

European Foulbrood Disease

A Threat to the New Zealand Beekeeping Industry

Author : Robert N. Rice
Apiculture Services Manager (South Island)
MAF Quality Management
Lincoln, New Zealand

Commissioned By : MAF Quality Management
Wellington, New Zealand

Submitted : June 1996

EXECUTIVE SUMMARY

European foulbrood (EFB) is a honey bee disease exotic to New Zealand caused by the bacterium *Melissococcus pluton*. The disease is widely distributed in most areas of the world, including Australia. In countries where the disease exists, it is generally regarded as a disease of minor importance. However, on introduction to a susceptible honey bee population, the impact of the disease may be severe. The immediate impact of an incursion of EFB into New Zealand would be as follows:

Export of Honey Bee Products

The presence of *M. pluton* in New Zealand should not result in lost of market access to most overseas countries. However, because many of the countries have existing sanitary requirements requiring "area freedom from" listed bee diseases including EFB, individual beekeepers may find it impossible to export honey from specific apiaries to a number of these overseas markets.

Live Bee Exports

The majority of live bee exports are to Korea and Canada. Korea would immediately ban all imports of bees, whereas Canada requires an "area freedom from" declaration. Live bees enroute to Canada are trans-shipped through the U.S.A.. The Americans therefore have the option of revoking these trans-shipment rights. With the exception of Israel, which requires that production hives are disease free, other countries require export certification similar to that required for honey exports. As a result of "area freedom from" requirements beekeepers may therefore not be able to export bees from specific apiaries to a number of these countries.

Honey Production

If the Chief Veterinary Officer initiates an EDPR response, procedures for EFB (ie. MQE6) allow for the immediate use of antibiotics for the eradication of EFB. This use of antibiotics during the initial outbreak will limit the production losses to hives exhibiting symptoms of the disease.

Pollination

If an EFB outbreak occurs during crop pollination (eg. kiwifruit and apples), the outbreak may result in a significant reduction in crop yields. EFB will cause a significant loss in hive populations, thus reducing the effectiveness of these hives as pollinators.

Long-Term Antibiotic Use

It is unclear whether after the EDPR response, the continued use of antibiotics would be allowed. This will be the single greatest issue resulting from an incursion of EFB.

Extensive consultation with industry is required. In the long-term, a "knee-jerk" response to allow the on-going use of antibiotics may in fact cause more problems for the beekeeping industry than the disease that it is meant to control. The use of antibiotics may have a dramatic impact on reducing export market access, especially to Japan (which requires certification that antibiotics are not used in the production of the honey to be exported).

Disease Biology and Diagnosis

There is an absence of quantitative information in the scientific literature detailing the epidemiology of EFB and the damage it causes (eg. the minimal effective dosage of *Melissococcus pluton* is not known). The disease often persists as a sub-clinical infection with outbreaks occurring in spring and in response to environmental conditions such as inclement weather or colony stress. The disease is dispersed quickly by both natural and artificial means, and in particular by the activities of the beekeeper during normal hive management practices.

The larvae of worker bees, drones and queens are susceptible to infection by *M. pluton*. Dying and dead larvae change colour, initially turning yellowish and then yellowish-brown. Dead larvae are often found lying across the mouth of the cell or twisted in a spiral stretched out along the length of the cell. The larvae collapse as if melted and eventually dry up to form a loosely attached brown scale. Surveillance and EDPR delimiting surveys currently rely on searching for these visual symptoms of EFB.

Halfmoon Disorder (HMD), a nutritional disorder of honey bees, exists in New Zealand, and exhibits symptoms very similar to those of EFB. This similarity in visual symptoms may lead to the misdiagnosis of EFB as HMD. Suspect samples of EFB are microscopically examined and if they contain "streptococcus-like particles" they are plate cultured. Positive identification requires an additional 4 to 22 days.

Two alternate methods for detection of *M. pluton* exist, although neither are currently available as commercial products. An enzyme-linked immunosorbent assay (ELISA) has been developed that detects the presence of *M. pluton* at levels of $\geq 10^5$ cells per ml of sample. Also under development is a polymerase chain reaction (PCR) based technology that will detect the presence of *M. pluton* at levels of ≥ 100 cells per ml of sample. Both of these techniques can return a positive identification in one day, although in terms of sensitivity of diagnosis only the PCR-based technology is applicable for surveillance and import product testing.

M. pluton will most likely enter New Zealand via illegally imported bee products, although entry via legally imported bee products is a potential risk. The sensitivity and reliability of current detection techniques is questionable. These techniques form the basis of New Zealand's honey bee surveillance system and bee product import testing procedures for this disease, and for this reason it is possible that *M. pluton* could become endemic in New Zealand before it is "officially" detected.

RECOMMENDATION SUMMARY

As a consequence of this review on the threat that EFB poses to the New Zealand beekeeping, horticultural and agriculture industries, the following recommendations are made:

- 1) That a full review of the current EFB surveillance procedures should be conducted, particularly regarding field sampling and laboratory diagnostic procedures, to ensure that these procedures are reliable in detecting *Melissococcus pluton*.
- 2) That the current EFB diagnostic procedures used to test imports should be evaluated with regards to their sensitivity, reliability, speed of diagnosis and cost-effectiveness.
- 3) That funding should be continued for the development of the EFB PCR-based diagnostic procedure, and that the use of PCR-based diagnostics should be investigated for the detection of exotic honey bee pests.
- 4) On completion of the development phase of the PCR-based EFB diagnostic procedure, this procedure replace the existing procedures of microscopic examination and confirmation by plate culturing.
- 5) That a review of procedures should be conducted for the delimiting survey function of an EFB EDPR response (MQE6: Honey Bee.Exotic Disease and Pest Response Procedures) to ensure that these procedures will be effective in determining the actual spread of *M. pluton*.
- 6) That a review of procedures should be conducted for the interpretation of survey data by the HQ technical advisor.
- 7) That in response to an EFB incursion, all relevant industries should be consulted extensively before a decision is made regarding the use of antibiotics for the control of EFB.

TABLE of CONTENTS

EXECUTIVE SUMMARY	ii
RECOMMENDATION SUMMARY	iv
TABLE of CONTENTS	v
1. BACKGROUND	1
2. BIOLOGICAL FEATURES	2
2.1 Life cycle	2
2.1.1 Secondary Infections	2
2.2 Population Dynamics and Epidemiology	2
2.2.1 The Infection Cycle	2
2.2.2 From Endemic to Epidemic, An EFB Outbreak	3
2.3 Mode of Dispersal Between Hives and Apiaries	4
2.4 Rate of Dispersal	4
2.4.1 The Australian Experience	4
2.4.2 Threat to New Zealand	6
2.4.3 Rate and Mode of Dispersal by Season	8
2.4.3.1 Artificial Dispersal	8
2.4.3.2 Natural Dispersal	8
3. IDENTIFICATION	10
3.1 Clinical Symptom	10
3.1.1 Halfmoon Disorder or EFB	10
3.2 Culturing Techniques	11
3.2.1 Isolation of Bacteria	11
3.2.2 Speed and Accuracy of Diagnosis	11
3.2.3 Cost of Diagnosis	12
3.3 New Developments in Identification Technology	12

4. SURVEY AND SAMPLING TECHNIQUES	13
4.1 Within The Apiary	13
4.2 Importation of Apiary Products	13
5. MANAGEMENT STRATEGIES	14
5.1 Breeding for Resistance	14
5.2 Thermal Destruction	14
5.3 Antibiotic Control	15
5.3.1 Success of OTC Control Techniques	16
5.4 Cleaning and Disinfection	16
5.4.1 Cleaning and Disinfection between Hives	17
5.4.2 Cleaning and Disinfection between Apiaries	17
5.5 Movement Control Strategies	17
6. ANTIBIOTIC VERSES NON-ANTIBIOTIC MANAGEMENT	18
6.1 EFB - The Immediate Impact	18
6.1.1 The Impact on Honey Production	18
6.1.2 The Impact on Queen Bee and Package Bee Production	21
6.1.3 The Impact on Pollination	21
6.2 Management of Endemic EFB	22
6.2.1 Antibiotic Management of EFB	22
6.2.1.1 Benefits of Antibiotic Management	22
6.2.1.2 Costs of Antibiotic Management	23
6.2.1.3 Loss of Income	23
6.2.2 Non-antibiotic Management of EFB	24
6.2.2.1 Benefits of Non-antibiotic Management	24
6.2.2.2 Costs of Non-antibiotic Management	25
6.2.2.3 Cost of Colony Replacement	25
6.2.2.4 Loss of Income	27
7. CONCLUSION	29
BIBLIOGRAPHY	31

1. BACKGROUND

European foulbrood (EFB) is caused by the bacterium *Melissococcus pluton*. *M. pluton* is a Gram-positive bacterium, lanceolate in shape, occurring singly, in clusters or in chains of various lengths. The organism was initially suspected as the causative agent of European foulbrood by White (1912, 1920), and was named *Bacillus pluton*. Subsequent isolation and the characterisation of *B. pluton*, based on morphology and its inability to form spores (characteristic of the genus *Bacillus*), resulted in the bacterium being renamed *Streptococcus pluton* (Bailey, 1957). Most recently, determination of the guanine + cytosine content of its nucleic acids at about 29% (Bailey and Collins, 1982a) has resulted in the organism being excluded from the genus *Streptococcus*. As now defined (Hardie, 1986), the organism has become the type species of a new genus *Melissococcus* (Bailey and Collins, 1982b, 1983).

2. BIOLOGICAL FEATURES

2.1 Life cycle

The larvae of worker bees, drones and queens are susceptible to infection by *Melissococcus pluton*. Nurse bees transmit *M. pluton* among larvae during feeding, the bacteria contaminating the mouth parts of the nurse bee and hence the food fed to larvae. Larvae are susceptible to infection while they remain uncapped and are still being fed. The bacteria are ingested by the larvae, ingestion usually occurring 48 to 72 hours after the eggs have hatched. Multiplication of *M. pluton* occurs close to the surface of the peritrophic membrane of the larval gut in contact with the food of the larvae. *M. pluton* actively competes with the larva for food secreted by the nurse bees, absorbing its nutritional requirements from this food. As growth of the bacterial mass continues, it extends toward the centre of the lumen of the peritrophic sac. Unable to compete for food with the expanding bacterial mass, the larva dies of starvation. Larvae usually pass through the fourth day of larval development before death occurs, which is 2-3 days post infection.

