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www.publish.csiro.au/journals/ajea *Australian Journal of Experimental Agriculture*, 2007, **47**, 883–886

Survey of feral honey bee (*Apis mellifera*) colonies for *Nosema apis* in Western Australia

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Abstract. The parasite, *Nosema apis*, was found to be widespread among feral populations of honey bees (*Apis mellifera*) in the south-west of Western Australia. The location, month of collection and whether the feral colony was enclosed in an object or exposed to the environment, all affected the presence and severity of infection. There was no significant difference in the probability of infection between managed and feral bees. However, when infected by *N. apis*, managed bees appeared to have a greater severity of the infection.

Additional keywords: metropolitan and rural bee colonies, swarms.

Introduction

The spore-forming microsporidian, *Nosema apis* Zander, is an intestinal parasite of honey bees. The aetiology of the protozoan is published in reviews by Bailey (1981) and Shimanuki *et al*. (1997).

The parasite can reduce honey bee (*Apis mellifera* L.) longevity by up to 50% and queen bees can supercede within 2–6 weeks (Moeller 1956; Bailey 1981). The supercedure may be associated with the malformation of the hypopharyngeal glands, where a 17–45% reduction in the size of the secretory globules within the gland is found in worker bees after inoculation with nosema spores (Wang and Moeller 1969). Honey yields can be significantly negatively correlated with nosema spore levels where the number of infected bees has a predictive value on honey yield (Fries *et al*. 1984).

Foraging honey bees can carry in excess of 16 million *Nosema* spores/bee (L'Arrivee 1963*a*). However, flying bees can average 8.9 million spores/bee; bees collected from the entrance average 6.8 million spores/bee and bees sampled from central brood combs, where there is a mix of newly emerged bees free of nosema, average 0.31 million spores/bee (El-Shemy and Pickard 1989). The incidence of the disease in hives is high. In Minnesota, USA and New Zealand, the incidence can be as high as 80% (Furgala *et al*. 1973; Malone *et al*. 1999) and 94% in South Australia (Doull and Cellier 1961).

The present study is the first survey of *N. apis* in a feral honey bee population in Western Australia (WA). The aims of the study were to investigate: (1) the parasite's prevalence in wild populations of honey bees resident in areas where both the amateur and commercial beekeeping industry operate, (2) whether managed bees have a different rate and severity of infection to feral honey bees and (3) if these are affected by month of year, location or colony habitat (exposed or enclosed) or colony type.

Methodology

Collection of feral bees

Stored frozen samples of up to 200 bees (each collected from the entrance of 107 feral colonies) remained after an earlier survey for the bee disease, American Foulbrood, which was conducted between October 2002 and July 2003. L'Arrivee (1963*a*) and L'Arrivee (1963*b*) determined that colonies of honey bees should be sampled for nosema from among their foraging populations in order to detect the highest number of diseased bees. Time of day at which collections can be taken had no significant effect on the numbers of infected bees in samples (Doull 1965).

The colonies were located in the south-west of WA and grouped to the nearest towns or cities based on latitude along the coast. From south to north the groupings were Albany, Bunbury, Perth (capital city), Gingin-Toodyay, Lancelin, Watheroo and Jurien-Leeman/Stockyard Gully. Feral bee colonies, particularly those sampled outside of the metropolitan area of Perth, are regularly subjected to periods of pollen dearth, which occur when plants are not in flower or when droughts prevail.

Colonies were found to occupy a range of habitats and various sized cavities including trees (both dead and living), farm rubbish such as old cars, water heaters, caravans, houses, sheds and letterboxes. A few feral colonies were subterranean. Samples were also taken from established open-air colonies located in trees and caves. Where some difficulty was encountered in collecting bees, particularly on cold days, a stick was jiggled into the entrance and a plastic bag immediately placed over the entrance to catch the exiting bees.

Samples from the entrance of 16 commercial hives over the same period were also collected for comparison. Commercial

 10.1071/EA04222 0816-1089/07/070883

beekeepers in WA are not permitted by regulation to use Fumagillin to treat for the control of nosema. Varroa and small hive beetle, both honey bee pests, which can influence bee population and, therefore, nosema status, are not yet recorded in WA.

Laboratory measurement

Twenty whole bee abdomens from each sample were crushed in a mortar with 5 mL distilled water, and the pestle was then rinsed with another 15 mL of water. The crushed mass was pushed to one side and the mortar tilted, to allow fluid to drain to the other side. The fluid was stirred and a sample taken and placed into a Hycor Biomedical Inc. Kova Glasstic slide with a 10-grid chamber. This slide was then placed under a 400× microscope and *N. apis* spores counted. Five squares of the slide were counted (see Cantwell 1970), averaged and multiplied by 89 991 to calculate the number of *N. apis* spores/bee.

