998, some Australian apiarists treated colonies with doses lower than those stipulated on OTC product labels. These apiarists believed that this might reduce the potential for OTC residues to occur in their extracted honey.

It is important that the dose of OTC applied to a honey bee colony is sufficient to effectively inhibit or control *Melissococcus pluton*. The dose must deliver sufficient antibiotic to meet or exceed the minimum inhibitory concentration (MIC) of OTC to *Melissococcus pluton*. The MIC is the minimum dose capable of inhibiting development of *M. pluton*.

Hornitzky and Smith (1999) conducted OTC sensitivity assays on 104 isolates of *M. pluton* obtained from four Australian States and determined the MIC for all isolates to be 1-2 µg/mL.

The following experiments were conducted to determine the potential for reduced doses of OTC to deliver enough antibiotic in individual honey bee larvae to meet the MIC as established by Hornitzky and Smith (1999).

Experiment 1 – Concentration of OTC in honey bee larvae following application of the antibiotic in summer and spring

Aim

To determine the concentration of OTC in honey bee larvae following application of OTC in caster sugar to honey bee colonies in summer and spring.

Methodology

Pre-treatment samples of at least 20 larvae were taken from the trial colonies and analysed to confirm that hives were free of OTC contamination. The hives of each treatment group were standardised to ensure similar numbers of adult bees, brood and honey. A very light nectar flow was in progress during these experiments. OTC was applied to hives according to Table 1.

Table 1. Oxytetracycline treatment, number of combs of brood and adult bees for each treatment group.

Treatment		Number of combs of brood and bees, and hive size	
OTC (g active)	Caster sugar (g)	6 combs of brood and 8 combs of bees (single storey)	6 combs of brood and 14-16 combs of bees (two storey)
0.3	33.3	5 colonies*	5 colonies*
0.5	50	5 colonies*	5 colonies*
1.0	100	5 colonies*	5 colonies*

* *4 colonies were used in the spring experiment*

Larvae were sampled from each colony 24 hours after treatment and thereafter on a daily basis. They were stored in a -20^{o}C freezer for later analysis to determine the concentration of OTC.

Results

The mean concentration of OTC in larvae sampled after treatment is presented in Table 2.

Table 2. Mean concentration of OTC in larvae for each treatment group over six days from medication.

Days after medication	OTC concentration (mg/kg) for each treatment and size of colony					
	0.3 g OTC		0.5 g OTC		1.0 g OTC	
	(1)*	(2)*	(1)	(2)	(1)	(2)
1	12.6	8.2	25.1	10.5	41.9	29.0
2	6.1	3.0	7.6	6.4	19.2	11.1
3	1.2	1.0	2.2	1.4	12.3	5.1
4	1.6	0.3	1.7	1.0	11.3	2.7
5	1.0	0.2	1.6	0.5	2.6	1.2
6	0.2	0	1.0	0.3	3.8	0.8

** denotes size of colony in storeys*

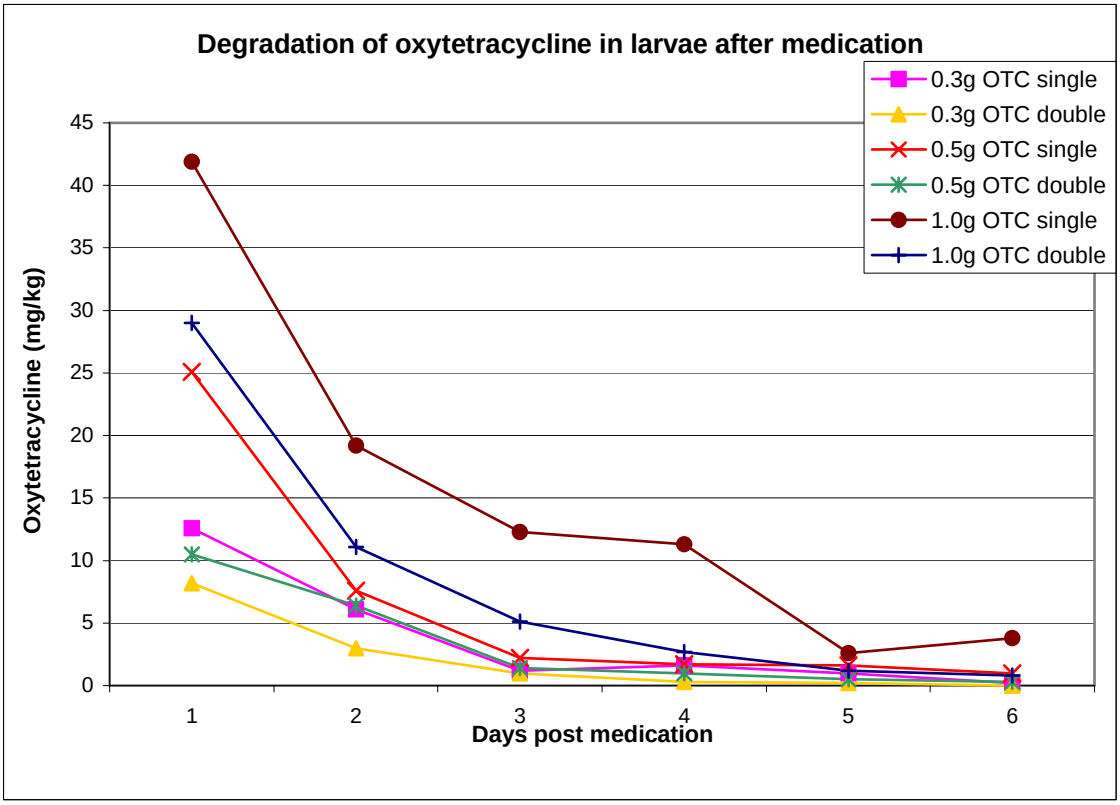

Figure 1 - Mean concentration of OTC in larvae for each treatment group over six days from medication

Conclusions

- The results, based on whole larval assays rather than the gut only, show that all treatments delivered sufficient antibiotic to exceed the MIC by a minimum of 4 times on day 1 after treatment and a minimum of about 10 times at the time of treatment (ie immediately after treatment).
- We suggest some caution in interpreting these results on the basis that our previous work demonstrated a lower concentration of OTC in the gut when compared to the whole larva. Readers are referred to the additional conclusion and recommendation on the following page.

Experiment 3 – Application of the OTC in water

Aim

To determine the concentration of OTC in honey bee larvae following application of the antibiotic in water.

Methodology

Ten double-storey eight frame hives were treated with either 0.3 g or 0.5 g OTC dissolved in 200 mL of distilled water on 20 November 2000. The medication was poured on the bees and top bars of the broodnest frames after first removing the queen excluder. Hives were elevated at the front so that liquid did not flow out the entrances. Larvae were sampled from each hive daily for 9 days after treatment.

Results

Although the mean level of OTC in larvae was generally higher for the 0.5 g treatment than the 0.3 g treatment, the difference was not statically significant on any day.

Table 3. Mean concentration of OTC in larvae for each water treatment for three days from medication.

Days after treatment	Concentration of OTC in larvae (mg/kg)	
	0.3 g OTC/hive	0.5 g OTC/hive
1	11.0	11.3
2	4.3	6.4
3	0.24	0.48

Conclusion

- Both the 0.3 g and 0.5 g OTC water applications delivered antibiotic above the minimum inhibitory concentration for *M. pluton* for 2 days.
- These treatments gave equal or higher OTC levels on the first two days post-treatment when compared with the same doses applied in caster sugar to two-storey colonies.
- By day 3, the concentration of OTC in larvae treated with OTC in water was considerably lower than that in larvae fed with medicated caster sugar.

Additional conclusion and recommendation for experiments 1 and 2

- The 1 g dose of OTC for double storey colonies has proven effective in controlling EFB in Australia since the disease became a problem in 1997. On that basis the 1 g dose provides sufficient antibiotic to meet or exceed the MIC.
- The ability of 0.5 g and 0.3 g doses (administered to double storey colonies) to deliver sufficient OTC to the larval gut (the site where *M. pluton* develops) to meet the MIC has not been shown by this work. We suggest that until further experimentation, the 1 g dose should not be reduced. In addition, any reduction in OTC may also encourage *M. pluton* to develop resistance to the antibiotic. The current 0.5 g and 1.0 g doses administered to single-storey and double-storey colonies respectively are therefore appropriate for control of EFB in Australia.

