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Nosema disease

Literature review and survey of beekeepers

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by Michael Hornitzky

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Foreword

Nosema apis causes one the most important diseases of honey bees but is mostly overlooked by beekeepers as there are no obvious characteristics or symptoms of disease. Hence, the infection caused by *N. apis* (nosema disease) is also 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.

This report provides the beekeeping industry with information about the disease as well as an indication as to its significance and control strategies under Australian conditions.

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

This report, an addition to RIRDC's diverse range of over 1200 research publications, forms part of our Honeybee program, which aims to improve the productivity and profitability of the Australian beekeeping industry.

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Contents

Executive Summary

Nosema apis is a serious parasite of adult honey bees. It has been reported to cause significant production losses as a result of a range of effects it has 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.

The aim of this project was to increase the awareness of the beekeeping industry to this serious infection. This was achieved by preparing a literature review that provides information on the effects of the disease, current control methods and the laboratory diagnosis of the infection. The laboratory diagnosis can be carried out by beekeepers so that they can monitor *Nosema* levels and apply appropriate management strategies to minimise infection levels.

There is very little published information on the prevalence and significance of *N. apis* in Australian honey bees. The second part of this study was a quantitative survey of 800 honey bee colonies at Robinvale, Victoria for *N. apis*. The colonies owned by 20 beekeepers from New South Wales, Victoria and South Australia were congregated in one area for almond tree pollination and provided a unique opportunity to sample colonies from diverse origins.

This survey provided good data on the prevalence of *Nosema* spores in Australia. There was a broad range of infection levels ranging from 10,000 to 12,236,000 spores per bee. This range suggested that some beekeepers were better able to control nosema disease than others, although they were probably not aware what impact these practices were having on *N. apis* levels. Analysis of the completed questionnaires that were filled in by the beekeepers that took part in the survey indicated likely management strategies to control *N. apis*.

These were (i) packing of hives down tight for the winter, (ii) no manipulation of hives during the winter (including no supplementary feeding of individual hives), (iii) no shifting of bees during the winter and (iv) hives with low *Nosema* levels were mostly ¾ to full of honey. A survey of the same hives will be carried out at the same time of next season to determine whether these factors continue to remain important in minimising the effects of *N. apis*. More details of hive location and management prior to the survey will also be determined.

This work has demonstrated that nosema disease is common in Australian honey bee colonies and that further work should be carried to determine what impact this disease has on the beekeeping industry in Australia.

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).

