Queen Honey Bees[1] in Colonies: Susceptibility to Nosema Disease[2]

by NORBERT M. KAUFFELD

Entomology Research Division,
Agr. Res. Serv., USDA,
Baton Rouge, Louisiana 70803

ABSTRACT

Queen honey bees, APIS MELLIFERA L., were not easily infected with NOSEMA APIS Zander, as might have been expected, even when the bees, combs, and the interior of the hives were sprayed regularly with 100X10⁶ spores. In the area around Baton Rouge, colonies are able to maintain brood rearing throughout the winter despite a heavy Nosema infection because the bees can forage almost daily and fecal matter is voided.

DURING the past years, much research has been done to determine the effects of *Nosema apis* Zander on worker and queen honey bees, *Apis mellifera* L.

Furgala (1962b) found that queen bees individually inoculated with *N. apis* and introduced into nuclei hives were often superseded; their ventriculi showed infections; he, Furgala (1962a) also found that 10-12% of 465 queens in packages shipped into Canada were infected with Nosema. Jay (1967) reported almost similar levels of infection in a survey of queens shipped into Manitoba, Canada. Fyg (1964) in Switzerland reported Nosema disease in 41% of 310 queens from colonies infected with disease and in 61% of 164 queens from colonies multiply infected with Nosema disease and amoeba, and Foote (1971) found that 16 of 145 queens dead in queen bank colonies were infected with Nosema disease. L'Arrivée (1965) reported that when queens artificially infected with spores of *N. apis* were introduced into "disease-free" colonies, a rapid rise in infection occurred among the worker bees within 2 months. Wang and Moeller (1969) reported no difference in the pattern of distribution in a hive between diseased and healthy bees. Since young bees usually were in the brood area occupied by the queen, Nosema was not readily transmitted to the queens. Wang and Moeller (1970) reported that worker bees infected with *N. apis* were less frequently found in the queen's royal circle. This behavior reduced the probability of a queen becoming infected with the disease. Hassanein (1951) also found that queens may remain free of infection even when the colony was heavily infected.

However, the mode of transmission from worker bee to queen is still not well understood. Nosema disease was reported by Bailey (1955) and L'Arrivée (1965) to be acquired by workers from spores on contaminated bee equipment and from deposits of fecal matter, contaminated honey, combs, and water. In addition, L'Arrivée (1965) observed that worker bees can transmit the disease from sugar syrup containing spores of *N. apis* to other workers in the hive. However, the degree of transmission of the spores from infected workers and contaminated equipment to queen bees under natural conditions has not been well demonstrated. Oertel (1964) found that the spores were absent or found in only an occasional worker in samples taken in the area of Baton Rouge in December and January. This test was made at the Bee Breeding Investigations Laboratory, Baton Rouge, La., 1970-1971 to determine whether spores of *N. apis* could be transmitted to queens by repeatedly infecting young and old worker bees and contaminating hive parts during the winter months in Louisiana.

Methods and Materials. — Colony and Queen Manipulations. — The test colonies (48 11-frame 2-story nuclei hives with about 17,500 bees each) were established on October 28, 1970 from nuclei and standard Langstroth hives maintained during the summer at Baton Rouge. They had not been treated to produce an infection of Nosema disease nor was fumagillin fed to make them disease-free. Food stores and brood were equalized in all of the nuclei hives so that each of the 48 nucleus hives had approximately 45-46 square inches of brood, 7 frames of honey, no frames of pollen, and a No-