4. Efficacy of low doses of OTC applied to honey bee colonies to control European Foulbrood disease

Aim

To determine the efficacy of low doses of OTC and antibiotic extender patties to reduce the incidence of clinical symptoms of EFB in honey bee brood.

Methodology

Forty honey bee colonies with EFB disease signs were selected. Ten colonies, were located at Dunolly, Victoria for the entire trial where yellow gum (*Eucalyptus leucoxylon subsps*) provided a little nectar. The other colonies were on a non-nectar flow site at Mt. Lonarch, Victoria where capeweed (*Arctotheca calendula*) provided light quantities of pollen. All colonies occupied a single box although some located at Dunolly had begun to expand into the second box.

The number of sick and dead honey bee larvae, including scale (dried remains of larvae) showing typical EFB disease signs were counted.

Colonies were allocated to the following treatment groups and treated (as per accepted industry practices) on 2 October 1999:

a. 0.3 g OTC in 19 g of icing sugar
b. 0.4 g OTC in 46 g of caster sugar
c. 0.5 g OTC in 45 g of caster sugar (standard dose for single storey colonies)
d. 0.8 g OTC in antibiotic extender patties made from 150 g hydrogenated cotton oil and 300 g caster sugar (Delaplane and Lozano, 1994)
e. control – no OTC treatment.

The antibiotic extender patties were oval and about 10 mm thick. They were placed on a sheet of paper of identical size and shape positioned centrally on the top bars of the brood nest frames. A 12 mm wooden riser (rim) was placed on the brood box to accommodate the thickness of the patty when the excluder or upper box was replaced on the hive.

On the night of 5 October 1999, the colonies at Mt Lonarch were moved to a yellow gum (*Eucalyptus leucoxylon* sp) nectar flow near Maryborough, Victoria.

Results

The number of larvae showing EFB disease signs and time of inspections are detailed in Table 1.

Table 1. OTC dose and mean number of diseased larvae per brood comb at each inspection.

OTC treatment	Observation and mean number of diseased honey bee larvae per brood comb			
	Day before	Days post-treatment		
	treatment	12	22	41
0.3 g	59.7	2.61a	3.40a	3.79a
0.4 g	39.8	1.48a	3.74ab	2.72a
0.5 g	73.0	0.88a	2.53a	1.97a
Extender patties	40.0	9.82b	16.39bc	12.20a
Control - no OTC	35.9	12.16b	11.83b	8.64a

Note: Means with different letters are significantly different at $P < 0.05$.

The patties were of no benefit in reducing the numbers of diseased larvae. In fact, the patties were unattractive to bees and virtually remained untouched; the greatest consumption per hive being 0.97 g for the duration of the experiment.

Conclusions

- The colonies treated with either a 0.3 g, 0.4 g or 0.5 g dose had statistically significantly less diseased and dead larvae than the controls at the 12 and 22 day post-treatment observations, but not at the 41 day post treatment observation.
- At the beginning of the trial, EFB was well established in some of the colonies. If OTC had been applied at an earlier time when the disease was not so advanced, we may have had a better clean up of symptoms. We chose colonies with well established EFB because colonies with only a light infection (a few diseased larvae) can often spontaneously clear up. A spontaneous clear-up of disease symptoms may have wrongly attributed success to one or more of our treatments.
- The antibiotic extender patties proved to be unattractive to bees. The amount consumed by the bees was insignificant and the uptake of OTC was negligible. Consequently there was no effect on the incidence of EFB. The unattractiveness of the patties may be partly due to the fact that we used hydrogenated cotton oil in our patties. Crisco® vegetable oil used in USA patties was unavailable in Australia and after considering expert advice provided by a chemist of an Australian firm trading in vegetable oils, we chose cotton oil.

Recommendations

- Australian apiarists should continue to use 0.5 g and 1.0 g doses of OTC for single-storey and double-storey colonies respectively until further investigations are conducted. This is in line with our conclusions of the previous chapter. Until the efficacy of low doses of OTC for control of EFB can be demonstrated by trials using *M. pluton* infected laboratory reared honey bee larvae and back-up field investigations, these doses should not be reduced.
- The research team *strongly* urges apiarists not to feed OTC antibiotic extender patties to colonies. Antibiotic extender patties provide a prolonged exposure of OTC in low concentrations over the period of consumption by hive bees. This results in a potential for the development of antibiotic resistance in bacteria (Delapane 1997) associated with EFB and American Foulbrood (AFB). It is believed that strains of *Paenibacillus larvae* sub-species *larvae* (the causal bacterium of AFB) in the USA have resulted from use of OTC extender patties.

5. Incidence of European Foulbrood in commercially managed honey bee colonies fed a dietary supplement

In Australia, apiarists locate honey bee colonies near high quality pollen and nectar resources to decrease the potential for outbreaks of EFB. Some apiarists also feed colonies with a high protein dietary supplement to reduce the risk of outbreaks.

Apiarists have indicated that the incidence of EFB is generally greatest in the colder districts of Victoria where frequent inclement weather can restrict honey bee foraging and collection of nectar and pollen. To date, there have been no formal scientific trials to confirm these observations.

Identification of environmental factors that might trigger EFB outbreaks could provide the basis for changes of apiary management that may, in turn, help to reduce the occurrence and impact of the disease.

Aim

To determine the effect of nutritional supplements fed to honey bee colonies and environmental factors on the incidence of EFB.

Methodology and results

Note: The methods and results are presented together to provide ease of comparison.

One hundred and twenty commercially managed honey bee colonies with no history of severe outbreaks of EFB or antibiotic treatment were selected. Each hive had an 8-frame single box broodnest and queen excluder. All colonies had sister queens to ensure standard genetics throughout the apiary.

The colonies were allocated in equal numbers to one of the following treatment groups:

- Group 1 OTC treatment
  OTC was applied on 17 August 2000 and 13 August 2001 before EFB symptoms were evident in the brood. OTC was applied in caster sugar to the hive broodnest at a rate of 0.5 g (active ingredient) per single-storey hive and 1g (ai) per two-storey hive.
- Group 2 nutritional supplement
  The supplement was made with: red gum (*Eucalyptus camaldulenis*) irradiated pollen obtained from Western Australia (53.6%); torula yeast (8.9%); soyabean flour (8.9%); honey (28.4%) and vitamin-mineral supplement (Roche, Australia) (0.2%). The mixture was made into flattened cakes of 100g, each measuring approximately 11.25 x 5 x 1.25 cm, and then stored at -20^{o}C until required.
- Group 3 control - no treatment.

In season 2002, there were 40 colonies in each treatment group and in season 2001, there were 31 colonies per group. Throughout the season, colonies that died or became queenless were removed from the trial.

The location of each apiary site used in this trial and the nectar and pollen resources available to bees at each site are presented in Table 1.

Table 1. Apiary site over time and the date of nutritional supplement feeding.