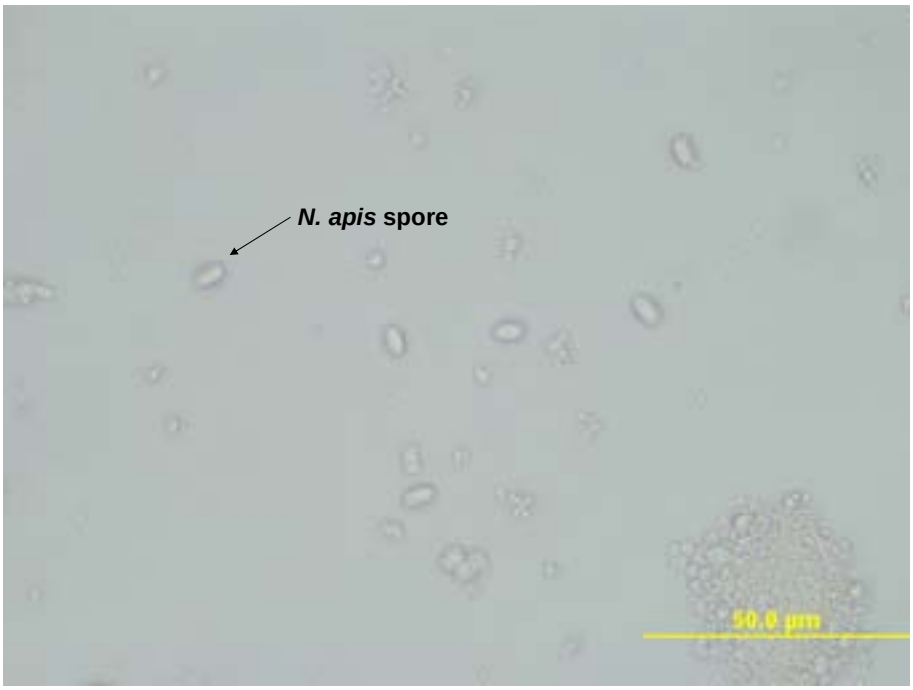

Figure 1: Wet preparation of *N. apis* spores prepared from whole adult bee sample

In a recent study it has been suggested 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 is has been suggested that molecular studies such as the use of micro-satellites (small repetitive sequences) may prove to be useful in identifying strains and hence assist in determining whether there are virulent and non-virulent strains of *N. apis* (Rice, 2001).

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). There are considerable variations reported between its incidences 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 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.

As the package bee colony is without emerging bees for 3 weeks, this type of colony is especially vulnerable to *Nosema.* 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 the bee 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). Worker bees, queen bees and 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 5 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.

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 are only characteristic of 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 2 to 8 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 seasonal trend of typical infections exhibits low levels during the summer, a small peak in autumn and a slow rise of infection during the winter. In the spring, the level of infection increases rapidly as brood rearing starts and while bee flight is limited (Fries, 1995). In South Australia, Doull (1961) reported a well defined peak of infection in the spring followed by a decline in summer. The disease then remains at a relatively low level of infection until the following spring. Doull (1961) also made the observation that the relatively high levels of infection throughout the autumn and winter, of both years of his study, and the autumn rise in infection appeared to confirm the suggestion that in South Australia the disease may rise to severe levels at any time of the year.

- 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
- Covers 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* 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 2)
    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 3 minutes)

v. Then count the spores in 5 large (60 small squares) Figures 3 and 4.
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 5 large squares (80 small squares) x 50,000

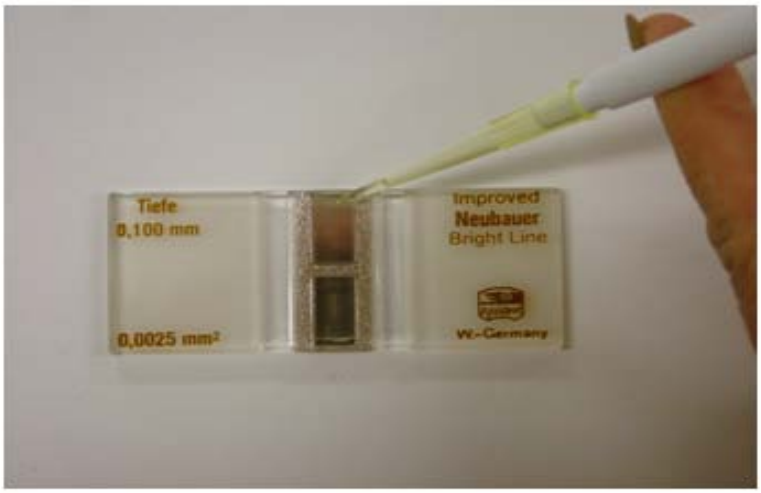

Figure 2: Inoculation of Improved Neubauer Counting to facilitate the counting of *N. apis* spores