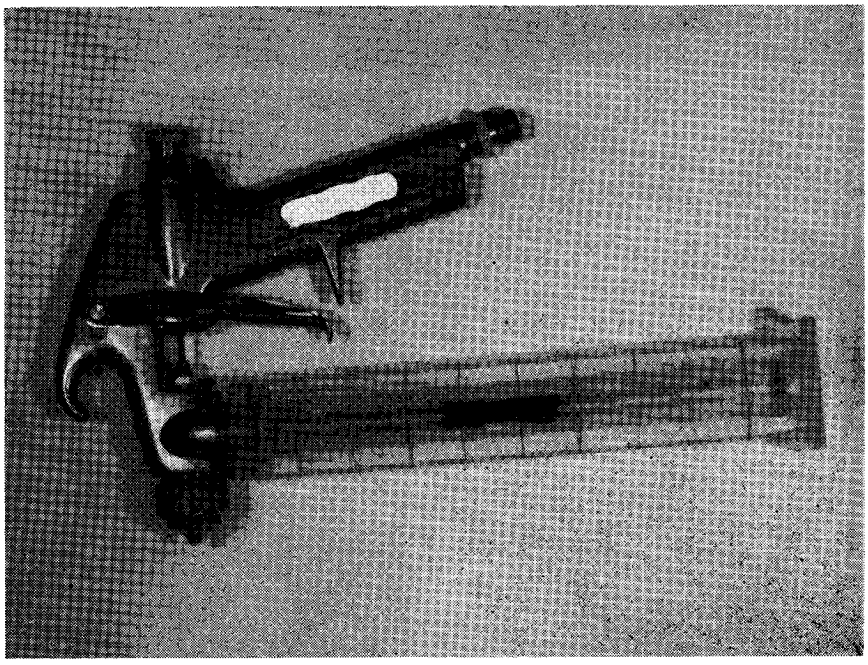

Fig. 1. — Plastic spray tube and paint gun used to apply the spore-infected sugar syrup.

FOOTNOTES

[1] Hymenoptera: Apidae.

[2] In cooperation with Louisiana State University Agricultural Experiment Station.

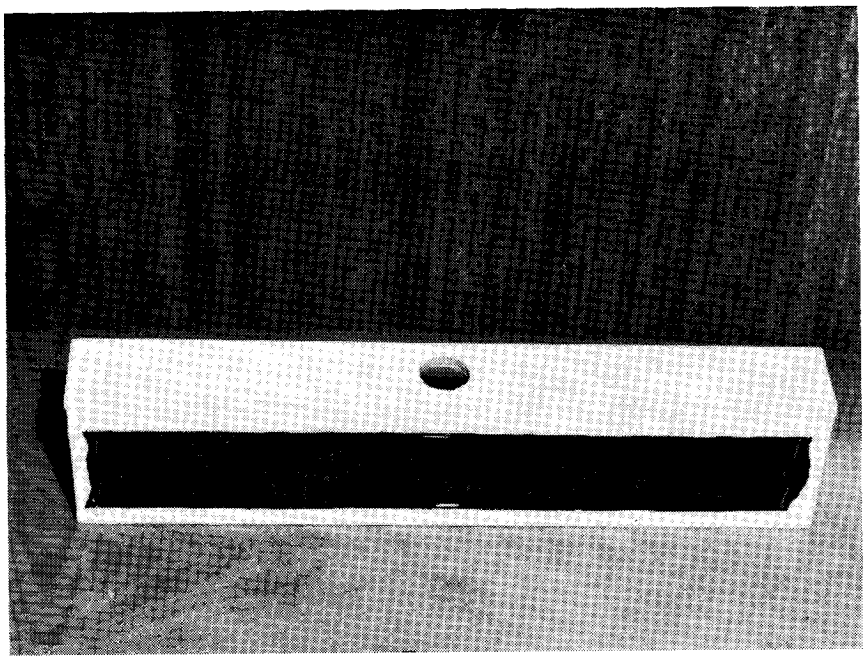

Fig. 2. — Trap without cylindrical cage.

sema count of 0.00-0.01X10^6 spores per bee. The queens (all sisters, instrumentally inseminated) were introduced and accepted by the bees. From October 28 to the initial data collection on December 9, colonies were permitted to become settled with brood reared from eggs laid by the introduced queens. No additional food was provided the colonies during the test period (December 1970-April 1971).

After the successful introduction of the queens, the 48 nuclei hives were randomly divided into 2 unequal groups, viz., 1 group of 12 served as controls and the other group of 36 was sprayed with spores of **N. apis** at regular intervals. The 48 nuclei hives were placed in a timbered site in a randomized complete block design with entrances alternated to reduce drifting and tree trunks furnished orientation objects. In an effort to collect all superseded queens, queen excluder material was placed over all hive entrances.

Spore Solution. — The spores of *N. apis* needed for the test were mass produced by spraying 1500-4000 bees in commercial shipping packages with a spore inoculum previously prepared in a 50% sugar syrup. Also, the bees were fed sugar syrup containing spores through perforated lids of inverted pint jars. After 2 weeks, abdomens were detached from the thoraxes and homogenized with an equal volume of water, and the solution was filtered and put into a deep freeze at -20° C for future use.

