

Original article

The influence of *Nosema apis* on young honeybee queens and transmission of the disease from queens to workers

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Abstract – *Nosema apis* inoculated and non-inoculated honeybee (*Apis mellifera*) queens were introduced into mating nuclei. The workers accepted all queens from both groups. During the 26 days of the experiment, 47.4% of the inoculated and 50% of the non-inoculated queens were lost. Queens from both groups started egg-laying after 15.8 days. Workers did not perceive the presence of *N. apis* in their queens and did not supersede inoculated queens significantly more often than healthy ones. Significantly more workers were infected in the mating nuclei with inoculated queens (61%) than in those with non-inoculated ones (5.3%).

Apis mellifera / *Nosema apis* / queen-worker transmission / spore / supersedure

1. INTRODUCTION

The protozoan *Nosema apis* Zander develops within the epithelial cells of the midgut of honeybee (*Apis mellifera*) workers, drones and queens [1, 4–6]. In queens infected with *N. apis* the ovaries do not develop properly, and this can lead to infertility [8, 9, 13]. Infected queens are more often superseded than uninfected ones [3, 7, 11]. Furgala [7] inoculated queens with different doses of *N. apis* (0.001 – 0.1×10^6 spores) and observed

supersedure as early as 6 days after inoculation. The mean number of days before supersedure was observed varied from 15 to 48 days. Supersedure of infected queens seems very important for colony health because workers attending the queen ingest its feces, which can contain parasites [1, 2]. It is not clear if workers are prompted to supersedure by the presence of *N. apis* in their queen or by pathological changes caused by the parasite. Perception of infection of the queen and its supersedure would

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prevent the spread of the parasite in the colony. This study investigated whether workers supersede infected queens more often than healthy ones even though the effects of infection are low or not yet apparent. The influence of *N. apis* on queens during mating flights and on the start of egg-laying was also examined. Additionally, the mode of infective spread from queens to attending workers was verified.

2. MATERIALS AND METHODS

The experiment was conducted in June 1999. Virgin queens of *A. m. carnica* derived from a queen inseminated with semen of a single drone were used. The queens emerged within 9 hours of each other and weighed 215–246 mg. They were divided into two groups, inoculated and non-inoculated, each consisting of 20 individuals. The queens from the inoculated group were individually fed 10 µl 50% sugar syrup containing 0.1889×10^6 *N. apis* spores on average. The queens were kept individually in Zander cages with two attending workers, and then introduced into mating nuclei one day after the nuclei were established.

To obtain young workers for the mating nuclei, combs with capped brood (no bees) were placed in the upper hive bodies of large colonies. The upper hive bodies contained no workers and were separated from the rest of the hive with wire mesh. Workers that emerged in the upper hive bodies during 8 days were used to form the mating nuclei, each of which contained 1/3 l of workers, two small frames with foundations, and one newly built comb. Just before formation of mating nuclei a sample of 30 workers was taken to verify the presence of *N. apis*. The mating nuclei were new and did not require disinfection. They were placed in four rows 3.3 m apart. The distance between mating nuclei within rows was 1.6 m. During the experiment the mating nuclei were examined daily to verify the presence of queens and eggs. Food was replenished if neces-

sary. After 26 days the queens and 15 workers from each mating nucleus were frozen for further analysis.

To assess the infection level in queens, the midgut was macerated in 0.5 ml distilled water; to assess the infection level in workers, the whole abdomen was macerated in 1 ml distilled water. The spores were counted in a hemacytometer in 0.025 mm³ suspension. If less than 10 spores were found in the sample they were recounted in 0.2 mm³ suspension. The results are expressed as spore counts per individual. For statistical analysis the Mann-Whitney test and χ^2 test were used.

