Journal of Invertebrate Pathology **77,** 258–268 (2001)
doi:10.1006/jipa.2001.5028, available online at http://www.idealibrary.com on IDEAL®

Effects of Time, Temperature, and Honey on *Nosema apis* (Microsporidia: Nosematidae), a Parasite of the Honeybee, *Apis mellifera* (Hymenoptera: Apidae)

Louise A. Malone,*[,1] Heather S. Gatehouse,† and Emma L. Tregidga*

**Horticulture and Food Research Institute of New Zealand Limited, Mt. Albert Research Centre, Private Bag 92169, Auckland, New Zealand; and †Horticulture and Food Research Institute of New Zealand Limited, Palmerston North Research Centre, Private Bag 11030, Palmerston North, New Zealand*

Received November 22, 2000, accepted April 20, 2001

Newly emerged adult bees were fed with *Nosema apis* spores subjected to various treatments, and their longevity, proportions of bees infected, and spores per bee recorded. Spores lost viability after 1, 3, or 6 months in active manuka or multifloral honey, after 3 days in multifloral honey, and after 21 days in water or sugar syrup at 33°C. Air-dried spores lost viability after 3 or 5 days at 40°, 45°, or 49°C. Increasing numbers of bees became infected with increasing doses of spores, regardless of their subsequent food (active manuka honey, thyme honey, or sugar syrup). Final spore loads were similar among bees receiving the same food, regardless of dose. Bees fed with either honey had lighter infections than those fed with syrup, but this may have been due to reductions in their longevity. Bees fed with manuka honey were significantly shorter lived, whether infected or not.

***Key Words:* *Apis mellifera* L.; honeybee; *Nosema apis* Zander 1909; spore viability; infectivity; spore load; bee longevity; thyme honey; active manuka honey; heat treatment.**

INTRODUCTION

Nosema apis is a microsporidian pathogen of honeybees (*Apis mellifera*). It occurs in nearly every country where honeybees are kept (Matheson, 1996) and it has a significantly negative impact on hive productivity (Goodwin *et al.*, 1990; Anderson and Giacon, 1992; Wilde and Bratkowski, 1995). *N. apis* is a parasite solely of the gut tissue of adult bees (de Graaf and Jacobs, 1991) and its spores are expelled in large numbers with the feces of infected individuals (Bailey, 1981). The disease is transmitted among bees via the ingestion of contaminated comb (Bailey, 1981) and water sources (L'Arrivee, 1965), trophallaxis (Webster, 1993), and perhaps also honey stores and crushed infected bees (Fries, 1993). Consequently the two "reservoirs" of *N. apis* inoculum within a bee colony are live infected bees and deposits of viable spores on or in wax, honey, and the interior surfaces of the hive. The relative importance of each reservoir is unknown. However, the results of a study using fumagillin feeding, which acts on the pathogen in live bees, combined with heat sterilization, which acts on spore deposits on comb, suggest that both need to be targeted for effective disease control (Cantwell and Shimanuki, 1970).

With a view to developing additional methods of nosema control, this study aimed first to better understand the effects of time and the presence of honey on *N. apis* spore viability. Further experiments were then conducted to determine the feasibility of using moderate temperatures for heat sterilization and the potential for two different honeys (active manuka, *Leptospermum scoparium*, and thyme, *Thymus vulgaris*) to be used as medications to suppress the development of *N. apis* infections in bees.

MATERIALS AND METHODS

Honeybees were obtained from an apiary in Mt. Albert, Auckland, New Zealand. A sample of New Zealand multifloral honey was obtained from a retail outlet and samples of active manuka honey and thyme honey from New Zealand commercial beekeepers. The active manuka honey sample had been shown to have total antibacterial activity equivalent to 11.7% phenol and nonperoxide activity equivalent to 11.0% phenol in tests with *Staphylococcus aureus* (G. Cammell, personal communication; see Molan and Russell, 1988, for test methods). *N. apis* spores were obtained in July 1998 from adult honeybees taken from hives with high levels of nosema disease. A purified spore suspension

[1] To whom correspondence and reprint requests should be addressed. Fax: (64) 09 8154211. E-mail: LMalone@HORT.CRI.NZ.

0022-2011/01 $35.00

was prepared by crushing these bees in sterile distilled water (SDW), filtering the resultant suspension through nylon bolt cloth, washing twice in SDW by centrifugation and resuspension, centrifuging through colloidal silica, followed by washing and final resuspension in SDW. This suspension remained free of bacterial contamination and was stored at 4°C until use (14 months maximum).

Assessing N. apis Viability

The viability of *N. apis* spores was assessed by feeding them to groups of newly emerged adult honeybees kept in small, mesh-sided wooden cages (9 × 8 × 6 cm) in darkness at 33°C. To obtain bees, beehive frames containing capped brood cells from which adults had been observed emerging were brought into the laboratory. To prevent bees from ingesting wax, which may have been contaminated with *N. apis* spores, their emergence from cells was aided by removing the wax cappings with fine forceps. These healthy young adult bees were then placed in cages, each containing a 2-mL plastic cup with 0.5 mL of sugar syrup (50% w:v) or 0.5 g of undiluted honey to which approximately 5×10^6 *N. apis* spores had been added. Each cage was also provided with gravity feeders containing water and 50% sugar syrup and a small cup containing about 6 g of protein food (sodium caseinate 0.12 parts, brewer's yeast 0.24 parts, and sucrose 0.64 parts, mixed to a paste with water). These food supplies were replenished as necessary.

In all but the first experiment described below, barriers consisting of sheets of acetate were placed between cages to minimize the possibility of cross-cage fecal contamination. Each cage was checked three times per week for bee survival and removal of cadavers, which were kept in individually labelled tubes at −17°C until all bees in the experiment had died. Each cadaver was then crushed in 1 mL of water and a 10-μL subsample examined for 1 min using phase-contrast microscopy. If one or more *N. apis* spores were seen, the bee was recorded as infected. Assuming a Poisson distribution of spores on the microscope slide, the probability of failing to detect an infection of 100,000 or more spores per bee using this method is e^{-333}, i.e., extremely low. The suspensions from all the infected bees in each cage were then pooled and a hemocytometer count (van Laere *et al.*, 1980) was made to estimate the number of spores per infected bee in that cage. Four different experiments were conducted as follows.