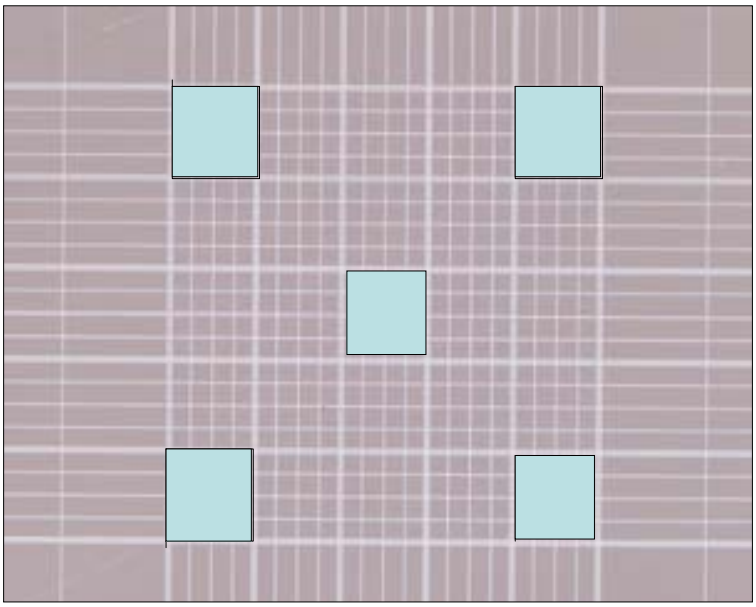

Figure 3: Five large squares (each containing 16 smaller squares) in which *N. apis* spores are counted to determine infection levels of adult bees.