Period at apiary site	Site location	Date of feeding and weight of supplement (group 2 only)	Nectar and pollen resource at apiary site
17 May - 10 July 2000	Moyston	17 May, 200 g	Longleaf box (*Eucalyptus goniocalyx*)
11 July - 30 August, 2000	Roses Gap	17 August, 200 g	Thryptomene (*Thryptomene calycina*), Correa (*Correa reflexa*), Wattle (*Acacia* sp.)
31 August - 1 October, 2000	Beulah	12 September, 200 g	Canola (*Brassica napus*)
2 October - 27 October, 2000	Callawadda	3 October, 200 g 21 October, 200 g	Canola, Capeweed (*Arctotheca calendula*)
28 October -15 November, 2000	Lexton	6 November, 200 g	Canola, Capeweed, Thistle (*Cirsium vulgare*)
16 November 2000 - 1 January 2001	Guildford	17 November, 200 g	Dandelion (*Taraxacum officinale*), Yellow box (*E. mellidora*)
2 January - 16 January, 2001	Grampians	None	Tea-tree (*Leptospermum sp.*), Messmate (*E. obliqua*)
17 January - 17 February, 2001	Hastings	None	Messmate
18 February - 19 March, 2001	Violet Town	None	Stringy bark (*E. macrorhynhca*)
20 March - 9 May, 2001	Rushworth	2 May, 200 g	Grey box (*E. microcarpa*), Ironbark (*E. sideroxylon*)
10 May - 5 August, 2001	Nhill	15 May, 300 g 27 June, 300 g 5 August, 300 g	Banksia (*Banksia ornata*), Yellow Gum (*E. leucoxylon*)
6 August - 31 August, 2001	Robinvale	13 August, 300 g	Almond orchard (*Amygdalus communis*), Turnip (*Brassica tourneforti*)
1 September - 16 October, 2001	Stawell	6 September, 300 g 18 September, 300 g 2 October, 300 g 11 October, 300 g	Capeweed, Onion weed (*Asphodelus fistulosus*), Yellow Gum, Salvation Jane (*Echium lycopsis*))
17 October - 30 November, 2001	Duneworthy	20 October, 200 g 31 October, 300 g 15 November, 300 g	Capeweed Onion weed (*A. fistulosus*), Yellow Box, Dandelion

Effect of nutritional supplement on crude protein of bees
On 30 November, 2001, at the end of the trial, samples of 30 adult bees were taken from five randomly selected colonies in each treatment group to determine the effect of feeding nutritional supplement on crude protein of adult bees. The mean crude protein of the samples of bees was 16.34, 16.22 and 15.86 g/100 g for the OTC group, nutritionally supplemented group and control group respectively. These differences were not statistically significant.

Effect of nutritional supplement on crude protein in adult bees and larvae – additional trial
In a separate trial conducted in autumn 2002, ten colonies of bees were selected to determine the effect of feeding nutritional supplement on crude protein of adult bees and larvae. The colonies were located at a site where maintenance nectar and pollen supplies were available from tea tree (*Leptospermum sp*), scent bark (*Eucalyptus aromaphloia*) and messmate (*E. obliqua*). Five colonies were fed supplement at a rate of 350 g/week and the other five colonies were not fed supplement (controls).

Samples of 50 larvae and 30 nurse bees were taken from each colony at days 8, 16, 24 and 32 after the commencement of feeding.

There were no statically significant differences in crude protein levels of adult bees or larvae sampled from colonies fed nutritional supplement and control colonies (Table 2).

Table 2. Mean crude protein levels of samples of adult bees and larvae – additional trial - 2002.

Sample and treatment	Mean protein content of sample (g/100g) for each sampling day				
	0	8	16	24	32
Adult bees from nutritionally supplemented colonies	14.7	14.4	15.2	17.4	17.1
Adult bees from control colonies	14.3	14.9	15.2	18.3	18.0
Larvae from nutritionally supplemented colonies	8.6	9.1	8.1	8.9	8.9
Larvae from control colonies	8.9	9.1	8.6	8.9	9.22

Spring honey production and mean number of combs of bees
The production of honey and the mean number of combs of bees in each colony was determined at the completion of each spring (Table 3). There were no significant statistical difference in honey production between the groups. The adult bee to honey ratio is a measure of the mean number of combs of adult bees and the weight of honey produced. Colonies having a low ratio were more efficient honey producers than those colonies with a higher ratio.

At the completion of spring 2001, those colonies infected with EFB (only) in treatment groups 1, 2 and 3 had means of 6.0, 7.0 and 10.7 combs of bees respectively. This result did not include healthy colonies.

Table 3. Spring honey production for each year of the experiment.

Year and attribute	Treatment		
	Group 1 (OTC)	Group 2 (supplement)	Group 3 (control)
Spring 2000			
Number of colonies in treatment group	27	26	28
Mean number of combs of adult bees	11.9	8.96	9.80
Mean weight of honey per colony (kg)	9.87	8.73	9.31
Adult bee to honey ratio	1.21	1.03	1.05
Total honey production for all hives (kg)	266.5	227.0	260.7
Spring 2001			
Number of colonies in treatment group	13	23	23
Mean number of combs of adult bees	13.1	13.7	13.8
Mean weight of honey per colony (kg)	11.6	11.7	10.7
Adult bee to honey ratio	1.13	1.17	1.29
Total honey production for all hives (kg)	150.4	268.3	247.0

Effect of nutritional supplement on the number of combs of brood and adult bees
There was no significant statistical difference in the amount of brood between treatments at any time during the trial. Group 1 colonies (OTC treatment) had a higher degree of swarming than the other groups.

Effect of nutritional supplement on the incidence of Nosema apis in adult bees
On 24 August, 14 September and 9 October 2001, samples of 30 adult bees were taken from the same 10 randomly selected colonies of each treatment group to determine the effect of nutritional supplement on the number of *Nosema apis* spores in adult bees. There was no significant statistical difference in the number of spores, except between the OTC and control groups on 9 October 2002.

Effect of nutritional supplement on the incidence of EFB
There was a significant statistical difference in the incidence of EFB only between Treatments 1 and 2, and Treatments 1 and 3 for the inspections of brood on 10 October and 28 October 2001. In spring 2000, EFB occurred in only one colony in each of Treatments 2 and 3 and because of this no statistical conclusion was reached.

Effect of weather on incidence of EFB
There was a significant statistical relationship between the number of days with rain per month and incidence of EFB in groups 2 and 3. No statistical relationship was found between temperature and incidence of EFB in groups 2 and 3 even though maximum temperatures were on average lower in spring 2001 than those of 2000.

Table 4. Monthly mean temperatures and days with rainfall during winter and spring for each year of the trial.

Year and weather attribute	Month					
2000	June	July	Aug	Sept	Oct	Nov
Maximum temperature (^{o}C)	13.2	12.6	14.7	18.3	19.6	23.4
Minimum temperature (^{o}C)	4.98	4.79	2.94	5.25	5.76	11.5
Days with precipitation	6	16	14	8	7	4
2001						
Maximum temperature (^{o}C)	15.2	14.3	17.7	18.1	16.7	19.09
Maximum temperature (^{o}C)	5.74	3.66	5.8	6.89	6.7	6.83
Days with precipitation	15	12	10	11	21	14

Discussion and conclusions

- The feeding of nutritional supplement to honey bee colonies in this trial did not result in a statistically significant reduction in the incidence of EFB when compared to untreated controls. This may be due to the prevailing conditions under which the trial was conducted.
- It is possible that under different conditions (eg other districts, species of flora and/or years) the feeding of nutritional supplement could significantly reduce the incidence of EFB.
- During 2001, the mean incidence of EFB in colonies fed nutritional supplement was 9% lower than the controls, but this was not statistically significant.
- The incidence of EFB was significantly related to the number of days of rain. While our colonies had ample pollen and honey stores, a shortage of fresh nectar and pollen supplies caused by poor weather and restricted honey bee foraging seems to have facilitated the onset of EFB.
- In 2000, the low incidence of EFB may have been the result of the:
  i. young age of the queens, recognised by apiarists as helpful in minimising the severity of EFB
  ii. favourable weather conditions for honey bee foraging and good supplies of nectar and pollen
  iii. little, if any, colony stress.

Recommendations

- That controlled laboratory trials, using honey bee larvae, be conducted to ascertain the potential of various dietary additives to control or prevent EFB.
- That formal surveys be conducted over a number of years to obtain information on the incidence of EFB and the interaction between environmental factors including weather/climate and apiary management practices.

6. The influence of pH in the honey bee larval gut on *Melissococcus pluton*

Honey bee larvae are fed brood food comprised of secretions from the mandibular and hypopharyngeal glands of nurse bees. This food has a pH of 4.0.

The gut of the honey bee larva is the primary site where *M. pluton*, multiplies and causes infection. Variations in pH of the larval gut could influence the multiplication of *M. pluton* and therefore its pathogenic virulence on larvae.