N. apis Spore Viability after Storage in Two Different Honey Samples for 1, 3, or 6 Months

Purified *N. apis* spores in SDW were taken from storage at 4°C after 6 months, removed from suspension by centrifugation, and resuspended in either multifloral or active manuka honey as described above. These preparations, and control samples of each honey without spores added, were placed in darkness at 33°C. After 1, 3 or 6 months, the honey samples were stirred and sufficient was removed to furnish three cages with 0.5 g of each of the four honeys (multifloral or manuka, with or without spores added). Additionally, at each time point, spores were taken from 4°C storage and resuspended in 50% sugar syrup to provide enough for three cages, each containing 0.5 mL of suspension. A further three cages were set up with 0.5 mL of 50% (w:v) sugar syrup without spores. Thus, at each of the three time points, three replicates of six different treatments were set up. Each cage in this experiment contained 20 newly emerged adult bees.

Viability of N. apis Spores after Storage in Honey, Sugar Syrup, or Water for 3, 7, 14, or 21 Days and the Effects of Resuspension in Fresh Syrup after Being Stored in Water for That Time

Purified *N. apis* spores that had been stored in SDW at 4°C for 14 months were resuspended in multifloral honey (12 cups), 50% sugar syrup (12 cups), or distilled water (24 cups) and placed in darkness at 33°C. At 3, 7, 14, and 21 days, three honey cups, three syrup cups and six water cups were removed from the incubator. The mixtures in the honey and syrup cups were stirred to ensure suspension of spores and each cup was placed directly into a cage containing 25 bees as described above. Sugar (0.25 g per cup) was added to three of the water cups to make the suspension palatable, and each was stirred thoroughly and placed in a cage of bees. The suspensions in the three remaining water cups were centrifuged, the water supernatent was removed and replaced with 0.5 mL of fresh sugar syrup (50%), and the cups were given to bees in cages. Thus 48 cages in total were set up, comprising four treatments (referred to as honey, syrup, water, and fresh) and four storage periods, replicated three times.

Viability of N. apis Spores after Being Dried onto Glass Slides and Stored at Four Different Temperatures for 3 or 5 Days

Aliquots of a purified spore suspension in SDW, stored at 4°C for 10 months (0.5 mL, each containing approximately 5×10^6 *N. apis* spores), were spread evenly over the surfaces of glass slides. These were dried at room temperature and three slides kept at 33°, 40°, 45°, or 49°C in darkness for 3 or 5 days. As a positive control, three replicate slides with spores were kept at room temperature (approximately 25°C) for 24 h. After 24 h or 3 or 5 days, the dried spores on each slide were resuspended in 0.5 mL of 50% sugar syrup in a small cup and put into a cage with 25 newly emerged adult bees.

Effects of Feeding Active Manuka or Thyme Honey to Bees Dosed with N. apis

Newly emerged bees were dosed individually by force-feeding each with 1 μL of sugar syrup (50%) containing 0, 10, 10^2, 10^3, or 10^4 *N. apis* spores, which had previously been stored in SDW at 4°C for 14 months (for method see Malone and Stefanovic, 1999). Bees that received the same number of spores were then placed in groups of 25 in cages as described above and supplied *ad lib.* with water, protein food, and one of three feed types: active manuka honey, thyme honey, or sugar syrup (50%). Forty-five cages were set up in total, comprising five different dosages and three different treatments replicated three times.

Statistical Analysis

For each experiment, survival curves, plotting number of live bees versus time, were constructed using data from each cage and compared using Mantel-Haenzel (log-rank) tests (Kalbfleisch and Prentice, 1980). Median survival times were calculated for each cage of bees and compared using χ^2 tests. Data describing proportions of infected bees in each cage were arcsine square root-transformed and compared for each experiment using ANOVA. Spore count data (spores per dead infected bee) from each experiment were log-transformed and compared using residual maximum likelihood (REML). For the fourth experiment, in which bees were given different doses of spores and then fed with syrup, thyme honey, or manuka honey, spore count data were subjected to a second REML analysis, using bee longevity as a covariate. This was to determine whether the observed differences in spore counts at the time of death could be explained by individual differences in bee longevity.

RESULTS

N. apis Spore Viability after Storage in Two Different Honeys for 1, 3, or 6 Months

Bee survival was very variable (median longevity ranged from 25 to 50 days), with significant differences among replicates for all treatments ($P < 0.01$, log-rank test), except for those bees given multifloral honey stored without spores for 1 month. There were no clear patterns to indicate that dosing with *N. apis* affected bee survival. For example, in the 1-month trial dosed bees died sooner than undosed bees ($P < 0.01$, χ^2 test), in the 3-month trial they lived longer ($P < 0.001$), and in the 6-month trial there were no significant differences in longevity attributable to dosing. Neither were there any consistent impacts on bee survival that could be attributed to the medium in which the spores had been stored. In the 1-month trial, bees given 0.5 g of multifloral honey (with or without spores) lived significantly longer than the others ($P < 0.001$, χ^2 test), in the 3-month trial there were no significant differences, and in the 6-month trial, bees given multifloral or manuka honey lived longer than those given syrup ($P < 0.001$).

There were no significant differences ($P < 0.05$) among the proportions of dosed bees that became infected that could clearly be attributed to honey type or storage time (Fig. 1a). Obviously, dosing had a highly significant impact on the proportions of bees that became infected ($P < 0.001$, ANOVA). Two bees of 20 in one replicate given multifloral honey without spores in the 1-month trial and 1 bee given fresh sugar syrup in the 6-month trial became infected.

Honey type had a highly significant impact on the numbers of spores per infected bee ($P < 0.001$, REML), with bees receiving spores that had been stored in either multifloral or manuka honey having significantly lighter infections (4.4×10^4 or 4.7×10^4 spores per bee on average, respectively) than the control bees given 4°C-stored spores in fresh syrup (1.27×10^7 spores per bee on average) (Fig. 1b).

Viability of N. apis Spores after Storage in Honey, Sugar Syrup, or Water for 3, 7, 14, or 21 Days and the Effects of Resuspension in Fresh Syrup after Being Stored in Water for That Time

As in the previous experiment, bee survival varied widely (median longevity, 18 to 32 days), with highly significant differences among replicates of the same treatment ($P < 0.001$, log-rank tests). There were no clear patterns demonstrating either treatment or time effects on the longevity of bees fed with spores subjected to the different storage regimes.

Significantly fewer bees became infected when fed with the honey-treated spores than with the other preparations (data pooled across all storage times, $P < 0.05$, ANOVA). This effect was particularly marked among bees fed with spores stored in the various media for 3 days (Fig. 2a). Furthermore, there was a significant downward trend in the numbers of bees infected with spores stored at 33°C for increasing lengths of time, regardless of the medium in which they were stored ($P < 0.01$)(Fig. 2a).