Frozen *Nosema* spore suspension (had been stored for 1 to 2 years) was thawed and mixed with a 50% (by volume) sucrose solution. Then the inoculum was filtered through a Buchner funnel to reduce the amount of foreign material that might clog the jets of the spray gun applicator. Also, at this time, the spore concentration was calculated with a grid haemocytometer.

The procedure for application of 20 ml sugar solution with 100X10^6 spores was as follows: the 1st frame was removed from 1 of the 2 brood chambers and both sides of the comb with adhering honey bees were sprayed and then each side of each of the other frames was sprayed without removing them from the hive. Both top and bottom brood chambers were sprayed in this manner. The sugar syrup was used as a carrier for the Nosema spore inoculum and as the bees readily licked each other (queens were licked off by the bees) the hive parts all were infected with Nosema. The 20 ml amount of syrup (equivalent to 1⅓ tablespoonful or 1.14 ul/bee) was not considered enough to stimulate or cause a difference in broodrearing. Therefore, the 12 control colonies were not sprayed with plain sugar syrup. With the air pressure set at 1.81 kg at the air tank, the special spray gun produced a fine mist leaving the dorsal surfaces of the bees, queens, and the combs and hive parts with minute droplets of inoculum. No injury to the bees and queens was observed at anytime. Thereafter, every 3 weeks as winter weather permitted, each of the 36 test colonies was retreated in the same way.

Data Collection.—Data consisted of reading the square inches of brood, frames of honey and pollen, and the collection of samples of bees from hive entrances every 3 weeks the week prior to that when the colonies were sprayed with *N. apis* spores. On December 9, samples of foragers were collected at the entrances during the morning hours with sampling traps designed and tested at this laboratory. Then immediately after this collection of the foragers, the 36 test colonies were inoculated with the inoculum. Levels of infection were determined from 20-bee samples and spore counts made with the aid of a haemocytometer grid.

Sampling Device and Spray Unit.—The spray gun (Fig. 1) was a paint applicator with a cylindrical plastic tank (OD of 3.81 cm and length of 21.59 cm; ID 2.54 cm and 19.69 cm in length) with a liquid capacity of ca. 95 ml. The top of the tank had an 0.79-cm threaded hole for attachment to the paint applicator. A small portable air tank with hoses and regulatory valves was used to furnish the necessary air pressure.

The overall exterior measurements of the sampling device are 4.44 (width) x 8.25 (height) x 36.8 (length) cm (Fig. 2); sides (ends) measure 4.44 (width) x 7.62 (height) cm; and the top has a 2.54-cm hole cut through the center of it. A cylindrical cage constructed of hardware cloth measuring 3.81 x 8.89 cm in diameter and height, respectively, with a small funnel inserted at one end and cork at the other was placed over the hole in the top board of the trap (Fig. 3).

A piece of 0.32-cm mesh hardware cloth with some weather-resistant black tape was stapled across the front of the trap to cut out light (Fig. 3).

The traps were placed in the opening of the Langstroth hive bottom

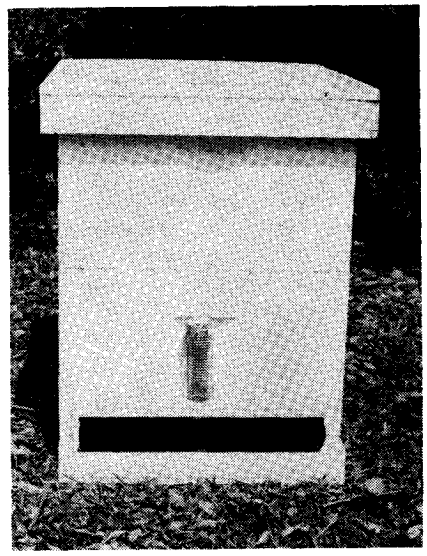

Fig. 3. — Trap and cylindrical cage in position on hive.

Table 1. — Brood, pollen, honey, spores per bee, % colony infected, and queens lost per sampling date in 36 colonies tested for transmission of spores of N. APIS by repeated inoculation* (avg. for 12 untreated colonies in parenthesis). Baton Rouge, La.