2.1.1 Secondary Infections

Death of the host may be accelerated by secondary bacterial infection. *Achromobacter* (*Bacterium*) *eurydice* (White, 1912) is the most common secondary bacteria. Other bacteria found in association with this disease include *Streptococcus faecalis*, formerly *Streptococcus apis*, (Maassen, 1907), a bacterium which is similar in appearance to *M. pluton* but serologically and culturally distinct (Bailey and Gibbs, 1962; Gubler, 1954); *Bacillus alvei* (Cheshire and Cheyne, 1885) and *Bacillus laterosporus* (McCray, 1917).

2.2 Population Dynamics and Epidemiology

2.2.1 The Infection Cycle

Once ingested, *Melissococcus pluton* multiplies rapidly in the anaerobic environment of the larval midgut. Not all larvae infected with *M. pluton* die. Larvae that become infected later than the third day of development may survive and pupate. The infected larvae discharge *M. Pluton* in their faeces. Faecal deposits occur mainly at the base and capping of the cell. The bacteria contained within the faeces remains infective for several years. Non-lethal infections of larvae result in pupae of sub-normal weight. These pupae generally have poorly developed silk glands which cause them to spin incomplete cocoons. As a result, the bacteria are spread from the exposed faeces of infected larvae. The faeces of healthy larvae generally remain sandwiched between the layers of the completed cocoons (Bailey and Ball, 1991).

The majority of bacteria are removed by house-cleaning bees. However, remaining bacteria contaminate the larval food supply, thus maintaining the infection and often at a sub-clinical level. The presence of microscopically undetectable, subclinical infections of *M. pluton* in

apparently healthy Australian beehives was demonstrated using an ELISA test (Pinnock and Featherstone, 1984). The results of Pinnock and Featherstone (1984) showed that an infection may persist for many years without visible signs of disease.

Sub-clinical infections of *M. pluton* occur when a balance develops within infected colonies between the rate of production and dissemination of the bacteria and the removal of bacteria and infected larvae. Infected larvae are often removed before symptoms of the disease are visible to humans. The level of *M. pluton* within the colony fluctuates, and is linked to the availability of brood food secreted from the hypopharyngeal glands of the nurse bees. It has been shown experimentally that when larvae receive excess amounts of glandular food, infected colonies keep proportionately more infected larvae than usual. Conversely, when larvae are starved for glandular food, infected colonies remove more infected larvae than usual (Gontarski, 1953; Bailey, 1960; Simpson, 1958).

2.2.2 From Endemic to Epidemic, An EFB Outbreak

With the unfolding of spring hive activity increases and the colony expands. Optimal conditions for colony expansion are influenced by many factors such as the availability of food and weather patterns. These conditions in turn influence the behaviour patterns of the worker bee population. For example, recently emerged worker bees tend to perform internal hive functions such as cleaning or brood feeding while older worker bees forage. However, during conditions such as nectar flows, many of the younger bees are recruited into foraging activities.

During colony expansion, environmental conditions such as inclement-weather or other events that interrupt nectar flow can impede colony growth. If *Melissococcus pluton* is present in the colony as a sub-clinical infection, an interruption in colony growth can lead to an increased level of the disease (Bailey and Ball, 1991). This increase is in response to the temporary abundance of nurse bees and hence food available to the larvae.

When the nectar flow begins again, brood rearing increases and the amount of glandular food available to individual larvae decreases. The decrease in availability of glandular food is linked to 1) the lag between the increase in egg laying by the queen and the availability of young nurse bees to feed the developing larvae, and 2) the recruitment of nurse bees to foraging activities. Under these conditions the infected larvae, in competition with *M. pluton* for food, starve and die. The rate of larval death now exceeds the bees' ability to detect and remove infected larvae. This results in an outbreak of European foulbrood (EFB) as evidenced by the clinical symptoms of dead larvae within the brood combs. Infections may be exacerbated if an infected colony is not genetically predisposed to uncapping and removing infected larvae, a trait known as "hygienic behaviour" (Rothenbuhler, 1964a 1964b; Moritz, 1988).

2.3 Mode of Dispersal Between Hives and Apiaries

Melissococcus pluton is dispersed both by natural and by artificial means (Hornitzky, White and Ward, 1978). Typically though, *M. pluton* is spread by artificial means, particularly through the activities of the beekeeper during normal hive management practices (Hornitzky, White and Ward, 1978).

The reported modes of disease dispersal are:

- a) The practice of interchanging hive components, particularly brood combs between hives,
- b) Robbing of honey from diseased colonies by healthy colonies (*M. pluton* is transmitted in honey),
- c) Drifting of infected bees from diseased to healthy hives,
- d) Contamination of healthy colonies by forager bees through the use of common watering places or open sugar feeders (infected bees may fall into or defecate in the water or feeders),
- e) Feeding of contaminated honey and pollen.

2.4 Rate of Dispersal

There is no available scientific data concerning the rate of dispersal for EFB within hives, between hives or between apiaries. There has been a large body of anecdotal evidence published however, the majority of which is found in unrefereed industry journals. In addition, the minimal infective dose of *Melissococcus pluton* has yet to be determined. In order to evaluate the potential rate of dispersal of EFB in New Zealand, we turn to Australia to provide the model.

2.4.1 The Australian Experience

Spread of EFB

The presence of *Melissococcus pluton* was confirmed in eastern Australia in September 1977 (Tham, 1978). Within 12 months of the initial confirmation, EFB was identified in beehives in all eastern states except Tasmania. The spread of EFB was attributed to the migratory nature of the Australian beekeeping industry, with the disease spreading from apiary to apiary by both natural and artificial means.

A 30 km wide quarantine corridor was established between Victoria and New South Wales to stop the spread of EFB northwards. Unfortunately, the quarantine corridor was not respected by all beekeepers and so EFB spread to New South Wales. Quarantine measures

also failed to stop EFB spreading from New South Wales into Queensland and eventually into Tasmania in July 1984 (Ward *et. al.*, 1985). EFB has been reported as present in Western Australia (Matheson, 1993). This report however, was based on an absence of information about the presence of the disease, and not as the result of a confirmed laboratory diagnosis.

The introduction of *M. pluton* into Australia caused extensive losses in both amateur and commercial apiaries. One report states that "The beekeeping industry in South Eastern [Sic] Australia has been rocked back onto it's economic heels by an infection known as European brood disease. It has put beekeepers out of business, broken up families and devastated the economics of bee-keeping [Sic] in South Australia, Victoria and parts of New South Wales" (Walton, 1979). In Walton's report he states that EFB had spread through 60,000 hives in the two years following the introduction of *M. pluton*. EFB is now endemic throughout eastern Australia (Ward *et. al.*, 1985).

Suppression and Spread of AFB

The cost of EFB to the eastern Australian honey industry can be assessed in two ways. First, there is the direct cost of antibiotic treatment for each beehive to control EFB. Second, there are the indirect costs associated with the spread of American foulbrood (causative agent *Bacillus larvae*). Clinical symptoms of the more damaging honey bee disease American foulbrood (AFB) are disguised by antibiotic treatment to control EFB (Moffet *et. al.*, 1970; Wilson *et. al.*, 1971; Hoopingarner and Nelson 1988; Bailey, L. 1981; Oldroyd *et. al.*, 1989). Oldroyd *et. al.* (1989) demonstrated the effects of oxytetracycline on AFB. They showed that:

- a) clinical symptoms of AFB disappeared within 30 days following treatment with 1 gram of active ingredient,
- b) AFB infected colonies were not all cured by OTC therapy, and the clinical symptoms of the disease recurred up to 14 months after treatment (in the trial, 60% of colonies developed AFB symptoms after OTC therapy),
- c) OTC treatment did not provide colonies with complete protection from a transitory high level of *B. larvae* spores for more than a few weeks.

In addition, Gilliam and Argauer (1981) demonstrated that OTC degrades in the brood nest in less than four weeks. This supports Oldroyd *et. al.*, (1989) conclusion that OTC only provides temporary repression of AFB clinical symptoms, not a cure.

The suppressive effect of OTC on AFB is believed to allow *B. larvae* to spread unnoticed and unchecked (Rendall 1981; Braybrook, 1985). In this case, the cost to the beekeeper will be the destruction of productive hives should symptoms of AFB become visible (as prescribed by the Australian laws). Oldroyd *et. al.* (1989) showed that once OTC treatment for EFB was withdrawn, endemic *B. larvae*, if present in hives, could develop into AFB (as diagnosed by its visible symptoms). It was predicted by Oldroyd *et. al.* (1989), that as a

consequence of using OTC for EFB control, the incidence of AFB would increase in Australia. A similar situation was thought to exist in the United Kingdom (Rendall, 1981). AFB is now wide-spread in Queensland even though it was uncommon in this state until 1987 (Warhurst, 1996). It appears that as a consequence of the use of antibiotics, beekeepers have changed their management practices and no longer try as hard to control AFB.

The increase in the incidence of AFB is due to a number of factors including:

- a) antibiotics do not kill AFB spores which are present in honey and on hive equipment, and can cause reinfection,
- b) beekeepers may not be using antibiotics correctly (with respect to dosage and timing),
- c) the development of antibiotic resistant strains of *B. larvae* can occur,
- d) there is only limited protection from AFB when OTC is fed for EFB control.

2.4.2 Threat to New Zealand

EFB was reported in Australia as being highly contagious and once established it spread rapidly throughout eastern Australia. The Australian experience also demonstrated that while the spread of EFB between hives and apiaries of an individual beekeeper occur by natural means, it is accelerated by artificial means (ie. the transfer of equipment between hives). On the other hand, the spread of EFB between apiaries belonging to different beekeepers generally occurs by natural means such as bees robbing hives in nearby apiaries (Hornitzky *et. al.*, 1978; White and Ward, 1978).