These trends were even more apparent with the spore count data (Fig. 2b). Spores stored in honey produced significantly lighter infections (2×10^5 spores per bee on average) than those stored in syrup (6.85×10^6 spores per bee), which in turn produced significantly less severe infections than those stored in water, regardless of whether the water was simply sweetened with sugar (1.1×10^7 spores per bee) or replaced with fresh syrup before feeding to the bees (8.46×10^6 spores per bee) (data pooled across all storage times, $P < 0.001$, REML). Once again, this effect was particularly pronounced among the bees fed with spores stored for 3 days (Fig. 2b). Storage time had a signifi-

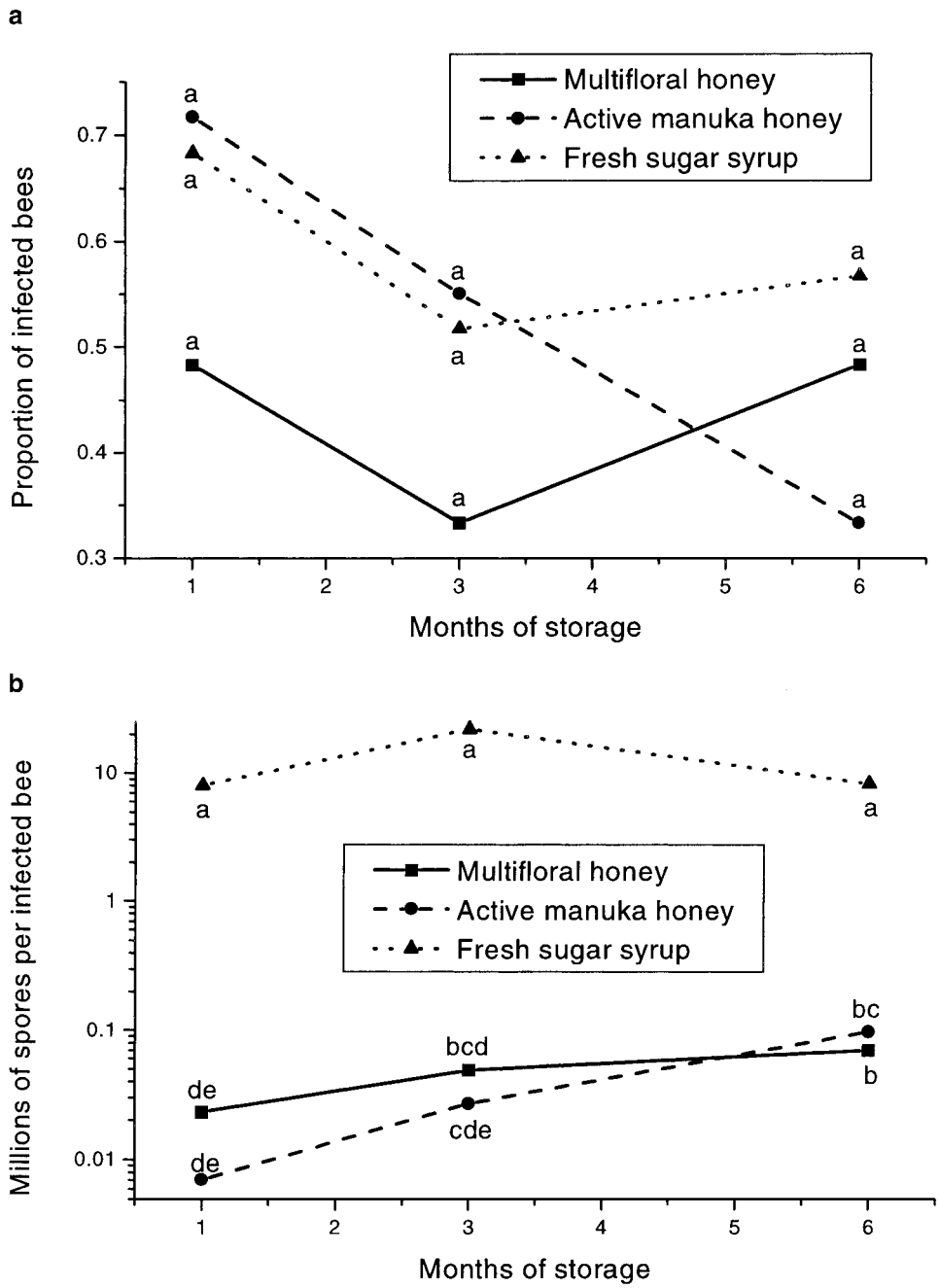

FIG. 1. (a) Proportions of adult honeybees infected with *N. apis* and (b) mean numbers of *N. apis* spores carried by adult honeybees at the time of death after being fed spores that had been stored in either active manuka or multifloral honey at 33°C for 1, 3, or 6 months. Numbers without a letter in common are significantly different ($P \leq 0.05$, ANOVA of arcsine square root-transformed proportion data (a) or REML of log-transformed spore-count data (b)). Control bees were fed with spores taken from cold storage and suspended in sugar syrup. Three replicates were run for each treatment.

cant impact on the severity of infections produced, with spores stored for 14 or 21 days resulting in significantly lighter infections (4.36×10^6 and 9.6×10^5 spores per bee, respectively) than those stored for 7 days (6.98×10^6 spores per bee), which in turn produced significantly lighter infections than those stored for 3 days (1.43×10^7 spores per bee) (data pooled across all storage media, $P < 0.001$).

Viability of N. apis Spores after Being Dried onto Glass Slides and Stored at Four Different Temperatures for 3 or 5 Days

As in the previous two experiments, bee survival was very variable (median longevity, 26 to 50 days) and no clear patterns related to temperature or storage time could be discerned.

