Australian Government
Rural Industries Research and Development Corporation

Nosema Disease

Literature review and three year survey of beekeepers

Part 2

by Michael Hornitzky

March 2008

RIRDC Publication No 08/006
RIRDC Project No DAN-228A

NSW DEPARTMENT OF PRIMARY INDUSTRIES

ISBN 1 74151 595 5
ISSN 1440-6845

Nosema Disease: *Literature review and three year survey of beekeepers - Part 2*

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Published in March 2008

Foreword

Nosema apis is one of the most important parasites of honey bees, but it is mostly overlooked by beekeepers as there are no classic signs of disease. Hence, *N. apis* (nosema disease) is referred to as 'the silent killer'. The repercussions of infection with this parasite have been considered to equal or exceed the losses caused by all of the other diseases, including the more easily diagnosed brood diseases.

There have been very few published studies of nosema disease in Australia and there is a general lack of understanding of the impact that *N. apis* has on honey bee health and hive production. The aim of this study was two fold. The first aim was to prepare a literature review which outlines the different facets of *N. apis* infections including a section on the methodology for counting *N. apis* spores in adult honey bees. The second aim was to conduct a survey of honey bee colonies to determine the prevalence and severity of *N. apis* under Australian conditions.

In May 2005 a report entitled "Nosema disease – literature review and survey of beekeepers" was produced following a survey involving 800 hives (owned by 20 beekeepers) for *N. apis* spores. One of the recommendations of that report was that a further survey of the apiaries used in the 2004 study be carried out to better access the links between management practices and nosema levels. This report includes the findings for follow up surveys carried out in 2005 and 2006.

The key findings of the three year survey were; (i) *N. apis* is commonly found in apiaries, (ii) the number of infected hives in an apiary increases as the average *N. apis* spore count per bee in an apiary increases, (iii) there is a clear association between hive manipulation (including supplementary feeding) and increased spore counts, (iv) the adult bee population in hives with very high nosema spore counts decreased or stagnated while the bees were pollinating almonds, indicating a reduced pollination efficiency of those hives, (v) low nosema spore counts were related to hives which were packed down tight for winter and were full or nearly full of honey, (vi) there was a clear relationship between bee colonies that were working spotted gum and high nosema spore counts, (vii) some flora which are associated with poor pollen production, e.g. grey box and iron bark, were used by beekeepers who had both high and low nosema spore counts, indicating that factors other than floral type influenced the levels of nosema spore counts and (viii) in some apiaries that were heavily infected with nosema spores, some hives were completely free of *N. apis* spores, suggesting that there may be specific unrecognised factors which impart resistance to this disease.

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

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Peter O'Brien
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Acknowledgements

We thank Trevor Monson for organising the nosema survey, Doug Somerville for his suggestions and assistance with the project, and Idris Barchia for the statistical analysis. We also thank the beekeepers who participated in the survey: Jim Picker, Glen Watson, Grant Lockwood, Laurie Fry, Alan Smith, Bob McDonald, Troy Pedretti, Mark Ipsen, Ray Phillips, Ian Clarke, Ashley Hazelman, Michael Leahy, Phillip McPhersen, Andrew Nicklaus, Craig Scott, David Mumford, Noel Taylor, Andrew Boyd, Dale Richards, Steve Williamson, Bert Kilpatrick, Darryl Mann, Troy Stidiford, John Edmunds and Kevin Emmins.

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Executive Summary

What the report is about
This report gives is a literature review the cause, occurrence, multiplication and spread, and effects on adult bees and honey bee colonies of the honeybee disease *Nosema apis.* The report also presents a three year survey into the occurrence of *Nosema apis* and how it is linked to different management practices. Results of this study directly impact beekeepers and their management techniques.

Background
Nosema apis is a serious disease of adult honey bees. It has been reported to cause significant production losses as a result of a range of effects on adult bee longevity, queen bees, brood rearing, bee biochemistry, pollen collection and other bee behaviour. Despite these effects there are no classic signs of infection and hence most infections are unrecognised. There is no comprehensive current data on the prevalence of nosema disease in bees in Australia and no work which links nosema disease with beekeeper management practices.

Aims/Objectives
The aims of this project were to provide a literature review and a three year survey of *Nosema apis* linked with management practices.

Methods used
The literature review was prepared from information obtained from relevant literature, some of which was obtained from computer searches.

The *N. apis* survey was carried out over a period of three years. Each year adult bee samples (25 bees per hive) were collected from about 800 hives owned by 20 beekeepers (40 hives per beekeeper). These samples were collected in the August of 2004, 2005 and 2006 while beekeepers from New South Wales, Victoria, Queensland and South Australia were using their bees to pollinate almond trees at Robinvale, Victoria. Bee samples were examined for *N. apis* spores using standard techniques. The participating beekeepers were asked to complete a questionnaire which was analysed in relation to the *N. apis* counts detected in their bees.

Results
The literature review is provided in the first section of the report. It provides details on the cause, occurrence, multiplication and spread, and effects on adult bees and honey bee colonies. Information is also provided on the diagnosis, factors affecting nosema spore counts and control methods. The section on diagnosis has been described in sufficient detail so that beekeepers can determine the level of infection in their hives using simple light microscopy and apply appropriate management strategies to minimise infection levels. A description of a new *Nosema* species *Nosema ceranae*, which has recently been identified in the European honey bee in the USA, Taiwan and Europe has also been included.

The three-year survey demonstrated that *N. apis* was present in every apiary in each of the three years. However, there was great variation in the average nosema spore counts for the three year period. These counts ranged from 12,236,000 to 10,000 spores per bee and the number of infected hives ranged from 40 (100%) to 1 (2.5%) per apiary.

Key findings of the survey were; (i) hive manipulation (including supplementary feeding) were related to increased spore counts, (ii) the adult bee population in hives with very high nosema spore counts decreased or stagnated while the bees were pollinating almonds, indicating a reduced pollination efficiency of those hives, (iii) low nosema spore counts were associated with hives which were packed down tight for winter and were full or nearly full of honey, (iv) there was a clear relationship between colonies that were working spotted gum and high nosema spore counts, (v) some flora which are

associated with poor pollen production, e.g. grey box and iron bark, were used by beekeepers who had both high and low nosema spore counts, indicating that factors other than floral type were involved in determining the levels of nosema spore counts and (vi) in some apiaries heavily infected with nosema spores, *N. apis* spores were not detected in one, three or four of the 40 hives examined, suggesting that there may be specific unrecognised factors which impart resistance to this disease in those hives.

No assessment was made on the importance of queen age or comb replacement, as most beekeepers used queens which were less than 12 months old and had a comb replacement strategy in place.

Implications for relevant stakeholders

Nosema apis was identified as an important parasite of adult honey bees in the literature review. The disease it causes, nosema disease, may result in significant production losses as a result of its effects on adult bee longevity, queen bees, brood rearing, bee biochemistry, pollen collection and other bee behaviour. The degree of loss in an individual bee colony is a function of environmental conditions and beekeeper management - strategies at critical times in the infection cycle. Unfortunately, there are no clear typical signs of nosema disease which allow easy confirmation of infection. The literature review provides a step-by-step procedure for the detection of *N. apis* spores using standard light microscopy. This is an option that beekeepers can use to access whether the infection is present and the degree of infection. *N. apis* spores are much larger than bacterial spores and can be readily identified using relatively low magnifications (X 200-X 400) compared to infections such as European and American foulbroods which require high magnification (X 1000) for identification and are more complicated to diagnose than nosema disease. A number of control options have also been listed in the literature review which can help reduce the impact of this disease.

The three-year survey clearly demonstrated that *N. apis* is commonly found in bees in Australia. It also demonstrated that there is a broad range of infection levels within apiaries and that there are management practices which can be used to minimise the effects of disease. Beekeepers need to consider their management strategies especially in autumn and winter as these are the critical times when nosema levels can rapidly increase and cause bee and production losses. The benefits of working autumn and winter flows should be weighed against not, or reduced working of bees, which would result in better bee health for spring.

No severe losses of bees or loss of hives due to nosema disease were reported in this study probably due to the generally dry weather that was experienced from 2004-2006. However, severe losses due to nosema disease have been previously reported. Had conditions been suitable for the development of nosema disease it is likely that the beekeepers with high nosema spore counts would have suffered significant losses. By minimising hive manipulation and maintaining a high nutritional plain for hives, nosema spore counts will be kept down, reducing the possibility of large bee losses in the event that conditions favour the proliferation of nosema spores.

Recommendations

Beekeepers can influence nosema spore counts in their bee colonies by using appropriate management strategies. Beekeepers working autumn and/or winter flows will increase nosema spore counts in their bees. The economic benefit of working these flows should be evaluated against not working such flows which would provide bees with low counts and a better health status at the beginning of spring.

One tool that beekeepers can use to gauge the progress of nosema disease is to examine their bees microscopically for the presence of spores at critical times of the *N. apis* cycle. Being aware of the level of infection can assist in making management decisions which can influence strategies that may affect the development of nosema disease.

1. *Nosema apis* literature review

1.1 Introduction

Nosema disease is the most important adult bee disease but is mostly overlooked by beekeepers as there are no characteristic obvious symptoms. Hence, nosema disease is also referred to as 'the silent killer'. The repercussions of this infection have been considered to equal or exceed the losses caused by all of the other diseases, including the more easily diagnosed brood diseases (Furgala and Mussen, 1990).

1.2 Cause

Nosema disease is caused by the microsporidian *Nosema apis* (Zander) which produces spores that are 4 to 6 µm in length and 2 to 4 µm in width (Figure 1). The disease is by far the most widespread of the adult honey bee diseases (Nixon, 1982).