Factors such as variation in plant species, soil type, pH, moisture and fertility may influence the pH of pollen. These factors could ultimately induce changes of pH in the larval gut and thereby influence multiplication of *M. pluton*.

Aim

To determine the pH of guts of larvae of honey bee colonies foraging on different flora.

Methodology

In autumn 2001, five commercial apiaries situated in central and western Victoria were selected. The location of each apiary and the flora targeted by bees were:

Grampians	tea-tree (*Leptospermum* species) messmate (*Eucalyptus obliqua*) apple box (*E. bridgesiana*)
Lismore	sugar gum (*E. cladocalyx*)
Pyrenees Ranges	longleaf box (*E. goniocalyx*)
Trentham	manna gum (*E. viminalis*)
Mount Cole	manna gum (*E. viminalis*)

Ten larvae, aged approximately 4-5 days, were sampled from each of 5 randomly selected colonies at each location. The gut was dissected from each larva and the pH of its contents was measured.

Laboratory studies were conducted to determine the effect of pH on the development of *M. pluton*. The bacterium was cultured on culture media having the following pH:

- 4.0 - adjusted medium
- 6.6 - conventional medium (Hornitzky & Smith, 1998)
- 8.0 - adjusted medium.

Five groups of larvae were reared in the laboratory (method described later in this report) and fed larval food of pH 3.1, 3.6, 4.1 (control), 4.6 and 5.1. The development and mortality of larvae was recorded.

Results

The grand mean of pH for all larval gut contents sampled at all five sites was 6.329. The mean pH of larval guts varied from site to site and ranged from 6.258 to 6.506 (Table 1).

Table 1. Mean pH of honey bee larval gut contents sampled from five colonies at various Victorian locations in autumn 2001.

Site number and location	Honey bee colony number and mean pH of ten larval guts					Mean pH for each location
	1	2	3	4	5	
1. Grampians	6.556	6.419	6.197	6.205	6.177	6.311
2. Lismore	6.448	6.244	6.226	6.255	6.279	6.290
3. Pyrenees	6.651	6.474	6.497	6.434	6.476	6.506
4. Trentham	6.451	6.165	6.238	6.229	6.302	6.277
5. Mt. Cole	6.315	6.212	6.273	6.262	6.230	6.258

The difference in pH between all five sites was statistically significant.

In the laboratory *M. pluton* grew well on culture media of pH 6.6 but did not grow on culture media of pH 4.0 or pH 8.0. The difference in mortality between the five groups of larvae was low and not statistically significant. There was no difference in the development of larvae fed food of various pH.

Discussion and Conclusions

- The greatest difference of pH measured at the 5 sites was 0.248 and occurred between sites 3 and 5.
- The pH of gut contents of honey bee larvae varied significantly between apiary locations. We suggest that this was a result of honey bee foragers having access to different pollen and nectar resources at sites 1-4. Interestingly, sites 4 and 5 had the same nectar and pollen yielding plant species and even though they were approximately 100 km apart, the mean pH of larval guts was similar at 6.277 and 6.258 respectively (Table 1).
- We found a high variability between the pH of guts removed from larvae of a given colony. The variability may have resulted from sampling larvae of different age (4 days versus 5 days of age); difficulty in obtaining sufficient volume of gut contents for pH determination and/or slight differences in the constituent of food fed to larvae by the nurse bees of the colony.
- Laboratory studies showed that *M. pluton* is sensitive to changes of pH.
- The laboratory studies also indicated that in the laboratory, honey bee larvae have a capacity to buffer their diet and consequently may not be adversely affected by changes in the pH of larval food, as previously suggested by other researchers.

Recommendation

That the influence of pH changes on the viability and growth of *M. pluton* within the honey bee larval gut be further investigated using laboratory reared larvae as described later in this report.

7. Detection of *Melissococcus pluton* in honey bees and their products

Apiarists are usually able to diagnose EFB in their hives with relative ease based on the presence of specific disease signs (symptoms) in the brood. When symptoms do not provide a clear indication of the disease, apiarists may seek laboratory confirmation of the presence of *M. pluton*. This is usually done by microscopic examination of stained smears of diseased larvae to view the bacterium or by culture of diseased larvae to grow the organism.

Other laboratory techniques that may be used include the polymerase chain reaction (PCR). PCR is a more sensitive assay than the culture. The PCR identifies and confirms the presence of specific fragments of DNA. Djordjevic et al. (1998) developed a PCR for the detection of *M. pluton* in bee larvae and pure cultures of the bacterium, but the assay was not successful for the detection of *M. pluton* in adult bees, honey and other apiary products.

Aim

To modify Djordjevic's hemi-nested PCR and to evaluate the usefulness of the modified assay for the detection of *M. pluton* in honey, pollen, adult bees and whole honey bee larvae.

Results

The hemi-nested PCR of Djordjevic *et al.* (1998) was successfully modified to produce a more sensitive PCR.

The modified PCR detected *M. pluton* in the following samples collected from a hive showing typical EFB disease signs:

- front and rear legs, wings, proboscis, ventriculus, rectum and honey crop of adult honey bees
- whole honey bee larvae
- pollen and honey collected from the hive broodnest
- washings (using distilled water) of individual broodnest cells that contained a freshly laid egg.

The PCR also confirmed the presence of *M. pluton* in the following samples collected from an apparently healthy hive located adjacent to an EFB infected colony:

- front and rear legs and rectum of adult honey bees
- whole honey bee larvae
- honey collected from the hive broodnest.

M. pluton was not found in adult bee mouth parts and legs, cell washes or pollen sampled from this same healthy hive.

Of 82 samples of extracted honey sourced from different states (Table 2), the modified PCR detected *M. pluton* in 59 (68.6%) of samples, whereas culture only detected *M. pluton* in 22 (27.9%) of the same samples. *M. pluton* was present in 46% of honey samples sourced from central and northern Queensland honey producing districts.

Table 2. The modified PCR and honey culture results of extracted honey samples from various Australian states.

Source of honey sample	Number of samples	No. and % *M. pluton* positive samples by PCR	No. and % *M. pluton* positive samples by culture
Victoria	27	24 (89)	11 (40)
New South Wales	24	16 (67)	9 (39)
Queensland	13	6 (46)	2 (15)
South Australia	11	11 (100)	2 (17)
Tasmania	7	2 (29)	0

Conclusions

- The modified PCR proved to be more sensitive than the culture method for the detection of *M. pluton.*
- All individual body components of adult bees, pollen, brood comb cell washes and broodnest honey from the infected colony were shown to contain *M. pluton.* This suggests that bees and bee products as well as diseased brood are a source of infection in diseased colonies.
- *M. pluton* may be present in larvae, adult bee digestive tracts and broodnest honey sampled from apparently healthy colonies.
- Honey was also shown to be commonly infected with *M. pluton* and therefore may be a constant supply of the organism to infect larvae.
- The Australian states with the highest percentage of samples of apiarist extracted honey infected with *M. pluton* were South Australia (100%), Victoria (89%) and New South Wales (67%).
- The modified PCR will be extremely useful for use in studies designed to evaluate the efficacy of potential new treatments for control of EFB.

8. Laboratory rearing of honey bee larvae for European Foulbrood studies

Honey bees freely forage on a variety of nectar and pollen plants within their general flight range of 5-6 kilometres from their hive. This is extremely beneficial to the health of the honey bee colony because it facilitates a balanced diet through a supply of various pollens, each contributing different amino acids, fatty acids, vitamins and minerals.

However, the same free flying honey bee behaviour can make it difficult for some scientific experiments to deliver reliable information. For example, studies investigating supplementary diets for commercially managed honeybee colonies may be adversely influenced by various pollens which foraging bees bring to the hive.

To alleviate this problem, some scientists have conducted experiments in insect proof enclosures to prevent bees gaining access to food sources that may adversely influence the outcomes of the research.