Field observations in Australia suggest that regional hive density influences the rate at which EFB spreads. An increase in regional hive density accelerates the rate at which EFB spreads (Hornitzky *et. al.*, 1978; White and Ward, 1978). It was observed that when infected apiaries were placed in close proximity to healthy apiaries, the healthy apiaries also quickly became infected (Hornitzky *et. al.*, 1978; White and Ward, 1978). A similar experience occurred in New Zealand when chalkbrood was first detected in Northland in 1983. Chalkbrood spreads by the same modes of dispersal as *M. pluton* (McKenzie, 1985).

As in Australia, many regions of New Zealand contain a large number of beehives. For example, about 75,000 beehives are domiciled in the Bay of Plenty for the pollination of kiwifruit. In Australia, the highly productive regions are ever-changing and seasonal, so beekeepers move their hives into productive areas for several weeks as they follow the nectar flows. Unlike Australia, New Zealand beekeepers not involved in paid pollination are mostly non-migratory and so keep their beehives in permanent apiaries. Large numbers of permanent apiaries are found in the highly productive regions of New Zealand, for example the clover pastures of the Canterbury Plain. There are, however, some exceptions to this general movement pattern, including shifts of hives to ling heather and honeydew.

While most beekeepers in New Zealand are non-migratory, many of the larger beekeepers maintain apiaries in several regions and in so doing mimic the migration of beehives by moving beekeeping equipment between regions. The dispersal of EFB in New Zealand is therefore likely to parallel the dispersal of EFB in Australia, spreading throughout New Zealand very quickly. Also, regardless of which island initially becomes infected with EFB, without an effective inter-island quarantine system for honey bees and bee products, the other island will also become infected. This occurred in Australia with the spread of EFB from mainland Australia to Tasmania. As in Australia, the most likely avenue by which the disease would spread between the islands is via the shipment of live bees, and in particular queen bees.

2.4.3 Rate and Mode of Dispersal by Season

2.4.3.1 Artificial Dispersal

Spring

- Rapid dispersal rate,
- Spread within and between apiaries by interchange of hive equipment and brood nest manipulation,
- Outbreak likely in same season as the initial infection occurs.

Summer

- Moderate dispersal rate,
- Spread within and between apiaries potentially by the interchange of honey boxes (supers),
- Outbreak possible in same season but more likely in following spring as clinical symptoms may not be obvious.

Autumn

- Moderate to low dispersal rate,
- Spread mostly within apiaries by hive manipulations in preparations for winter (such as adding frames of contaminated honey or feeding of contaminated honey),
- Outbreak in following spring.

Winter

- Low to extremely low dispersal rate,
- Spread unlikely and confined to apiary,
- Outbreak in following spring.

2.4.3.2 Natural Dispersal

Spring

- Rapid dispersal rate,
- Spread within and between apiaries by drifting of bees between hives, robbing of diseased hives by healthy colonies, cross-contamination of floral sources and contamination of communal watering sites,
- Outbreak likely in same season.

Summer

- High dispersal rate,
- Spread within and between apiaries by drifting of bees between hives, cross-contamination of floral sources and contamination of communal watering sites,
- Outbreak possible in same season but more likely in following spring.

Autumn

- Moderate dispersal rate,
- Spread mostly within apiaries by robbing of diseased colonies by healthy colonies, drifting of bees between hives and contamination of communal watering sites.
- Outbreak in following spring.

Winter

- Low to extremely low dispersal rate,
- Spread within apiaries by robbing of diseased colonies by healthy colonies,
- Outbreak in following spring.

3. IDENTIFICATION

3.1 Clinical Symptom

- a) Diseased brood has a mottled, "peppered" appearance. In heavy infections larvae may die after capping, in which case the cell caps will appear sunken and may be perforated.
- b) Drone brood is very susceptible.
- c) Larval death usually occurs at four to five days of age. During the initial stages of infection, larvae are unusually active in the brood cell. Dead larvae are found in unnatural positions, often lying across the mouth of the cell or twisted spirally or stretched out along the cells.
- d) Dying and dead larvae change colour, initially turning yellowish and then yellowish-brown. The larvae collapse as if melted and eventually dry up to form a loosely attached brown scale. Scales may be removed by bees.
- e) The consistency of recently dead larvae varies and may be either sticky, sloppy or ropy. Larvae may contain lumps.
- f) The smell of infected brood varies from no odour to either sour or putrid.
- g) The symptoms of the disease may be more pronounced in the outer combs of the brood nest, and may show symptoms earlier than in the inner combs.
- h) Uninfected adult bees may appear agitated.
- i) Diseased hives rapidly become unproductive, while smaller colonies in an apiary are usually heavily infected and often queenless (Hornitzky, 1978).

3.1.1 Halfmoon Disorder or EFB

Halfmoon Disorder (HMD) is a syndrome of honey bees believed to be a nutritional disorder (Anderson, 1988, 1993). Halfmoon Disorder exhibits symptoms very similar to that of EFB. Larvae dying of HMD are found coiled and turn yellow. The dead larvae eventually dry into 'crescent' or 'halfmoon' shaped brown scales. The scale is often positioned half-way around the lip of the cell (Matheson, 1984).

To the trained eye EFB and HMD can be differentiated by associated symptoms--if present. Typically, multiple egg-laying (with the eggs attached in strings) or the presence of unfertilised eggs (that develop into drone brood) are observed in conjunction with the other symptoms of HMD (Anderson, 1988). Without the presence of these associated symptoms, it is impossible in the field to reliably differentiate EFB and HMD.

MAF officers rely heavily on the vigilance of beekeepers to report the presence of suspicious bee disease symptoms. New Zealand beekeepers who have not travelled overseas will have never seen clinical symptoms of EFB first hand. Because of the similarity in symptoms between EFB and HMD it is possible that a beekeeper may make a misdiagnosis. Beekeepers may believe that the clinical symptoms they are seeing are caused by HMD when in fact they are caused by EFB. The potential for the misdiagnosis of field symptoms EFB by beekeepers could result in the disease becoming endemic before it is "officially detected" by MAF's current surveillance system.

Because of this possibility of an incorrect diagnosis by a beekeeper, it is recommended that a revision of the current New Zealand MAF surveillance programme for disease be undertaken. It is of paramount importance that the MAF surveillance system is capable of quickly detecting an incursion of *Melissococcus pluton*.

3.2 Culturing Techniques

3.2.1 Isolation of Bacteria

Bacteria are isolated from diseased larvae in 0.5 ml of sterile 0.85% sodium chloride. A smear is made on a glass slide and negatively stained with 0.2% carbol fuchsin. The smear is examined for the presence of bacteria by light microscopy at a magnification of x1000. Extracts that contain cells resembling *Melissococcus pluton* (single, pairs and short chains of lanceolate cocci) are inoculated onto plates of basal medium consisting of 1% Oxoid L37 peptone, 1% starch, 1% glucose and 0.1% cysteine in 0.1M potassium phosphate buffer pH 6.7 (Bailey and Collins, 1982b). Two plates are prepared for each extract and inoculated, one plate incubated aerobically; and the other anaerobically at +10%(vol/vol) carbon dioxide at 35°C for 3 to 6 days. Individual bacterial colonies exhibiting anaerobic growth are sub-cultured. Their identity is confirmed by negative staining and light microscopy. It should be noted that some strains of *M. pluton* have required incubation periods of up to 22 days to grow colonies (Allen & Ball, 1993).

3.2.2 Speed and Accuracy of Diagnosis

The microscopy diagnostic technique used in New Zealand is sensitive down to 10^7 cells/ml (Pinnock and Featherstone, 1984) and as such, is useful only for the identification of *Melissococcus pluton* in larval samples with confirmation by plate culturing. Detection by plate culturing is time-consuming requiring as long as 22 days for confirmation. Wootton *et. al.* (1981) demonstrated that honey bee colonies naturally infected with *M. pluton* can harbour *M. pluton* cells at levels below 3.3×10^3 viable organisms/ml of honey. At this concentration the only method of detection for *M. pluton* currently available is plate culturing.

During an EDPR response the delimiting survey could be compromised if this diagnostic technique is relied upon. MAF would only be able to detect the presence of *M. pluton* quickly when clinical symptoms are present, and so it is likely that the actual spread of EFB would be faster than the observed spread (based on this current diagnostic procedure).

Also, as demonstrated by the Nelson EDPR response, the technique currently used is prone to misdiagnosis and potentially inaccurate.

3.2.3 Cost of Diagnosis

The cost of the plate culturing technique for the identification of *Melissococcus pluton*, is dependent on the number of samples, and ranges from \$200 per sample for individual samples tested to \$25 per sample for large numbers of samples (as was encountered during the Nelson EDPR response for EFB).

3.3 New Developments in Identification Technology

Two alternate methods for detection of *Melissococcus pluton* exist, although neither are currently available as commercial products. Pinnock and Featherstone (1984) developed an enzyme-linked immunosorbent assay (ELISA) test capable of detecting the presence of *M. pluton* in adult bees at a level of 10^5 cells per ml of sample. Currently under development is a polymerase chain reaction (PCR) based diagnostic test. The technique has the potential to detect very low levels of *M. pluton* in different sample types (eg. honey or larvae); theoretically detecting the presence of just a few cells of *M. pluton* in a sample (Dancer, 1996). In addition to the sensitivity of this test, it is possible to obtain a diagnosis in just one day. Both of these techniques are adaptable to present technology available in New Zealand. The PCR-based diagnostic technique is currently under evaluation by MQM.

Because of the sensitivity and speed of PCR-based diagnostics, it is recommended that funding for the development of EFB PCR-based diagnostic test technique continues. It is also recommended that following the completion of the development of the PCR-based EFB diagnostic test, that this test replace the existing EFB diagnosis test.

4. SURVEY AND SAMPLING TECHNIQUES

4.1 Within The Apiary

Suspect samples of EFB are routinely collected by apiary inspectors and beekeepers. The samples are collected during the National Beekeeper's Association (NBA) AFB contract inspections and during the exotic disease surveillance program conducted by MAF - Quality Management (MQM). Single larvae are removed from the brood comb and put into plastic sample bottles. The samples are sent to the Animal Health Laboratory at Invermay for diagnosis (section 3.2.1). The cost of the survey and sampling program is absorbed primarily by the MAF Regulatory Authority (MAF-RA) surveillance contract and partially by the NBA AFB inspection contract.