Figure 1: Wet preparation of *N. apis* spores prepared from whole adult bee sample

In a recent study it has been suggested that this microsporidian can be divided into a number of different groups. There is no data available to indicate whether virulent or avirulent strains of *N. apis* exist. However, it has been suggested that molecular studies such as the use of micro-satellites (small repetitive DNA sequences) may prove to be useful in identifying strains and hence assist in determining whether there are virulent and non-virulent strains (Rice, 2001).

In 1996 a parasite similar to *N. apis* was found in the Asian honey bee *Apis cerana* (Fries et al., 1996). This parasite, called *Nosema ceranae*, was found in European honey bees in Taiwan in 2005 (Huang et al., 2005) and more recently in the European honey bee in the USA, Spain and Germany. To date non-specific symptoms, such as a gradual depopulation, higher autumn/winter colony death or low honey production have been associated with the presence of this parasite. None of the dysentery or crawling bee behaviour which is sometimes associated with *N. apis* infection has been reported (Fries et al., 2006).

N. ceranae spores are usually a little smaller than *N. apis* spores but they cannot be definitively differentiated from each just by using microscopy. Molecular techniques are necessary to differentiate the two species. It is possible that *N. ceranae* exists in honey bees in Australia although limited testing of bees in Australia, using these molecular techniques, have been carried out without detecting this new pathogen. Further testing needs to be done confirm the *N. ceranae* status of bees in Australia.

1.3 Occurrence

Nosema disease is probably the most widespread of the diseases of adult bees. White (1919) cited reports of the presence of nosema in Australia, South America, North America and Europe. The disease has been reported on every continent (Furgala and Mussen, 1990). Considerable variation has been reported in its incidence in different countries. but this is probably a function of the scale and timing of investigations. The true incidences are probably considerably greater than the values that have been reported. These range from less than 2% of colonies infected in Italy to more than 60% in the Black Forest regions of Germany (Bailey and Ball, 1991).

Doull (1961) carried out a study of *N. apis* in hives in South Australia. He determined that *N. apis* was present in all hives at all times. He concluded that no hive is likely to be completely free from either spores or infected bees for any appreciable length of time. Langridge (1961) reported that during the summer months colonies in Victoria generally carry a few infected bees (usually a small fraction of 1%).

'Package bee colonies' are especially vulnerable to nosema, as they are without emerging bees for three weeks. A survey in the USA showed that most queens in "package bee" colonies were infected with nosema (Farrar, 1947). However, in a follow-up study he found very few infected bees probably because of treatment for nosema. In a recent study in Australia it was demonstrated that infection with *N. apis* did not have a significant affect on the introduction success and early performance of queen bees (Rhodes and Somerville, 2003).

1.4 Multiplication and spread

N. apis spores are ingested by susceptible worker bees via contaminated water or food, by food exchange with other bees or in their duties of cleaning contaminated combs. The spores are passed quickly into the midgut by the proventriculus. A single spore of *N. apis* can cause infection. However, the mean infective dose is reported to be between approximately 20 and 90 spores per bee. When they enter the mid-gut they each extrude their hollow polar filament and inject the germ through it onto an epithelial cell (Kramer, 1960b; Morganthaler, 1963). Adult worker bees, adult queen bees and adult drones are all susceptible.

In the human, digestive juices are secreted in the stomach and gut to facilitate digestion. However, the honey bee does not secrete digestive juices into the ventriculus. Under normal conditions honey bee epithelial cells shed into the ventriculus (stomach), burst, and release their contents including digestive juices. However, when the cells are infected with *N. apis* the parasite develops and multiplies in the cytoplasm and form after about five days. The spore-filled cells are shed into the lumen. Some cells pass into the rectum and are voided. The spore-filled cells burst and release infective spores rather than digestive juices. If the cells burst in the lumen they may release spores that quickly germinate, infecting additional epithelial cells (Morse and Nowogrodzki, 1990).

All the cells of the mid-gut are eventually parasitized, possibly by reinfection with newly-produced spores that have been cast off into the gut cavity, or by invasion of vegetative forms from adjacent cells as described for *Nosema bomycis* in the silkworm (Isihara, 1969). If infection of other epithelial cells is not blocked, the digestive function of the epithelium is repressed in about 14 to 21 days. About

30-50 million spores are in the gut of a bee when infection is fully developed (Bailey and Ball, 1991) although 180 million spores per bee have been reported.

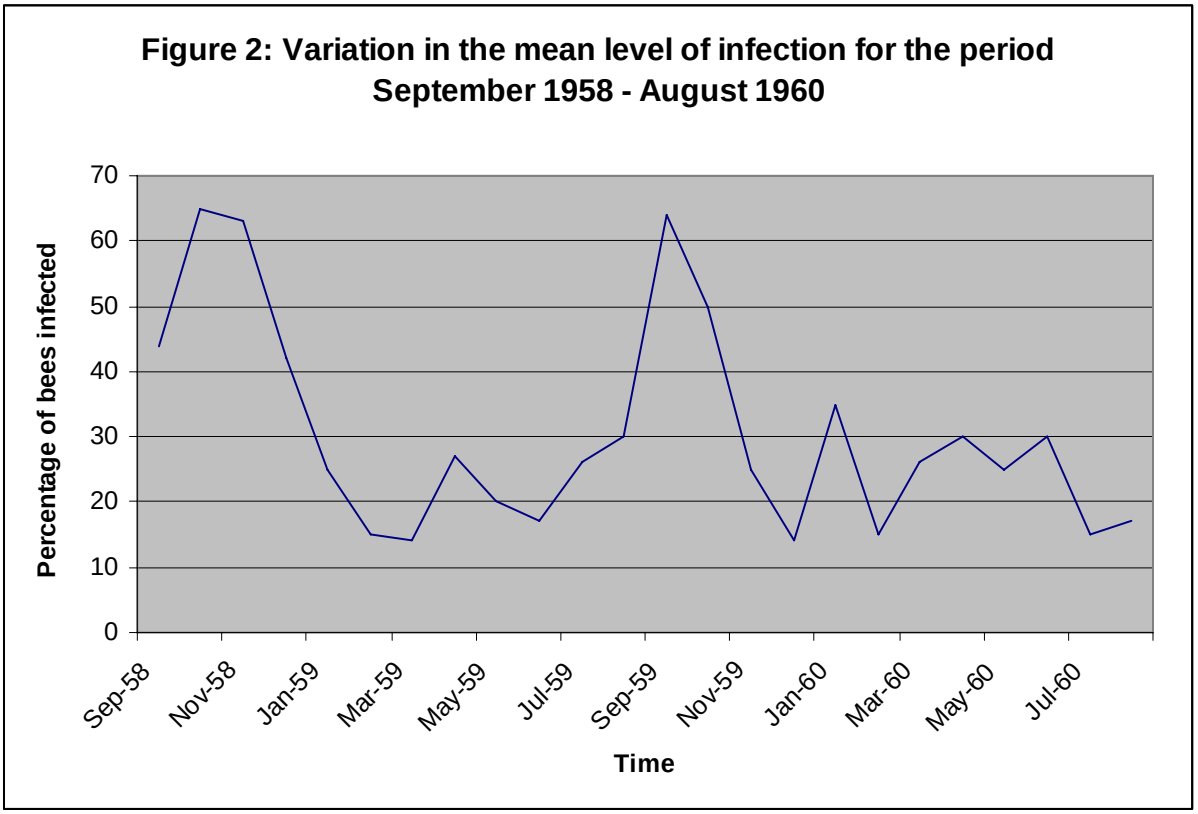
There is a well defined cycle of infection for nosema disease in Victoria (Langridge (1961). During the summer months colonies generally carry a few infected bees. There is no outward evidence of disease. However, at the break of the weather, which usually occurs about mid-March the small pool of infection spreads rapidly through the colony and assumes the proportion of an epidemic. In ten days, strong three-storied colonies have been reduced to the size of small nuclei (Langridge, 1961).

As the colder winter conditions set in, the disease becomes relatively dormant, until late August or early September when temperatures begin to rise. At this stage a further upsurge of mortality is usual, and it may last until December. From this point hot dry summer conditions reduce the incidence of the disease to negligible levels (Langridge, 1961).

Langridge (1961) identified two main periods of heavy mortality, March-May and August-November and that serious outbreaks seem to be initiated in autumn and terminated in spring, the actual timing depending on seasonal conditions.

In a study of six apiaries in South Australia where bee samples were collected from four hives per apiary from September 1958 to August 1960 a spring peak of infection was observed in both 1958 and 1959. A smaller infection peak was detected in March – May in each year (Figure 2) (Doull, 1961). This data is somewhat similar to the observations made in Victoria.

Figure 2: Variation in the mean level of infection for the period September 1958 - August 1960



Adapted from (Doull, 1961)

N. apis does not infect honey bee larvae (Hassanein, 1951). Newly-emerged bees are always free of infection, but they are susceptible as older bees (Bailey and Ball, 1991).

1.5 Effect on:

1.5.1 Adult bees

Infected bees do not show any signs which are exclusively characteristic of infection with *N. apis* and infected mid-guts show little evidence of damage. However, there are a number of signs of infection that are not readily observable but have a marked impact on the function of the hive:

- Infected bees live only about half as long as non-infected bees in colonies in spring and summer (Kang *et al.*, 1976). This is due to pathological changes in epithelial cells in the gut and derangement of digestive processes which lead to malnutrition and premature death (Morse and Nowogrodzki, 1990)

- Crawling bees may indicate the disease during the first few days of a heavy honey flow; however, this may also be caused by pesticide poisoning or viral infections. These crawling bees, which may have their hind wings unhooked from the front wings and held at unusual angles, are apparently too weak to handle heavy loads of nectar (Moeller, 1962). They may also have a sickly look with greasy-looking abdomens (Somerville, 2002).

- Infected bees do not fully develop their hypopharyngeal glands resulting in up to 15% of eggs in severely infected colonies not producing mature larvae in early summer (Moeller, 1969).