There are other examples of factors that can influence experiments. Studies on EFB in beehives may be somewhat hindered because it is not possible to guarantee that the disease will occur in an apiary in any given year. Attempts to get around this problem by inoculating colonies with *M. pluton* have met with little success and no reliable inoculation method had been developed.

Laboratory reared larvae have been used in studies of the honey bee brood diseases, chalkbrood and American foulbrood. However at the time of writing, there were no published reports indicating that laboratory reared larvae have been used in EFB studies.

Peng *et al.* (1992) developed a method for rearing larvae in American Foulbrood disease studies. They used a basic larval diet (BLD) that contained protein (10.5%), solids (22%) and had a physical consistency to maintain moisture content while placed in incubation. The consistency enabled larvae to float on the BLD without drowning. This reduced the likelihood of mechanical damage that might have resulted from excessive handling and feeding of the larvae.

Objectives

To develop a method for the laboratory rearing of honey bee larvae and their inoculation with *M. pluton* for future studies of potential EFB control measures.

Outcomes

- Newly hatched honey bee larvae were successfully reared in the laboratory using the diet developed by Peng *et al.* Larvae were reared from the age of less than 24 hours to the stage of defecation and prior to pupation.
- Laboratory reared larvae were successfully inoculated with *M. pluton* and they exhibited EFB disease signs identical to those of naturally infected larvae. The mortality of individual larvae increased with the dose of *M. pluton*.
- At all times, larvae were given a supply of food surplus to their requirements. Despite this, larvae died due to infection with *M. pluton*. This finding seems contrary to the suggestion of Bailey and Ball (1991) that larvae infected with *M. pluton* primarily die because of the inability of nurse bees to adequately feed individual larvae.

Conclusion

The successful of rearing of honey bee larvae in the laboratory and their inoculation with *M. pluton* will enable fully controlled experiments to be conducted without the unwanted influences experienced in field trials. Such laboratory studies may include the effect of nutrition and other potential treatments for the control of EFB. In comparison to field trials, investigations involving laboratory reared larvae will deliver results more quickly and at less cost.

9. A survey of apiarists' use of oxytetracycline hydrochloride and management of European foulbrood

The survey comprised the following two questionnaires:
1. apiarists' use of oxytetracycline hydrochloride (OTC)
2. non-antibiotic management of European Foulbrood disease.

The questionnaires were mailed during winter 1999 to 190 Victorian apiarists who owned 100 or more hives. In addition, the Australian Honey Bee Industry Council kindly arranged for copies of the two questionnaires to be distributed to selected Tasmanian apiarists in spring 1999.

Information obtained from the Victorian and Tasmanian responses was collated on an individual State basis and is presented in the following pages.

Department of Natural Resources and Environment

A SURVEY OF APIARISTS' USE OF OXYTETRACYCLINE (OTC)

Introduction

This survey is being conducted to identify reasons why some apiarists don't have residues in their honey when they use oxytetracycline hydrochloride. The information will help to determine industry's current 'Best Practice' use of OTC. This in turn may help industry to avoid OTC residues in the future and thereby protect valuable domestic and export markets.

Note: The information you provide is ***"Commercial-in Confidence"*** and will be kept strictly confidential. Individual apiarists will not be identified in any report arising from this survey.

Use of OTC

1. Do you use OTC to prevent or control Foulbrood in your hives?

	No. of respondents Victoria	No. of respondents Tasmania
Yes. *Please proceed with the questionnaire*	54 (79%)	12 (100%)
No. *The remaining questions do not apply to you; there is no need to proceed*	14 (21%)	0

Time of OTC treatment of hives

2. What are the approximate months you would usually treat your hives with OTC?

The number of respondents by State and month in which the primary application of OTC for the beekeeping season was applied are presented in the following table.

Victorian respondents				Tasmanian respondents	
July	4	September-October	4	July-September	1
July-August	2	September-November	4	August-September	1
August	11	October	3	August-October	3
August-September	7			September	1
August-October	1			September-October	5
September	9			October-November	1

3. At the beginning of the season in late winter or early spring, when would you usually treat your hives with OTC?

	No. of respondents Victoria	No. of respondents Tasmania
Before EFB disease signs (symptoms) are seen in your hives	19 (38%)	5 (42%)
Only when EFB disease signs (symptoms) are present in your hives	31 (62%)	7 (58%)

4. At the beginning of the season, during late winter or early spring, when you observe EFB disease signs (symptoms) in any one load or yard, what hives do you treat with OTC?

	No. of respondents Victoria	No. of respondents Tasmania
Spot treat only those hives showing EFB symptoms	24 (49%)	0
Blanket treat all hives whether symptoms are present or not in every hive?	25 (51%)	12 (100%)

5. What is the approximate average period of time from date of treatment of hives at the beginning of the season in late winter or early spring to the first extraction of honey?

The number of respondents by State and period (weeks) between treatment of hives and first extraction of honey are presented in the following table.

Victorian respondents				Tasmanian respondents	
4-8 weeks	1	8 weeks	9	7 weeks	1
5-6 weeks	2	8-10 weeks	5	8 weeks	1
6 weeks	6	10-12 weeks	1	8-10 weeks	3
6-7 weeks	1	11 weeks	1	12 weeks	3
6-8 weeks	4	12 weeks	3	16 weeks	1
6-9 weeks	1	12-16 weeks	1	18 weeks	1
7 weeks	2	15-20 weeks	1		
7-8 weeks	1	16 weeks	2		
7-10 weeks	1	16+ weeks	1		
7-17 weeks	1				

6. When there is a need to treat hives with OTC at any other time of the year (that is, apart from late winter or early spring) what hives do you treat?

	No. of respondents Victoria	No. of respondents Tasmania
Spot treat only those hives showing EFB symptoms	45 (94%)	10 (100%)
Blanket treat all hives whether symptoms are present or not in every hive?	5 (6%)	0

Method of feeding OTC

7. When you treated your hives with OTC in late winter or spring did you apply the antibiotic in a single feed or did you divide the dose and apply it over three feeds?

	No. of respondents Victoria	No. of respondents Tasmania
One feed - single dose	39 (83%)	3 (25%)
Three feeds - multiple dose	8 (17%)	9 (75%)

8. How did you feed OTC to your hives?

		No. of respondents Victoria	No. of respondents Tasmania
Liquid - wet treatment	*Go to question 9*	16 (31%)	1 (8%)
Powder - dry treatment	*Go to question 14*	34 (65%)	11 (92%)
In pollen supplement	*Go to question 18*	1 (2%)	0
In a patty	*Go to question 18*	1 (2%)	0

Feeding OTC in liquid 'wet treatment' form

9. How did you mix and apply OTC as a liquid "wet treatment"?

	No. of respondents Victoria	No. of respondents Tasmania
Dissolved in sugar syrup	12 (75%)	1 (100%)
Dissolved in water	4 (25%)	0

10 If you fed OTC to your hives in sugar syrup how much sugar and water did you use to make the syrup?

	No. of respondents Victoria	No. of respondents Tasmania
One part sugar, one part water (1:1)	6 (76%)	1 (100%)
Two parts sugar, one part water (2:1)	1 (12%)	0
One part sugar, four parts water (1:4)	1 (12%)	0

11. Where did you pour or sprinkle the liquid 'wet' treatment in the hive. (*See also next question*).

	No. of respondents Victoria	No. of respondents Tasmania
On the bees and top bars of the frames in the broodnest	11 (85%)	1 (100%)
In the honey super(s)	0	0
Into brood combs	2 (15%)	0

12. If you treated a hive that had a double box broodnest, into which box did you apply the liquid?

	No. of respondents Victoria	No. of respondents Tasmania
Lower box	9 (69%)	0
Lower and second box	1 (8%)	0
Second box	3 (23%)	1 (100%)

13. Did you lift the queen excluder before you put the liquid into the broodnest?

	No. of respondents Victoria	No. of respondents Tasmania
Yes, lifted queen excluder and replaced it immediately after treatment	8 (53%)	0
Queen excluder not lifted	0	0
Queen excluder not used	7 (47%)	1 (100%)