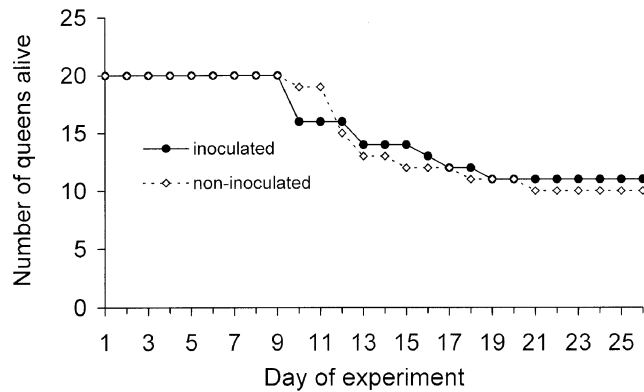
3. RESULTS

All queens from both groups were accepted by the workers. Only one of the inoculated queens that survived to the end of the experiment contained no spores. This queen was excluded from further analysis. The other inoculated queens contained from 6.6×10^6 to 22.6×10^6 spores (mean $15.3 \pm 4.3 \times 10^6$, Tab. I). The non-inoculated queens contained no spores.

Workers superseded one of the inoculated queens and none of the non-inoculated ones. This difference was not statistically significant ($\chi^2 = 0.045$, $p > 0.05$). The only supersedure that was observed started on the 17th day of the experiment. Four queen cells were found in the mating nuclei and remained there until the end of the experiment. During the experiment, 47.4% of the inoculated queens and 50.0% of the non-inoculated ones were lost. This difference was not statistically significant either ($\chi^2 = 0.00$, $p > 0.05$). During the first 15 days of the experiment there was unfavorable weather, with rain and overcast. This could have caused loss of some queens during mating flights (Fig. 1). Another reason for queen loss could have been a gradual decrease in the number of workers in some mating nuclei. Inoculated queens started

Table I. *N. apis* infection of inoculated and non-inoculated honeybee queens and workers attending them.

	Groups	Number of individuals		Millions of spores per bees (mean \pm SD)
		examined	infected	
Queens	Inoculated	10	10	15.3 \pm 4.3
	Non-inoculated	10	0	0 \pm 0
Workers	Inoculated	150	92	7.9 \pm 9.4
	Non-inoculated	150	8	7.5 \pm 6.4
Workers before experiment		30	0	0 \pm 0

**Figure 1.** Losses of inoculated and non-inoculated queens on consecutive days of the experiment.

egg-laying after 15.77 ± 1.64 days on average (\pm SD), and non-inoculated queens after 15.82 ± 1.47 days ($U = 69.0, p > 0.05$).

No *N. apis* spores were found in the workers used to prepare the mating nuclei (Tab. I). After 26 days, infected workers were found in all mating nuclei with inoculated queens, but in only 50% of the mating nuclei with non-inoculated queens. The proportion of infected workers found in mating nuclei with inoculated queens (61.3%) was statistically greater than the corresponding proportion in mating nuclei with non-inoculated queens (5.3%; $\chi^2 = 0.35, p < 0.001$) (Tab. I). The spore counts in infected workers did not differ statistically between the groups ($U = 351.50, p > 0.05$) (Tab. I).

4. DISCUSSION

There were no differences in losses between inoculated and non-inoculated queens. This suggests that during the 26 days after inoculation with *N. apis* the pathological changes were not marked enough to cause losses of queens. The results concerning queen losses in both groups are similar to those of Konopacka [12], who observed most losses during mating flights. In this experiment those losses probably were affected by unfavorable weather, which differed markedly from the optimal weather for mating flights described by Soczek [16] and Ruttner [15]. Workers from most mating nuclei where the queens were lost either absconded or drifted and their analysis for

presence of *N. apis* at the end of the experiment was not possible.