The composite sample number of bees tested follows the standard Department of Agriculture and Food's Animal Health Laboratory diagnosis, which references the methodology of Cantwell (1970). Though for statistical accuracy, 60 bees should be examined individually (Fries *et al*. 1984), this can, for a laboratory, place the test beyond the capacity of research budgets.

Statistical analyses

The non-managed bees were allocated to four variables: (1) location of sample (nearest town for managed hives), (2) colony type (whether an enclosed or open colony), (3) hive category (whether feral, swarm or managed) and (4) month. The statistical analysis required the assignment of reference groups for the four variables. Relative stable parameter estimates are obtained when the number of records for each of these groups is large. The reference group for location was the Perth metropolitan area (most records), colony type was enclosed (most records), hive category was feral (most records) and month was January (a central month, which also had the second highest number of records).

The probability of infection by nosema was investigated by creating a binary variable, which was given the value 0 if no nosema spores were found and value 1 if spores were identified. Using SAS (2002), a generalised linear model (GLM) with a logit link [Logit(p) = log(p/1 − p)] was fitted to the data. The model was:

$$\text{Logit(p)} = \text{location} + \text{type} + \text{hive} + \text{month} + \text{intercept} \quad (1)$$

Analysis of deviance was used to test which terms were significant in the model.

The severity of infection was determined from the counted number of nosema spores/20 bees. A GLM was constructed which included all four factors: location, month, colony type, hive category and their interactions. Using ':' to denote the interaction of two factors, the model developed was:

$$\begin{aligned}\text{Log (number of spores counted)} = {} & \text{location} + \text{type} + \text{month} \\ & + \text{hive} + \text{location:type} + \text{location:month} + \text{location:hive} \\ & + \text{type:month} + \text{type:hive} + \text{month:hive} \\ & + \text{location:type:month} + \text{location:type:hive} \\ & + \text{location:hive:month} + \text{type:hive:month} \\ & + \text{location:hive:type:month} \quad (2)\end{aligned}$$

The severity of infection was also analysed for the enclosed feral honey bee population only, as this was the only group where a reasonable amount of data was available. The model for this subset was:

$$\begin{aligned}\text{Log (number of spores counted)} = {} & \text{location} + \text{month} \\ & + \text{location:month} \quad (3)\end{aligned}$$

Spore counts from infected feral colonies was compared with infected managed honey bees by fitting a Poisson GLM to the spore counts where the only variable in the model was whether or not the reading came from a managed or feral honey bee colony.

Results

A total of 123 samples were collected during the survey: 93 from feral colonies, 14 from settled swarms with no comb and 16 from beekeepers' hives. Of all the bees sampled, 77% were infected with *N. apis*. The infection of feral honey bees ranged from 0 to 25 million spores/bee, with average spore counts for feral, swarm, managed bees, feral colonies in exposed and enclosed environments. This can be seen in Fig. 1.

Probability of infection

Location of colonies had a significant effect ($P = 0.001$) on infection levels in non-managed bees. Colony type also played a significant role in nosema infection. Whether the colony was enclosed in natural or man-made hollow objects, exposed from a cave wall or from a tree branch or in a swarm, the effect on nosema infection was significant ($P = 0.033$). The time of year for sampling bees for nosema was also significant ($P = 0.030$), as was whether the colony was feral, a swarm or a managed hive ($P = 0.002$). It was expected that interaction between the variables would be present. However, there was not enough data to fully test these. The proportion of infected bees from feral and swarm (non-managed) was not significantly different ($P = 0.81$) to the proportion of managed bees that were infected.

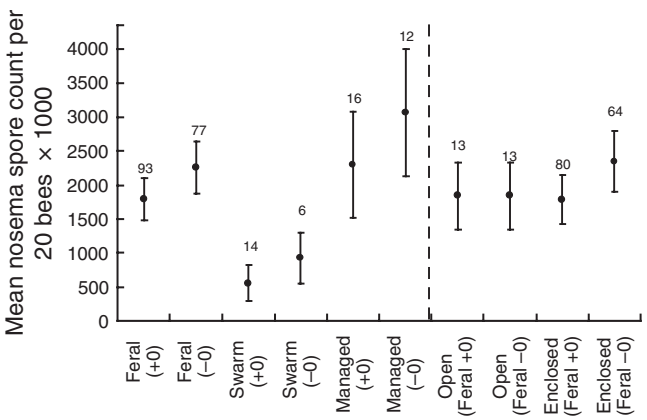

Fig. 1. Mean number of nosema spores in bees from feral, recently swarmed or managed colonies and of colonies of feral bees exposed (e.g. cave wall) or enclosed in natural or man-made objects. Vertical bars indicate the standard deviation: (+0) = all samples, (−0) = only those bees with nosema present.