4.2 Importation of Apiary Products

The current system of risk assessment and laboratory testing is intended to screen out high risk category imports. Bee products are approved for import on the basis that they are not likely to come into contact with bees, are in a form not attractive to bees, and/or are deemed by laboratory test or processing to be "disease free". Such products include royal jelly, honey coated fruit and beverages. The New Zealand market for royal jelly based products is currently worth approximately \$3 million per annum. In addition, there have been recent proposed changes to the import regulations concerning Australian honey. Clearly, there is a large market in New Zealand for imported bee products, many of which originate from countries known to harbour pest and diseases not present in New Zealand.

To maintain New Zealand's agricultural security, the diagnostic test used to test imports for exotic diseases must reflect the sensitivity and cost effectiveness of new technologies as they become available. This is of particular importance given that the primary requirement for the importation of a product is that it is "disease free" or at least considered "safe". MQM must remain vigilant in its efforts to maintain agricultural security and in doing so be prepared to validate the reliability and sensitivity of current diagnostic procedures.

As discussed, the current diagnostic test in use can only detect (microscopically) the presence of *Melissococcus pluton* down to 10^7 cells/ml of sample. Additionally, the end-point of sensitivity for plate culturing of *M. pluton* has not been empirically determined, nor is the minimal infective dose of *M. pluton* known. It is therefore of some concern that the true potential danger for the introduction of EFB into New Zealand (via imports) may have been underestimated. This situation warrants an evaluation of current diagnostic procedures for the detection of *M. pluton*.

5. MANAGEMENT STRATEGIES

5.1 Breeding for Resistance

Honey bees can be bred for disease resistance (Rothenbuhler, 1964a, 1964b; Moritz, 1988) by selecting and developing suitable strains of honey bees. However, the effect of EFB on susceptible bee populations tends to be severe initially, and antibiotics may be needed for several years until resistant strains of bees can be selected and distributed commercially.

There is little doubt that a breeding programme could develop *Melissococcus pluton* resistant stock. However, in reality this may be difficult to achieve. If breeding of disease resistant stock is possible, then why are antibiotics used to control EFB and AFB in many countries throughout the world? It may be that the soft option of drug control verses a focused breeding programme for disease resistance is a better "business" alternative, at least in the short term. In New Zealand where *Bacillus larvae* is endemic, there are no breeding programmes focused on developing *B. larvae* resistant strains, only strains that demonstrate "hygienic behaviour".

Beekeepers wishing to maintain organic (BioGro) registration or continue to use drug-free beekeeping practices are most likely to seek out EFB-resistant strains of honey bees. Other beekeepers are likely to lobby for the introduction and use of antibiotics to control bee diseases.

5.2 Thermal Destruction

Honey contaminated with *Melissococcus pluton* can be sterilised by heating the honey to 80°C for 30 minutes. Sterilisation can also be achieved at low temperatures but the duration of heating is substantially increased (Wootton *et. al.*, 1981). Honey contaminated with *Bacillus larvae* can also be sterilised by heating the honey to 104.5°C for 60 minutes or alternately to 140°C for 50 seconds (White and Stutevant, 1954). The difference between the temperatures and duration of heating is indicative of the difference in these two genera of bacterium. *B. larvae* is an endospore-forming bacteria and like many spore-forming bacteria resistant to heat sterilisation (VanDemark and Batzing, 1987). *M. pluton* on the other hand does not form endospores and as such is heat labile (Wootton *et. al.*, 1981).

The technique of dipping wooden beehive components in hot paraffin wax to preserve the timber against wood rot has been used in New Zealand for at least 50 years (Matheson, 1985). It is also an effective method of sterilizing hive parts contaminated with *B. larvae*. Hive components are immersed for at least 10 minutes in paraffin wax heated to 160°C (Matheson and Reid, 1992). The work of Wootton *et. al.* (1981) suggest that this method would prove effective in sterilizing EFB contaminated hive parts. This method of sterilization is part of the current EDPR response procedure for EFB as described in MQE6: Honey Bee Exotic Disease and Pest Procedures.

5.3 Antibiotic Control

Melissococcus pluton manifests itself in spring when brood rearing increases and there is a proportional drop in the number of nurse bees. In countries where EFB is endemic, it is controlled with the antibiotic oxytetracycline hydrochloride (OTC), commercially marketed as Terramycin™ by Pfizer Agricare Pty. Ltd. Treatment should occur when the symptoms of EFB first appear in an apiary. However, the drug is usually administered as a prophylactic. Treatment of infected hives is by direct application of OTC to the broodnest. Treatment of hives involves the application of 1 g of OTC active ingredient per hive, with all hives in the apiary being treated (whether they show signs of EFB or not). Hives are routinely treated one and sometimes two times per year by one of the following methods:

- a) In a pattie or cake consisting of 1 g of OTC, and materials attractive to bees such as castor sugar, pollen and yeast,
- b) By dissolving 0.330 g of OTC in 200 ml of cold 2:1 sugar/water solution, the mixture being poured over the tops of the frames of the broodnest once a week for three consecutive weeks (this method is no longer recommended in Australia),
- c) By adding 1g of OTC base to 500 ml of a cold 2:1 sugar/water solution which is poured into an internal feeder,
- d) By mixing 1 g of the OTC with 80 g of castor sugar, and sprinkling the mixture over the top bars of the frames of the broodnest (the mixture should be prepared fresh each day and administered immediately otherwise it forms hard "cakes" and the antibiotic properties of the mixture degrade),
- e) Administered as in (d) above, but with the castor sugar replaced with dextrose.

The methods of drug application have been devised to minimise the risk of contaminating honey with OTC. Beekeepers however, do not always use antibiotics correctly and at the correct dosage rate. It is possible that when antibiotics are not correctly administered, they can end up as residues in honey produced for export and local markets (Matsuka and Nakamura, 1990). Gilliam and Argauer (1981) demonstrated that OTC degrades in the broodnest in less than four weeks. Pfizer Agricare (1991) therefore recommend a withholding period of 8 weeks (between the last treatment of OTC and the extraction of honey) to allow contaminating antibiotics to degrade.

Mixing and application of OTC is of minimal risk to the operator, as the drug itself is not toxic to humans. However, caution should be exercised by the operator to minimise contact with the drug. Simple precautions as required by Occupational Safety and Health (OSH) in the wearing protective clothing (ie. overalls, safety glasses, boots and gloves) should be taken while dispensing antibiotic mixtures. Nevertheless, in countries where OTC is used regularly by beekeepers, protective clothing is rarely worn.

Before antibiotic control of EFB could occur in New Zealand, the Director of Agriculture would be required to approve the use of the antibiotic by way of a *New Zealand Gazette* notice, since the section of the Apiaries Act relating to drugs (Section 25 - "Prohibition on use of drugs") is retained by Section 171 of the Biosecurity Act.

5.3.1 Success of OTC Control Techniques

OTC is the common and preferred method used to control EFB internationally. If used in accordance with the manufacturers directions, OTC poses little risk as a contaminant to honey, or as a threat to human welfare. However, it has not been demonstrated that OTC will eradicate *M. pluton*. The drug only controls the organism, and then only as part of a disease management strategy.

The use OTC for EFB control requires changes in beekeeping management practices. If the drug is administered correctly, beekeepers will apply one application of OTC in early spring as a prophylactic, with perhaps a subsequent application of OTC if symptoms of EFB are observed. Beekeepers who experience both EFB and AFB outbreaks soon discover the utility of OTC for suppressing AFB. Although AFB control is an illegal use of OTC in a number of countries, the use of the drug to suppress AFB symptoms becomes a "soft option" for the beekeeper, especially when compared to the traditional method of AFB control (burning infected hives). In order to continue the suppression of AFB, beekeepers must further modify their management practices and apply the drug on a routine basis as a prophylactic.

Recently, OTC resistant strains of *Bacillus larvae* have been found in Argentina (Alippi, 1994). These OTC resistant strains of *B. larvae* have developed probably as a consequence of the misuse of OTC in suppressing AFB. It is therefore possible that if OTC control of EFB was permitted in New Zealand, then similar OTC-resistant strains of *B. larvae* could develop here.

The use of antibiotics to control EFB in New Zealand would pose a significant threat to the proposed National Beekeeper's Association AFB Pest Management Strategy (AFB PMS). The goal of the strategy, which is the eradication of AFB from New Zealand, is dependant on the beekeepers being able to identify the presence of *B. larvae* by its clinical symptoms. If *B. larvae* is present, but its clinical symptoms are suppressed by antibiotics, eradication of the disease would be impossible. AFB would be disguised, either as a consequence of the antibiotic treatment of beehives with concurrent infections of EFB and AFB, or as the result of the illegal treatment of AFB. In either case, the goal set by the NBA to eradicate AFB from New Zealand would not be achievable if antibiotics were gazetted for the control of EFB.

5.4 Cleaning and Disinfection

These procedures are based on the work of Hitchcock (1936) and Goodwin *et. al.*, (1992) and are those currently recommended for use in MQE6: Honey Bee Exotic Disease and Pest Response Procedures (MAF Quality Management, 1995).

5.4.1 Cleaning and Disinfection between Hives

After completing the inspection of a hive:

- a) scrub the outside of gloves with disinfectant or, if using disposable gloves, place them in a plastic rubbish bag,
- b) scrub the bellows and base of the smoker,
- c) scrape all propolis off the hive tool and scrub the hive tool in disinfectant.

Disinfectant is 1% hypochlorite, made by mixing two parts water and one part Janola®.

5.4.2 Cleaning and Disinfection between Apiaries

At completion of work at each apiary and before leaving the site:

- a) scrub the outside of gloves with disinfectant or, if using disposable gloves, place them in a plastic rubbish bag,
- b) scrub the bellows and base of the smoker,
- c) scrape all propolis off the hive tool and scrub the hive tool in disinfectant.