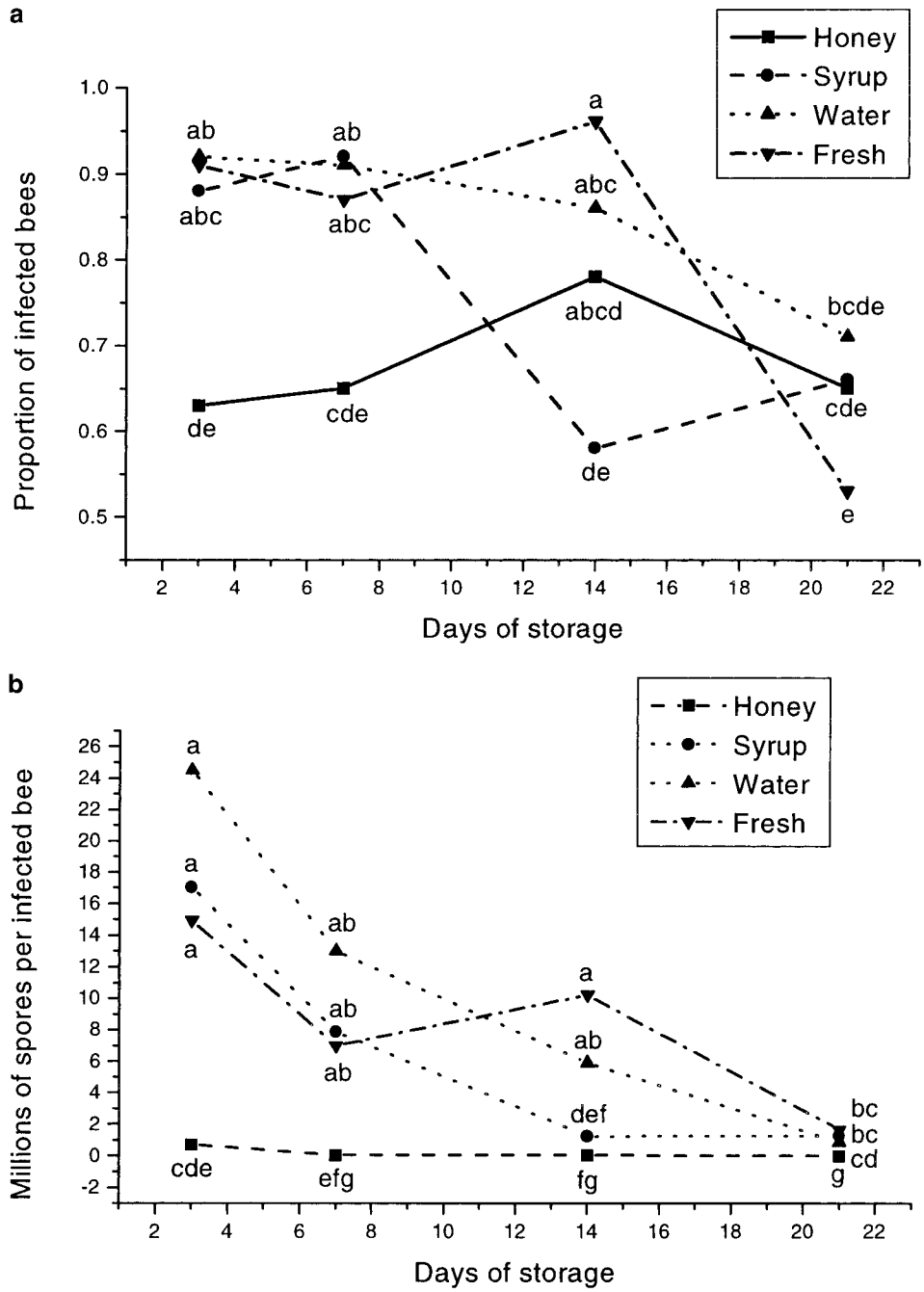

FIG. 2. (a) Proportions of adult honeybees infected after being fed with *N. apis* spores that had been stored in multifloral honey, sugar syrup, or water at 33°C for 3, 7, 14, or 21 days. Half of the water-stored spores were mixed with sugar and fed directly to bees ("water"). For the rest, the water was removed and replaced with fresh sugar syrup ("fresh") before feeding. (b) Mean numbers of *N. apis* spores carried by these adult honeybees at the time of death. Numbers without a letter in common are significantly different ($P \leq 0.05$, ANOVA of arcsine square root-transformed proportion data (a) or REML of log-transformed spore-count data (b)). Three replicates were run for each treatment.

When compared with the 25°C, 24-h controls, significantly fewer bees became infected when fed with spores that had been stored at 40°, 45°, or 49°C for 3 or 5 days ($P < 0.01$, ANOVA) (Fig. 3a). The storage time of 3 or 5 days did not have a significant impact on the proportions of bees infected within each temperature group (including 33°C). Heat treatment at 49°C for 3 or 5 days had a similar impact on spore viability, measured by proportions of bees infected, as did treatment at 40°C or 45°C for either 3 or 5 days ($P < 0.05$).