Table 1. Significance levels from analysis of deviance performed on the model (Eqn 2)

The analysis also showed that the seven terms created involving those interactions with 'hive category' (not shown, but see Eqn 2) were over-parameterised, i.e. where the data did not sufficiently capture the variation between the effect of different factors in the model because many of the interaction combinations have no data associated with them

Source of variation	d.f.	Deviance	*P*-value
Location	6	2471.0	<0.001
Type	1	1112.6	<0.001
Month	7	1244.6	<0.001
Hive	1	0.7	0.40
Location × type	3	193.2	<0.001
Location × month	5	496.1	<0.001
Type × month	1	51.3	<0.001
Location × type × month	1	13.2	<0.001

Severity of infection

The analysis of deviance model indicated that location, month and colony type were important in predicting the severity of nosema infection and some interaction was found between location, colony type and month (Table 1).

Severity of infection was significantly related to location and month of collection. All months and location represented in the data (except Watheroo and December) were found to be significantly ($P < 0.004$) different to the reference group. No significant difference in severity of infection was found at Watheroo and in the month of December ($P = 0.539$, $P = 0.762$, respectively) to spore numbers in bees from enclosed feral populations in reference groups: Perth and January (mid summer), respectively. Only 4 of the 30 possible interactions (Gingin-Toodyay: April and with December; Jurien-Leeman/Stockyard Gully April and with March) had enough data associated with them to calculate parameter estimates that were all significant interactions ($P < 0.001$). We hypothesise that given more data, we would be able to calculate more interaction terms but as it currently exists, the model (as can be seen in Eqn 3) is inadequate to describe what is happening. This is due to more data being required. The relationship of variables such as location and month of collection on the prevalence of nosema in bees, should be explored further with a designed experiment, as many of the possible combinations of location, colony type, hive category and month were not present in our data.

Spore counts from infected managed colonies had a significantly higher level ($P < 0.001$) of infection than non-managed bees (feral and swarm). However, as mentioned before, factors such as location and month of collection affect the results of the non-managed bees, which could bias the overall mean of non-managed bee/spore level.

Discussion

N. apis is evidently a widespread parasite of feral honey bee colonies in WA. The strong association of nosema incidence with location and month, may be correlated with the disease's sensitivity to temperature. Nosema infectivity develops within the temperature range of 30–35°C. The upper limit does equate to the general bee colony temperature (Shimanuki *et al*. 1997). When temperatures are increased in a colony to between 37 and 38°C, re-infection of nosema is prevented and honey bees kept at 42°C for 9 days prevented the development of *N. apis* (see Goetze and Zeutzschel 1959).

For open-air feral colonies, the regulation of colony temperature would be more difficult than if they found residence in a hollow object. However, in warmer weather, when bees are flying or fanning, their abdomen temperatures can rise from 37 to 44°C (Goetze and Zeutzschel 1959). Jaycox (1960) noted that infection levels decreased over summer in California. Doull and Cellier (1961) found the level of infection is at a minimum in mid summer and winter but may rise in autumn in southern Australia in managed colonies. Supporting this relationship to temperature is the observation of Langridge (1961) of a heavily infected colony of bees placed in a sun-trap (allowing maximum exposure to sunlight), which was found to be clear of nosema within 42 days Further support is the research of Cantwell and Shimanuki (1970), where they heat treated combs to 48.9°C for 24 h and found nosema spore count was reduced from 2.4 million (unheated combs) to 1.2 million/bee, which subsequently increased average honey production.

From earlier research (see Manning 1993) conducted in the Stockyard Gully area, 277 km north of Perth, commercial honey bee colonies that were moved into this warmer northern sand-plain from apiary sites 300 km south (south of Perth), showed an average reduction in the number of nosema spores from 8.9 to 0.7 million spores/bee without any chemical intervention. Perhaps this was further evidence of the influence of temperature on this disease or a combination of temperature and the beneficial nutritional aspect of the sandplain's renowned diversity of plant pollen. Honey bees with good nutrition inoculated with nosema had the same length of life as healthy non-infected bees but when the supply of protein (from pollen) was insufficient, the life-spans of infected bees were shortened (Hirschfelder 1964). However, honey alone from the nectar of different species can also be the cause of shortened longevity (Malone *et al*. 2001).

Domestication of the honey bee allows for constant human intervention into colonies. The disturbance can cause the percentage of bees infected with nosema to significantly ($P < 0.01$) increase over undisturbed colonies (Oertel 1967). However, we found no difference in the proportion of infected bees between managed and feral colonies, only a significantly higher level or severity of infection in managed hives.

The high rate of nosema infection indicated from surveys and the marked reduction in the size of the bees' hypopharyngeal glands, which supply the colony with royal jelly following nosema infection (Wang and Moeller 1969), the substantially reduced pollen intake into infected hives noted by Anderson and Giacon (1992) in addition to reduced life-spans from nosema infection noted by Moeller (1956) and Bailey (1981), suggest that *N. apis* is having a deleterious effect on feral colonies in south-west WA.