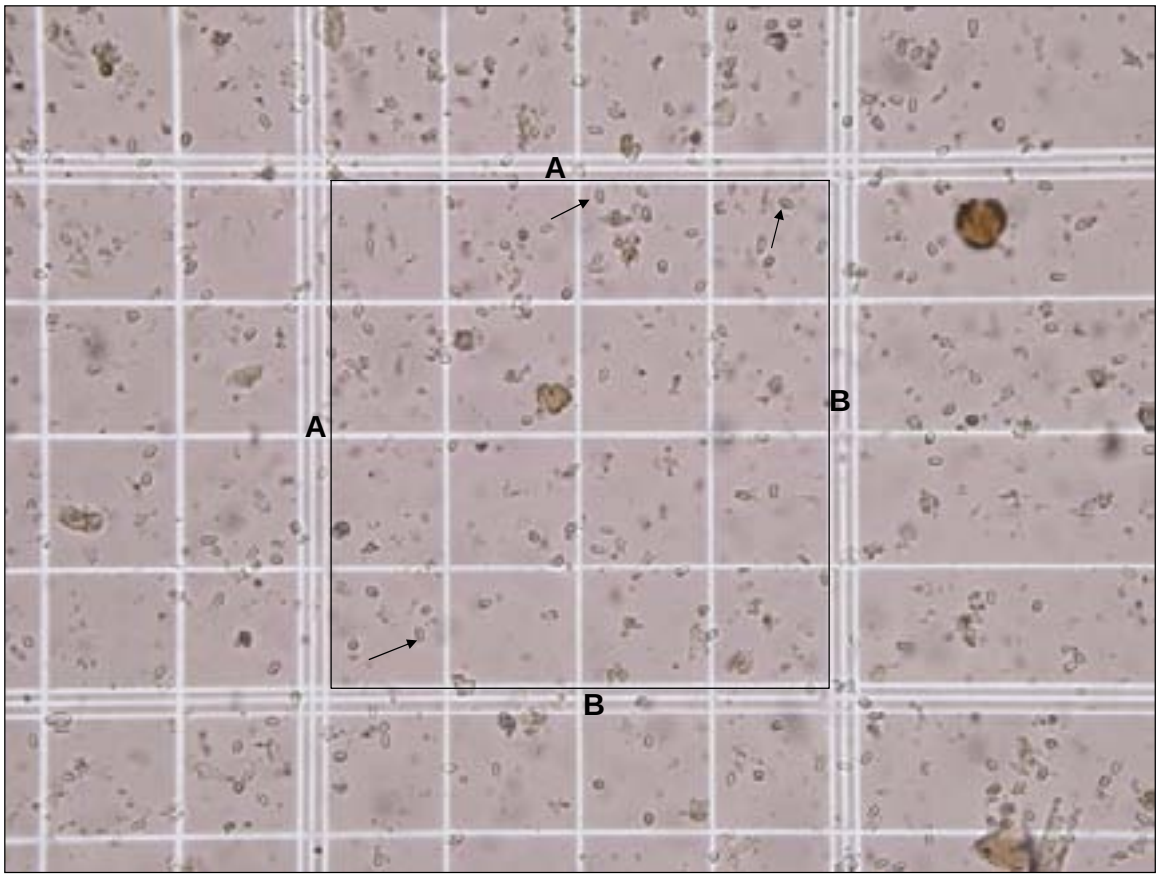

Figure 4: One large square containing 16 smaller squares and *N. apis* spores.
Small arrow heads point to 3 *N. apis* spores.
There are 47 spores in these 16 small squares. Assuming that the other 4 large squares (see Figure 3) also contained 47 spores the *N. apis* spore count would be:
47 x 5 x 50,000 = 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 counts were recorded at regular intervals. Before half of the colonies were moved to winter ironbark, the original apiaries had recorded nosema 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 3 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 2-3 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).

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 3 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* – 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 which have been conducted to determine the extent of the disease in Australia. Doull and Cellier, (1961) carried out a 2 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.

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. 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.

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 Questionnaire

A questionnaire was sent to each of the participating beekeepers requesting information on the hive movements, supplementary feeding and general hive management.

2.2.4 Statistical analysis

A preliminary analysis showed the *Nosema* spore counts approximately follow a negative binomial distribution. Hence, a generalized linear model was fitted to the data with an assumption that errors followed a negative binomial distribution with the aggregate parameter of 0.336. A logarithmic

function was used to link the observed values to the beekeeper effects. The comparisons between beekeepers were made using the least significant difference (LSD) test on the logarithmic means.

2.3 Results

2.3.1 Hives

Eight hundred hives owned by 20 beekeepers (40 hives per beekeeper) were sampled for this study. The beekeepers home bases were in New South Wales, Victoria and South Australia. Bees were collected on 16th and 17th August 2004. The weather was fine.

2.3.2 Statistical analysis

A summary of the results is provided in Table 1. The average number of *N. apis* spores per bee for all 40 hives for each beekeeper is provided. 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). The 3 beekeepers (B, A, D) with highest spore counts per bee had all their hives infected. Beekeepers Q, S and G had the least number of infected hives (1, 5 and 3 respectively).

2.3.3 Questionnaire

There were a number of management practices which were associated with low *Nosema* counts:

- Packing of hives down tight for the winter
- No manipulation of hives during the winter (including no supplementary feeding of individual hives)
- No shifting of bees during the winter
- Hives mostly ¾ to full of honey

High *Nosema* counts were associated with:

- Multiple shifting of hives during winter
- Manipulation of hives during winter
- Hives generally about ½ full or less of honey

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

Beekeepers	Spores per bee	Number of hives infected
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	30,125	1
19 - S	17,500	5
20 - G	10,000	3

2.4 Discussion

This study demonstrated that there can be a broad range of *N. apis* infection levels in beehives congregated in one area. This indicates that conditions prior to movement to almonds and management practices are likely to have a significant impact on *Nosema* levels.

There were four key factors noted in the questionnaires which appeared to play a role in minimising infection levels.

(i) Packing of hives down tight for the winter. This minimises the energy required by the bees to maintain hive and brood temperature, if brood is present. The less energy expended by the bees the less stress which assists in keeping *N. apis* numbers down.
(ii) The manipulation of hives during winter. This has been recognised for some time as aggravating *Nosema* levels (Bailey and Ball, 1991). Bees are often crushed when colonies are opened examined. These are removed by other bees who become infected when they ingest the remains of infected bees promoting the proliferation of more spores.
(iii) Shifting of bees during winter. Moving colonies to new sites encourages bees to deposit faecal matter within the hive which further contaminates the hive and facilitates the spread of *Nosema* (Bailey, 1955).
(iv) Hive honey content: Hives with low *Nosema* spore counts were generally ½ or more full of honey. Having good supplies of honey reduces the need for bees to forage, reducing stress.