- Queens are generally superseded within two to eight weeks after becoming infected (Moeller, 1962). Queens become infected when confined with infected workers in queen mailing cages in queen banks and packages (Lehnert *et al.*, 1973; Foote, 1971).

- Infections of *N. apis* have a negative effect on the protein build-up of the fat body (Bailey and Ball, 1991).

- Nosema infected bees start their foraging activity at a younger age than healthy bees (Fries, 1995).

- Bees from colonies infected with *N. apis* spores collect significantly less pollen than uninfected colonies (Anderson and Giacon, 1992).

- The honey bee ventriculus is normal when straw-brown and the individual circular constrictions are clearly seen. Nosema disease can be implicated when the ventriculus is white in colour, soft in consistency, and swollen to the extent that constrictions are obscured (Shimanuki *et al.*, 1992).

1.5.2 Honey bee colonies

- Late winter and early spring dwindling of adult bee populations may be caused by nosema. In severe infections the death rate may exceed the birth rate.

- Dwindling populations makes it more difficult for bees to maintain brood nest temperature

- Decreased honey production. Farrar (1947) demonstrated that colonies that were not infected or lightly infected with *N. apis* produced on average 24.5 kg more honey than from severely infected colonies. When the queens in the severely infected group were superseded, the loss in production averaged about 50 kg.

- Decreased brood production. Infected queens are less productive and infected colonies have been reported to produce 12% less brood than apparently healthy colonies (Moeller, 1962).

- In severe cases nosema disease may kill the colony.

1.6 Diagnosis

The microscopic examination of bees or their faecal samples is the only method that provides a definitive diagnosis of nosema regardless of the level of infection. There are a number of methods by which infection can be determined and these are all based on the detection of *N. apis* spores (Figure 1).

Materials required for carrying out counts:

- Compound microscope with X400 objective
- Microscope slides
- Cover slips
- Mortar and pestle or equivalent
- Bacteriological loops
- Pipettes
- Counting chamber.

Quick, routine examinations can be carried out by examining whole bees or the abdomens from 10 bees or more.

The following procedure based on that reported by Cantwell (1970) is a reliable method for determining the *N. apis* spore count of infected honey bees.

- Collect from 10 to 25 bees from under the top lid, from outside the cluster or from the hive entrance just before or after flight. (Newly emerged bees have not had time to become infected and do not contain spores. These are unsuitable for nosema spore counts).

- The bees can be collected in 70% alcohol (methylated spirits can also be used) if they need to be stored or submitted to a laboratory).

- After the bees have been immobilised by freezing they are placed in a mortar or dish with one millilitre of water per bee. Alternatively, the abdomens of the bees can be removed and used as the sample rather than whole bees.

- The bees are then ground with a pestle or other suitable implement until an even suspension is formed. (The mortar and pestle should be thoroughly cleaned before being used again.)

- A wet preparation is prepared by placing a drop of the resulting suspension on a microscope slide, covering the drop with a cover slip and examining the resultant preparation under the high dry objective (X400) of a compound microscope. *N. apis* spores. This provides a non-quantitative assessment of spore numbers but is adequate for determining whether infection is present.

- Alternatively, a counting chamber such as an Improved Neubauer Chamber (approximate cost $50.00 and available from scientific suppliers) can be used to determine the number of spores per bee. This chamber consists of a cover glass and a chamber that holds a specific volume of fluid and is marked with a grid pattern for ease of counting.

    i. Ensure chamber is clean before use
    ii. Inoculate suspension using a loop or pipette under the cover glass (Figure 3)
    iii. The material will flow under the cover glass and fill the chamber (do not overload and avoid producing bubbles)
    iv. Let the suspension settle (about three minutes)
    v. Then count the spores in five large (60 small) squares, Figures 4 and 5.
    vi. The number of spores per bee is determined according to the following formula.

Calculation:

$$\frac{\text{Total number of spores counted}}{\text{Number of squares counted.}} \times 4 \times 10^6 = \text{Number of spores /bee}$$

Or more simply:
Number of spores per bee = Number of spores in five large (80 small) squares x 50,000

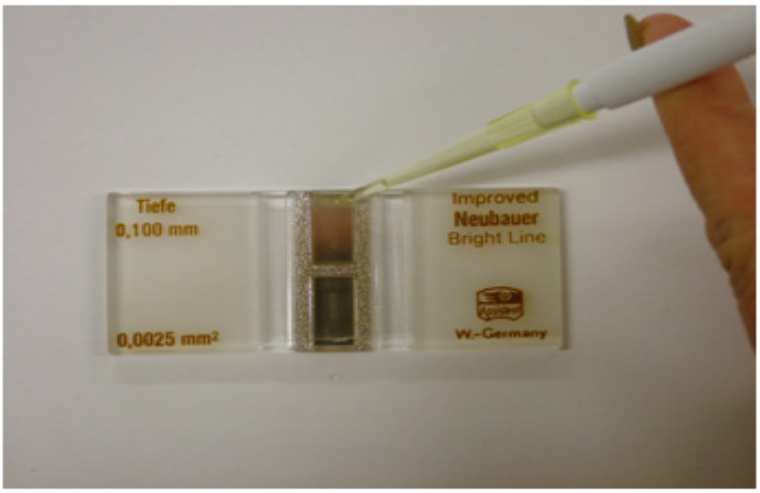


Figure 3: Inoculation of Improved Neubauer Counting Chamber to facilitate the counting of *N. apis* spores

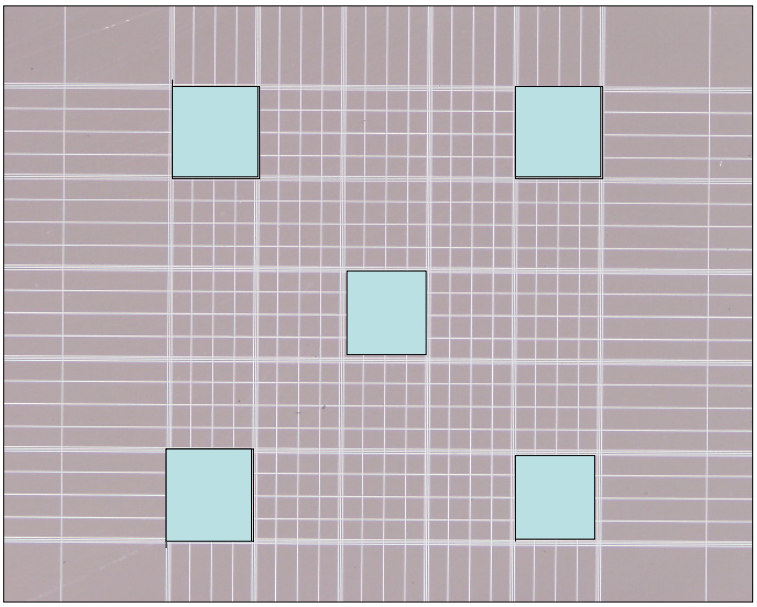


Figure 4: Five large squares (each containing 16 smaller squares) in which *N. apis* spores are counted to determine infection levels of adult bees.

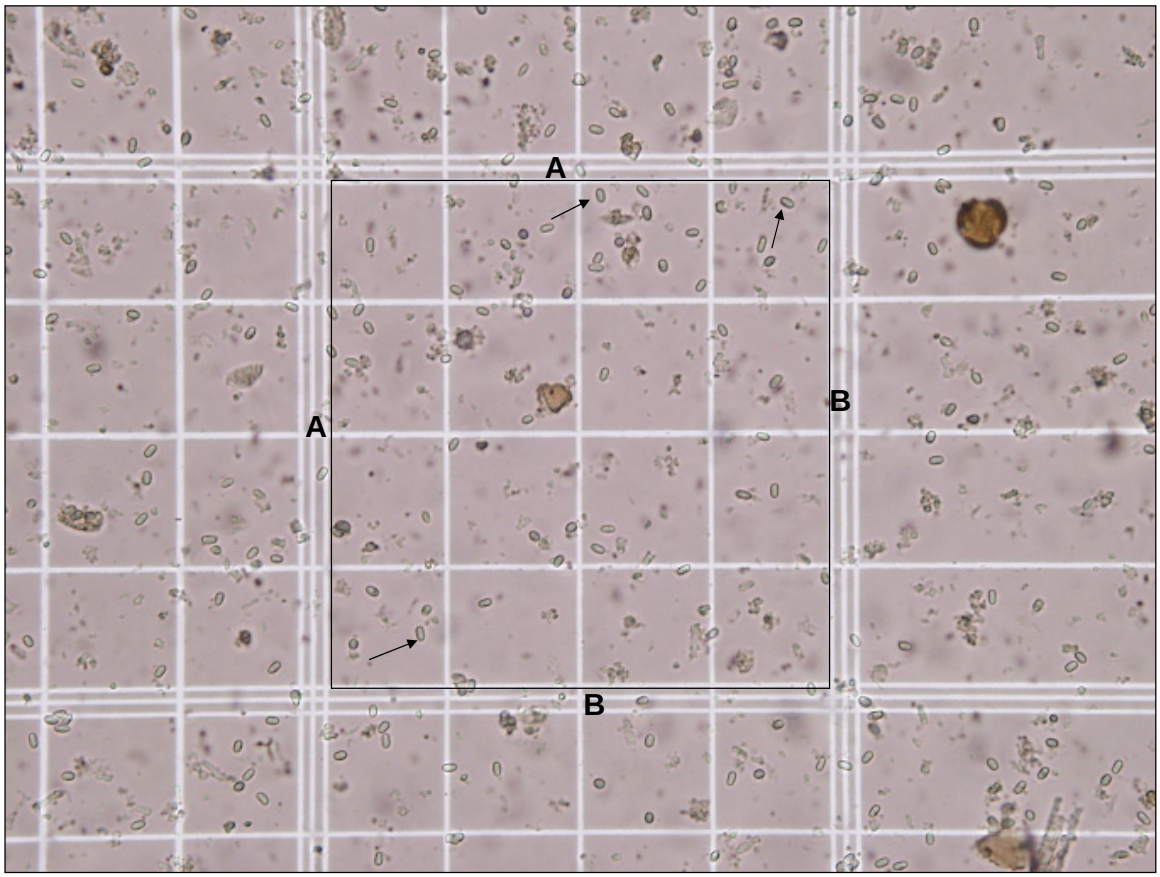


Figure 5: One large square containing 16 smaller squares and *N. apis* spores.
Small arrow heads point to 3 *N. apis* spores.
There are 47 spores in these 16 small squares. Assuming that the other 4 large squares (see Figure 3) also contained 47 spores the *N. apis* spore count would be:
47 x 5 x 50,000 = 11,750,000 spores per bee
NB: Spores that touch lines A should be included in the count. Spores that touch lines B should not be counted.