Disinfectant is 1% hypochlorite, made by mixing two parts water and one part Janola.

Replace any pieces of comb that are broken off or dislodged during inspection in the hive or secure them in a plastic bag and return them to HQ for destruction.

Before leaving the apiary site, kill any bees which have entered the vehicle and dispose of them in the plastic rubbish bag.

5.5 Movement Control Strategies

In an EDPR response, the movement control strategies for *Melissococcus pluton* will depend on the findings of the delimiting survey. Based on the Australian example, if the disease is well established and widespread, movement controls of beehives and bee products will have little effect, since the disease will spread rapidly as a consequence of normal beekeeping activities. The only exception would be a limitation on the movement of all apiary products and equipment between the islands. This would potentially delay the

spread of *M. pluton* for several years, but would be dependant on the honesty of the beekeeping industry in maintaining regional quarantines. If the disease is confirmed shortly after incursion, and the area infected is quickly identified by a delimiting survey, then the control measures for eradication as outlined in "MQE 6: Exotic Disease and Pest Response Procedures" should be adopted.

6. ANTIBIOTIC VERSUS NON-ANTIBIOTIC MANAGEMENT

Beekeeping activities in New Zealand can be categorised into two groups: 1) production of honey and other bee hive products such as live bees, wax and honey comb, and 2) pollination of fruit, vegetable and seed crops. Of the 300,000 beehives in New Zealand, approximately 108,000 are used for pollination. Many of these pollination beehives are also used for honey production. Beehives used for both pollination and honey production will on average produce less honey than those used strictly for honey production. The gross income per hive for pollination and non-pollination hives is \$132.90 and \$103.89 respectively, with an average income of \$114.77. The production costs per hive for pollination and non-pollination hives is \$82.36 and \$63.26 respectively, with an average production cost of \$70.42 (Meister and Wilson-Salt, 1995).

The cost of *Melissococcus pluton* to New Zealand would be cumulative and attributable to three sources. These costs are 1) direct operational, 2) loss of income and 3) direct non-apicultural. Operational and loss of income costs impact directly on the beekeeper, while direct non-apicultural costs impact on the profitability of New Zealand horticulture and agriculture. Proportional distribution of the three costs is dependant on the control method used, but direct non-apicultural costs have the potential to be the largest attributable cost.

6.1 EFB - The Immediate Impact

The immediate impact from the detection of EFB in New Zealand will depend on the timing and location of the initial find and the extent to which the disease has spread. The initial incursion will result in either the death of honey bee colonies or their weakening to the extent that they will be unproductive for the remainder of that beekeeping season. The sectors of the beekeeping industry which would be at greatest risk are honey production, package bee and queen bee production, and pollination.

There will be an EDPR response as prescribed in MQE 6: Honey Bee Exotic Disease and Pest Procedures. The procedures include provision for the use of antibiotics to control the outbreak. However, by the time the response is in full swing and the antibiotics distributed, the initial damage caused by EFB is likely to already have occurred.

6.1.1 The Impact on Honey Production

The Australian response to EFB during 1977-78 is an interesting model which may be useful in predicting the affect of EFB on New Zealand honey production. The following statistics were derived from the Australian Bureau of Statistics and cover honey production for the period 1967-1993.

In eastern Australia during the year of epidemic EFB (1977-78), honey production declined from 55.2 kg to 41.7 kg per hive (see Graph 1), recovering in the following year to 54.8 kg. The observed drop in eastern Australian honey production figures may be in part associated with the EFB epidemic, but is more likely the result of other external factors such as weather patterns. Coincidentally in that year, Western Australian honey production

dropped from 86.5 kg to 45.3 kg (1977-78), recovering in the following year to 60.3 kg (see graph 1). The Western Australian decline in honey production was not a consequence of EFB as Western Australia was (and continues to be) free from *M. pluton*.

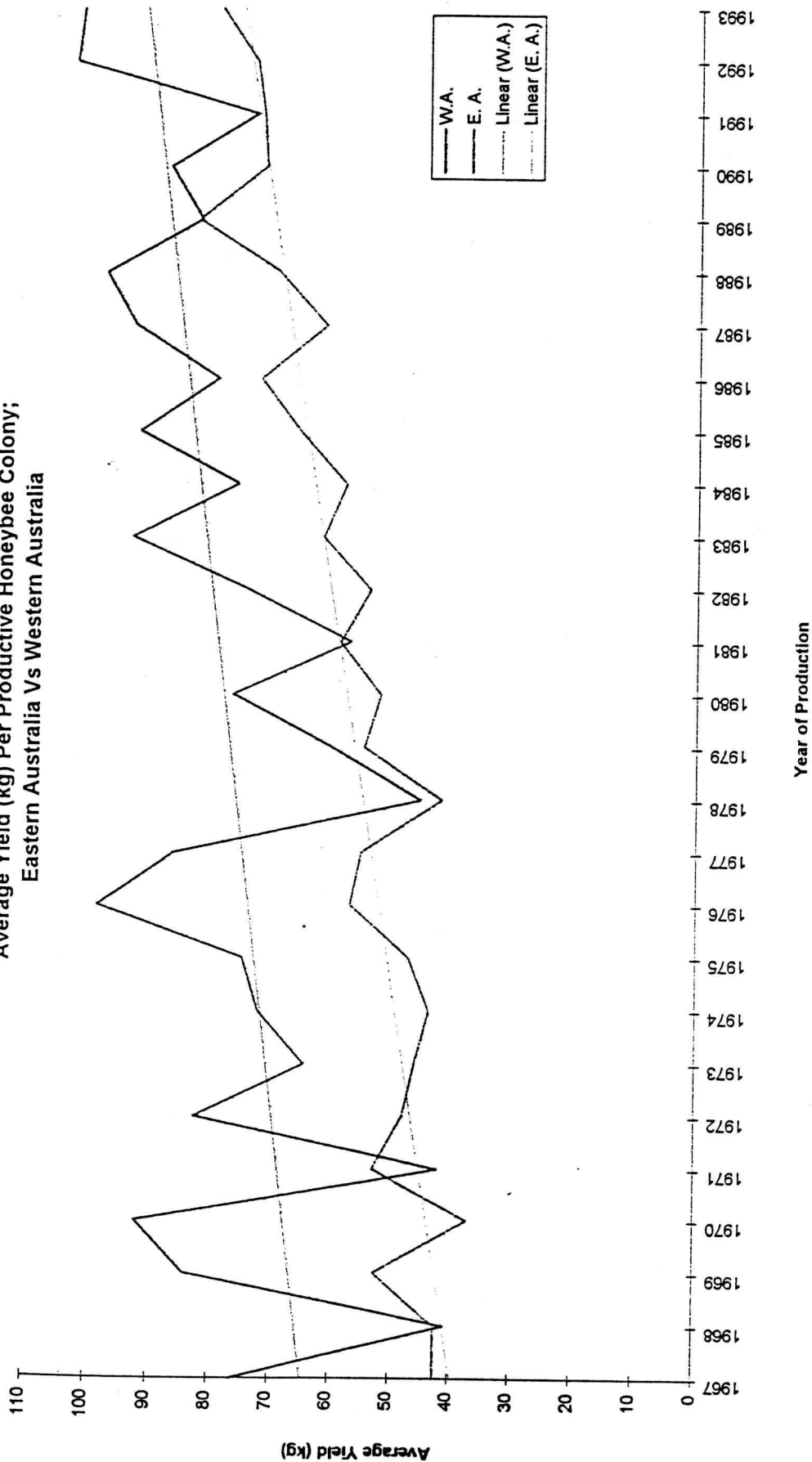
If there was a significant impact from EFB on eastern Australia honey production during the epidemic, this impact was reduced by the use of antibiotics. Once it became apparent to government authorities that eradication of EFB would not be possible in Australia, antibiotic control was gazetted. This quick response to legalise antibiotic control of EFB may have been a "knee jerk" reaction related to the concurrent drop in honey production experienced during the Australian epidemic.

The initial outbreak and spread of EFB in New Zealand may result in a significant drop in honey production within the affected region although the Australian experience suggests otherwise. The EDPR response will result in the destruction of colonies found to have clinical symptoms of EFB, while hives potentially infected with *M. pluton*, but not showing visible symptoms, will be treated with antibiotics. Honey production will be totally lost for all hives destroyed, and reduced for hives infected with *M. pluton* but not having visible symptoms. A quick EDPR response, including the application of antibiotics, should limit honey production losses regionally, while losses in honey production from the national perspective will likely be small.

If EFB is discovered in New Zealand, it is likely that sectors of the beekeeping industry will be proactive in seeking permission to use antibiotics as a prophylactic treatment for the disease. Some beekeepers might also argue that use of the drug will serve as a "general tonic" and that this will increase overall production. The comparison in graph 1 shows an increasing trend in honey production for both eastern Australia and Western Australia during the period 1967-1993. The increase in honey production for eastern Australia after 1977 could possibly be attributed to the antibiotics used in EFB control having an additional "tonic effect" in suppressing *B. larvae* and other bacteria. It may be possible that colonies treated with antibiotics are not only more healthy, but also produce more honey. However, the Western Australian trend suggests otherwise, with average yields per hive increasing in that state at an equivalent rate to eastern Australian. The statistical information before 1977 also indicates that an average annual increase in honey production occurred throughout Australia without the aid of antibiotics.

Antibiotic usage may increase honey production above the current levels, but this unlikely, particularly in regions deemed to be already over-populated with beehives. In the event that EFB became endemic, antibiotic control of the disease would at least maintain the "status quo" in terms of honey production in New Zealand. It would, however, increase operational costs and have a serious impact on the ability of the NBA to achieve its PMS goal of eradication of AFB.

Graph 1
Average Yield (kg) Per Productive Honeybee Colony;
Eastern Australia Vs Western Australia



E.A. - Eastern Australia; W.A. - Western Australia; Linear - Yield Trend.
Data Sourced From The Australian Bureau Of Statistics.

