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Original article

The effect of *Apis mellifera carnica* Polm worker bee source for populating mating nuclei on degree of infection by *Nosema apis* Zander

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Summary — The influence of worker bee source for populating mating nuclei on the degree of infection with *Nosema apis* Zander spores was examined. One-day-old bees were obtained by inserting brood combs into an incubator at 34–35 °C. Older worker bees were taken from the same donor colony. Bees from the incubator were free from *Nosema* infection. The average infection level in donor colonies was 26.5% infected bees. At the end of the experiments the *Nosema* infection level was significantly lower in mating nuclei which were populated with bees from the incubator. Out of 23 examined queens, 2 were *Nosema*-infected; both came from mating nuclei with young worker bees. No statistically significant difference in *Nosema* disease level was found between the 2 sizes of nuclei used, populated with 1/3 l and 3/4 l bees respectively.

***Nosema* disease / mating nucleus**

INTRODUCTION

Nosematosis is a protozoan disease of adult honeybees, *Apis mellifera* L in which a microsporidium, *Nosema apis* Zander, infects the midgut epithelial cells. Colonies of honeybees which are used for commercial production, especially in queen rearing apiaries, are manipulated more often and show a higher level of infection with *N apis* than unmanipulated colonies (Oertel, 1967; Taber and Lee, 1973). In queen rearing apiaries there are conditions favourable for causing honeybees and young queens in the mating nuclei to become infected with *N apis* spores (Shimanuki *et al,* 1973). In a normal colony, a laying queen is surrounded by healthy attendants (Wang and Moeller, 1970); thus, healthy queens can be found even in heavily infected colonies. In mating nuclei, however, the high proportion of infected bees significantly affects the risks of disease transmission, as does the fact that young queens feed themselves (Kauffeld, 1973).

To raise healthy queens, the contact of young queens with *N apis* spores in mat-

ing nuclei must be prevented. The present paper investigates the effect of populating the mating nuclei with young bees hatched in an incubator or with bees from a field colony on the subsequent level of infected worker bees and queens about 2 weeks after nuclei formation.

MATERIAL AND METHODS

On 10 July 1990 a group of small (Zander) and larger mating nuclei were stocked with newly emerged bees from an incubator and with young bees from bee colony. Virgin queens emerged in an incubator were added. Samples of worker bees and queens were taken 14 days later.

At the end of July and beginning of August, Zander's and a larger mating nuclei were stocked with emerged bees from an incubator and with young bees from bee colonies.

A total of 20 Zander's nuclei with 1-day-old bees and 9 with hive bees was produced. Five larger nuclei with 1-day-old bees and 5 with hive bees were also included in the experiment.

Before use, the mating nuclei were mechanically cleaned and disinfected with chlorine (Cl, 0.1%). Small Zander mating nuclei (interior dimensions 12 x 13 x 5.5 cm) were stocked with 1/3 l of bees, and bigger mating nuclei (14.5 x 11 x 16.5 cm) were stocked with 3/4 l of bees. Sugar honey patties were available in the nuclei throughout the experiment.

Samples of 10 worker bees were taken from each mating nucleus and 20 bees from the bee source colonies. The macerated alimentary canal of individual bees was microscopically examined at 600 x and the number of spores per infected bee calculated as described by Cantwell (1970).

Queens were removed from mating nuclei and confined to Petri dishes for a few minutes to defecate. The excrements were examined microscopically and the presence or absence of *N apis* spores was determined. The statistical evaluation was based on the method of least square mean (LSQ) (Harvey, 1987).

RESULTS AND DISCUSSION

Spores of *N apis* were not found in the 1-day-old bees hatched in an incubator. This result is in agreement with Bailey's (1963) findings. In the 3 experiments, 20, 18 and 3.5% of these bees were infected after 14, 18 and 13 days respectively. The average infection percentage was 11.2. Two possible sources of *N apis* spores for the incubator-hatched bees were the apiary environment and the comb with the sealed brood.

The average infection level in nuclei formed from colony bees was 19.5-fold higher compared to nuclei formed from incubator-hatched bees. This difference was significant ($P < 0.01$). The smallest difference was found in August when the weather permitted intensive flight from the nuclei. At that time, when sampling for terminal infection, the average percentages of infected bees were 11.2 ± 3.3 and $56.4 \pm 9.6\%$ for nuclei formed from incubator-hatched bees and from bees taken from a colony, respectively. Thus, establishing mating nuclei with bees hatched in an incubator significantly reduces the percentage of *Nosema*-infected bees ($P < 0.01$). These results are in agreement with data presented by Loskotova *et al* (1980). Of the 23 queens examined, 2 were found positive for *N apis* spores (table I). Unexpectedly, both these queens were from nuclei stocked with 1-day-old bees. Thus, our results also indicate that of infected bees in a nuclei, young queens easily become infected, irrespective of the source of bees.

The acceptance of virgin queens amounted to 84% in the nuclei with newly emerged bees and 50% in the nuclei with bees from a colony. The influence of *Nosema*-infected worker bees in nuclei on virgin queen acceptance was not determined.