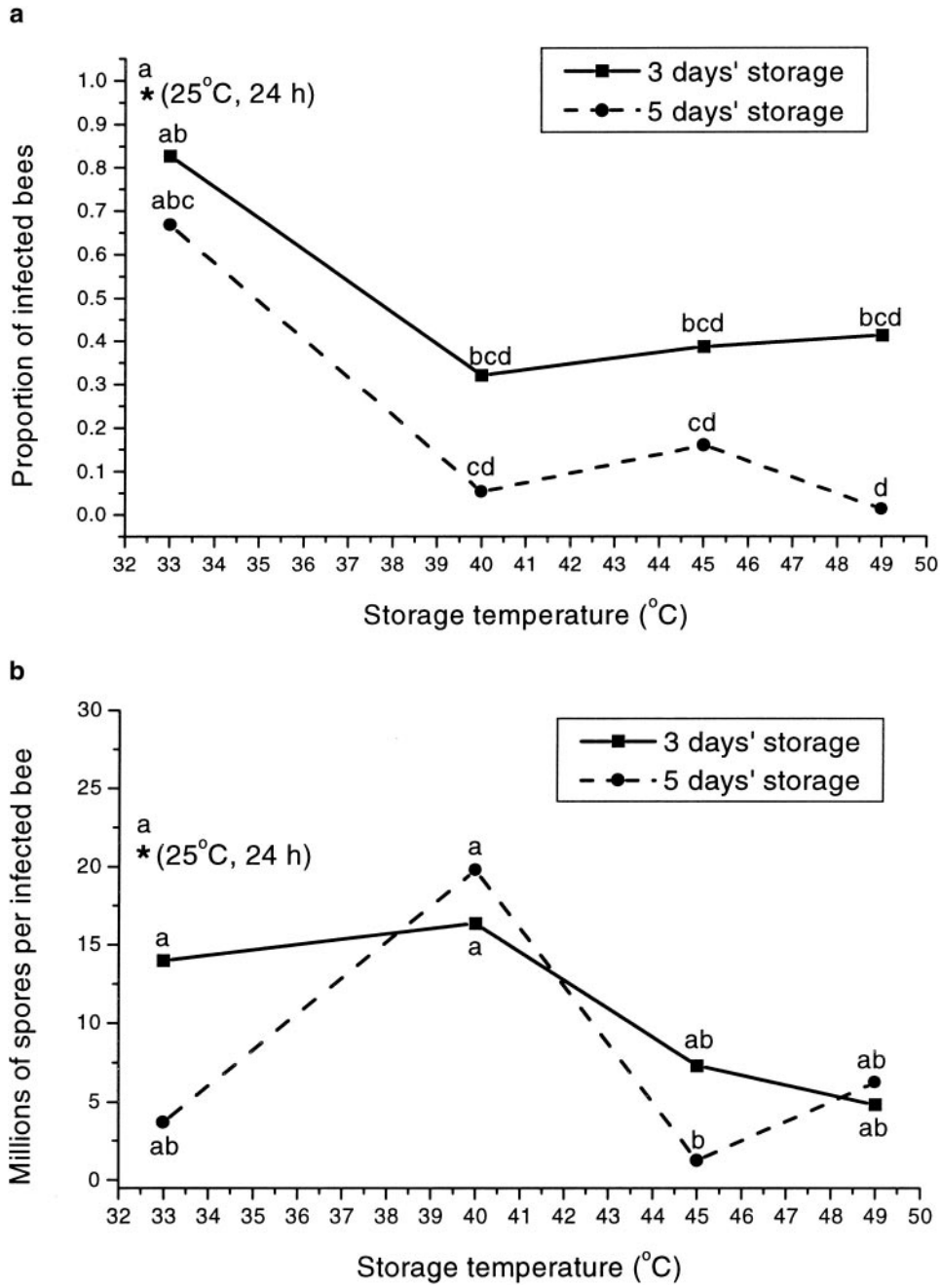

FIG. 3. (a) Proportions of adult honeybees infected with *N. apis* after being fed with spores that had been air-dried onto glass slides, kept at 33°, 40°, 45°, or 49°C for 3 or 5 days and then resuspended in sugar syrup. A control group of bees were fed with air-dried spores that had been kept at 25°C for 24 h. (b) Mean numbers of *N. apis* spores carried by these adult honeybees at the time of death. Numbers without a letter in common are significantly different ($P \leq 0.05$, ANOVA of arcsine square root-transformed proportion data (a) or REML of log-transformed spore-count data (b)). Three replicates were run for each treatment.

Only heat treatment at 45°C for 5 days resulted in spores which produced significantly lighter infections in bees than those kept at 25°C for 24 h (1.27×10^6 cf. 2.17×10^7 spores per bee) ($P < 0.05$, REML) (Fig. 3b). The effects (on final spore loads in infected bees) of storing spores at 49°C for 3 or 5 days were not significantly different from those of storing them at 33°, 40°, or 45°C for 3 or 5 days.

Effects of Feeding Active Manuka or Thyme Honey to Bees Dosed with N. apis

Bee survival was very variable, with significant differences among some replicates. There was a dose effect, in that bees that received 10 *N. apis* spores had significantly better survival than those given the highest spore dose or even, surprisingly, than the undosed

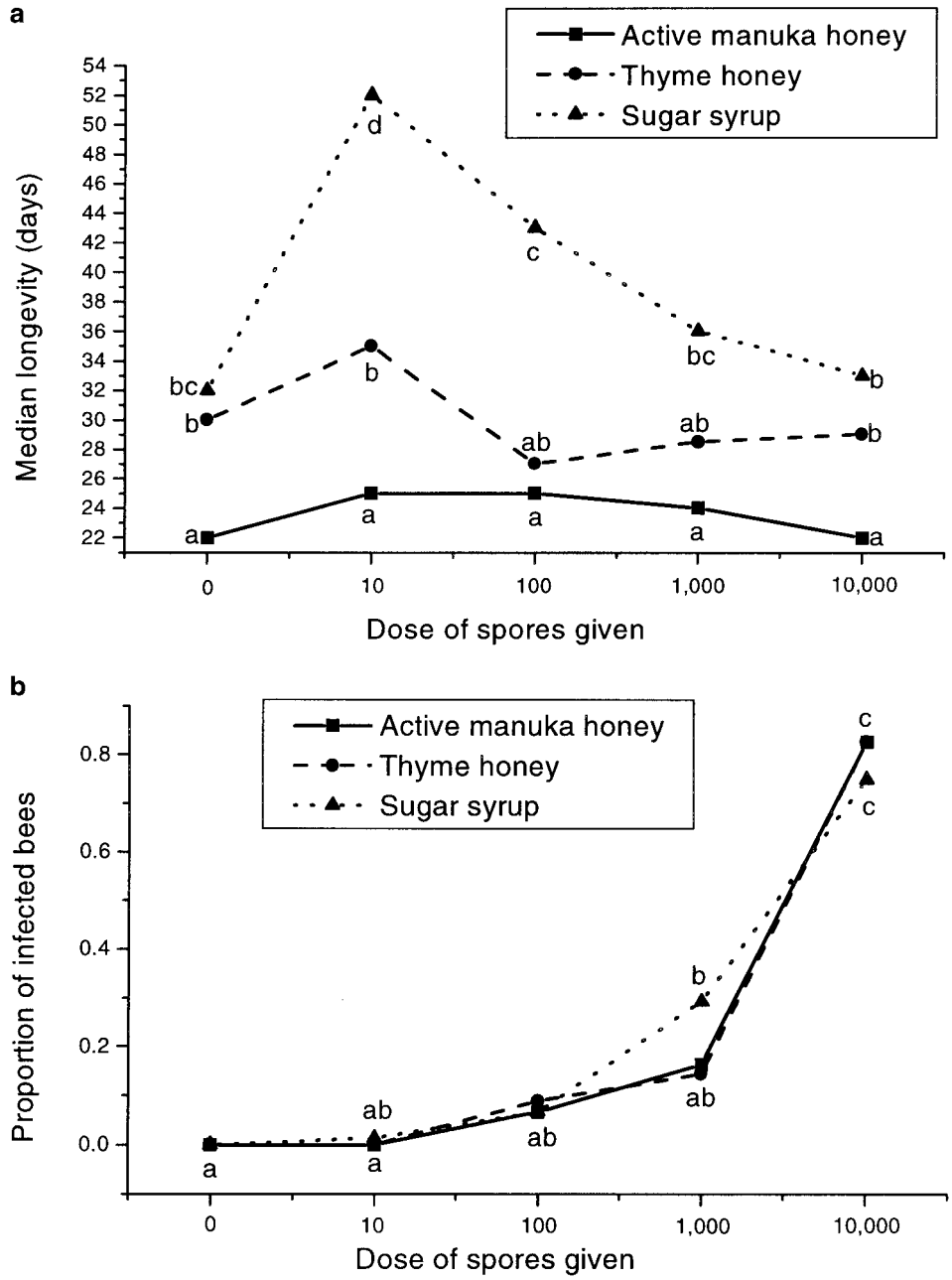

FIG. 4. (a) Median longevities of adult honeybees individually dosed with 0, 10, 10^2, 10^3, or 10^4 *N. apis* spores and then fed *ad lib.* with active manuka honey, thyme honey, or sugar syrup. Medians without a letter in common are significantly different ($P \leq 0.05$, χ^2 test). (b) Proportions of adult honeybees infected with *N. apis* and (c) mean numbers of *N. apis* spores carried by adult honey bees at the time of death after being dosed as described above. Numbers without a letter in common are significantly different ($P \leq 0.05$, ANOVA of arcsine square root-transformed proportion data (b) or REML of log-transformed spore-count data (c)). Three replicates were run for each treatment.