1.7 Factors affecting nosema infection levels

- If contaminated with *N. apis* spores, combs placed in colonies towards the end of summer, which is done to take advantage of late nectar flows, will introduce infection too late for bees to clean them adequately by autumn. This may result in bee numbers dwindling more rapidly than usual or the death of the colony (Bailey and Ball, 1991).

- Colony disturbance in the winter as well as in spring increases the risk of detectable disease levels. Individual bees are frequently crushed when colonies are opened and examined. They are removed by other bees which ingest the liquid remains, which if contaminated with *N. apis* spores will infect the cleaning bees (Bailey and Ball, 1991). Stimulating bees by feeding sugar syrup may also stress bees and increase nosema levels.

- In a case study of an apiary during the 1977 and 1978 winter and spring periods, 48 colonies were divided into two groups. One group was used to work winter honey and the other remained at the original site in a build area. Nosema spore counts were recorded at regular intervals. Before half of the colonies were moved to winter ironbark, the original apiaries had recorded nosema spore counts ranging from 0-100,000. The heavy flow necessitated regular colony management during June. By August 1977, spore counts increased to about 4,500,000 per bee and in September 1977, counts were still between 650,000 and 1,300,000. Under this infection level, colonies dwindled from three stories to one while the nosema levels in colonies left at the original site varied from 0 to 650,000 per bee and were kept in two to three hive bodies (Kleinschmidt, 1984). This example illustrates the effect of nosema disease on hives worked in the winter and the spore levels associated with hive dwindling.

- Moving colonies to new sites may encourage bees to deposit faecal matter within the colony facilitating the spread of nosema spores within the colony (Bailey 1955).

- Protein deficiency has been identified as a key reason for increased nosema spore counts, especially when working late autumn and winter flows. Managing colonies to avoid protein deficiency has been shown to be beneficial. Supplementary feeding of protein or moving colonies to areas with a good supply of pollen has been shown to reduce infection levels in colonies, probably due to the large number of bees being produced (Fries, 1995).

1.8 Control

Heat treatment: *N. apis* spores on combs and other hive equipment can be killed using heat treatment. The treatment involves heating the equipment at 49^{o}C for 24 hours. This is best conducted in a room where the temperature is uniform and thermostatically controlled. Hot spots should be avoided, as higher temperatures may melt combs or cause them to sag (Morse and Shimanuki, 1990).

Fumigation: Fumigation with acetic acid is effective, especially when the bees are transferred as early as possible in the season from contaminated equipment to fumigated equipment. An efficient method is to intersperse absorbent materials between piles of hive bodies containing the combs. Pour 150 ml of acetic acid (80% strength) onto the material between each box. The stacks should be left outside in a warm corner and protected from direct winds for about one week. It is also recommended that the material be aired for one day prior to use (Bailey and Ball, 1991; Shimanuki *et al.*, 1992). Fumigation with ethylene oxide (ETO) has also been demonstrated to kill spores on combs (100 mg ETO/l for 24 hours at 37.8^{o}C). However, there are a number of safety issues associated with the use of ETO (Shimanuki *et al*. 1992).

Chemotherapy: Fumagillin is the only drug that has been found to be effective against *N. apis* (Katznelson and Jamieson, 1952). Fumagillin inhibits DNA replication of the microsporidian without affecting the DNA of the host cell. Studies of freeze-etched healthy and nosema-infected cells, confirmed this finding (Hartwig and Przelecka, 1971; Liu, 1973). The activity of the fumagillin remains high in honey kept at 4^{o}C for several years (Furgala and Gochnauer, 1969) and for at least 30 days at 30^{o}C (Furgala and Sugden, 1985). Thymol (3-Hydroxy-*p*-cymene) a constituent of the essential oil derived from thyme and many other plant species is effective in suppressing *Nosema vespula* infection in the *Helicoverpa armigera* caterpillars under laboratory conditions (Rice, 2003). He recommended that three lines of research be followed; (i) to determine the suitability of thymol as an additive to dietary supplements used in commercial beekeeping, (ii) to examine the effectiveness of other organic substances derived from essential oils for their activity against *N. apis* and (iii) to test the pollen and nectar of a range of economically important floral species for the presence of thymol and other substances that are shown to be active against *N. apis*.

Management techniques: Keep colonies as populous as possible by supplying adequate ventilation and protection from prevailing winds, and by avoiding cool, humid, shady locations. Minimise colony manipulation in cool weather, particularly during winter and early spring.

2. *Nosema apis* – a three year survey of Australian honey bee colonies

2.1 Introduction

Nosema disease is recognised as the most serious adult honey bee disease. However, there are few Australian studies to determine the extent of the disease in Australia. Doull and Cellier, (1961) carried out a two year survey of the incidence of nosema disease of the honey bee in South Australia. They concluded that a spring peak of infection may be expected each year. The level of infection was at its minimum in mid-summer and winter but that a rise may occur in the autumn. They concluded that the disease, either in the form of a few infected bees or of spores on the combs, was present in all hives throughout the survey. They also made the suggestion that there may be factors varying in some way from hive to hive, which determine the level of infection within each hive.

Langridge (1961) identified nosema disease as being a serious problem to the honey industry in Victoria. His observations over a period of seven years indicated that serious outbreaks of the disease were generally associated with a break in fine weather which normally occurred in the mid-autumn period (mid-March to early April) when bees were working flows from grey box (*Eucalyptus hemipholia*), red ironbark (*Eucalyptus sidaeroxylon*) and later white box (*Eucalyptus albens*).

Langridge (1961) also observed that as the colder conditions set in, the disease becomes relatively dormant, until late August or early September when temperatures begin to rise. At this stage a further upsurge of mortality is usual, and it may last until December after which the hot dry summer conditions reduce the incidence of the disease to negligible proportions. This indicated that there are two main periods of mortality, March-May and August-November. All serious outbreaks seem to be initiated in autumn and terminated in spring, the actual timing depending on seasonal conditions.

More recently a trial was carried out to maximise bee populations by using a range of bee supplements. A surprising finding was that any benefit from the various supplements provided to the colonies was overridden by *N. apis* infection which was most likely to have been exacerbated in the test hives by manipulation associated with supplementary feeding. The control hives (not supplementary fed) performed best (Somerville, pers coms).

Little is known about the prevalence of *N. apis* in Australian honey bee colonies. There are no classic clinical signs which are characteristic of infection such as there are with European and American foulbroods. The only robust method of confirming *N. apis* infections is by the microscopic examination of infected bees (Bailey and Ball, 1991).

The aim of this study was to survey hives used for the pollination of almond trees at Robinvale, Victoria, in the Augusts of 2004, 2005 and 2006. The hives sampled originated from New South Wales, Victoria and South Australia.

2.2 Materials and Methods

2.2.1 Hives

The hives used in this study were located at Robinvale, Victoria and were being used for the pollination of almond trees in 2004, 2005 and 2006.

2.2.2 Collecting bees and counting *N. apis* spores

Twenty five bees were collected from under the top lid or from the outside of the cluster from each hive. Counts were carried out as described by Cantwell (1970) (see previous section).

2.2.3 Observations

In 2005 and 2006, as well as collecting adult bees, the bee strength of each hive was estimated and a record was made of whether the hives were spotted with bee faeces.

2.2.4 Questionnaires

In each of the three years following the collection of adult bee samples for nosema testing a questionnaire was sent to each of the participating beekeepers.

In 2004, the questionnaire sought information on:

- Hive manipulation during the three months prior to sampling
- Supplementary feeding in the five months prior to sampling
- Flora utilised during the five months prior to almond pollination
- Honey content of hives going onto the almonds
- Honey production from September to December

In 2005 and 2006, the questionnaires were expanded to include questions on:

- Bee strength of test hives at the beginning of almonds, end of almonds (August), end September and end October.
- Comb replacement strategies
- Age of queens
- Beekeepers were also invited to make comments especially on what they thought might effect nosema spore levels.

2.3 Results

2.3.1 Hives

Eight hundred hives owned by 20 beekeepers (40 hives per beekeeper) were sampled in August 2004. Eight hundred and forty hives, also owned by 20 beekeepers, were sampled in August 2005 including two lots of 40 hives (one lot of singles and one lot of doubles) owned by one beekeeper. A further 800 hives owned by 20 beekeepers (40 hives per beekeeper) were sampled in August 2006. The beekeepers' home bases were in New South Wales, Victoria and South Australia. Every effort was made to use the same apiary from the same beekeeper each year. It was not feasible to sample the same hives from each apiary.

2.3.2 Nosema spore counts and number of infected hives per apiary

The results for the 2004 sampling are provided in Table 1. This data includes the average number of *N. apis* spores per bee for all 40 hives for each beekeeper and the number of hives with positive *N. apis* samples. Beekeeper B had the highest number of spores per bee (12,236,000) and beekeeper G had the least number of spores per bee (10,000).

In 2005 the highest nosema spore count (6,190,000) and lowest was about half that of 2004 (12,236,000) and the counts were generally lower than those of 2004 (Table 2). The highest count for 2006 (3,836,500) was about 60% of the highest count of 2005 and only about 30% of the highest 2004 count (Table 3).