Table I. *N apis* infection level in nuclei established from 1-day-old incubator-hatched bees or from bees collected from a bee colony.

Bee source	N	*Initial infection*	*Initial infection (%)*	*Terminal infection*	*Terminal infection (%)*	*Exam queens*	*Inf*
Incub	25	0	0	0.7 ± 0.6	11.2 ± 3.3	16	2
Colony	14	0.9 ± 0.4	20.4 ± 3.6	14.0 ± 4.4	56.4 ± 9.6	7	0

Source of bees, number of nuclei (*N*), average initial spore count x 10^5 (x ± SE), average initial percentage of infected bees, average source count x 10^5 13–18 days after nuclei formation (terminal infection, x ± SE), average percentage of infected bees 13–18 days after nuclei formation (percentage of terminal infection, x ± SE), number of examined queens, number of infected queens. Incub = incubator.

The effect of nuclei size or the quantity of stocking bees on the percentage of *Nosema*-infected worker bees or queens was not statistically significant ($P > 0.05$).

Résumé. — Influence de l'origine des ouvrières d'abeilles *(Apis mellifera carnica* Polm) constituant les nuclei de fécondation sur le taux d'infection par *Nosema apis* Zander. On a étudié l'influence de l'origine des ouvrières d'abeilles utilisées pour former des nuclei de fécondation sur le degré d'infection par les spores de *Nosema apis* Zander. Des ouvrières âgées d'un jour ont été obtenues en plaçant des rayons de couvain dans une étuve à 34–35 °C. Des ouvrières plus âgées ont été prélevées dans la même colonie donneuse. Les abeilles venant de l'étuve étaient indemnes de nosémose. Le taux moyen d'infection dans les colonies donneuses a été de 26,5%. À la fin de l'expérience, le niveau de nosémose était significativement plus bas dans les nuclei de fécondation qui avaient été peuplées avec des ouvrières provenant de l'étuve. Sur 23 reines examinées, 2 étaient atteintes de nosémose; curieusement elles provenaient toutes 2 de nuclei peuplés d'ouvrières d'un jour. On n'a trouvé aucune différence statistiquement significative dans le niveau de nosémose entre les nuclei peuplés avec 1/3 l d'abeilles et ceux peuplés avec 3/4 l.

nucleus de fécondation / nosémose

Zusammenfassung. — Nosemainfektion von Begattungsvölkchen und die Herkunft der Arbeitsbienen *(Apis mellifera carnica* Polm) bei ihrer Bildung. Es wurde der Einfluß der Herkunft der Arbeiterbienen für die Bildung von Begattungsvölkchen auf den Infektionsgrad mit Sporen von *Nosema apis* Zander untersucht. Eintägige Arbeiterinnen wurden durch Einstellen von Brutwaben in einen Brutschrank bei 34–35 °C gewonnen. Ältere Arbeiterinnen wurden aus demselben Spendervolk genommen. Die Bienen aus dem Brutschrank waren frei von Nosemainfektion. Der mittlere Infektionsgrad der Spendervölker war 26.5% infizierte Bienen. Am Ende des Versuchs war der Befallsgrad der Begattungsvölkchen, die aus Brutschrankbienen gebildet worden waren, signifikant niedriger. Von 23 untersuchten Königinnen waren zwei Nosemabefallen; beide stammten aus Völkchen aus eintägigen Arbeiterinnen. Zwischen Begattungsvölkchen, die aus 1/3 Liter und

aus 3/4 Liter Bienen gebildet worden waren, bestand kein signifikanter Unterschied im Grad des Nosemabefalls.

Begattungsvölkchen / *Nosema*

REFERENCES

Bailey L (1963) *Infectious Diseases of the Honey Bee.* Land Books Ltd, London

Cantwell GE (1970) Standard methods for counting *Nosema* spores. *Am Bee J* 110, 222-223

Harvey WR (1987) *User's Guide to LSMLMW. Mixed Model Least-Squares and Maximum Likehood Computer Program.* Ohio State Univ, OH

Kuffeld NM (1973) Queen honey bees in colonies: susceptibility to *Nosema* disease. *Am Bee J* 113, 12-14

Loskotova J, Peroutka M, Vesely V (1980) *Nosema* disease of honeybee queens (*Apis mellifica* L). *Apidologie* 11, 153-161

Oertel E (1967) Colony disturbance and *Nosema* disease. *J Apic Res* 6, 119-120

Shimanuki H, Lehnert T, Knox D (1973) Transmission of *Nosema* disease from infected honey bee workers to queens in mating nuclei. *J Econ Entomol* 66, 777-778

Taber S, Lee H (1973) Seasonal variations in levels of *Nosema* infection in honey bees in Arizona. *Am Bee J* 113, 281-282

Wang I, Moeller FE (1970) The division of labor and queen attendance behavior of *Nosema*-infected worker honey bees. *J Econ Entomol* 63, 1539-1541