control bees (data pooled across all feed types, $P < 0.001$, log-rank test). Bees had significantly poorer survival when fed with active manuka honey rather than with sugar syrup (data pooled across all dosage groups, $P < 0.001$, log-rank and χ^2 tests). This effect was significant even for the undosed control bees (Fig. 4a). Thyme honey-fed bees had intermediate survival times in all cases (Fig. 4a).

Spore dose had a significant effect on the proportion of bees infected, with increasingly more bees infected when given higher doses of spores ($P < 0.001$, ANOVA) (Fig. 4b). However, the subsequent feeding regimes had no impact on this relationship between the dose given and the likelihood of a bee becoming infected.

Infected bees within each feed type group had similar spore loads at death, regardless of the dose received

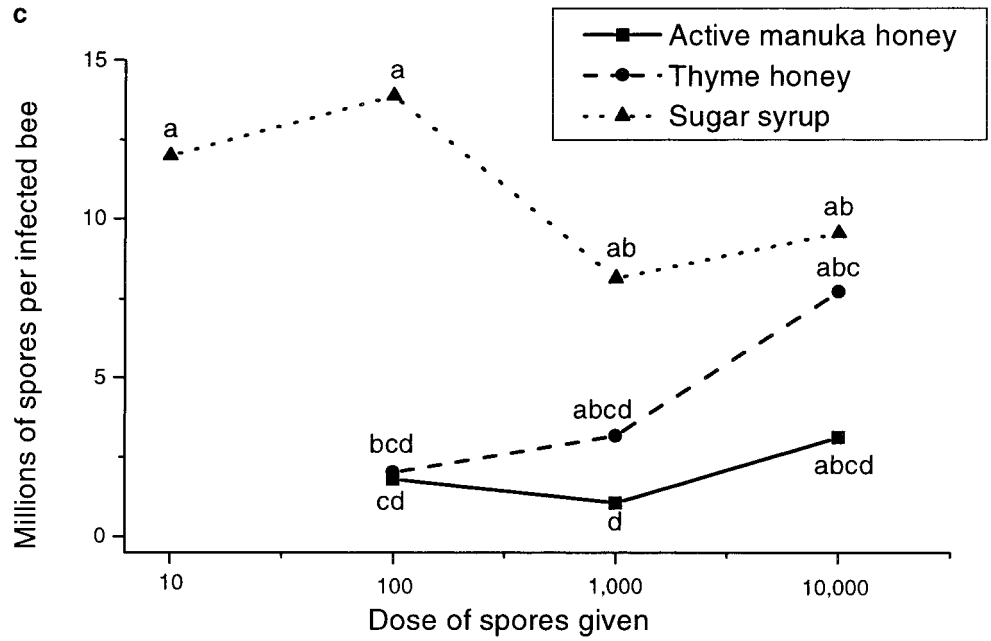

FIG. 4—*Continued*

(Fig. 4c). However, bees fed with manuka honey tended to have lower spore loads (4.22×10^5 spores per bee on average) than bees fed with syrup (2.3×10^6 spores per bee), but this trend was statistically significant only for the infected bees that had received 10^2 or 10^3 spores each ($P < 0.05$, REML) (Fig. 4c). Bees fed with thyme honey had intermediate spore loads (1.17×10^6 spores per bee), which did not differ significantly from those carried by either manuka honey-fed or syrup-fed infected bees, except in the case of bees receiving the 10^2-spore dose. These had significantly lower loads than the corresponding syrup-fed bees ($P < 0.05$, REML) (Fig. 4c). However, all of these significant differences were lost when a second REML analysis using bee longevity as a covariate was carried out.

DISCUSSION

Previous studies have shown that the proportions of bees infected with *N. apis* (or the likelihood of a bee becoming infected) can be directly correlated to the dose of spores received by each bee (Fries, 1988). Thus it is not surprising that treatments capable of killing some of the *N. apis* spores in a preparation would result in fewer bees becoming infected in a viability check trial. This was the case for the honey treatment and all treatments after 21 days in the second experiment and some of the high temperature treatments in the third experiment. However, it was not the case in the first experiment, where prolonged storage of spores in two honeys had no effect on the proportions of bees infected, even though each had a highly significant effect on the subsequent spore loads carried by infected bees at their deaths (i.e., the development of the parasite). This is difficult to explain, but one possibility is that there was some cage-to-cage transmission in this experiment, since even three of the undosed bees became infected. Bee feces may have passed through the mesh sides of adjacent cages in this experiment, but barriers to prevent this were used in subsequent experiments.

Further variability may have been introduced by the "mass dosing" technique used in the first three experiments, i.e., bees received *N. apis* spores from a communal cup, rather than being force-fed spores individually. However, in each experiment all bees were subjected to the same dosing technique and so any differences due to treatments should still be evident. Since bees cannot be kept individually in cages, opportunities for bee-to-bee transmission via trophallaxis within each cage cannot be eliminated and thus exactly uniform dosages of spores cannot be administered to bees even with the force-feeding method.

In each of the honey, time, and temperature experiments, most of the effective treatments resulted in bees with significantly lighter infections, as measured by spore loads at the time of death. In the first three experiments this variation could not be explained by variations in bee longevity, i.e., the bees dying before the infection had a chance to develop fully. This suggests that when spore viability is severely compromised, the resultant *N. apis* infections may not always develop to the point where the midgut epithelium becomes "filled" with the parasite. This reduction in final spore load may occur only when extremely low numbers of viable spores are ingested by a bee, since bees

force-fed 10, 10^2, 10^3, or 10^4 spores each and maintained on sugar syrup had all developed, by the time they died, infections of similar magnitude.