The cycle of infection for *Nosema* levels has been well documented. Typically there are low levels during the summer and autumn, a slow rise of infection during winter but in the spring, the level of infection increases rapidly as brood rearing starts and bee flight is limited. Most hives survive the infection and may prosper; however, a reduced bee population in spring will reduce honey production substantially compared to bees that have no or little infection with *N. apis*.

Nosema disease has been long identified as a serious disease of honey. It affects adult bee longevity, queen bees, brood rearing, bee biochemistry, pollen collection and other bee behaviour. Although it has broad ranging effects these may not be recognised by beekeepers as being caused by disease and any losses may be put down to unfavourable conditions rather than the effects of *Nosema.*

If colonies are required to be of a certain size population in late winter or early spring, which is the case for pollination purposes, then management strategies should be implemented during the autumn period prior to winter, giving sufficient time to expand the population of the colonies to the required size prior to winter. This should be done with little or no management activity on the hives during the winter period, except for the possibility of sugar supplementation in the event of imminent starvation although this may take the form of dry sugar rather than syrup.

The broad range of infection levels in this study indicate that there are beekeepers who operate their hives in such a way that infection is very low compared to other beekeepers hives. However, the beekeepers with low infection levels were just as unaware of their *N. apis* infection levels as those with high infection levels.

It would be useful for beekeepers to monitor the *Nosema* levels in their hives. This monitoring could be achieved using the quantitative technique as described previously or it could be achieved using more simple techniques such as examining wet preparations of processed bees as long as it was done in a consistent manner. Regular monitoring coupled with modifications in management techniques to reduce *N. apis* infection will no doubt have beneficial effects on bees and hive production.

3. Recommendations

- Beekeepers be encouraged to monitor their hives for *N. apis* spores in order to determine their levels of infection at various times of the year. Monitoring should also be linked to specific management practices to identify what actions promote or minimise nosema disease.

- That a further survey of the apiaries used in the August 2004 study be carried out so that *Nosema* infection levels can be compared over a 2 year period to more strongly access the links between management practices and *Nosema* levels.

- To conduct a comprehensive survey of the beekeepers used in this study to better determine management practices that minimise the proliferation of *Nosema* in honey bees. This would include requesting information about productivity of hives between sampling times in an attempt to access production losses caused by *N. apis.*

- To conduct trials to determine what impact specific management practices identified in this study have on *N. apis* infection levels.

- To better determine the significance of different *N. apis* levels and the significance of these levels at different times of the year.

4. References

1. Anderson, D.L. and Giacon, H (1992) Reduced pollen collection by honey bee (Hymenoptera: Apidae) colonies infected with *Nosema apis* and sacbrood virus. *Journal of Economic Entomology* 85(1):47-51.

2. Bailey, L. (1955a) The infection of the ventriculus of the adult honey bee by *Nosema apis* (Zander). *Parasitology* 45: 86-94.

3. Bailey, L. (1955b) The epidemiology and control of Nosema disease of the honey bee. *Annals of Applied Biology* 43: 379-389.

4. Cantwell, G.E. (1970) Standard methods for counting nosema spores. *American Bee Journal.* 110: 222-223.

5. Bailey, L. and Ball, B. (1991) Honey bee pathology. Second Edition, Academic Press p64-72; p141-143.

6. Doull, K. (1961) Nosema disease. *The Australasian Beekeeper* 62: 228-235.

7. Farrar, C.L. (1947) Nosema losses in package bees as related to queen supersedure and honey yields. *Journal of Economic Entomology.* 40: 333-338.