Proceed to question 18

Feeding OTC in powder 'dry treatment' form

14. How did you mix and apply OTC as a powder (dry treatment)?

	No. of respondents Victoria	No. of respondents Tasmania
Mixed in caster sugar	18 (51%)	5 (45%)
Mixed in plain sugar	2 (6%)	0
Mixed in icing sugar	5 (14%)	6 (55%)
Commercial OTC product that required no further mixing	10 (29%)	0

15. Where did you sprinkle or place the dry OTC mixture or commercial pre-mix product the broodnest?

	No. of respondents Victoria	No. of respondents Tasmania
On the top bars of all the broodnest frames	6 (19%)	1 (10%)
Between the combs and on the top bars of all the broodnest frames	10 (31%)	0
On the top bars of the broodnest frames containing brood only	13 (41%)	7 (70%)
At the ends (lugs) of the frame top bars only	1 (3%)	2 (20%)
On top of the queen excluder	2 (6%)	0
In or on frames/combs in honey super(s)	0	0

16. Did you lift the queen excluder before putting dry OTC mixture or commercial pre-mixed product in the broodnest?

	No. of respondents Victoria	No. of respondents Tasmania
Yes, lifted excluder and replaced it immediately after treatment	24 (86%)	0
No, queen excluder left in position	4 (14%)	0
Queen excluder not used (*Data not included in percentage calculations*)	4	11

17. If you treated a hive that had a double box broodnest, into which box did you put the dry OTC mixture or commercial pre-mixed product?

	No. of respondents Victoria	No. of respondents Tasmania
Lower box	2 (50%)	3 (60%)
Second box	1 (25%)	0
Lower and second box	1 (25%)	2 (40%)

Withholding periods and residues

18. Please answer this question if you observed a withholding period before selling the honey that was removed and extracted hives treated with OTC. How long was this withholding period from the date of treatment of the hives?

The number of respondents by State and period (weeks/months) between treatment of hives and sale or delivery of honey to a packing plant are presented in the following table.

Victorian respondents				Tasmanian respondents	
3-4 weeks	1	8-10 weeks	2	6 weeks	2
4-6 weeks	2	8-12 weeks	5	8 weeks	2
5 weeks	1	12 weeks	3	8-10 weeks	2
6 weeks	5	12-16 weeks	1	12 weeks	3
6-12 weeks	1	16-26 weeks	4	1-12 months	1
6-8 weeks	3	6-12 months	1		
8 weeks	19				

Department of Natural Resources and Environment

A SURVEY OF EUROPEAN FOULBROOD DISEASE

Introduction

This survey is designed to gather information that may provide a better understanding of European Foulbrood disease (EFB). We hope the information will help to explain:

- why EFB is more prevalent in Victoria than in other Australian States
- possible links between EFB, honeybee nutrition and various pollen plants
- how EFB can be a better controlled without using oxtetracycline (OTC).

Note: The information you provide is ***"Commercial-in Confidence"*** and will be kept strictly confidential. Individual apiarists will not be identified in any report arising from this survey.

Total number of respondents

	No. of respondents Victoria	No. of respondents Tasmania
EFB disease signs present in one or more hives managed by respondent	58 (94%)	9 (90%)
No EFB disease signs present in one or more hives managed by respondent	4 (6%)	1 (10%)

Frequency of EFB outbreaks

1. In which years did you find disease signs (symptoms) of EFB in any of your hives? (You may *tick one or more boxes*).

	No. of respondents Victoria	No. of respondents Tasmania
1999-2000 (this season)	38	5
1998-99 (last season)	47	5
1997-98	43	5
1996-97	44	6
1995-96	47	6
No EFB in any of these years	4	1

2. What month of the beekeeping season do you usually observe the first symptoms of EFB?

	No. of respondents Victoria	No. of respondents Tasmania
July	3 (5%)	0
August	17 (30%)	3 (33%)
September	24 (42%)	6 (67%)
October	11 (19%)	0
November	1 (2%)	0
February	1 (2%)	0

Location of apiary site and pollen/nectar flora targeted by bees

3. In what district was the apiary site that you used for pollen and nectar in the autumn prior to the spring outbreak of EFB?

Respondents indicated the following districts:
Arnold, Avoca, Baringhup, Barkly, Bealiba, Beechworth, Benalla, Bendigo, Blackwood, Broadford, Bullarto, Castlemaine, Chiltern, Cowwarr, Daylesford, Big and Little Desert, Echuca, Edenhope, Eldorado, South and East Gippsland, Goulburn Valley, Lower Goulburn Valley, Grampians, Heathcote, Henty, Howlong, Inglewood, Kingower, Lakes Entrance, Landsborough, Mt Cole, Mafeking, Menindee, Mildura, Mornington Peninsular, Muckleford, Mudgegonga, Nhill, Nyah, Red Cliffs, Rheola, Rushworth, Skipton, Smeaton, St Arnaud, St Leonards, Stawell, Swan Hill, Tarnagulla, Tatong, Tawonga, Trentham, Tooan, Wangaratta, Wedderburn, Wehla and Yarram

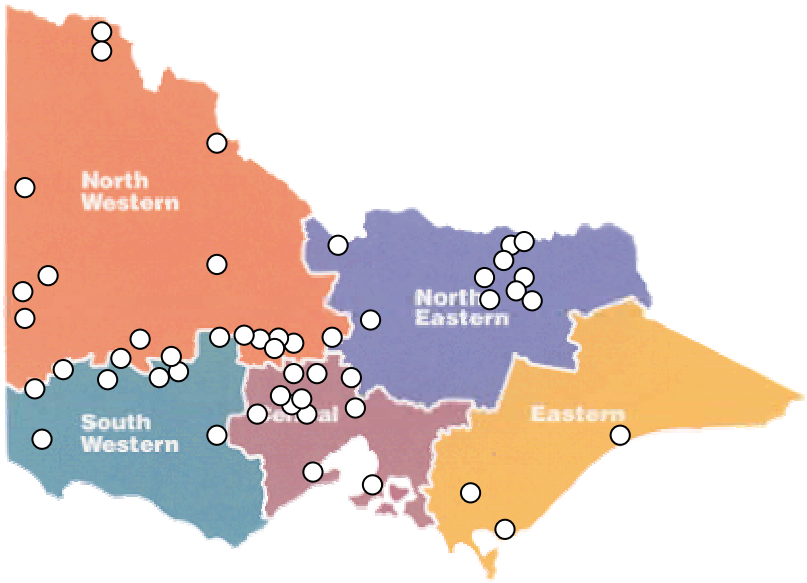

Figure 1. - Districts in which respondents' apiary sites were located during the autumn prior to the spring outbreak of EFB.

Nectar and pollen flora targeted by bees in autumn prior to the spring outbreak of EFB								
Victoria						Tasmania		
Apple Box	4	np*	Mallee	1	n	Leatherwood	6	
Banksia	3	np	Messmate	5	np	Prickly box	1	
Black Box	2	np	Prickly Moses	1	n	Stringybark	1	np
Blue Mallee	5	np	Red Stringybark	1	np	Banksia	1	
Flatweed	6	np	Silver Banksia	1	np	Snow gum*	1	
Gorse	2	np	Sugar Gum	1	np			
Grey Box	16	np	Stringybark	4	np			
Red Ironbark	5	n	Swamp Gum	1	np			
Longleaf Box	2	np	Tea Tree	1	n			
Manna Gum	4	np						

One respondent indicated no incidence of EFB when bees were foraging on Capeweed.
* n = nectar; p = pollen

4. In what district was the apiary site that you used for pollen and nectar in the winter/early spring prior to the spring outbreak of EFB?