The results suggest that workers did not perceive presence of *N. apis* in their queens because they did not supersede inoculated queens significantly more often than non-inoculated ones. Only one of the inoculated queens was superseded during 26 days of the experiment. It can be expected, however, that workers eventually would have superseded the queens if the experiment had not been ended. In Furgala's [7] experiments queens inoculated with 0.1×10^6 *N. apis* spores were all superseded. For this inoculation dose the average number of days before supersedure in the first trial was 16 and in the second trial was 28. Only in the first trial of Furgala's [7] experiment was the infection level of superseded queens similar to the infection level observed in the experiment presented here. It has been suggested that queens infected with *N. apis* delay the start of egg-laying [14]. This was not confirmed in this study; inoculated and non-inoculated queens started egg-laying at the same time.

To reduce the risk of infection of workers with *N. apis* it has been suggested to use one-day-old workers that emerged in incubators [10, 14]. The workers used in the present experiment emerged in isolation from the workers in the nest and were not infected with *N. apis* before the experiment. After the experiment only a small proportion of workers attending non-inoculated queens were infected. Probably some of the infected workers drifted from the mating nuclei with inoculated queens to mating nuclei with non-inoculated queens. Furgala [7] observed that workers inoculated with *N. apis* spores become heavily infected. It was observed also in the experiment presented here. In the mating nuclei with inoculated queens there were many more infected workers. This is probably because the workers ingested the queen's feces containing spores. Because the infected queen can live for a long time it can be a serious source of

N. apis spores in a colony. Despite this, the workers apparently did not perceive the presence of *N. apis* in their queens.

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Résumé – Influence de *Nosema apis* sur les jeunes reines d'abeilles et transmission de la maladie des reines aux ouvrières. Nous avons recherché si les jeunes reines d'abeilles (*Apis mellifera* L.) infestées par le protozoaire *Nosema apis* Zander étaient plus souvent remplacées que les saines. Nous avons examiné l'influence de *N. apis* sur les reines durant le vol de fécondation et au début de la ponte, ainsi que la façon dont l'infection est transmise des reines aux ouvrières accompagnatrices. Les reines ont été divisées en deux groupes, comportant chacun 20 reines : (i) les reines inoculées et (ii) les reines non inoculées. Les reines inoculées ont été nourries individuellement avec 10 µl de sirop à 50 % renfermant $0,1889 \times 10^6$ spores de *N. apis*. Toutes les reines ont été introduites dans des nuclei de fécondation avec des ouvrières âgées de huit jours au plus. Au bout de 26 jours, les reines et 15 ouvrières de chaque nucleus de fécondation ont été prélevées et la présence de *N. apis* a été vérifiée.

Les reines des deux groupes ont été toutes acceptées par les ouvrières. 47,4 % des reines inoculées et 50 % des reines non inoculées ont été perdues au cours des vols de fécondation (Fig. 1, différence non significative), principalement à cause du mauvais temps. Les reines inoculées ont commencé à pondre à l'âge de $15,77 \pm 1,64$ jours (moyenne \pm écart-type) et les non inoculées à $15,82 \pm 1,47$ jours. Les reines inoculées renfermaient entre $6,6 \times 10^6$ et $22,6 \times 10^6$ spores (moyenne $15,3 \pm 4,3$, Tab. I). Les reines non inoculées ne contenaient aucune spore.

Les ouvrières ont remplacé une reine inoculée et neuf reines non inoculées.

Aucune spore de *N. apis* n'a été trouvée chez les ouvrières utilisées pour constituer les nuclei de fécondation (Tab. I). Après l'expérimentation, des ouvrières infestées ont été trouvées dans tous les nuclei de fécondation avec reines inoculées, mais seulement dans 50 % des nuclei avec reines non inoculées. Un nombre significativement plus grand d'ouvrières a été infecté dans les nuclei avec reines inoculées (61,3 %) que dans les nuclei avec reines non inoculées (5,3 %). Le nombre de spores par ouvrière ne différait statistiquement pas d'un groupe à l'autre.

Les résultats suggèrent que les ouvrières ne perçoivent pas la présence de *N. apis* chez les reines, puisque la supersédure des reines inoculées n'est significativement pas plus élevée que celle des reines non inoculées. Le nombre beaucoup plus grand d'ouvrières infestées dans les nuclei avec reines inoculées est probablement dû au fait que les ouvrières ingèrent les spores avec les fécès des reines. Les reines infectées peuvent vivre longtemps et constituer pour la colonie une source importante de spores de *N. apis*.