Because the present study was conducted with a single collection of spores that had been cleaned and kept at 4°C, caution must be exercised in extrapolating these findings to the field situation, where spores are deposited about the hive in feces or cadavers. Furthermore, there may be genotypic or phenotypic variations among natural populations of *N. apis* spores that could alter their durability in the field. An analysis of *N. apis* ribosomal RNA gene sequences has shown marked differences between isolates from different countries, but little genetic variation among isolates from different New Zealand locations (Gatehouse and Malone, 1999). By using a relatively uniform spore preparation, these sources of variation may have been minimized, allowing treatment effects to become apparent.

Even though the spores were stored at 4°C for different periods (6 to 14 months) before use in experiments, this is unlikely to have altered subsequent changes in their viability attributable to the treatments in honey or at different temperatures. Other studies with microsporidian spores have shown that storage in clean aqueous suspension at approximately 4°C or in liquid nitrogen results in only a very slow change in viability over several years (Kramer, 1970; Vavra and Maddox, 1976). Revell (1960) found that *N. apis* spores stored in clean, distilled water at 5°C for 12 months produced infections in 75% of bees dosed and those stored for 24 months produced infections in 72% of bees. (Initial infectivity data were not presented.) Even after 7 years, 60% of bees became infected. In contrast, *N. apis* spores stored in a refrigerator naked and dried, in bacterially contaminated aqueous suspensions, or in bee cadavers, lost viability completely after a matter of days or months (White, 1919). *N. apis* spores kept at −20°C also lost viability significantly over 2 years (Bailey, 1972), as has been noted for other microsporidia (Vavra and Maddox, 1976).

Time elapsed, temperature, and submersion in honey appear to be important factors affecting *N. apis* spore viability. Results from the first two experiments show that *N. apis* spores may lose viability when submerged in honey at hive temperature after only 3 days. Interestingly, *N. apis* spores stored in honey at room temperature have been shown to retain their infectivity for 2 to 4 months (White, 1919), suggesting that temperature alters this response. Comparisons with spores in sugar syrup in the present study suggest that the honey effect is not due solely to high osmolarity, but to other properties of the honey samples tested, perhaps high pH or peroxide activity. That both the multifloral and active manuka honey samples had similar effects on spore viability suggests that the special nonperoxidase antibacterial properties of active manuka may not be necessary for this effect, but that any honey could have a similar impact on *N. apis* spores. Assuming that the honey samples tested in the present study were typical of multifloral or active manuka honey, this suggests that honey in many hives housing infected bees would not be a significant source of *N. apis* inoculum. However, there are no published data on the presence or absence of *N. apis* spores in honey. Fries (1993) notes that "robbing of honey from weak colonies spreads the disease since stores of heavily infected colonies often contain spores," but does not provide experimental data. It seems likely that fecal contamination of wax, especially in combs used for brood rearing, or other hive interior surfaces provides sufficient inoculum for *N. apis* to be successfully transmitted to the next generation of bees.

Additionally, *N. apis* spores in liquid suspension steadily lost viability over 21 days at 33°C, even in the absence of honey. Microsporidian spores seem to lose viability faster at temperatures above 35°C and retain their viability better under cold conditions (Kramer, 1970). White (1919) found that *N. apis* spores in water contaminated with bacteria and kept at 37.5°C lost viability after only 2 to 4 days, but those in similar suspensions at room temperature or under refrigeration survived between 6 days and 3 months. In a hive, *N. apis* spores may well be contained within a cadaver or coated with feces. Bailey (1962) found that *N. apis* spores in fecal deposits at 20°C and 50% relative humidity retained infectivity for more than a year. The higher temperature within a hive may well alter this, however. *N. apis* spores in bee cadavers at room temperature have been recorded as losing viability after either 4.5 years (Kulikov and Akramovsky, 1961) or 1 month (White, 1919). However, *N. apis* spores in bee cadavers kept at 37.5°C remained infective for only 6 days (White, 1919). Faecal deposits or cadavers may provide some protection, but our results and those of others suggest that at brood chamber temperature the viability of a population of *N. apis* spores will decrease steadily over time.

The results from the experiment to assess the effects of various temperatures on the viability of dried spores indicate that some useful degree of heat sterilization may be obtained at lower temperatures and longer times than the current standard method of 49°C for 24 h (Shimanuki *et al.*, 1992). However, further tests with wax combs are needed to confirm this. Cantwell and Lehnert (1968) found that *N. apis* spores dried onto glass slides and kept at 49°C for 6 h failed to produce infections in bees 14 days after dosing with 5×10^4 spores each. In contrast, spores kept at 43°C for 6 h did result in infections. Further experiments showed that treating beehive frames at 49°C for 24 h resulted in a significant reduction in the incidence of *N. apis* infections (Cantwell and Shimanuki, 1970). In the present study, dried spores kept at 49°C for 3 or 5 days were still capable of producing infections in bees, but

these bees were fed with approximately 2×10^5 spores each, four times the dosage in the study reported by Cantwell and Lehnert (1968). From the present results we can conclude that keeping dried spores at 45°C is as effective in reducing spore viability as keeping them at 49°C for 3 or 5 days. This suggests that significant reductions in *N. apis* infections in bee colonies might be obtained by subjecting frames and other hive parts to 45°C for 3 or 5 days, but further tests are required to confirm this.

Only honeydew honey has been tested as a treatment for nosemosis. Gregorc (1993) found that colonies fed with honeydew honey had lower levels of *N. apis* infection (2×10^7 spores per bee) than colonies fed with sugar syrup (3×10^7 spores per bee). Many honeys have antibacterial properties, usually attributable to their high acidity and peroxide activity (Molan and Russell, 1988). Some batches of manuka honey have especially strong antibacterial activity (not due to acidity or peroxide) in tests with a range of bacteria pathogenic to mammals (Molan and Russell, 1988; Allen and Molan, 1997; Willix *et al.,* 1992). This is known as "active manuka honey" and is sold at a premium price as both a wound dressing and a healthy food. There are no previous reports of its effects on bees infected with *N. apis*. Thyme honey was chosen as a second potential medication for bees with *N. apis* infections. Although its antibacterial activity is low compared with other New Zealand honeys (Molan and Russell, 1988), there is anecdotal evidence that bees kept in regions of New Zealand where thyme honey is produced may have a lower incidence of nosemosis than those kept in other regions.

