Worker Honey Bee[1] Response to Infection with *Nosema apis*: Influence of Diet[2,3]

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ABSTRACT

In 2 experiments, adult workers of *Apis mellifera* L. were individually fed either spores of *Nosema apis* Zander or a sucrose solution and then transferred on the basis of common treatment to cages. The 2 treatment groups were each subdivided into 2 further groups. One was fed only syrup and the other was fed syrup and proteinaceous food. In one experiment, the proteinaceous food was mixed-floral-source pollen; in the other experiment, food was a pollen substitute. Protein food increased both the longevity of bees, whether or not they received spores, and also increased *Nosema* spore development. While spore feeding generally reduced longevities in both experiments, the numerical difference between longevities of spore-fed-bees and bees not fed spores was greater when the bees were fed protein. Thus, protein food increased the usefulness of mortality as a measure of worker bee response to Nosema infection.

The number of *Nosema apis* Zander spores present in midguts of adult worker honey bees, *Apis mellifera* L., has been used as a measure of the incidence and level of parasitism and interpreted as a measure of the response of bees to infection. Gochnauer et al. (1975) reviewed a variety of such investigations. Since nosematosis involves not only the parasite but also its host, use of spore counts alone as a measure of the response of bees to infection probably is inadequate. Spore counts cannot measure possible differences in tolerance by the host to a given level of infection. This deficiency underlines the desirability of expanding investigations to include mortality as a measure of worker bee response to infection.

Beutler and Opfinger (1950) and Hirschfelder (1964) suggested that protein may be useful in a standardized infectivity test by more closely approximating the nutritional status of bees in the field and by helping to separate more distinctly the response of experimentals from controls. Reported here is an experiment designed to explore further the influence of pollen on honey-bee longevity associated with Nosema infection and also a 2nd experiment to investigate the potential of protein feeding as a component in a standardized test of honey bee response to Nosema infection.

MATERIALS AND METHODS.—The 1st experiment used 16 cages patterned after those described by Kulinčević et al. (1973) fitted with a gravity feeder containing 50% sucrose solution and one containing deionized water. Half of these cages contained bees fed Nosema spores and half contained bees not feed spores. To achieve this, adult worker bees, ages 0–24 h were collected from brood combs held in an incubator at 35°C. These bees were individually fed, without CO_2 treatment, by the method of Rinderer (1976) either a 5.0 μl droplet of a 33% sucrose solution or a 5.0 μl droplet of a 33% sucrose solution containing 6.2×10^5 *N. apis* spores obtained as described by Rinderer and Elliott (1977a).

The bees in half of the cages in each group were continually supplied through the course of the experiment with containers of a paste-like mixture of mixed-floral-source pollen and 50% sucrose solution while the bees in the other half of the cages did not receive this material.

These cages of bees were held in an incubator at 32°C and 50% RH and inspected daily until all the bees died. Dead bees were removed, recorded, and stored at −20°C prior to dissection. The number of Nosema spores in nearly every bee's midgut was determined with a haemocytometer after the midgut was removed and its contents were suspended in one ml of water. A few midguts were too fragile to dissect. Longevity data, represented by the days required for 50% of the bees in each cage to die, were analyzed by analysis of variance and an LSD test. Spore count data were analyzed by a *t* test.

The 2nd experiment, using the same colony as a source of bees, had a similar design and used similar materials with 2 exceptions. First, the spore dose per individually fed bee was 4.3×10^5. Second, instead of pollen, the protein food was a mixture of Yeaco 20®[4], sucrose, α-cellulose, and alcohol extract of pollen formulated as described by Rinderer and Elliott (1977b).

RESULTS AND ANALYSIS.—Overall, mortality tended to occur close to the number of days required for 50% of the bees in each cage to die. While spore feeding generally reduced longevities in both experi-

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[2] In cooperation with Louisiana Agricultural Experiment Station. Received for publication Dec. 6, 1976.

[3] Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

[4] Obtained from Milbrew Inc., Juneau, Wisc. 53039.

