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NOSEMA DISEASE OF HONEYBEE QUEENS *(APIS MELLIFICA L.)*

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SUMMARY

The feasibility of coprological examination for nosema disease was studied in 442 young queens. Possible age-linked resistance of queens was investigated by selecting 50 aged from 1 to 10 days and infecting them with spores of the protozoan *Nosema apis* Z. The quantity of spores eliminated with the faeces was then determined.

Experiments showed an infestation rate of 18.4 % amongst queens taken from nuclei (206 queens examined) and an infestation rate of 9.1 % amongst queens reared in the bee flight room (186 queens examined). Queens 1 to 10 days old inoculated with nosema spores showed no indication of age-linked resistance to the disease. Taking into consideration the high degree of the nosema infestation of young queens, their average life-span of 25.3 days, and the improbability of young queens recovering, we believe that nosema disease is the main cause underlying requeening in colonies where a queen has already been accepted.

Experiments under the hygienic standardized conditions of the bee flight room proved conclusively that the incidence of nosema disease can be reduced by applying strict prophylactic measures (particularly the use of comb foundations instead of finished combs and the stocking the nuclei with young bees hatched in an incubator).

INTRODUCTION

The problem of nosema disease has mostly been associated with worker bees, whereas drones and queens are generally considered to be more resistant or less susceptible to infection and the danger to them appears to have been somewhat underestimated. Although experiments by other researchers have proved that nosema can infect also the drones (BAILEY, 1972) and queens (POLTEV, 1960; FURGALA, 1962) the results of the examination of queens from nosema-infected colonies indicates that

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the percentage of diseased queens in colonies is not high. The finding of diseased queens in only about 20-30 % of the diseased colonies can be attributed to the resistance of the queens to the disease (FURGALA, 1962) and to the protective system based on the different behaviour of healthy and diseased bees. The nosema- infected bees suffer from an atrophy of the pharyngeal glands, consequently they do not feed the brood and, moreover, they do not accompany the queen (WANG & MOELLER, 1970). The mature queen is normally surrounded by healthy bees preventing her from coming in contact with nosema spores. This is the reason why young queens, which depend on themselves for feeding are more liable to come in contact with spores in the colony (KAUFFELD, 1973).

Nosema disease in queens of different age was studied by BOBRZECKI (1975). He found that in nosema-infected colonies the higher the age of the queens the higher the frequency of nosema disease in them.

The purpose of our present study was firstly to ascertain the degree of nosema infection in the queens dispatched from commercial bee farms producing marketable queens. A simple method for making a preliminary examination of the queens prior to dispatch was sought. Secondly we wished to verify the existence or non-existence of age-linked resistance as described by GONTARSKI (1952) and HANKO (1964), and queens of different ages were inoculated for this purpose. In addition experiments in a bee flight room, using cages equivalent to nuclei, and in the colonies in an apiary were designed to study the possibilities of restoring diseased queens to health.

MATERIAL AND METHODS

Various methods of coprological examination were tested and the most suitable chosen, involved the placement of queens on slides under halves of Petri dishes or 50 ml beakers (Fig. 1). Thermal shock was used with advantage, the slides being cooled in a refrigerator or in a cellar before use. The queens in beakers were placed on the lids of the nuclei. When the queens had calmed down they usually defecated within 5 minutes and only rarely did a queen defecate after longer than 15 minutes. Immediately after capture in the nucleus the queens had to be placed in the prepared beaker. The transfer of the queens to the laboratory implied the possibility of undesired defecation during transportation.

The queens mostly defecated on the slide and therefore the faeces did not need to be handled. When faeces were deposited on the wall of the beaker a sharp jerk was enough to make them drop onto the slide. The excreta of the queens were thin and light yellow in colour. Without any further treatment we covered them with a micro-cover slip and examined them under a microscope at 450 x magnification. In all 206 queens from five different localities and 186 queens from the bee flight room were examined.

The queens in the bee flight room (VESELÝ, 1977) were studied for nosema disease after natural infection with spores of the protozoan.