Neither of the samples of active manuka or thyme honey used in this study had an impact on the proportion of bees infected. The likelihood of a bee becoming infected was strongly linked to the dosage each bee received, rather than its subsequent treatment. This is not surprising, as the establishment of a *N. apis* infection is probably determined within a short time of the bee ingesting the spores, as the polar filaments emerge and pierce the gut epithelium. Feeding honey to bees after they have been force-fed with *N. apis* spores is perhaps more likely to influence the subsequent development of the disease, either by the action of antibiotic properties absorbed by gut cells or via nutritional effects on the bee. Surprisingly, the active manuka honey sample used in this study caused significant reductions in bee survival, compared with sugar syrup, among both undosed and dosed bees. Bees fed with thyme honey had intermediate survival. Covariate analysis suggested that these longevity effects alone could explain the observed reductions in the severity of infections sustained by bees receiving either type of honey sample, i.e., these bees did not live long enough to develop full *N. apis* infections. Since manuka honey has long been used to feed bees in New Zealand without apparent harm, the abiotic effect demonstrated in this experiment either has no obvious impact at the colony level or is present only in some types of manuka honey. Even though the active manuka honey sample used in this study affected bee survival, it did not have a significant impact on the development of *N. apis* in dosed bees, and neither did the thyme honey sample. Although only a single sample of each honey type was tested, these results suggest strongly that neither honey would be useful as a medication against *N. apis*.

ACKNOWLEDGMENTS

We thank Graeme Cammell, of Cammell's Honey, Auckland, New Zealand, for supplying us with active manuka honey and Ernest Adamson, of Adamson's Honey, Alexandra, New Zealand, for the thyme honey. We also express our appreciation of the various members of the National Beekeepers Association of New Zealand, whose questions have prompted much of this research. We are most grateful to Anne Gunson, of the Horticulture and Food Research Institute of New Zealand Ltd., for carrying out all statistical analyses and to Mark Goodwin, of the same institute, for helpful comments on the manuscript. This work was supported by the Foundation for Research, Science and Technology, Contract C06807.

REFERENCES

Allen, K. L., and Molan, P. C. 1997. The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey. *N. Z. J. Agric. Res.* **40,** 537–540.

Anderson, D. L., and Giacon, H. 1992. Reduced pollen collection by honey bee (*Hymenoptera: Apidae*) colonies infected with *Nosema apis* and sacbrood virus. *J. Econ. Entomol.* **85,** 47–51.

Bailey, L. 1962. Bee diseases. *In* "Report of the Rothamsted Experimental Station for 1961," pp. 160–161.

Bailey, L. 1972. The preservation of infective microsporidian spores. *J. Invertebr. Pathol.* **20,** 252–254.

Bailey, L. 1981. "Honey Bee Pathology." Academic Press, London.

Cantwell, G. E., and Lehnert, T. 1968. Mortality of *Nosema apis* and the greater wax moth, *Galleria mellonella* L, caused by heat treatment. *Am. Bee J.* **108,** 56–57.

Cantwell, G. E., and Shimanuki, H. 1970. The use of heat to control nosema and increase production for the commercial beekeeper. *Am. Bee J.* **110,** 263.

Fries, I. 1988. Infectivity and multiplication of *Nosema apis* Z. in the ventriculus of the honey bee. *Apidologie* **19,** 319–328.

Fries, I. 1993. *Nosema apis*—A parasite in the honey bee colony. *Bee World* **74,** 5–19.

Gatehouse, H. S., and Malone, L. A. 1999. Genetic variability among *Nosema apis* isolates. *J. Apicult. Res.* **38,** 79–85.

Goodwin, M., Ten Houten, A., Perry, J., and Blackmann, R. 1990. Cost benefit analysis of using fumagillin to treat nosema. *N.Z. Beekeeper* **208,** 11–12.

de Graaf, D. C., and Jacobs F. J. 1991. Tissue specificity of *Nosema apis. J. Invertebr. Pathol.* **58,** 277–278.

Gregorc, A. 1993. The effects of honeydew honey on honey bee colonies (*Apis mellifera carnica*) and development of nosema disease (nosemosis). *In* "Prvi Slovenski Veterinarski Kongres, 18–20 November 1993, Portoroz, Slovenia."

Kalbfleisch, J. D., and Prentice, R. L. 1980. "Statistical Analysis of Failure Time Data." Wiley, New York.

Kramer, J. P. 1970. Longevity of microsporidian spores with special reference to *Octosporea muscaedomesticae* Flu. *Acta Protozool.* **15,** 217–224.

Kulikov, N. S., and Akramovsky, M. N. 1961. Sroki ziznesposobnosti spor mosey u pcel. *Pcelovodstov* **38,** 46.

van Laere, O., van der Weghe, L., Moermans, R., and Fordeyn, D. 1980. Quantitative determination of *Nosema apis* Z. in the individual honey bee *Apis mellifera* L. *Parasitica* **36,** 68–73.

L'Arrivee, J. C. M. 1965. Sources of nosema infection. *Am. Bee J.* **105,** 246–248.

Malone, L. A., and Stefanovic, D. 1999. Comparison of the responses of two races of honeybees to infection with *Nosema apis* Zander. *Apidologie* **30,** 375–382.

Matheson, A. 1996. World bee health update 1996. *Bee World* **77,** 45–51.

Molan, P. C., and Russell, K. M. 1998. Non-peroxide antibacterial activity in some New Zealand honeys. *J. Apicult. Res.* **27,** 62–67.

Revell, I. L. 1960. Longevity of refrigerated nosema spores—*Nosema apis*, a parasite of honey bees. *J. Econ. Entomol.* **53,** 1132–1133.

Shimanuki, H., Knox, D. A., Furgala, B., Caron, D. M., and Williams, J. L. 1992. Diseases and pests of honey bees. *In* "The Hive and the Honey Bee" (J. M. Graham, Ed.), pp. 1083–1151. Dadant, Illinois.

Vavra, J., and Maddox, J. V. 1976. Methods in microsporidiology. *In* "Comparative Pathobiology, Vol. 1, Biology of the Microsporidia" (L. A. Bulla and T. C. Cheng, Eds.), pp. 281–319. Plenum, New York.

Webster, T. C. 1993. *Nosema apis* spore transmission among honey bees. *Am. Bee J.* **133,** 869–870.

White, G. F. 1919. "Nosema—Disease." U.S. Dept. Agric. Bull., No. 780.

Wilde, J., and Bratkowski, J. 1995. Nosema disease in honey bee colonies and pollen trapping. *Pszczelnicze Zeszyty Naukowe* **39,** 99–105.

Willix, D. J., Molan P. C., and Harfoot, C. G. 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *J. Appl. Bacteriol.* **73,** 388–394.