N. apis was detected in one or more hives in every apiary. In 2004 three beekeepers with the highest counts had all 40 hives infected. In 2005 the two beekeepers with the highest counts had all 40 hives infected and in 2006 no beekeepers had all their 40 hives infected. The number of infected hives in any apiary generally increased as the average nosema spore count increased.

Table 1 – Beekeepers, spores per bee and number of hives infected - 2004

Rank / Beekeeper code	Spores per bee	Number of infected hives
1 - B	12,236,000	40
2 - A	6,066,300	40
3 - D	4,340,000	40
4 - H	2,228,800	37
5 - O	2,205,000	39
6 - E	2,092,500	36
7 - J	993,750	36
8 - C	875,000	27
9 - P	667,500	21
10 - R	640,000	24
11 - Y	585,000	24
12 - K	540,000	24
13 - F	281,250	18
14 - I	205,000	7
15 - N	195,000	7
16 - L	127,510	3
17 - X	102,500	9
18 - Q	31,250	1
19 - S	17,500	5
20 - G	10,000	3

Table 2 – Beekeepers, spores per bee and number of hives infected –2005

Rank / Beekeeper code	Spores per bee	Number of infected hives
1 - C	6,190,000	40
2 - N	5,645,000	40
3 - L	5,335,000	39
4 – R*	1,324,000	35
5 – F	841,000	11
6 – J	716,500	21
7 – M	577,500	21
8 – A*	552,500	21
9 - E	447,500	15
10 - O	410,000	16
11 - Q	361,250	12
12 - P	321,250	16
13 - X	282,500	8
14 - D	266,250	12
15 - H	207,500	10
16 - K	195,000	9
17 - S	147,500	13
18 - B	101,250	5
19 - I	42,000	5
20 - T	26,250	5
21- G	11,250	3

* R and A are apiaries from the same beekeeper. R = doubles
A singles

In 2005 one beekeeper had two apiaries tested (Table 2). The code for these apiaries are R and A. R consisted of double hives and A were singles. It is interesting to note that the doubles had nosema spore counts of 1,324,000, 2.4 times the number of spores in singles (552,500). The difference in the management of these two apiaries was that the doubles (R) were taken to the south coast to work the spotted gum and the singles (A) were left alone to winter.

Table 3 –Beekeepers, spores per bee and number of hives infected – 2006

Rank/Beekeeper code	Spores per bee	Number of infected hives
1 – Q	3,836,500	36
2 – L	2,230,500	32
3 – D	1,851,500	34
4 – K	1,629,000	33
5 – S	1,433,000	23
6 – F	957,000	32
7 – P	796,500	24
8 – E	793,000	15
9 – B	417,500	19
10 – A	314,500	7
11 – R	225,500	6
12 – H	221,000	10
13 – N	208,000	9
14 – G	203,000	12
15 – J	191,750	10
16 – T	174,250	9
17 – O	153,000	8
18 – C	104,250	5
19 – U	103,000	9
20 - M	55,500	6

Table 4 provides the nosema spore counts for each beekeeper for 2004, 2005 and 2006 where applicable. Two beekeepers who participated in the survey in 2004 were unable to participate in 2005 and 2006 and were replaced by another two beekeepers. Two beekeepers who participated in the 2004 and 2005 samplings did not participate in the 2006 samplings and one beekeeper who participated in the 2004 sampling but not the 2005 sampling was able to participate in the 2006 sampling. One beekeeper was also sampled in the 2006 exercise for the first time.

Table 4. Nosema spore counts for each beekeeper for 2004, 2005 and 2006

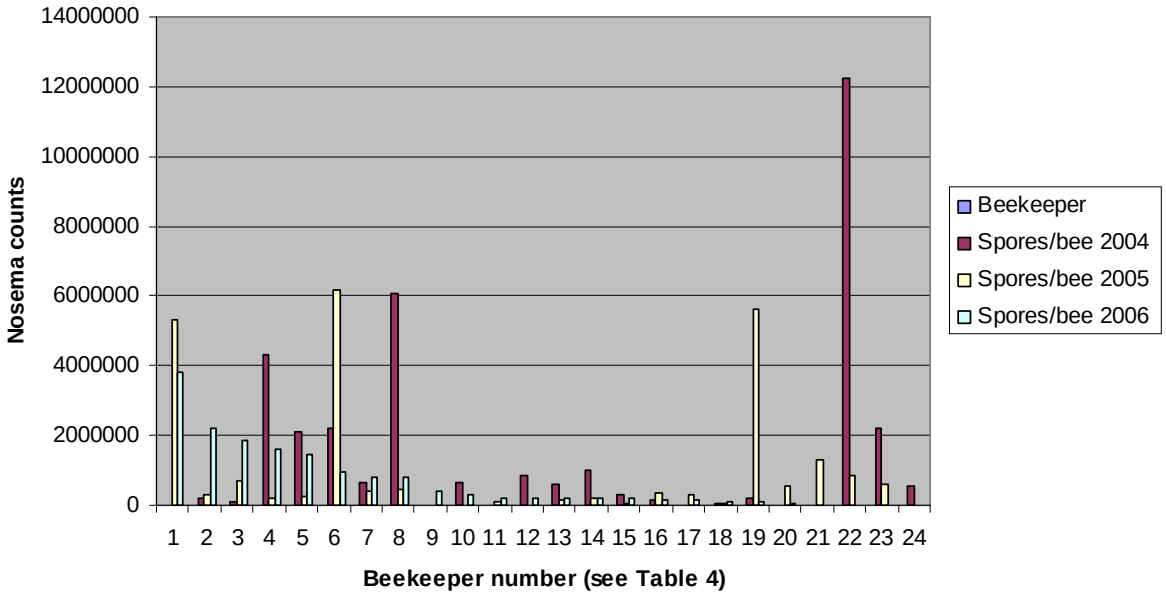
Beekeeper	Spores/bee 2004	Spores/bee 2005	Spores/bee 2006
1	N/A	5,335,000 – L	3,836,500 - Q
2	205,000 - I	321,250 – P	2,230,500 - L
3	102,500 - X	716,500 – J	1,851,500 - D
4	4,340,000 - D	207,500 – H	1,629,000 - K
5	2,092,500 - E	266,250 – D	1,433,000 - S
6	2,205,000 - O	6,190,000 – C	957,000 - F
7	640,000 - R	410,000 – O	796,500 - P
8	6,066,300 - A	447,500 – E	793,000 - E
9	N/A	N/A	417,500 – B
10	667,500 - P	N/A	314,500 - A
11	17,500 - S	101,250 – B	225,500 - R
12	875,000 - C	11,250 – G	221,000 - H
13	585,000 - Y	147,500 – S	208,000 - N
14	993,750 - J	195,000 – K	203,000 - G
15	281,250 - F	42,000 – I	191,750 - J
16	127,510 - L	361,250 – Q	174,250 - T
17	N/A	282,500 – X	153,000 - O
18	31,250 - Q	26,250 – T	104,250 - C
19	195,000 - N	5,645,000 – N	103,000 - U
20	10,000 - G	552,500[2] - A	55,500 – M
21	10,000 - G	1,324,000[1] – R	N/A
22	12,236,000 - B	841,000 – F	N/A
23	2,228,800 - H	577,500 - M	N/A
24	540,000 - K	N/A	N/A

[1]= 10 frame doubles
[2]= 10 frame singles
N/A = not applicable

The data for Table 4 is also presented as a histogram (Figure 6) which better shows that some beekeepers maintained a consistently low nosema spore count over the three year sampling periods (eg 11 and 18), relatively high counts for two of three years (during the survey (1, 4, 5, and 22) although beekeepers 1 and 22 were only involved in the survey for two years. Beekeeper 6 had the most consistently high counts over three years.

Beekeeper 18, who had the lowest average count over the three year period, did not manipulate his hives in the three months before going to almonds for all three years. He produced about 120 kg of honey (80 kg in 2004 and 40 kg in 2005). Beekeeper 6, who had the highest average count over the three year period, manipulated his hives in the three months prior to almonds in all three years but only supplementary fed in two of those three years. Total honey production was 148 (88 kg in 2004 and 60 kg in 2005).

Figure 6: Nosema counts for each beekeeper for 2004, 2005 and 2006



2.3.3 Questionnaires

The aim of the questionnaire was to link specific management practices and floral type with nosema levels.

(i) Hive manipulation in the three months prior to placing bees on almonds
In 2004 there was a clear association between manipulation of hives three months prior to almond pollination and nosema spore counts. Manipulation was considered to be any handling of hives such as taking honey off, checking brood and shifting bees. Supplementary feeding was also considered as hive manipulation but was treated as a separate category. The four beekeepers with the lowest nosema spore counts did not manipulate their hives. However, the five beekeepers with the highest counts manipulated their hives prior to almonds.

There was a similar observation for 2005 where the five beekeepers with the highest counts also manipulated their hives and the five beekeepers with the lowest counts did not. There was a similar but not as obvious a trend in 2006 where four of five beekeepers with the highest nosema spore counts manipulated their hives and two of four beekeepers with the lowest scores did not manipulate their hives.

(ii) Supplementary feeding in the five months prior to sampling
There was also a clear association between supplementary feeding and nosema spore counts in 2004. The eleven beekeepers with the highest counts supplementarily fed their hives whereas three of four of the beekeepers with the lowest counts did not supplementarily feed their hives. Interestingly the beekeeper with the lowest counts fed his hives but also used the hives to provide package bees in 2004.

In 2005 and 2006 the relationship between supplementary feeding and high nosema spore counts was not as clear as in 2004. The three beekeepers with the highest counts and the beekeeper with the sixth highest count did not supplementarily feed their hives. Although, four of the five beekeepers with the lowest counts did not supplementarily feed. In 2006 only one of the five beekeepers with the highest counts supplementarily fed his hives while none of the five beekeepers with the lowest counts supplementarily fed his hives.