Respondents indicated the following districts:
Avoca, Bairnsdale, Baringhup, Barkly, Benalla, Bendigo, Boundary Bend, Castlemaine, Dimboola, Edenhope, Echuca, South and East Gippsland, Goulburn Valley, Heathcote, Howlong, Inglewood, Jeparit, Kingower, Landsborough, Little Desert, Mt Cole, Mallee, Mildura, Mornington Peninsular, Mudgegonga, Murrayville, North -east Victoria, Newstead, Ouyen, Rheola, Rushworth, St Arnaud, St Leonards, Samaria, Sea Lake, Smeaton, Stawell, Sunraysia, Swan Hill, Tatong, Underbool, Wangaratta and Yungera

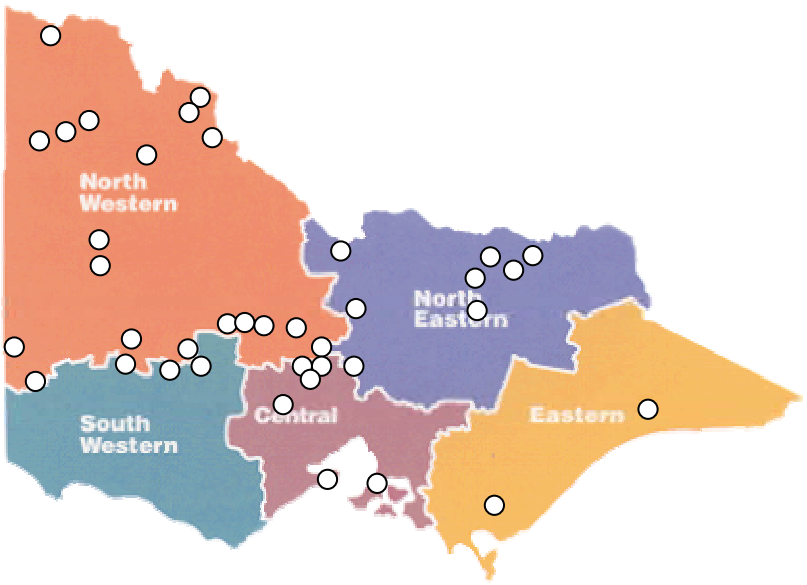

Figure 2. - Districts in which respondents' apiary sites were located in the winter/early spring prior to the spring outbreak of EFB.

Nectar and pollen flora targeted by bees in winter and early-mid spring prior to the spring outbreak of EFB								
Victoria						Tasmania		
Almonds	3	np*	Red Ironbark	2	n	Capeweed	1	p
Canola	14	np	Red Box	1	n	Fruit trees	1	np
Capeweed	20	np	Shrubs general	2	np	Heath	2	
Banksia	4	np	Turnip weed	10	np	Tea Tree	1	n
Eggs and bacon	1	np	Wattle	5	P	Wattle	1	p
Faba Beans	1	np	Wattle (black)	1	P	Wildflowers	1	np
Fruit trees	1	np	Wattle (Golden)	1	P			
Gorse	2	np	Wattle (silver)	1	P			
Paterson's Curse	6	p	Willow	1	np			
Onion grass	2	np	White Mallee	2	n			
Onion weed	8	np	Yellow Gum	5	n			
Orange blossom	1							

* n = nectar; p = pollen

5. When you noticed EFB, in what district was the apiary site and hives located?

Respondents indicated the following districts:
Bairnsdale, Baringhup, Barkly, Benalla, Bendigo, Boundary Bend, Castlemaine, Edenhope, Echuca, Geelong, South and East Gippsland, Goulburn Valley, Heathcote, Howlong, Inglewood, Jeparit, Kingower, Landsborough, Little Desert, Mt Cole, Mallee, Mildura, Mornington Peninsular, Mudgegonga, North-east Victoria, Natte Yallock, Newstead, Ouyen Rheola, Rushworth, St Arnaud, St Leonards, Sea Lake, Stawell, Swan Hill, Wangaratta and Yungera.

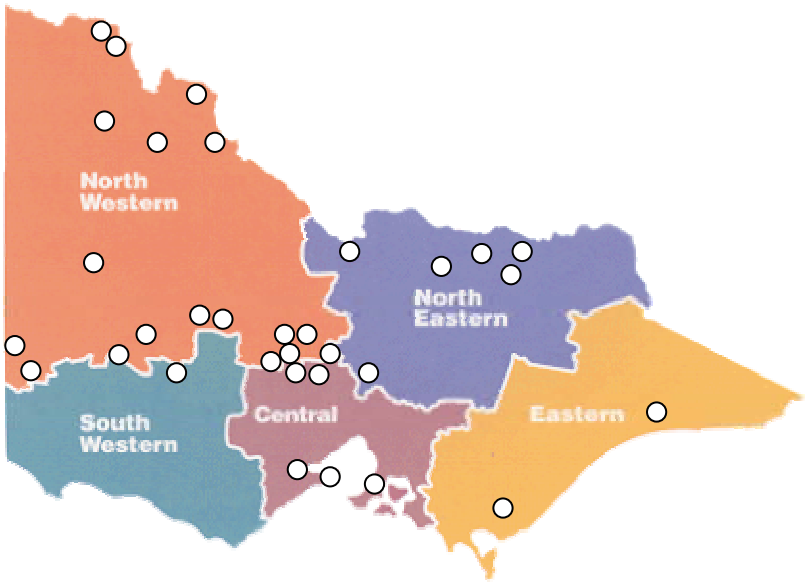

Figure 3. - Districts in which respondents' apiary sites were located when EFB was detected.

Nectar and pollen flora targeted by bees at the site where EFB was first noticed in the honey bee colonies								
Victoria						Tasmania		
Almond	2	np*	Orange Blossom	1	np	Cape weed	1	np
Canola	18	np	Paterson's Curse	6	np	Fruit trees	1	np
Capeweed	22	np	Red Box	2	n	Heath		n
Coast Tee Tree	1	n	Scrub/ground flora	5	np	Tea Tree	1	np
Faba beans	1	np	Turnip Weed	7	np	Wattle	3	p
Fruit trees	1	np	Yellow Gum	8	n	Wildflowers	2	np
Gorze	2	np	Wattle	9	p	Willow	1	np
Onion grass	2	p	White Mallee	1	n			
Onion Weed	8	np	Willow	2	n			

* n = nectar; p = pollen

Queen excluders

6. Were queen excluders on the hives at the time the EFB disease signs (symptoms) were found?

		No. of respondents Victoria	No. of respondents Tasmania
Yes	*Go to question 10.*	34 (64%)	0
No	*If you answered 'No' go to Question 8*	19 (36%)	9 (100%)

7. Please state the number of boxes you allow for the broodnest under the queen excluder?

	No. of respondents Victoria
One box	33 (100%)*
Two boxes	0

* Two respondents indicated that a few hives had 2 boxes below the excluder.

Honey bee queens

8. How old were the queens in the hives at the time the EFB disease signs (symptoms) were found?

Age of honey bee queens and number of respondents					
Victoria				Tasmania	
2 months	1	>1 year	1	1 year	1
6 months	1	1-1½ years	7	12-22 months	1
8 months	2	1-2 years	3	1-2 years	1
< 1 year	2	2 years	4	2 years	1
1 year	6	>2 years	2	1-4 years	1
< 15 months	1				
All ages		6		0	
Unsure of age		16		2	

Pollen supplements/substitutes

9. Had the hives with EFB disease signs (symptoms) been fed with a pollen supplement or substitute in the previous autumn, winter or spring immediately before symptoms of the disease were found?

	No. of respondents Victoria	No. of respondents Tasmania
Yes *Go to question 13*	9 (18%)	1 (11%)
No *If you answered 'No' go to Question 17*	42 (82%)	8 (89%)

Note: Apart from one Victorian respondent, all respondents who answered 'yes' to this question also indicated presence of disease signs in their hives (Question 1).

10. When did you feed the colonies with a pollen supplement/substitute? (*You may tick more then one box*).

	No. of respondents Victoria	No. of respondents Tasmania
Spring only	3	1
Winter only	4*	0
Autumn only	0	0
Autumn, winter and spring	2	0

* Two respondents indicated that the winter feed of pollen supplement was given prior to moving hives to almond pollination.

11. Where did you obtain the pollen supplement or substitute?

	No. of respondents Victoria	No. of respondents Tasmania
Mixed your own *Go to question 16*	6 (67%)	0
Purchased a pre-mixed powder, patty or cake. *Go to next question*	3 (33%)	1 (100%)

12. If you mixed your pollen supplement or substitute, please provide details of the ingredients and the amount (or weight) of each ingredient used in the mixture.