Table 1.—Longevity and spore counts of groups of honey bees individually fed or not fed Nosema apis spores and supplied or not supplied with a proteinaceous diet.[a]

Treatment	Longevity ($\bar{x}\pm SD$) [b,c]	% with spores at death[d]	Spores/bee at death ($\bar{x}\pm SE\times10^5$)
	Experiment 1		
Spores + pollen	21.0 1.4B	96.9	180± 6A
No spores + pollen	46.8 3.1C	2.5	e
Spores + no pollen	14.0 0.0A*	97.9	29± 3B
No spores + no pollen	21.3 8.3B	1.0	e
	Experiment 2		
Spores + pollen[f]	22.8 1.0B	96.6	140±10A
No spores + protein	33.3 5.7C	3.7	e
Spores + no protein	14.0 1.6A	95.7	95± 7B
No spores + no protein	22.3 3.9B	8.3	e

[a] Each treatment in each experiment has 4 replicates. Bee/replicate = 50.
[b] Longevity is measured as the time required for half the bees in a replicate to die. $\bar{x}$ is avg of this measure for all replicates in a treatment.
[c] For each experiment, numbers followed by a common letter are not significantly different. * = different at $P<0.05$; all other differences are at $P<0.01$.
[d] Based on numbers of bees examined for spore content. A few bees had fragile midguts and were not successfully examined for the presence of spores.
[e] Too few bees infected to justify averaging.
[f] A mixture including Yeaco 20, a yeast product, as the protein source.

ments, the numerical difference between the longevities of spore-fed bees and bees not fed spores is greater when the bees are fed proteinaceous food (Table 1). In experiment 1, bees receiving spores and pollen lived 25 days less than bees receiving pollen but no spores ($P < 0.01$). Among the groups not receiving pollen in experiment 1, those bees receiving spores lived 7 days less than those bees not receiving spores ($P < 0.05$). The same relationships occurred in experiment 2. Bees receiving spores and Yeaco 20 lived 11 days less than bees receiving Yeaco 20 but no spores ($P < 0.01$). Among the groups not receiving Yeaco 20 in experiment 2, those bees receiving spores lived 8 days less than those groups not receiving spores ($P < 0.01$).

Pollen and Yeaco 20 feeding increased the avg number of spores per bee at death ($P < 0.01$). Since this difference could arise as a consequence of longer longevity with pollen and Yeaco 20 feeding, a t test was conducted on spore count data taken from bees in the pollen and no pollen treatment groups which died from days 14–19 of the experiment. Pollen-fed dead bees from this time period contained an avg of 1.3×10^7 spores; non-pollen-fed dead bees from the same time period averaged 3.4×10^6 spores per bee ($P < 0.01$). These spore count means are almost identical to the overall spore count means for the respective treatments (Table 1). Lack of substantial mortality in an overlapping time period precluded such analysis of data from the experiment using Yeaco 20.

DISCUSSION.—These experiments show the usefulness of proteinaceous food as a component of tests using mortality for a measure of worker bee response to Nosema infection. While the precise nature of the active nutritional component is unclear, both the pollen and the Yeaco 20 formulation improved the ability of an infectivity test to distinguish between the response of bees fed spores and bees not fed spores. This was accomplished in 2 ways. First, the difference between the longevity of Nosema-fed bees and bees not fed Nosema spores was greater when protein is available to the bees. Second, proteinaceous food apparently reduced the occurrence of unexplained early mortality in bees not fed spores. Early mortality in a group of bees not fed either protein or spores was noted by Moffett and Lawson (1975) and also occurred in both experiments reported here perhaps since newly emerged workers do poorly on a carbohydrate diet (Haydak 1937). Early mortality did not occur in non-spore-fed groups fed pollen or Yeaco 20.

The use of a pollen substitute such as Yeaco 20 has decided advantages over the use of pollen in an infectivity test. The extraction of the pollen with ethanol and the filtration of the extract probably reduces contamination of the food with advantitious *N. apis* spores. Furthermore, a pollen substitute's semidefined nature provides for greater uniformity of food substance through time.

This experiment also provides insight into the relationship between bee nutrition and Nosema spore production. Gontarski and Mebs (1964) suggested that protein feeding results in an expansion of the honey bee midgut which leads to more infection sites and thereby causes greater spore production. While the 3-fold increase in midgut volume from newly-emerged bees to pollen-eating bees caring for brood (Schreiner 1954) can account for the 2-fold increase in spores noted by Gontarski and Mebs (1964), it cannot account for the 6-fold increase observed in our experiments. Apparently, feeding bees with proteinaceous food results in a nutritional enrichment, not only for bees, but also for their Nosema parasites.

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