(iii) Flora utilised during the five months prior to almond pollination
A broad range of flora was utilised in the five months prior to almonds in 2004, 2005 and 2006. In 2004 the most widely used flora was iron bark and manna gum. Other flora included red gum, white gum, tea tree, messmate, sugar gum and banksias. The nine beekeepers with the highest nosema spore count (from 12,236,000 nosema spores per bee to 667,500 spores per bee) most commonly used iron bark (five beekeepers) and manna gum (three beekeepers). However, iron bark was used by five of the six beekeepers and manna gum was used by three of the five beekeepers with the lowest nosema spore counts.

In 2005 the most commonly used flora were long-leaved box, mallee species and spotted gum. Other flora included stringy bark, apple box, tea tree and sugar gum. The four beekeepers with the highest nosema spore counts all worked the spotted gum from about April to July. None of the other beekeepers worked the spotted gum that year. Long-leaved box was the most common species used by beekeepers from the 5th highest nosema spore counts to 3rd lowest count.

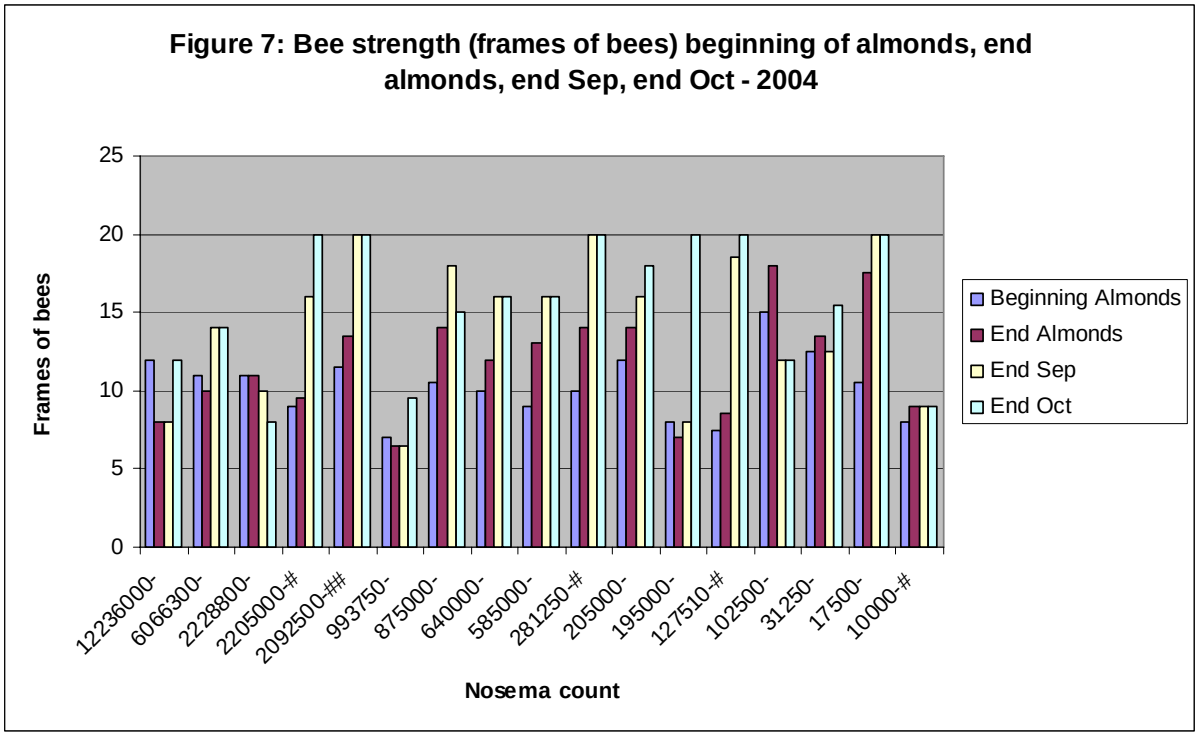
In 2006 the three most commonly used floral species were grey box, stringy bark and iron bark. Other flora included mallee species, white gum, turnip, apple box, white stringy bark and yellow gum. There was no clear association between any specific floral species and nosema spore counts.

(iv) *Honey content of hives going onto the almonds*

Hives which were full or nearly full of honey generally had the lowest nosema spore counts. Beekeepers with high counts often had less honey on their hives going on to the almonds than beekeepers with the lowset counts. This trend was also obvious in 2006 but not as clear in 2005.

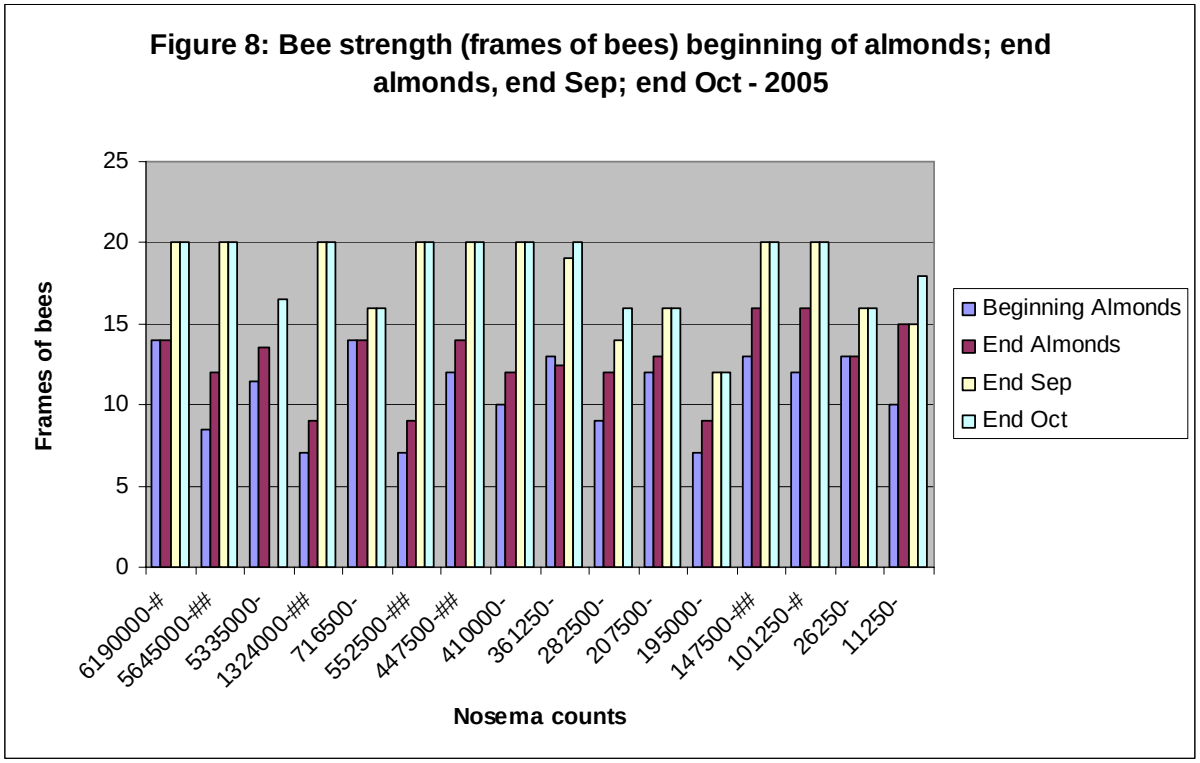
(v) *Bee strength of test hives at the beginning of almonds, end of almonds (end August), end September and end October.*

Bee strength in terms of frames of bees from the beginning of almonds, end of almonds, end September and end October were obtained for 2004 and 2005. In 2004 the number of frames of bees decreased at the beginning of almonds to the end of almonds for the two beekeepers with the highest nosema spore counts and the beekeeper with a count of 993,750. Beekeeper 1 (spore count 12,236,000) from 12 frames of bees down to eight frames and beekeeper 2 (spore count 6,066,300) fell from 11 frames of bees down to 10. The bee strength fell from an average of seven frames of bees down to 6.5 frames for the beekeeper with a spore count of 993,750 and remained the same at 11 frames for the beekeeper with a spore count of 2,228,000. The bee strength data from those beekeepers that responded to the questionnaires are provided in Figure 7.



 # indicates more than 20 frames of bees end October; ## indicates more than 20 frames of bees end September & end October

In 2005 the bee strength of the hives owned by the beekeeper with the highest nosema spore count remained the same while pollinating almonds. However, the highest spore count for 2005 was less than half the highest spore count for 2004. The bee strength of the hives of the beekeeper with a spore count of 361,250 decreased by half a frame of bees over this period and remained the same for the beekeeper with the second lowest spore count (26,250). The bee strength for the almond pollination to end October for all beekeepers is provided in Figure 8.



 # indicates more than 20 frames of bees end October; ## indicates more than 20 frames of bees end September & end October.

(vi) Comb replacement strategies

All beekeepers except one had a comb replacement strategy. This usually consisted of replacing two to four frames usually in spring. The beekeeper that did not have a replacement strategy had the 8th lowest nosema spore count in 2004, 4th lowest in 2005 and 6th lowest in 2006.

(vii) Honey production from September to December 2004 and 2005

Honey production for September to December 2004 and 2005 is provided in Figures 9 and 10 respectively.