6.1.2 The Impact on Queen Bee and Package Bee Production

Queen and package bee production is estimated to be worth about \$2.3 million per annum to the New Zealand beekeeping industry (National Beekeepers Association, 1995). There is some production in the South Island around Nelson and the West Coast. However, the predominance of queen and package bee production is located in the North Island, and centralised in several regions including Northland, Whangarei, Rotorua, Bay of Plenty and the Waikato.

Most of New Zealand's production of queen and package bees is for export markets such as Canada and Korea. The confirmation of EFB in New Zealand would immediately threaten exports to these markets. It is likely that the initial response from these markets would be to prevent the export of bees from New Zealand, at least for the season following discovery of the disease. MAF-RA would then be called upon to renegotiate export protocols with overseas government authorities.

6.1.3 The Impact on Pollination

New Zealand crops directly relying on pollination are worth more than \$1,216.1 million per annum, while pasture legumes pollinated by bees provide more than \$1,872.4 dollars worth of nitrogen each year to the country's soils (Matheson, 1987; National Beekeepers Association, 1995). Potentially the most significant loss to New Zealand from EFB is likely to be the reduced "effectiveness" of bees as pollinators. This loss in "effectiveness" is attributed to a decrease in fecundity of the queen from reduced nutrition, loss of hive/bee vigour, and reduction in hive populations. Beehives that develop clinical EFB lose their honey bee populations very quickly and are generally unproductive for the remainder of the season.

Colonies used for pollination are particularly susceptible to EFB outbreaks (Shimanuki, 1978; Crane, 1990). In New Zealand, intensive pollination of horticultural crops (e.g., kiwifruit) is typical with large concentrations of beehives being used to effect successful pollination of these crops. The risk of epidemic EFB, combined with high concentrations of beehives and the narrow window of opportunity associated with pollination, set the scene for a potential catastrophe for the horticultural industry. This is also true, but to a lesser extent, for the agricultural industries of which only a relatively small sector are involved in the intensive pollination of crops.

Agriculture will suffer from decreased honey bee pollination efficiency, although the effects are not as likely to be apparent. The value of bees to agriculture is attributed to the pollination of nitrogen producing legumes. The cost to agriculture would be the cost of supplementing nitrogen artificially through the use of fertilizers.

Horticulture is at a far greater risk because of the integral role that honey bees play in fruit, vegetable and seed crop production. A case in point is pollination of kiwifruit. The kiwifruit industry is dependant on the beekeeping industry for the provision of large numbers of honey bee colonies for pollination. Approximately 81,000 beehives are used

each year for the pollination of kiwifruit blossoms. Kiwifruit pollen is deficient in some of the amino acids essential to honey bees (Day *et. al.*, 1990). This deficiency in amino acids and the susceptibility of pollination hives to EFB (Shimanuki, 1978; Crane, 1990) potentially places the kiwifruit industry at considerable risk should an EFB epidemic occur during the peak pollination period. For example, the Bay of Plenty region is responsible for 75 to 80% of the annual \$500 million per annum kiwifruit industry. The potential losses caused by an EFB outbreak in the Bay of Plenty region could cost the kiwifruit industry millions of dollars in lost production from reduced pollination. If EFB were to become endemic in New Zealand, in the short term, the only way to protect the kiwifruit industry from the potential disaster would be to use antibiotics as a prophylactic. The same would hold true for other horticultural crops dependant on honey bees as pollinators, such as apples and stonefruit.

It is likely that beekeepers involved in pollination would be proactive in seeking permission for the use of antibiotics to control of EFB pollination hives.

6.2 Management of Endemic EFB

If the EDPR response is ineffective in eradicating the EFB outbreak, there are four possible outcomes for EFB control. These outcomes are, 1) unrestricted use of antibiotics by the beekeeping industry to control EFB, 2) a continued enforcement on the ban of antibiotic control of diseases in beehives, 3) a managed response where only certain sectors (e.g., queen and package bee producers) are permitted to use the antibiotics, or 4) the restricted use of antibiotic for the treatment of beehives exhibiting clinical symptoms of EFB.

The following sections look at benefits, costs and income loss in each of these four potential outcomes. The outcomes for managed response for specific sectors of the beekeeping industry and restricted use of antibiotics are discussed under the section on unrestricted use of antibiotics.

6.2.1 Antibiotic Management of EFB

6.2.1.1 Benefits of Antibiotic Management

There are three major benefits for the beekeeper in using antibiotic to control EFB.

- a) The development of a management strategy that allows for the prophylactic treatment of all hives each spring (This strategy fits with standard hive management routines without significant additional labour charges.),
- b) Savings in operational cost from not having to sterilise or destroy equipment (beehives) with clinical EFB symptoms,
- c) Supposed savings in operational costs from the inadvertent suppression of AFB (Without the presence of AFB symptoms there is no legal requirement to destroy *B. larvae* infected equipment.).

6.2.1.2 Costs of Antibiotic Management

a) Administration of Antibiotics

The annual cost for the antibiotic control of EFB in New Zealand's 300,000 honey production and pollination hives would be at least \$500,000. This cost would include the prophylactic treatment of each beehive at least once per year in spring, when an outbreak would be most likely to occur. In addition, a number of beehives, and especially those involved in pollination, would require additional antibiotic treatments to prevent/control EFB outbreaks. Potentially, 100,000 beehives could require this second antibiotic treatment at an additional cost of \$161,000.

The cost for antibiotic treatment is based on the current price of Terramycin (Terramycin 100 at \$169.50/5 kg), sugar (\$770/tonne) and labour/equipment (\$50.00/100 hives), provided that the antibiotic is administered as part of the usual management routine.

Cost Per Hive Per Treatment :-

Terramycin	\$0.34
Sugar	\$0.77
Labour/Equipment	<u>\$0.50</u>
Total/Treatment/Hive	\$1.61

b) Residue Testing

As a consequence of the use of antibiotics to control EFB in New Zealand, it is likely that many countries would require residue analysis of honey prior to export. Until 1983, for example, Japan required all honey imported from New Zealand to be tested for antibiotic residues. This testing was undertaken at considerable cost to the exporter. The cost included the fees for the test, plus storage and the occasional retesting of samples that the Japanese claimed to be contaminated, (but were later proven to be residue-free). Currently, depending on the type of residue test required, the cost is a minimum of \$200/sample.

6.2.1.3 Loss of Income

a) Decrease in Export Sales

The "clean, green" image is of significant benefit to New Zealand primary production industries. This image has a significant impact on market access for New Zealand bee products. New Zealand's antibiotic-free status is of particular importance to the "anti-drug" European and Japanese consumer. The potential growth in this market is of such importance that a number of New Zealand producer/exporters have obtained BioGro organic status for their businesses, guaranteeing them market access and a premium for their product. Any outbreak of EFB and subsequent use of antibiotics for its control would put New Zealand's honey export market at risk.

If the use of OTC for EFB control was permitted, then the possibility of residues in hive products would be a major concern to all honey producers and packers. The Japanese market, for example, has a zero tolerance level for OTC in honey. Domestically, the New Zealand Health Department could also set a zero tolerance level. Otherwise, an automatic limit of 0.1 parts per million would apply (Reid, 1990).

Currently 20 percent of New Zealand's annual honey production is exported, along with hive products destined for niche markets. By way of example, New Zealand comb honey attracts the highest price on the Japanese market, and is highly sought after in the European market (particularly Germany and Switzerland). In 1994, 251,616 kg of honey comb was exported, with 155,493 kg going to the Japanese market (Export News, 1995). This market is particularly vulnerable in light of Japan's zero tolerance drug residue level.

6.2.2 Non-antibiotic Management of EFB

As discussed, the initial infection of EFB in New Zealand could be very contagious, spreading rapidly and infecting many thousands of hives. In eastern Australia, an estimated 60,000 hives were infected within 2 years of the first confirmation of *M. pluton* (Walton, 1979). If the introduction of EFB into New Zealand and development of EFB as an endemic disease parallels Australia and many other countries, in the years following the initial outbreak the incidence of EFB should decline to the point where EFB becomes an occasional problem of little consequence (Shimanuki, 1978; Crane, 1990; Bailey and Ball, 1991; Shimanuki *et. al.*, 1992).

The response to the introduction of EFB and its establishment as an endemic disease could parallel the response made to chalkbrood when it was identified in 1984 (Heath, 1985). There was an initial EDPR response that determined the spread of chalkbrood. Chalkbrood was found to be widespread and was declared endemic. At the time there were no chemicals\drugs available for the control of chalkbrood and so beekeepers developed a management practice to control the disease. This practice involved the replacement of the queen bee in heavily infected hives by a queen bee from a strain that showed improved hygienic behaviour. This is still the practice used to deal with the disease.

6.2.2.1 Benefits of Non-antibiotic Management

Following an initial EDPR response to EFB, there would be a number of immediate benefits from not allowing the continued use of antibiotics to control EFB. New Zealand would retain its "drug free" status and could continue to strive for "AFB free" status once the AFB PMS is put into place. We would also still maintain our preferred supplier status for exports into "drug sensitive" markets, and be able to use our "drug free" status for honey bee products as a marketing tool. Our continued "drug free" status would also save on the costs of residue testing.

6.2.2.2 Costs of Non-antibiotic Management

In comparison to Australia, it is reasonable to assume that initially EFB would spread rapidly throughout New Zealand, or at the very least throughout the island where the disease is first identified. The initial response to limit the spread or eradicate the disease would involve the use of antibiotics for colonies in the region not showing EFB symptoms, and the burning of frames and heat sterilisation of beehive parts for colonies found to be infected.

Without the use of antibiotics, heat sterilisation of equipment would be the only option currently available to control EFB, and to be effective would require a uniform industry approach, as is the case for AFB control. Most desirable would be a control method capable of sterilising equipment for both EFB and AFB, as it is likely that there would be some instances where both diseases would occur concurrently. In addition, one method of sterilisation for both organisms would eliminate the need for the beekeeper to differentiate between the diseases, eliminating the potential for misdiagnosis.

Currently, the only cost-effective method of heat sterilization suitable for use by the beekeeping industry in New Zealand is hot paraffin wax dipping (see section 5.2).