8. Foote, L. (1971) California nosema survey, 1969-1970. *American Bee Journal* 111: 17.

9. Fries, I. (1988) Comb replacement and Nosema disease (*Nosema apis* Z.) in honey bee colonies. *Apidologie.* 19: 343-354.

10. Fries, I. (1988) Infectivity and multiplication of *Nosema apis* Z in the ventriculus of the honey bee. *Apidologie* 19: 319-328.

11. Fries, I. (1995) *Nosema apis* – a parasite in the honey bee colony. *Bee World* 74: 5-19.

12. Fries, I., Ekbohm, G. and Villumstead, E (1983) *Nosema apis*, sampling techniques and honey yield. *Journal of Apicultural Research* 23(2): 102-105.

13. Furgala, B. (1962). Factors affecting queen losses in package bees. *Gleanings in Bee Culture* 90: 294-295.

14. Furgula, B and Mussen, E.C. (1990) Protozoa *In:* Honey bee pests, predators, and diseases, Second Edition, Roger A. Morse and Richard Nowogrodzki., eds. Cornell University Press, Ithica and London, 48-58.

15. Furgala, B. and Sugden, M.A. (1985) Residual activity of bicyclohexylammonium Fumagillin in sucrose and high fructose corn syrup stored at two temperatures. *American Bee Journal* 125: 47-48.

16. Hartwig, A. and Przelecka, A. (1971) Nucleic acids in the intestine of *Apis meliffera* infected with *Nosema apis* and treated with fumagillin DCH; cytochemical and autoradiographic studies. *Journal of Invertebrate Pathology* 18: 331-336.

17. Kang, Y.B., Kim, D.S. and Jang, D.H. (1976) Experimental studies on the pathogenicity and development al stages of *Nosema apis*. *Korean Journal of Veterinary Research* 16: 180-184.

18. Katznelson, H. and Jamieson, C.A. (1952) Control of Nosema disease of honey bees with fumagillin. *Science* 115: 70-71.

19. Kleinschmidt, G (1984) Australian Development Assistance Course in Apiculture 1984. Queensland Agricultural College 6.1.2.1

20. Lehnert, T., Shimanuki, H. and Knox, D. (1973) Transmission of nosema disease from infected workers of the honey bee to queens in mailing cages. *American Bee Journal* 113: 413-414.

21. Liu, T.P. (1973) Effects of fumadil B on the spores of *Nosema apis* and on lipids of the host cell as revieal ed by freeze-etching. *Journal of Invertebrate Pathology* 22: 364-368.

22. Moeller, F.E. (1962) Nosema disease control in package bees. *American Bee Journal* 102: 390-392.

23. Morse, R.A. and Shimanuki, H (1990) Summary of control methods *In:* Honey bee pests, predators, and diseases, Second Edition, Roger A. Morse and Richard Nowogrodzki.,eds. Cornell University Press, Ithica and London, 341-354.

24. Nixon, M (1982) preliminary world maps of honey bee diseases and parasities. *Bee World* 63: 23-24.

25. Rhodes, J. and Somerville, D. (2003) Introduction and early performance of queen bees – some factors affecting success. RIRDC No 03/049.

26. Rice, R. (2001) Nosema disease in honeybees – Genetic variation and control. RIRDC N0 01/46.

27. Shimanuki, H, Knox, D.A., Furgala, B, Caron, D.M. and Williams, J.L. (1992) *In:* The hive and the honey bee, Tenth edition, Joe Grham and Dadant & Sons, eds, Dadant and Sons Inc, Hamilton, Illinois.

28. Somerville, D. (2002) Nosema disease in bees. Agnote DAI-124.

29. Wang, Der-I and Moeller, F.E. (1971) Ultrastructural changes in the hypopharyngeal glands of worker honey bees infected by *Nosema apis*. *Journal of Invertebrate Pathology* 17: 308-320.

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