Washed and disinfected cages 80 mm × 130 mm × 140 mm in dimension were used to accommodate the queen together with 400 bees either hatched in an incubator and up to 4 days old, or swept from combs with unsealed brood. A part of the brood comb or foundation was placed in the cage. The occupied cages, were then kept in an incubator for a week at 28 °C. When this time had elapsed the queens were artificialy inseminated and the cages were exposed in the bee flight room the next day. From the beginning of the experiment ground mixed pollen in an aluminium foil feeder and 66 % sugar solution in a

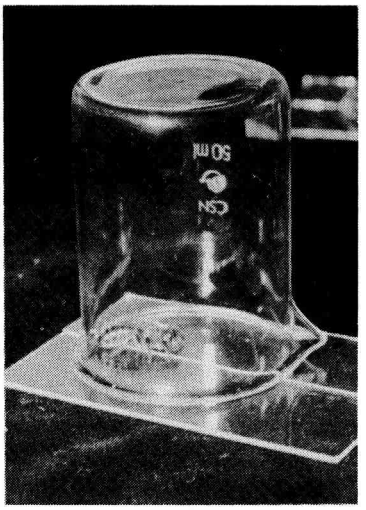

FIG. 1. — *The honeybee queen under a beaker.*

glass feeder were available to the bees in the cages. The stock in the cages was inspected every day. The behaviour of the queens, the commencement of oviposition, and — in the diseased queens — the length of life were the main factors under study. Each queen was subjected to coprological examination at the beginning of oviposition to determine the presence or absence of the spores of the nosema protozoan.

The following procedure was adopted in the study of the resistance of young queens to the disease. Queens which were obtained by rearing from the egg or young larvae were placed in nuclei and divided into 10 groups with five individuals in each. The groups were subjected to an inoculation of nosema spores at periods varying from 1 to 10 days. The inoculation dose was administered to the queens individually by means of a micropipette, the queen either being held in the hand and inoculated or being first placed in a small cage where it was left to calm down and fast for several minutes. This cage had a fine mesh and the dose was administered through the netting with the micropipette. The queens readily took the feed provided in this way and each received 3×10^4 nosema spores in 10 mm^3 of sugar solution. After inoculation they were returned to the nuclei. On the third day after infection nosema spores could be detected in the faeces. Therefore coprological examination of the excrements could be carried out daily from the third post-infection day. Excrements were obtained in the manner described earlier. The examination was, however, elaborated : the faeces were removed from the slide and transferred to a Bürker chamber where the spores in 1 mm^3 of faeces from each queen was determined quantitatively. The values obtained were analysed mathematically and the groups of queens were compared with one another by the S method. The experiments on queens for the determination of their age resistance to nosema disease were made from July 6 to July 29, 1974.

The possibility of restoring queens to health and the survival of diseased queens were studied by using only a small part of the infected stock : 10 queens from the bee flight room and 5 from colonies. The experiments in colonies comprised queens which showed the least clinical symptoms of disease. The queens were introduced to colonies on August 2, 1974 and were prepared for overwintering in the colonies so that their condition could be evaluated the following spring.

RESULTS

The examination of 206 queens from 5 localities on two farms producing queens for commercial purposes revealed that the morbidity of young queens was high,

ranging from 7.8 % in non-laying queens to 29.8 % in laying queens. An average of 18.4 % of the queens examined were infected by the nosema protozoan. Detailed results are shown in Table 1.

TAB. 1. — *The rate of nosema infection in young honeybee queens at the time of dispatch from bee-breeding farms.*

Locality	No. of queens examined	No. infected
A	34	8 (23.5 %)
B	10	2 (20.0 %)
C	25	7 (28.0 %)
D	47	14 (29.8 %)
E	90*	7 (7.8 %)
Total	206	38 18.4

* Non-laying queens examined.