6.2.2.3 Cost of Colony Replacement

AFB is endemic in New Zealand and is controlled in the absence of antibiotics by good hive management and proper hygiene practices. Only about 1% of New Zealand's 300,000 beehives develop clinical AFB annually. Unlike AFB, EFB spreads rapidly, and so it is likely that the percentage of hives that will develop EFB will be greater than those that develop AFB. From the statistics on AFB incidence in New Zealand, it would be reasonable to expect that without antibiotic control, at least 1% of New Zealand's beehives would develop EFB annually. Caution, however, is warranted in making calculations regarding colony loss since there is no model to compare potential outcomes for commercial beekeeping in the presence of endemic EFB without antibiotic control (i.e., in every country where EFB occurs, antibiotic control for disease is approved).

If at least 1% of New Zealand's 300,000 colonies developed clinical EFB annually, then the replacement cost (replacing destroyed beehives) would be between \$356,310 and \$635,000, at a current replacement value of between \$118.77 (2 box beehive) and \$211.91 (4 box beehive) per beehive (see Table 1.). The alternative would be to burn all combs in EFB infected beehives and sterilise the remaining beehive equipment by dipping in hot paraffin wax. The cost of this alternative would be between \$60.09 and \$120.19 per beehive (see Table 2.). These figures are based on costs of assembling 100 four box or two box beehives, and do not include the cost of the bees themselves.

Table 1. Costs to Build a 4 Box and 2 Box Beehive

		4 Box Beehive		2 Box Beehive	
Item	Cost	Number	Total Cost	Number	Total Cost
storeys	\$10.62 ea.	400	\$4,248.00	200	\$2,124.00
lids with iron	\$16.35 ea.	100	\$1,635.00	100	\$1,635.00
bottom boards	\$9.28 ea.	100	\$928.00	100	\$928.00
frames	\$659.56 /1000	3600	\$2,374.42	1800	\$1,187.21
kg wax foundation	\$10.76 /kg	206	\$2,216.56	103	\$1,108.28
reels frame wire	\$23.41 /reel	3.6	\$84.28	1.8	\$42.14
kg frame nails	\$17.33 /kg	8.3	\$143.84	4.15	\$71.92
kg storeys nails	\$7.72 /kg	28.6	\$220.79	14.3	\$110.40
units/wood preservative	\$0.40 /unit	600	\$240.00	300	\$120.00
units/paraffin	\$0.10 /unit	600	\$60.00	300	\$30.00
kg/sugar/draw foundation	\$0.08 /kg	1800	\$1,440.00	900	\$720.00
hrs/labour/hive	\$9.50 /hr	8	\$7,600.00	4	\$3,800
Total Costs			\$21,190.88		\$11,876.94
Costs Per Hive			\$211.91		\$118.77

(Mister and Wilson-Salt, 1995)

Table 2. Costs to Replace Combs and Sterilise a 4 Box and 2 Box Beehive

		4 Box Beehive		2 Box Beehive	
Item	Cost	Number	Total Cost	Number	Total Cost
frames	\$659.56 /1000	3600	\$2,374.42	1800	\$1,187.21
kg wax foundation	\$10.76 /kg	206	\$2,216.56	103	\$1,108.28
reels frame wire	\$23.41 /reel	3.6	\$84.28	1.8	\$42.14
kg frame nails	\$17.33 /kg	8.3	\$143.84	4.15	\$71.92
units/paraffin	\$0.10 /unit	600	\$60.00	300	\$30.00
kg/sugar/draw foundation	\$0.08 /kg	1800	\$1,440.00	900	\$720.00
hrs/labour/hive	\$9.50 /hr	6	\$5,700.00	3	\$2850.00
Total Costs			\$12019.10		\$6009.55
Costs Per Hive			\$120.19		\$60.09

(Meister and Wilson-Salt, 1995)

6.2.2.4 Loss of Income

a) Honey Production Losses

In the absence of antibiotic control of EFB it is possible that there would be a general decline in honey production due to the presence of the disease. This loss would vary from year-to-year and be subject, as previously discussed, to a number of factors relating to hive stress. The initial epidemic of EFB would cost, in terms of lost honey production, an estimated \$170,000 for each 1% drop in honey production. This figure is based on a six year average honey production of 7,698 tonnes/annum, and a wholesale price of \$2.20/kg for honey (National Beekeeper's Association, 1995). In concert with a drop in honey production, there would be a reduction in beeswax production. Beeswax is used extensively in the cosmetic and pharmaceutical industries. Current exports of beeswax average 150 - 200 tonnes per annum.

b) Losses Associated With Pollination

Beehives are supplied under negotiated pollination contracts which specify conditions such as delivery times of hives, stocking rates of hives per hectare, price per hive, colony strength and hive audit specifications. Currently the contract fee for the provision of hives for pollination ranges from \$50 to \$100 dollars per hive and is dependant on the location and type of target crop to be pollinated. EFB could pose a significant threat for beekeepers in terms of meeting these contractual requirements, and in particular, meeting hive strength requirements as a consequence of the affect of EFB on pollination hives.

Without the antibiotic control of EFB, outbreaks of the disease during fruit pollination would therefore be recurrent and could potentially be very costly for the beekeeping industry. The beekeeping industry stands to lose in several ways if hives used for pollination develop EFB:

- a) Hive numbers would drop rapidly and contract provisions relating to hive strength would not be met. Beekeepers would in some cases not be paid for hives failing to meet audit requirements,
- b) Audits of colonies supplied under pollination contracts will take more time, increasing audit costs,
- c) Beehives that developed EFB would have to be sterilised, and infected combs would have to be destroyed. This would possibly increase operational costs perhaps to the point where net profit from contract pollination would fall below the net profit for honey production. To recoup these costs, beekeepers would have to increase pollination contract charges.

c) Losses Associated With Queen\Bulk Bee Production

New Zealand exports around \$2,300,000 worth of queen and package bees per year. The production of queen bees in particular is labour intensive and requires the constant interchange of equipment, a common way to spread EFB. To export live bees, the exporter must meet stringent import conditions for the recipient country. These conditions place either "country freedom from" or "area freedom from" disease requirements on the export of bees.

The presence of EFB without the utility of antibiotic control would make it extremely difficult to meet the current disease requirements for some importing countries. Under these conditions, it is likely that current export levels for live bees (and in particular queen bees) would fall dramatically.

7. CONCLUSION

European foulbrood has the potential to be spread via the interchange of hive equipment and by the bees themselves. The causative organism *Melissococcus pluton* may remain undetected for years until prevailing environmental conditions result in an outbreak of the disease. Of particular concern is the similarity between the symptoms of this disease and Halfmoon Disorder. Halfmoon Disorder is believed to be a nutritional disorder of honey bees. This similarity between the symptoms of these two conditions is likely to cause confusion and misdiagnosis at early stages of an EFB infection.

The Australian experience indicates that if the initial introduction of EFB goes undetected, the disease may spread as a sub-clinical infection, and that once established it will be extremely unlikely that the disease will be eradicated. Current surveillance techniques for EFB practised in New Zealand are reliant on the presence of visible symptoms of the disease in larvae, and as such are inherently flawed. As demonstrated by Pinnock and Featherstone (1984), the disease has the potential to spread as a sub-clinical infection. Therefore, to be truly effective, surveys need to be conducted which compensate for the lack of visual evidence of this disease, using identification techniques far more sensitive than those currently employed.

The World Trade Organisation is helping to remove agricultural trade barriers and the world's population is becoming more mobile. Because of these changes in trade and the movement of people internationally, the threat of introducing exotic diseases into New Zealand increases. To maintain New Zealand's agricultural security in an era of increasing risks and costs, diagnostic tests must be more sensitive, faster and more cost-effective.

Since EFB is endemic in most of the world, and is controlled almost exclusively using the antibiotic oxytetracycline, little research is being undertaken on its detection or control. Therefore, the onus is on New Zealand to take a proactive role in developing more reliable and more efficient diagnostic techniques, if New Zealand wishes to protect its beekeeping industry and minimise the risk to horticulture and agriculture in general.

The weak point in the New Zealand quarantine and surveillance procedures for EFB lies in the level at which the disease can be detected. Alternative, more sensitive methods of detection such as PCR-based technologies should be explored and if suitable adopted. Funding for a PCR-based diagnostic technique for *M. pluton* has been provided and should be given continued support for four reasons: 1) this technique would allow extremely sensitive testing of imported materials for the presence of *M. pluton*; 2) the technique would allow for the testing of honey samples for surveillance, 3) the technique would allow the testing of adult bees or combs for preclinical diagnosis, and 4) the technique would allow for a reliable determination of the extent of disease spread during the EDPR response delimiting survey.

If the presence of EFB was confirmed in New Zealand, and its eradication was unlikely, then the decision whether or not to allow antibiotic control of EFB should be made after consultation with industry representatives. Research indicates that EFB may not be the problem that it has been perceived to be. It may prove to be of greater economic advantage to the beekeeping industry not to approve the use of antibiotics, and instead use other methods of control, such as sterilisation of equipment. On the other hand, the legal use of antibiotics for the control of EFB would at least maintain the current status quo regarding all apicultural production and the pollination efficiency of honey bees.

In not allowing antibiotic control, New Zealand's beekeeping industry could maintain its "clean, green" image and could continue to attempt to eradicate AFB. Neither of these outcomes will be possible if OTC control of EFB is gazetted. The challenge will be to protect beekeeping businesses from financial ruin after the initial infection until management techniques and resistant bee stocks can be developed.

(Note: Extension of a PCR-based diagnostic procedure would provide the added ability to test for *B. larvae* and Africanised honey bee (AHB) as a routine testing procedure should endpoint testing of export product become an integral part of future export certification protocols. In addition, a PCR-based diagnostic procedure would also be useful to uncover any masking of AFB if OTC control of EFB was permitted. An identification technique based on PCR-based technology should provide an accurate diagnosis within 24 hours and be far more sensitive in detecting *M. pluton* at much lower concentrations.)