Figure 9: Honey production from September to December 2004 vs Nosema spore counts

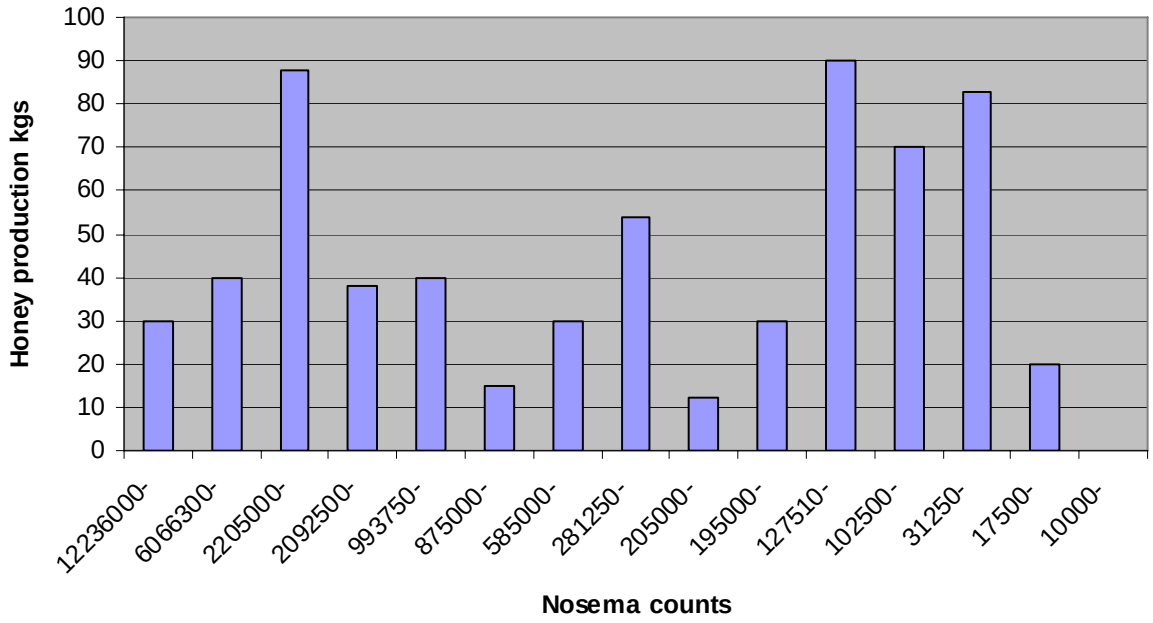
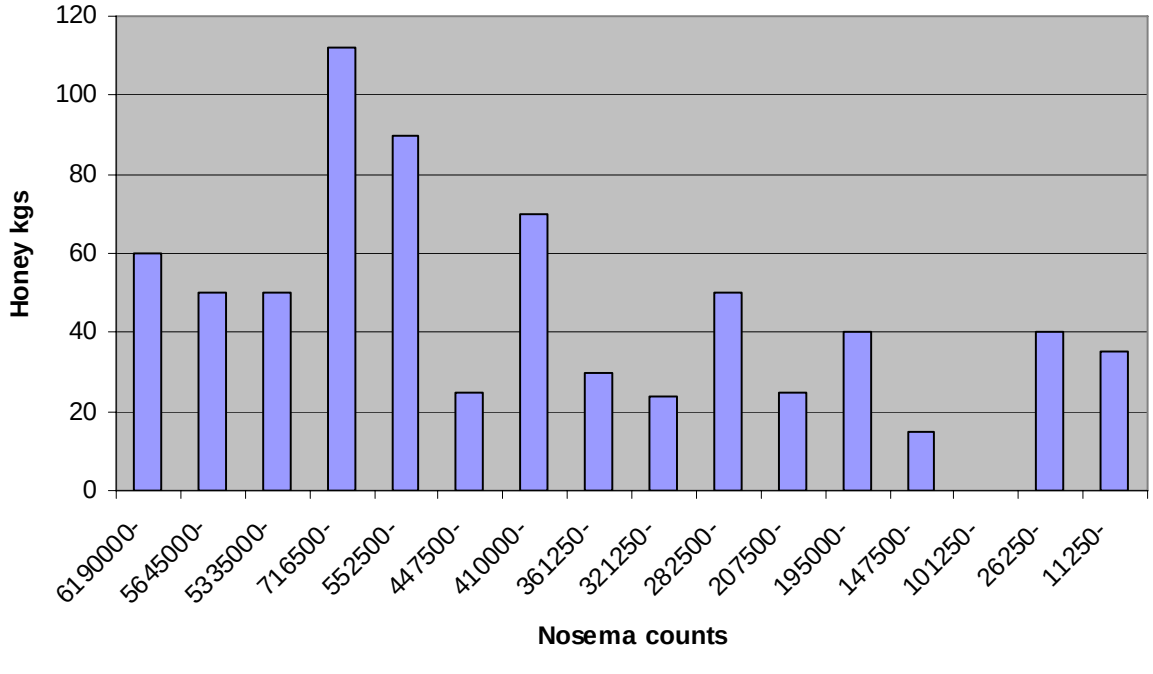


Figure 10: Honey production from September to December 2005 vs Nosema counts



There appeared to be a trend for greater honey production for beekeepers with lower spore counts in 2004 although the beekeeper with the lowest spore count (10,000) did not produce any honey. In 2005 the trend seemed to be reversed.

(viii) Age of queens
There was no detectable association between age of queens and nosema spore counts. However, most beekeepers used queens that were 12 months old or less.

(ix) Spotting on hives
In 2005 and 2006 we recorded faecal spotting on the test hives. This was to determine whether spotting could be an indicator of the presence of nosema in the hives. However, at least one hive in all apiaries was diagnosed with nosema disease. Spotting was detected on 12 of 20 apiaries in 2005 and 17 of 20 beekeepers in 2006 with no association of severity with spore count.

(x) Beekeepers thoughts on factors that may effect nosema levels
The beekeepers comments relating to nosema and pollinating almonds are listed below. Where comments deal with specific activities for that year's sampling rather than general comments nosema spore counts are also provided.

2004

'Bees' autumn crop was iron bark which came in thin towards the end'. Spore count 6,066,300.

'Capped iron bark honey went thin that's why top boxes were taken off". Spore count 667,500.

'Winter flowering eucalypts, unsealed honey'.

'Dry conditions with little ground flora pollen over winter'.

'With experience from the previous season we found grey box pollen and honey to leave the bees of poor constitution. This season we found our bees to come through the winter better from being on wild turnip and staying around Swan Hill after almond pollination. I feel the quality of autumn pollen is a big factor in preventing nosema. Also a good time during May, June and July to leave the bees alone.' Spore count 993,750.

'Ideally over wintered hives need to get pollen/nectar but in our area this is not always possible, despite it being desirable. I think the age of the queen is significant – older queens are more likely to succumb due to stress factors such as night ants, ants in general, cold weather, wind, lack of pollen/nectar, damp bases - internally and poorly maintained hive materials favour nosema. The more bees have water from drums rather than natural sources, the greater the chance of infections'.

'I think you need you have to leave bees as tight as you can with as much capped honey as possible and don't disturb the bees until they are ready to work'.

'I packed bees for winter mid-May while the weather was still warm, then only checked the bees twice before almonds'. Spore count 195,000.

'These hives had no honey harvested from mid-May. Nosema appears to be a greater problem in drought years. The previous year we fed sugar syrup on similar pollen flows. Bees appeared to have similar nosema levels. Brood was also manipulated prior to almonds'. Spore count 2,205,000

'Wet winter and thin honey eg: grey box and uncapped iron bark'.

'In Victoria we have a huge problem in that many of our autumn honey flows have no pollen or poor nutritional pollens and nectar. Beekeepers generally, in the past, have not worried about this to any extent. They rely on good breeding conditions in the spring to lift their bees and in the past were

prepared to forgo possible early honey, as poor bees coming into spring quite often don't create swarming problems. However, now with so many of these beekeepers providing beehives for August almond pollination there is an urgent need for changes in attitudes and a resolve to apply more attention to wintering of bees'.

'Honey was extracted in April and hives were topped up and left until almonds'. Spore count 540,000.

2005

'We worked the bees fairly hard until about three to four weeks prior to almond pollination. The bees then returned to the south coast for swarm control and one box of honey was then moved elsewhere for little canola and then the curse crop. The bees built up well after almonds. They didn't take off tested load but used other hives for nucleus producing hives. It didn't appear to make any difference come late October'. Spore count 6,190,000.

'Only replace queens as they show signs of failing. Hives were stressed at various times during 2004-2005 due to the lack of nectar'. Spore count 11,250.

'Try to keep hives on good pollen at all times and avoid thin honey at all times'. Spore count 42,000.

'All hives should be put into singles in the autumn and not manipulated. Should also be encouraged to be presented for almond pollination as single box hives'. Spore count 195,000.

'I do not tend to overwork bees except in spring time in an effort to control swarming. I tend to work half of my bees on an individual honey flow whilst the others are on breeding condition/pollination. If all are on honey, I tend to just under super half and forget about them a little until I catch up. Basically the bees are not overworked. I tend to produce 90-120 kgs of honey per season. Spore count 5,335,000

'A young queen gives the hive greater resistance to most of the common troubles of beehives. Our hives are requeened every year, sometimes twice a year. …and whether it is nosema or any of the common troubles that bees have, young queens will give your hives much greater resistance. Spore count 26,250.

'In April 2005, when the grey box was mid-flowering, the weather was wet and cold. This would have started the nosema in some hives'. Spore count 321,250.

2006

'Please convince almond pollinators to accept single eight framed hives. I believe it would be much better for the health of the bees by not having to heat a double hive while still coming out of winter in August. I also believe they would be better pollinators as more would fly rather than many of the worker bees staying at home for hive temperature control. Another plus is that we, as beekeepers, could carry more bees per load and therefore save a bit on transport costs'.

'This year (in my opinion) the grey box was exceptionally good due to dry conditions – no spotting – good pollen'. Spore count 191,750.

'In Victoria, many of our autumn flowering eucalypts yielded poor nutritional quality pollens, and nectar that is not ideal for the wintering of bees. Therefore the possibility of bad nosema outbreaks in the early spring is always there. The often experienced rapid depletion of field bees at the start of the almond flowering is caused by a lack of young bees in the hive at almond bud burst and the sudden expansion of flowers that are very attractive to bees. This excites the bees to such an extent that they go from a semi-dormant state to an overactive state too quickly.