In the bee flight room, where 186 queens were examined over a 2-year period, 17 queens were found to suffer from nosema disease, i.e. a morbidity rate of 9.1 %. A high degree of nosema infection was ascertained in queens during the first year of the experiments, when the cages were stocked with bees from colonies nursing the young brood and when part of the brood combs were placed in the cages. In this first year 74 queens were examined and 13 of them, i.e. 17.6 % were found to be infected.

In the second year of the experiments all cages were stocked with bees up to 4 days of age, hatched in an incubator, and only comb foundations were placed in the cages. Of the 112 queens examined coprologically, only 4 were found to be infected i.e. 3.6 %.

Seven of the total number of 17 queens infected with nosema failed to oviposit. Three of these died within 33 days of emergence and the other four were killed and dissected.

The remaining 10 diseased queens started laying eggs about 17.5 days after insemination, whereas in the healthy queens the interval from insemination to the beginning of oviposition averaged 14.2 days. These 10 nosema-infected queens were left in cages and none was found to get rid of the disease. All died after 25.3 days from commencement of oviposition, with an average age of 49.4 days. They exhibited typical symptoms of nosema disease, such as languor, swollen back and cessation of oviposition several days prior to death.

In the experiments to find whether there is resistance to the disease in young queens, the coprological examination of 50 laboratory infected queens proved negative. The experimental groups also showed no significant difference at alpha = 0.01 in the elimination of the spores of nosema in faeces contaminated during the 1st to

10th day of life. At alpha = 0.05 a statistically significant difference was found between the group of queens infected on the 2nd day after emergence and the group of queens infected on the 10th day. The differences between the remaining queen groups were statistically insignificant. The smallest amount of nosema spores was eliminated by queens of the group infected on the 2nd day after emergence (139.78 spores per 1 cu. mm of faeces). The largest amount of the spores was eliminated by queens infected on the 10th day after emergence (379.47 spores per 1 mm^3 of faeces). Detailed data are shown in Table 2.

TABL. 2. — *The number of nosema spores in 1 cu. mm of faeces of honeybee queens inoculated at various ages* (observation made from 1 to 11 days after inoculation; 5 honeybee queens in each group.).

Queen's age at inoculation, days	No. of observations	Mean $\bar{x}$	Standard deviation S_x
1	40	302.383	258.393
2	38	139.781	168.598
3	38	234.134	297.356
4	42	307.531	256.392
5	39	267.126	267.004
6	38	289.052	312.320
7	45	195.107	170.503
8	40	226.570	156.802
9	40	296.286	232.440
10	39	379.470	292.166

Fig. 2 shows the amount of nosema spores in 1 mm^3 of faeces eliminated from the 3rd to 11th day after infection. As may be seen from the graph, the elimination of the spores continued to increase up to the 8th day after infection and then stabilized.

When checking the possibility of the recovery of diseased queens, it was found that the five queens deposited in August 1974 for overwintering in colonies had been replaced by autumn of the same year.

DISCUSSION

Coprological examination of 206 queens from nuclei revealed the presence of nosema spores in 18.4 % of individuals. The occurrence rate of the disease in Czechoslovakia thus compares with that in other countries (JAY, 1967).

Examination of queens in the field suggested a higher incidence of the disease in laying queens (20 % to 30 %), as distinct from non-laying queens where the occurrence rate was only about 8 %. Similar conclusions had been drawn by LEHNERT, SHIMANUKI & KNOX (1973) who ascribed the different infestation rate of young and