Acknowledgements

The following beekeepers are thanked for their assistance in the collection of feral honey bee samples: Alan Baker, Neil Brown, Gordon Brown, Kim Fewster, Steven Fewster, Colin Fleay, Deb Mason, Glen Pattinson,

Steve Richards, Ken West and Wally Zajac. The Department of Agriculture and Food staff are also thanked for their input into the project: Jeff Mitchell, Joe King, and Dieter Palmer, Chris Hawkins, Jeff Beard, Jim Prince, Kate Ambrose and the library staff.

References

Anderson DL, 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.

Bailey L (1981) 'Honey bee pathology.' (Academic Press: London)

Cantwell GE (1970) Standard methods for counting nosema spores. *American Bee Journal* **110**(6), 222–223.

Cantwell GE, Shimanuki H (1970) The use of heat to control nosema and increase production for the commercial beekeeper. *American Bee Journal* **110**(7), 263.

Doull KM (1965) The effects of time of day and method of sampling on the determination of nosema disease in beehives. *Journal of Invertebrate Pathology* **7**, 1–4. doi: 10.1016/0022-2011(65)90143-6

Doull KM, Cellier KM (1961) A survey of the incidence of nosema disease (*Nosema apis* Zander) of the honey bee in South Australia. *Journal of Insect Pathology* **3**, 280–288.

El-Shemy AAM, Pickard RS (1989) *Nosema apis* (Zander) infection levels in honey bees of known age. *Journal of Apicultural Research* **28**(2), 101–106.

Fries I, Ekbohm G, Villumstad E (1984) *Nosema apis*, sampling techniques and honey yield. *Journal of Apiculture Research* **23**(2), 102–105.

Furgala B, Hyser RA, Mussen EC (1973) Enzootic levels of nosema disease in untreated and fumagillin treated apiaries in Minnesota. *American Bee Journal* **113**(6), 210–212.

Hirschfelder H (1964) Investigations concerning the nutrition, length of life and the nosema disease of honey bees. In 'Complete texts of lectures of the 19th congress of Apimondia in Prague, 1963'. (Ed. U Mzlvh) pp. 274–278. (Apimondia Publishing: Prague)

Jaycox ER (1960) Surveys for nosema disease of honey bees in California. *Journal of Economic Entomology* **53**(1), 95–98.

Goetze G, Zeutzschel B (1959) Nosema disease of honey bees and its control with drugs: a review of research work since 1954. *Bee World* **40**(9), 217–225.

Langridge D (1961) Nosema disease of the honey bee and some investigations into its control in Victoria, Australia. *Bee World* **42**(2), 36–40.

L'Arrivee JCM (1963*a*) The effects of sampling sites on nosema determination. *Journal of Insect Pathology* **5**, 349–355.

L'Arrivee JCM (1963*b*) Comparison of composite versus individual bee sampling for *Nosema apis* Zander. *Journal of Insect Pathology* **5**, 434–439.

Malone L, Stefanovic D, Gatehouse H (1999) Why is nosema such a common bee disease? *New Zealand Beekeeper* **16**(8), 5–6.

Malone L, Gatehouse HS, Tregidga EL (2001) Effects of time, temperature and honey on *Nosema apis* (Microsporidia: Nosematidae), a parasite of the honey bee, *Apis mellifera* (Hymenoptera: Apidae). *Journal of Invertebrate Pathology* **77**, 258–268. doi: 10.1006/jipa.2001.5028

Manning R (1993) The effect of using Fumagillin in queen candy as a treatment for the control of *Nosema apis* L. in Western Australian honey bee colonies. *The Australasian Beekeeper* **94**(7), 281, 287–288, 290.

Moeller FE (1956) The behaviour of nosema-infected bees affecting their position in the winter cluster. *Journal of Economic Entomology* **49**(6), 743–745.

Oertel E (1967) Colony disturbance and nosema disease. *Journal of Apicultural Research* **6**(2), 119–120.

SAS (2002) 'The SAS system for Windows. Release 9.1.' (SAS Institute: Cary, NC)

Shimanuki H, Knox DA, Furgala B, Caron DM, Williams JL (1997) Disease and pests of honey bees. In 'The hive and the honey bee'. (Ed. JM Graham) pp. 1083–1151. (Dadant and Sons: Hamilton, IL)

Wang DI, Moeller FE (1969) Histological comparisons of the development of hypopharyngeal glands in healthy and nosema-infected worker honey bees. *Journal of Invertebrate Pathology* **14**, 135–142. doi: 10.1016/0022-2011(69)90098-6

Manuscript received 26 October 2004, accepted 14 January 2007