The feeding of sugar syrup four weeks prior to almond flowering stimulates and activates these older bees slowly and they are stronger at the start of the almond flowering. The addition of hatching bees, which will not be weakened by nosema etc. ensures that the brood is fed well.

I believe that if the older field bees die off rapidly and the hive population becomes out of balance, nurse bees can become field bees at a few days of age, especially with the incentive of the heavy flowering.

Sugar feeding at about a week after the bees are placed in the almond orchard is a big aid in ensuring the continued stimulation of the bee hives towards pollination of the flowers."

"The levels of nosema really don't surprise me but I always try to keep bees on good nutrition and do a lot of kilometres to do so. The nosema counts have not hurt me – why? Good question isn't it? I do my best to look after the bees so they look after me. Spore count 3,836,500. (*Honey production 110+ kg per hive in 2005 – spore count in 2005 – 5,335,000).*

2.4 Discussion

Few studies have been carried out to determine the prevalence of *N. apis* in Australian honey bee colonies (Doull, 1961; Langridge, 1961; Kleinschmidt, 1979). These have involved relatively few hives compared to this study where about 800 hives were sampled each year for three years. In this survey the opportunity was taken to sample from a congregation of about 25,000 hives at Robinvale in Victoria. These hives had been moved to Robinvale for the prime purpose of pollinating almond trees for three weeks in August. This yearly event provided a unique opportunity to sample bees originating from diverse areas in one location. However, the limitation of this study was that the survey consisted of only one sampling per year for the three year period.

Most of the beekeepers (15) were based in various parts of Victoria; four were based in New South Wales and one in South Australia. Every attempt was made to sample the apiaries from the same beekeepers who were involved in the first survey in 2004. Bush fires and other factors prevented the sampling of all the apiaries from the same beekeepers; however, we were able to sample the same apiaries from 16 beekeepers for all three years.

The time of sampling was also a critical time in the nosema cycle where nosema infections are about to reach their peak for the year. August provides an ideal sampling time as it is in August when the spore numbers in hives begin to increase and usually reach a peak in spring. The August/September rise in nosema spore counts has been reported by Doull (1961) and Langridge (1961) to be peak infection times (see Figure 2). Sampling in August also provides a good indication as to the relative infection levels of the apiaries. This type of information would not be gleaned at times when infection levels were generally low such as in late summer or early autumn.

N. apis was found in every apiary examined in this survey. However, there was a broad range of infections in these apiaries spanning from 12,236,000 to 10,000 spores per bee in 2004, 6,190,000 to 26,250 spores per bee in 2005 and 3,836,500 to 55,500 spores per bee in 2006. There was also considerable variation in the number of hives infected in each apiary. In 2004 three beekeepers had all 40 hives infected and two beekeepers had all hives infected in 2005. In 2006 the highest number of infected hives was 36. In 2004 one beekeeper had only one hive infected while the minimum number of infected hives in 2005 and 2006 was five for both years. These substantial differences between years are likely to reflect differences in management techniques and the impact of different flora on bee health.

In 2004 and 2005 beekeepers provided the bee strength (frames of bees) of the test hives at the time they were taken to almonds, at the end of almond pollination, at the end of September and at the end of October. There was no correlation between hive strength and nosema *spore* counts. However, in 2004 the bee strength of hives in the two heaviest infected apiaries (12,236,000 and 6,066,300 spores per

bee) decreased from the time they were placed on almonds to the end of almond pollination. The bees with a count of 2,228.800 spores per bee only maintained their strength. Most of the bees with low counts increased their bee strength while on almonds. In 2005 this trend was not as clear, however, the counts were generally lower in 2005 compared to 2004. The highest count in 2005 (6,190,000 spores per bee) bee strength remained the same from being placed on almonds to the end of almond pollination. A similar situation was observed for a count of 716,000 spores per bee.

The reduction of bee strength associated with high nosema spore counts indicates bee deaths and an inability of these hives to replace bees as quickly as the field bees die. Anderson and Giacon (1992) have demonstrated that honey bees infected with *N. apis* collected significantly less pollen than colonies fed only sucrose solutions. This factor in association with the other effects of nosema on adult bees (see literature review 1.5.1 Effect on adult bees) will have a significant effect on the pollination efficiency of heavily infected bees.

It has long been recognised that colonies working late autumn and early winter flows are prone to develop high nosema spore counts when breeding becomes minimal as a result of protein deficiency. Under these conditions, deaths cannot be replaced by births (Kleinschmidt, 1979). The protein (pollen) availability and protein status of the bee affects the impact of nosema disease. Managing colonies to avoid protein deficiency has also been shown to be beneficial in reducing nosema spore counts (Fries, 1995; Kleinschmidt, 1979). Kleinschmidt (1979) demonstrated that colonies in Queensland which commence work in July would show peak nosema spore counts in September. He also noted that more rigorous breeding will compensate for increased mortality of bees on low quality pollen. The longevity of such bees was reduced to 20-26 days and when high nosema spore counts coincided with low quality pollen or an insufficient quantity of pollen, the average life span of bees was less than 20 days.

Other factors such as colony disturbance in winter and moving colonies to new sites especially during winter have also been reported to aggravate nosema disease. In this study there was a clear association between low counts and no manipulation of hives in the three months prior to moving bees to the almonds. The high counts were usually associated with hive manipulation. A similar observation was made with supplementary feeding. One interesting exception to this observation was associated with a beekeeper who provides package bees for export. In April 2004 bees were harvested from his hives to produce export packages. This would have resulted in the extraction of most of the nosema-infected bees, interrupting the normal infection cycle at a critical time when infection levels would have increased further over autumn and winter. Having significantly reduced the number of infected bees, the natural progression of the nosema cycle was interrupted, preventing the usual build up of nosema in August and September. When tested in August 2004 this apiary had the lowest nosema spore count of all (10,000 spores per bee). Bees were again extracted for packages after pollination in August 2004. However, the removal of bees at this time would have had little impact on the nosema spore counts in the following August. This was reflected in the nosema spore counts for these bees in 2005, which was 552,500 per bee.

In this study a range of flora were utilised by beekeepers during the five months prior to almond pollination. In 2004 iron bark and manna gum were associated with high nosema spore counts but were also associated with low counts. In 2006 the three most commonly used floral species were grey box, stringy bark and iron bark. As was the case in 2004 there did not seem to be any association of this flora with particular nosema levels as they were used by the beekeepers with the highest and the lowest nosema spore counts. The 2004 and 2006 floral utilisation patterns suggest that other factors rather than floral type were involved in determining nosema concentrations.

In 2005 the four beekeepers with the highest nosema spore counts all had their bees on spotted gum. This would suggest that spotted gum may have been the cause of these high counts. However, spotted sum is a good honey producer. With favourable weather, bees are able to ripen honey, but prolonged wet periods may cause deterioration. Spotted gum produces heavy supplies of pollen in favourable weather. The abundant pollen supplies, together with the stimulation provided by continuous supplies of nectar from this species, enable colonies of bees to maintain their numerical strength even while good honey crops are being harvested and, during favourable seasons, in mid-winter (Klemson, 1985).

However, one beekeeper made the comment that autumn flowering eucalypts in Victoria yield poor nutritional quality pollens and nectar that is not ideal for wintering bees. Doull (1961) also noted that nectar from the grey box was “thin” prior to significant losses from nosema disease. Together these comments suggest that the quality of nectar and pollens are influenced by environmental factors which may influence the nutritional value of floral species which are usually considered to be of high quality.

An interesting observation in some of the heavily infected apiaries was that *N. apis* was not detected in all hives. A small number of hives appeared to be *N. apis* free. Doull (1961) also observed this in his study of the incidence of *N. apis* in South Australia. Twenty five of the 417 samples he examined did not contain *N. apis*. However, in most of these cases infected bees were found in the preceding and subsequent samples. He postulated that the significant differences in the level of infection between hives in the same apiary (which was apparent in this study) suggested that there may be factors, varying in some way from hive to hive, which determine the level of infection.

In this study the beekeepers were asked whether they had a comb replacement program. All except one beekeeper had a system in place to replace old combs. This question was asked as Fries (1988) has demonstrated that keeping bees on old comb increases the risk of detectable *N. apis* spores and that nosema disease was significantly correlated with winter loss. The fact that most beekeepers had a comb replacement program makes it impossible to gauge what effect comb replacement has on nosema spore counts. However, the fact that such a strategy is very commonly undertaken indicates that beekeepers see benefit in replacing old combs.

The beekeepers in this study were also invited to provide comments about nosema. These responses, as well as some comments on pollination of almonds, have been provided in the results section. These comments provide a personal perspective from commercial beekeepers with a wealth of experience in their profession.

This is a unique study for a number of reasons. More apiaries and hives have been sampled than in other previous studies for nosema disease in Australia. The sampling of these hives on three different occasions in the same location at the same critical time of year, linked with a questionnaire provided a novel means of linking nosema spore counts with management practices. The fact that there were a range of management practices and flora associated with a range of nosema spore counts makes it difficult to definitively provide the precise input of any particular management practice, although clear links were identified with some activities.

The drawbacks of this study were that only a single sampling was possible per year and that all beekeepers at the time of sampling were working almonds, which provides useful pollen which counteracts the negative effects of nosema disease building up in spring. It also focused purely on commercial beekeepers that in general, had long standing and considerable beekeeping skills which would prevent the worst effects of nosema disease becoming apparent. Nevertheless, the survey provides a good indication of the extent and range of nosema infections in bees in most of the major beekeeping States in Australia. Had the drought conditions not prevailed during the three years of the survey, severe losses of bees and hives as been described by Doull (1961) may have been experienced by the beekeepers with the highest counts.

4. References

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