BIBLIOGRAPHY

- Alippi, A. (1994). Sensibilidad "in vitro" de *Bacillus larvae* frente a diferentes antimicrobianos. *Vida Apícola* 66:20-24.
- Allen, M.F., Ball, B.V. (1993). The cultural characteristics and serological relationships of isolates of *Melissococcus pluton*. *Journal of Apicultural Research* 32(2):80-88.
- Anderson, D.L. (1988). Halfmoon disorders of the honey bee (*Apis mellifera* L.) in New Zealand in Rhodes, J.W. *Proceedings of the Second Australian and International Bee Congress*. Department of Primary Industries, Brisbane pp. 77-79.
- Anderson, D. L. (1993). Pathogens and queen bees. *Australian Beekeeper*, 93:292-296.
- Bailey, L. (1957). The isolation and cultural characteristics of *Streptococcus pluton* (*Bacillus pluton* White) and further observations on *Bacterium eurydice* White. *Journal of General Microbiology*, 17:39-48.
- Bailey, L. (1960). The epizootiology of European foulbrood of the larval honey bee, *Apis mellifera* Linnaeus. *Journal of Insect Pathology*, 2:67-83.
- Bailey, L. and Gibbs, A.J. (1962). Cultural characters of *Streptococcus pluton* and its differentiation from associated enterococci. *Journal of General Microbiology*, 28:385-391.
- Bailey, L. (1981). *Honey Bee Pathology*. Academic Press.
- Bailey, L. and Collins, M. D. (1982a). Taxonomic studies on *Streptococcus pluton*. *Journal of Applied Bacteriology*, 53:209-213.
- Bailey, L. and Collins, M.D. (1982b). Reclassification of *Streptococcus pluton* (White) in a new genus *Melissococcus*, as *Melissococcus pluton*. rev.; comb. nov. *Journal of Applied Bacteriology*, 53:215-217.
- Bailey, L. and Collins, M.D. (1983). Validation list No. 11. *International Journal of Systematic Bacteriology*, 33:672-674.
- Bailey, L. and Ball, B. V. (1991). *Honey Bee Pathology* (2nd Edition).pp. 41-48, Academic Press.
- Braybrook, L.H. (1985). Queensland Beekeepers - Association Address to Conference. *Australasian Beekeeper* 87, 55-60.
- Cheshire, F.R. and Cheyne, W.W. (1885). The pathogenic history and history under cultivation of a new bacillus (*B. alvei*), the cause of a disease of the hive bee hitherto known as foulbrood. *Journal of the Royal Microscopical Society*, 5:581-601.

Crane, E. (1990). Bees and beekeeping : Science, Practice and World Resource. pp. 332. Cornell University Press.

Dancer, B. (1996) pers. comm.

Day, S., Beyer, R., Mercer, A. and Ogden, S. (1990). The nutrient composition of honey bee-collected pollen in otago, New Zealand. *Journal of Apicultural Research*, 29(3) 138-146.

Export News (1995). Honey Exports - NZ Comb honey queen of the hive. Export News.

Gilliam, M. and Argauer, R.J. (1981). Oxytetracycline residues in surplus honey, brood nest honey, and larvae after medication of colonies of honeybees, *Apis mellifera*, with antibiotic extender patties, sugar dusts, and syrup sprays. *Environmental Entomology*, 10:479-482.

Gontarski, H. (1953). Zur Brutbiologie der Honigbiene. *Zeitschrift für Bienenforschung*. 2:7-10.

Goodwin, R.M., Perry, J., Haine, H. Houten, A., and Brown, P.J. (1992). Ruakura apicultural research and advisory unit; annual report, July 1991- June 1992. Ruakura Agricultural Centre, Ministry of Agriculture and Fisheries, Hamilton, New Zealand.

Gubler, H.U. (1954). Bakteriologische Untersuchungen über gutartige Faultbrut der Honigbiene. *Schweizerische Zeitschrift für allgemeine Pathologie und Bakteriologie*, 17:507-513.

Hardie, J.M. (1986) Genus *Streptococcus* Rosenbach. In *Bergey's Manual of Systematic Bacteriology* (Peter H.A. Sneath *et al.*, eds),. Vol.2 1043-1071. Williams & Williams.

Heath, L.A.F., (1985). Occurrence and distribution of chalkbrood disease of honeybees. *Bee World*, 66(1) 9-15.

Hitchcock, J.D. (1936). Laboratory and field tests of chlorine treatment of honey combs. *Journal of Economic Entomology*, 29(5) 895-904.

Hoopingarner, R. and Nelson, K. (1988). American foulbrood cleanup rate using three Terramycin treatments. *American Bee Journal*, 128:120-121.

Hornitzky, M., White, B. and Ward, B. (1978). A disease of bees - European brood disease. *The Agricultural Gazette of N.S.W.*, Vol 89(5).

Maassen, A. (1907) Über die sogenannte Faulbrut der Honigbienen. *Mitteilungen aus der Kaiserlichen biologischen Anstalt für Land- und Forst- wirtschaft*, Hft.4: 51-53, fig6.

McCray, A.H. (1917) Spore-forming bacteria in the apiary. *Journal of Agricultural Research*, 8:399-420.

MAF Quality Management (1995). MQE6: Honey Bee Exotic Disease and Pest Response Procedures. New Zealand Ministry of Agriculture.

Matheson, A. (1984). Practical Beekeeping in New Zealand. Government Printer.

Matheson, A. (1985). Recent research into halfmoon disorder in honey bees. The New Zealand Beekeeper, 188(4) 15.

Matheson, A. (1987). The value of honey bees to New Zealand. The New Zealand Beekeeper, 194:29.

Matheson, A. and Reid, M (1992). Strategies for the prevention and control of American foulbrood. American Bee Journal, July:471-475.

Matsuka, M. and Nakamura, K. (1990). Oxytetracycline residues in honey and royal jelly. Journal of Apicultural Research, 29:112-117.

Meister, A. and Wilson-Salt, R. (1995). An Analysis of the Benefits and Costs of the Introduction of Pest Management Strategy to Eradicate American Foulbrood. Massey University.

McKenzie E.H.C. (1985). Proceedings of the combined 29th N.Z. microbiological society conference and 17th N.Z. biotechnology conference at Massey University, Palmerston North, May 27-39, 1985. (Michael Baxter Ed.). Department of Microbiology & Genetics, Massey University.

Moritz, R.F.A. (1988). A reevaluation of the two-locus model for hygienic behaviour in honeybees (*Apis mellifera* L.). Journal of Heredity 79:257-262.

Moffet, J.O., Hitchcock, J.D., Lockett, J.J. and Elliot, J.R. (1970). Evaluation of some new compounds in controlling American Foulbrood. Journal of Apicultural Research, 9:39-44.

National Beekeepers' Association. (1995). New Zealand beekeeping - an industry in profile. National Beekeepers' Association of New Zealand.

Oldroyd, B.P., Goodman, R.D., Hornitzky, M.A.Z. and Chandler, D. (1989). The effect on American brood disease of standard oxytetracycline hydrochloride treatments for the control of European brood disease of honeybees (*Apis mellifera*). Australian Journal of Agricultural Research, 40:691-697.

Pfizer Agricare Pty Ltd (1991). The safe use of Terramycin in honey bees. Technical Information Update, Reference Number 379. Pfizer Inc.

Pinnock, D.E. and Featherstone, N.E. (1984). Detection and quantification of *Melissococcus pluton* infection in honeybee colonies by means of enzyme-linked immunoabsorbent assay. Journal of Apicultural Research, 23:168-170.

- Rendall, G.F. (1981). Antibiotic risk to bees. *Australian Veterinary Journal*, 28:194.
- Reid, M. (1990). Feeding drugs to honey bees to control diseases - some of the issues. New Zealand Ministry of Agriculture and Fisheries.
- Rothenbuhler, W. C. (1964a). Behaviour genetics of nest cleaning in honey bees. I. Responses of four inbred lines to disease-killed brood. *Animal Behaviour*, 12:578-583.
- Rothenbuhler, W. C. (1964b). Behaviour genetics of nest cleaning in honey bees. IV. Responses of F₁, and backcross generations to disease-killed brood. *American Zoologist*, 4:111-128.
- Shimanuki, H. (1978). Honey bee pests, predators, and diseases. (Ed. R.A. Morse). pp. 54-58. Cornell University Press.
- Shimanuki, H., Knox, D.A., Furgala, B., Caron, D.M. and Williams, J.L. (1992). Diseases and pests of honey bees. *In* *Hive and the Honey Bee* (10th edition). 25:1091-1095. Dadant and Son.
- Simpson, J. (1958). The factors which cause colonies of *Apis mellifera* to swarm. *Insectes Sociaux*, 5:77-95.
- Tarr, H.L.A. (1938). Studies on European foulbrood of bees. IV. On the attempted cultivation of *Bacillus pluton*, the susceptibility of individual larvae to inoculation with the organism and its localization within the host. *Annals Applied Biology*, 25:815-821.
- Tham, V.L. (1978). Isolation of *Streptococcus pluton* from the larvae of european honey bees in Australia. *Australian Veterinary Journal*, 54:406-407.
- VanDemark, P. J. and Batzing, B. L. (1987). Bacteria:Groups *In* *The Microbes*. Chapter 12:550-552.
- Walton, T. (1979). Australian disease not wanted. *New Zealand Beekeeper*, September Issue.
- Warhurst, P. (1996) pers. comm.
- Ward, B., Hornitsky, M. and White., B. (1985) European brood disease of bees. *Agfacts* A8.9.4, Department of Agriculture, New South Wales.
- White, G.F. (1912) The cause of European foulbrood. Circular, US Bureau of Entomology, No. 157.
- White G.F. (1920) European foulbrood. Bulletin, US Department of Agriculture, No. 810.

White, J. W. and Sturtevant, A. P. (1954). Sterilization of honey for bee feeding. Gleanings in Bee Culture, November:658-661.

Wilson, W.T., Elliot. J.R. and Hitchcock, J.D. (1971). Antibiotic extender patties for control of American foulbrood. Journal of Apicultural Research, 10:143-147.

Wootton, M., Hornitzky, M., and Ryland, L. (1981). Thermal destruction of *Streptococcus pluton* in Australian honeys and its effect on honey quality. Journal of Apicultural Research, 20:115-120.