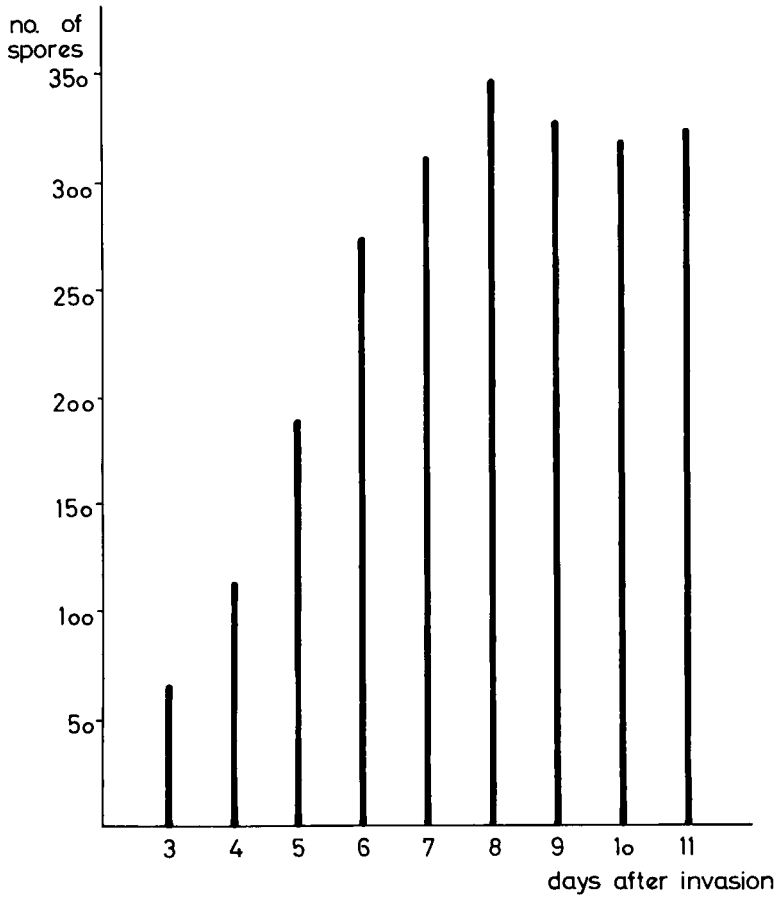

FIG. 2. — *Number of the spores of the protozoan* Nosema apis *Z. eliminated in 1 mm³ of faeces.*

older queens to difference in feeding. It is also very probable that under field conditions this factor is combined with the longer stay of laying queens in an infectious environment.

The experiments proved the necessity of using cell foundations and healthy young bees for stocking nuclei in which the queens are to be reared, instead of combs and bees from colonies since these are a potential source of nosema spores. This is proved by the results of the first and second experimental years in the bee flight room where nearly 18 % of the queens were infected by the protozoan when combs were used and the nuclei were stocked with bees nursing young brood. On the other hand, only about 4 % of the queens suffered from nosema disease when foundations were used and the nuclei cages were stocked with young bees hatched in an incubator. All these queens

came from a group which was, by mistake, placed in non-disinfected cages from previous experiments.

Taking into account the high incidence of infection in queens dispatched from farms and the length of their life, it can be assumed, as stated by FURGALA (1962), that in Czechoslovakia nosema disease is one of the main causes of the replacement of queens which have already been accepted by the colony.

Unlike FURGALA (1962), we did not find effective resistance in any of the infected queens : no instance of complete recovery was observed. Examination of the faeces of queens of different ages indicated hardly any age-linked resistance to the disease. However the results may have been partly distorted by the fact that the most severely affected individuals died at the end of the experimental period in a majority of the groups. Another distortive factor may be the higher frequency of defecation in severely infected individuals, leading to a decrease of the number of spores per 1 mm^3 of faeces.

On the other hand, a larger quantity of spores in the faeces of older queens might be due to difference in diet of laying and non-laying individuals. Younger queens mostly got glycidic foods whereas proteinaceous food prevailed in the diet of older queens; the proteins may have stimulated development of the protozoan spores in the queens, as it does in bees (PEROUTKA, 1975). In our experiments the older groups of queens infected mostly included queens which were already laying.

CONCLUSION

Experimental sampling indicates that up to 20 % of young honeybee queens reared commercially in Czechoslovakia are infected by the protozoan *Nosema apis* Z. at time of dispatch.

The method of coprological examination of honeybee queens to test for nosema infection is satisfactory and can be recommended as an effective means of surveillance on bee-farms.

Experiments indicate that the high infection rate of nosema in young honeybee queens, their approximately 50-day average lifespan and the improbablity of their recovering from the disease are the main causes in Czechoslovakia for the requeening in colonies where a queen had already been accepted.