Sampling date		Amt. of brood (in.²)	No. frames of Honey	No. frames of Pollen	No. lost queens	Spores/bee X10⁶	% colonies infected
1970							
10/28†		45(46)	7(7)	0(0)	....	0.01(0.00)	2.7(0)
12/9	S‡	48(42)	7(7)	0(0)	0(0)	1.01(0.01)	41.6(25)
12/22	S	62(52)	16(14)	3(4)			
1971							
1/21					2(0)	42(26)	100(100)
1/25	S	103(81)	14(14)	5(5)			
2/12	S	97(92)	10(14)	1(2)			
2/25					4(1)	21(16)	100(100)
3/2	S	171(138)	10(9)	6(6)			
3/10					2(0)	25(22)	100(100)
3/22	S				3(0)	22(16)	100(100)
3/30		288(239)	5(5)	3(2)			

* Treated colonies received 100X10⁶ spores/20 ml sugar syrup every 3 weeks.
† Values for colonies on October 28, 1970 when set up.
‡ Spray dates.

boards either late in the evening prior to or early in the morning of the sampling date (Fig. 3). Honey bees leaving the hive crawled to the opening in the trap's top board because of the light showing through it.

Results and Discussion.—The samples of foraging bees taken on December 9 just before the first spray treatment showed that the control colonies (12) and the test colonies (36) had average spore counts of 0.01×10^6 and 1.01×10^6/bee, respectively (Table 1). However, only 15 of the 36 colonies (41.6%) and 3 of the 12 colonies (25%) had infected bee samples. From previous laboratory records, this level of infection was high for the month of December in the Baton Rouge area.

The infection levels increased drastically after the 1st application of sugar syrup containing spores of *N. apis* and by the first sampling date after inoculation (January 21) spore counts/bee had risen to 77×10^6 spores in one of the inoculated colonies. The levels of infection increased in both the control and treated colonies, though they remained lower in the control group. Drifting probably caused the increased infection in the control group since laboratory records and previous investigation of the cycle of Nosema disease in the Baton Rouge area do not show a natural peak of infection until sometime in March or April.

The heavy, repeated inoculations of spores of *N. apis* caused 100% of the colonies to become infected and remain infected throughout the test (Table 1). Also, 8.3% and 30.5% of the queens (colonies) in the control and inoculated groups, respectively, were lost.

From these data (Table 1), it may be concluded that regular inoculation by spraying of large concentrations of spores of *N. apis* onto bees, queens, combs, and equipment increased the loss of queens. The 30.5% loss of queens in the test colonies is highly significant and is indicative of the problem facing the Northern and Canadian beekeeper. However, unfortunately, only 2 of the dead queens from the inoculated colonies were recovered, and these did not show Nosema infection.

On April 2, 1971, all living queens (25 from the test colonies and 11 from the controls) were removed from the colonies and examined for spores of *N. apis*. Three of the inoculated group were infected (avg. 0.18×10^6 spores); none of the control group were. Since only 30.5% of the queens in the 100% infected inoculated colonies were lost and only 3 of the remaining 25 queens in these colonies were infected, it is apparent that certain factors in a colony do not permit queens to be easily infected with Nosema disease. Therefore, it is theorized that queens may be more easily infected while they are still virgin and are feeding themselves from food stores in the combs or when they are being transshipped (Farrar 1947) as mated queens with infected attendants.

Data in Table 1 also show more square inches of brood present in the inoculated colonies than in the control colonies and a continuation and increase in brood rearing. This result is somewhat opposed to those reported by other investigators who found that Nosema infection reduced brood rearing (Farrar 1947). However, Hassanein (1953) and Moeller (1962) showed that on given days diseased colonies had higher percentages and square inches of brood than healthy or Fumidil-B fed colonies. Perhaps, with the stress of repeated heavy Nosema infection, above certain unknown levels, the workers or queen, or both, are stimulated to increase brood rearing, to offset the loss of adult bees. In any event, in the area of Baton Rouge, La., colonies with heavily infected worker bees can maintain brood rearing throughout a year, even during the winter months (Table 1). Quantities of pollen and nectar may be limited, and weather conditions such as a rain may reduce or hinder foraging but some supplies are collected regularly and the bees usually can forage for nectar and pollen during winter months. Since that situation exists, the bees in this part of the United States can void fecal accumulations almost every day. Therefore, contamination of hive parts and combs by spots of fecal matter containing spores of *N. apis* is rarely observed, even in colonies heavily infected with Nosema disease. ●

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