- Pollen, soy flour, Torula yeast, honey and sugar
- Irradiated pollen-½ litre, soy flour-5 litres and sugar syrup-4 litres (1:1 sugar/water)
- Soy flour-3kilograms, Torula yeast-1kilogram, Kalavit 50 grams and sugar syrup
- Soy flour, Torula yeast, Kalavit, sugar and mountain honey
- Soy flour, pollen, sugar and some yeast.

Comb replacement

13. Did the hives with EFB disease symptoms have some or all of the old combs in the brood nest replaced with foundation or newly drawn comb within the last 12 months?

		No. of respondents Victoria	No. of respondents Tasmania
Yes	*Go to Question 18*	48 (83%)	4 (44%)
No	*If you answered 'No' go to Question 20*	10 (17%)	5 (56%)

14. How old were the combs that you removed from the brood nest? (*An average age will do*).

Age of combs removed from the brood nest and number of respondents					
Victoria				Tasmania	
1 year	1	5 years	2	4 years	2
1-1½ years	1	5-6 years	1	4-6 Years	1
2 years	3	5+ years	1		
2-3 years	2	5-10 years	2		
2-4 years	1	6-8 years	1		
3 years	4	7-8 years	1		
3-4 years	5	7-10 years	1		
4 years	4	15 years	1		
4-5 years	3	Until worn out	1		
All ages		3		0	
Unsure of age		3		1	

15. Did you put foundation or newly drawn combs in the brood nest to replace the old combs?

	No. of respondents Victoria	No. of respondents Tasmania
Newly drawn combs	15 (33%)	2 (50%)
Comb foundation	10 (22%)	0
Combination of comb foundation and newly drawn combs	20 (45%)	2 (50%)

Treatment of colonies with oxytetracycline (OTC)

Questions 20 and 21 refer to the time you last saw EFB in your hives.

16. Did you treat your hives with OTC some time before EFB disease signs (symptoms) were evident?

		No. of respondents Victoria	No. of respondents Tasmania
Yes.	*Go to question 21.*	20 (36%)	3 (38%)
No	*If you answered 'No' go to question 22.*	35 (64%)	5 (62%)

17. Please indicate when the colonies were treated with OTC.

	No. of respondents Victoria	No. of respondents Tasmania
Previous autumn	4	0
Previous Late winter/early spring	8	2
Previous autumn and late winter/early spring	7	0
Spot fed throughout the season as EFB disease signs became evident in individual hives	4	1

Note: Three respondents indicated that after a blanket feed was given to all hives, spot treatment was also practiced throughout the season whenever diseased colonies were detected.

Management of EFB

18. What do you find is the best method to control EFB without using OTC? Please describe. This question refers to methods, other than OTC treatment, that you have used to prevent or control EFB.

Responses detailing use of management practices for control EFB

The number of apiarists who responded to this question by indicating use of one or more management practices to minimise or prevent EFB outbreaks are presented in the table below. It should be noted that these practices may be used in addition to the application of OTC.

	No. of respondents Victoria	No. of respondents Tasmania
Apiarists indicating (in Question 1) presence of EFB in hives	38 (90%)	2 (66%)
Apiarists indicating (in Question 1) absence of EFB in hives	4 (10%)	1 (34%)

Management practices for control of EFB

Responses provided by Victorian and Tasmanian respondents are combined unless otherwise indicated. The number of respondents is indicated by the figures in brackets.

- requeen colonies regularly to ensure presence of young queens (23)
  Some of the above respondents also indicated that:
  - queens were no older than 2 years of age (4)
  - some colonies were requeened twice a year (1)
  - failing queens and drone layers were immediately replaced (1)
  - individual EFB infected colonies were immediately requeened when the disease was detected (5)
  - infected colonies were requeened by introducing a young nucleus colony headed by a

new queen (1)
    - a break in the brood cycle caused by requeening enabled colonies to overcome EFB (3)
- replace some broodnest combs annually with comb foundation or newly drawn comb (22)
- maintain healthy, strong colonies (ie populous with adult bees) (6)
    - infected hives were given combs of sealed or emerging (hatching) brood to increase the numbers of adult bees (2)
- ensure good honey bee nutrition (4) and feeding of pollen supplements (2)
- place colonies on good conditions (honey and pollen flows) (13)
    - avoid tail end of flowering where conditions deteriorate as flowers dry (1)
    - avoid wintering sites with sloppy nectar, (eg, blue mallee and/or ironbark) (1)
    - feed sugar syrup to colonies during spring (3 Tasmania only)
- keep hives compact (5), warm (4) and avoid over supering (2)
    - use queen excluders to confine the queen to a one box broodnest (1)
    - provide good hive ventilation (1 Tasmania only)
    - provide sheltered winter apiary sites (1) and dry sunny spring sites (1)
- EFB was considered to be a problem in cooler climates (3). One apiarist indicated that there was less EFB in the north of Victoria (eg Mallee) when compared to southern districts of the state. Another indicated that EFB was initiated more easily by the cold wet windy weather of Gippsland than any other factor.
- avoid shifting hives in spring immediately prior to a weather change (1)
- avoid colony stress (4)
- sterilise hives that had housed dead or sick colonies by gamma irradiation (3).

Discussion and conclusions

- Preliminary results of this project have resulted in the withdrawal of directions on OTC product labels for wet-treatment of honey bee colonies. Consequently, it is likely that at the present time, the number of respondents using wet-treatment (if any) could be much lower than indicated by the survey.
- The incidence of EFB in Victoria was higher in the central and western districts than elsewhere. However, this may simply demonstrate that the majority of respondents had located their colonies to target flora in these areas. Four respondents indicated that they considered the disease to be a greater problem in the cooler areas of Victoria when compared to the warmer, northern districts of the state.
- This survey did not confirm a relationship between the flora on which bees foraged and outbreaks of EFB. Many respondents placed colonies in stands of grey box (*Eucalyptus microcarpa*), a late summer-autumn nectar and pollen yielding tree. In spring, canola (*Brassica napus* cv) and capeweed (*Arctotheca calendula*) were commonly targeted. Manning (2001) reviewed the literature on fatty acids in pollen and their importance to honey bees. He stated that linoleic acid had antimicrobial properties that inhibited growth of *M. pluton*. Linoleic acid formed approximately 50% and less than 10% of the total lipid of capeweed and *Brassica* species pollen respectively. In Victoria, any relationship between these two plants and spring outbreaks of EFB may be entirely coincidental, but it is possible that the majority of districts in which these plants grow also have cool and frequently changing weather. This weather restricts honey bee foraging and impacts on the ability of colonies to access uninterrupted supplies of nectar and fresh pollen (as opposed stores in the hive). Our study of the incidence of EFB in commercially managed honey bee colonies fed a dietary supplement also indicate this.

Recommendations arising from the survey

The research team recommends that:

- OTC portion control packs containing a single hive dose be developed to ensure that colonies receive the correct dose
- scientific studies be conducted to determine the effect of:
  - fatty acids on the incidence of EFB and their application to honey bee colonies to prevent - and/or control the disease
  - feeding of protein supplements to improve nutrition and minimise the incidence of EFB under different field conditions
  - inclement weather and restricted honey bee foraging for nectar and pollen on the incidence of EFB
  - young honey bee queens on the incidence of EFB.

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A beekeepers' guide to understanding control measures for European Foulbrood

The antibiotic oxytetracycline hydrochloride (OTC) is used for the control of European foulbrood in all Australian states except Western Australia, but the potential for residues to remain in honey poses a serious threat to the honey's future marketability.

This publication presents outcomes of investigations concerning the application of OTC to honey bee colonies, degradation of OTC in honey extracted from treated hives and efficacy of lower doses of OTC to control European foulbrood.

The report also discussed studies on *Melissococcus pluton,* the causal organism of the disease.

Publication No 04/091
RIRDC Project No DAV-157a

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