Inoculation with nosema spores of 50 queens on the 1st to 10th day after emergence showed no obvious age-linked resistance as described in young bees.

Experiments conducted under hygienically standardized conditions in a bee flight room show that the risk of nosema infection can be significantly reduced by applying strict prophylactic measures (particularly the use of foundations instead of finished combs and by stocking of nuclei with young bees hatched in an incubator).

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RÉSUMÉ

LA NOSÉMOSE CHEZ LES REINES D'ABEILLES (*APIS MELLIFICA* L.)

On a étudié sur 442 jeunes reines s'il était possible d'utiliser l'examen coprologique pour détecter la nosémose. On a recherché une éventuelle résistance des reines liée à l'âge en sélectionnant 50 reines âgées de 1 à 10 jours et en leur inoculant des spores du protozoaire *Nosema apis* Z. On a alors déterminé la quantité de spores éliminées par les faeces.

Les expériences ont montré un taux d'infestation de 18,4 % parmi les reines provenant des nuclei (206 reines examinées) et un taux de 9,1 % parmi celles élevées en chambre de vol (186 reines examinées). Les reines âgées de 1 à 10 jours, auxquelles on avait inoculé des spores de *Nosema,* n'ont montré aucun signe de résistance à la maladie liée à l'âge. En tenant compte du fort degré d'infestation des jeunes reines par *Nosema,* de leur durée moyenne de vie de 25,3 jours et de l'improbabilité pour les jeunes reines de guérir, nous pensons que la nosémose est la principale cause de supersédure chez des colonies qui ont déjà accepté une reine.

Les expériences menées dans les conditions standardisées d'hygiène de la chambre de vol ont prouvé de manière décisive que l'incidence de la nosémose peut être réduite par application de mesures prophylactiques strictes (en particulier en utilisant des feuilles de cire gaufrée à la place de rayons construits et en peuplant les nuclei avec de jeunes abeilles écloses en étuve).

ZUSAMMENFASSUNG

NOSEMA-ERKRANKUNG VON BIENENKÖNIGINNEN *(APIS MELLIFICA)*

An 442 jungen Königinnen wurde untersucht, ob eine koprologische Untersuchung auf das Vorliegen einer Nosema-Infektion möglich ist. Um die Möglichkeit einer altersabhängigen Resistenz zu untersuchen, wurden 50 Königinnen im Alter von 1-10 Tagen ausgewählt und mit Sporen des Protozoen *Nosema apis* Z. infiziert. Nachher wurde die mit den Faeces eliminierte Sporenmenge bestimmt.

Die Experimente ergaben eine Infektionsrate von 18,4 % bei Königinnen aus Begattungskästchen (206 untersuchte Königinnen) und eine Infektionsrate von 9,1 % bei Königinnen, die im Flugraum gezüchtet worden waren (186 Königinnen). Die Königinnen, die im Alter von 1-10 Tagen mit Nosemasporen infiziert worden waren, erbrachten keinen Hinweis auf eine altersabhängige Resistenz gegenüber der Krankheit. Wenn man die grosse Häufigkeit von Nosema-Infektionen von jungen Königinnen in Betracht zieht, ferner ihre durchschnittliche Lebenserwartung von nur 25,3 Tagen und die Unwahrscheinlichkeit, dass junge Königinnen wieder ausheilen, so neigen wir zu der Ansicht, dass die Nosemaerkrankung die Hauptursache für eine Umweiselung bald nach der Annahme einer zugesetzten Königin ist.

Versuche unter den standardisierten hygienischen Bedingungen des Flugraumes bewiesen in überzeugender Weise, dass das Auftreten einer Nosema-Infektion durch strenge vorbeugende Massnahmen verringert werden kann (vor allem durch Verwendung von Mittelwänden anstelle von ausgebauten Waben und der Aufbau der Begattungsvölkchen mit Jungbienen aus dem Brutschrank).

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