***Apis mellifera* / *Nosema apis* / transmission reine-ouvrière / spore / supersédure / nucleus de fécondation**

Zusammenfassung – Wirkung von *Nosema apis* auf junge Königinnen und die Übertragung der Infektion von Königinnen auf Arbeiterinnen bei Honigbienen (*Apis mellifera* L.). Es wurde untersucht, ob junge, mit *Nosema apis* Zander infizierte Königinnen (*Apis mellifera*) häufiger umgeweiselt werden als gesunde. Die Auswirkung von *N. apis* auf Hochzeitsflüge und Beginn der Eilage, sowie die Ansteckung der Arbeiterinnen durch die Königin wurden überprüft. Dazu wurden Königinnen in zwei Gruppen mit je 20 Tieren eingeteilt, eine Gruppe wurde mit *N. apis* geimpft, die andere nicht. Für die Impfung

wurden die Königinnen individuell mit 10 µl 50 % Zuckerwasser gefüttert, das im Durchschnitt $0,1889 \times 10^6$ *N. apis* enthielt. Alle Königinnen wurden Begattungskästchen mit Arbeiterinnen, die nicht älter als 8 Tage waren, zugesetzt. Nach 26 Tagen wurden die Königinnen und je 15 Arbeiterinnen aus jedem Begattungskästchen genommen und der Infektionsgrad mit *N. apis* bestimmt.

Bei beiden Gruppen wurden alle Königinnen von den Arbeiterinnen angenommen. Während des Versuchs gingen 47,4 % der geimpften und 50 % der nicht geimpften Königinnen wegen ungünstiger Witterung auf dem Hochzeitsflug verloren (Abb. 1). Die geimpften Königinnen begannen im Durchschnitt im Alter von $15,77 \pm 1,64$ Tagen, die nicht geimpften $15,82 \pm 1,47$ Tagen mit der Eilage. Nach 26 Tagen enthielten die geimpften Königinnen im Durchschnitt $15,3 \pm 4,3$ Millionen Sporen, bei den nicht geimpften konnten keine Sporen nachgewiesen werden (Tab. I). Es wurde in dieser Zeit nur eine der geimpften und keine der nicht geimpften Königinnen durch die Arbeiterinnen umgeweiselt.

Am Beginn der Versuche wurden bei den Arbeiterinnen, mit denen die Begattungskästchen gefüllt wurden, keine *N. apis* Sporen gefunden (Tab. I). Nach dem Versuch enthielten alle Begattungskästchen mit geimpften Königinnen infizierte Arbeiterinnen. Bei den anderen waren es dagegen nur 50 %. Außerdem waren signifikant mehr Arbeiterinnen in den Kästchen mit geimpften Königinnen infiziert als in denen mit gesunden Königinnen (61,3 % bzw. 5,3 %, Tab. I). Die Anzahl der Sporen von infizierten Arbeiterinnen unterschied sich nicht signifikant zwischen den Gruppen.

Da geimpfte Königinnen nicht häufiger umgeweiselt wurden als gesunde, wird angenommen, dass die Arbeiterinnen die Infektion ihrer Königinnen mit *N. apis* nicht wahrnehmen. In den Begattungskästchen mit geimpften Königinnen wurden sehr viel mehr Arbeiterinnen infiziert als in der Gruppe mit gesunden Königinnen. Wahrscheinlich nehmen Arbeiterinnen mit dem

Kot der Königinnen Sporen auf. Demnach kann eine infizierte Königinnen eine längere Zeit im Volk überleben und dadurch zu einer bedeutenden Infektionsquelle für Sporen von *N. apis* bleiben.

***Apis mellifera* / *Nosema apis* / Übertragung Königinnen-Arbeiterinnen / Begattungskästchen**

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