THE EFFECT OF TEMPERATURE ON *NOSEMA APIS* ZANDER (MICROSPORIDA, NOSEMATIDAE) INFECTION IN HONEY BEES (*APIS MELLIFERA*)

WOYCIECHOWSKI M.* & CZEKOŃSKA K.*

Summary :

Newly emerged honey bee (*Apis mellifera carnica* L.) workers infected individually with *Nosema apis* Z. spores were divided into three groups and kept in incubators at 25°, 30° or 35°C. After 48 h all workers were kept at 30° C. The numbers of parasite spores in individual bees were counted in all groups on the 11th, 16th, 21st and 26th days of life. Generally higher numbers of spores were observed in workers infected at 25° C. However, the numbers in workers infected at extreme temperatures (25° and 35°C) differed significantly. Choosing suitable temperature conditions might be employed by infected bees to reduce the parasite's fitness and prolong their life spans.

KEY WORDS : *Apis mellifera, Nosema apis,* nosema disease.

Résumé : EFFET DE LA TEMPÉRATURE SUR L'INFECTION À *NOSEMA APIS* ZANDER (MICROSPORIDA, NOSEMATIDAE) CHEZ L'ABEILLE (*APIS MELLIFERA*)

*De jeunes abeilles ouvrières (*Apis mellifera carnica *L.) individuellement infectées par des spores de* Nosema apis *ont été divisées en trois groupes et placées en incubateur à 25°, 30° ou 35°C. Après 48 heures, toutes les ouvrières ont été placées à 30°C. Le nombre de spores du parasite chez chaque abeille a été compté dans chaque groupe au 11e, 16e, 21e et 26e jour de vie. Globalement, un nombre plus élevé de spores a été observé chez les ouvrières infectées à 25°C. Cependant, le nombre d'ouvrières infectées aux températures extrêmes (25° et 35°C) diffère de manière significative. Le choix de conditions thermiques adéquates pourrait être utilisé par les abeilles infectées pour réduire la charge parasitaire et prolonger leur durée de vie.*

MOTS CLÉS : Apis mellifera, Nosema apis, *microsporidiose à* Nosema.

Nosema apis Zander (Microsporida, Nosematidae) develops within the epithelial cells of the honey bee (*Apis mellifera* L.) midgut. It is a common parasite throughout the beekeeping world, which not only limits the life spans of workers but also deeply influences their behavior (Wang & Moeller, 1970; Malone *et al.*, 1995; Woyciechowski & Lomnicki, 1995; Woyciechowski & Kozlowski, 1998). There is much evidence for a significant relation between the parasite's development and temperature. Karmo & Morgenthaler (1939) suggest that *N. apis* finds optimal conditions for development between 30° and 34°C, that at 20-25°C their development is slower, and that below 10°C and above 37°C development is stopped. Lotmar (1943) confirms these results and points to the adaptation of *N. apis* to the narrow temperature interval (30-35°C) prevailing in the brood region of the bee nest (Büdel, 1960; Vogt & Heinrich, 1985). In some parts of the nest distant from the brood the temperature may even come close to ambient temperatures. This might be one reason why individuals have different levels of infection. Choosing suitable temperature conditions might reduce the parasite's fitness and prolong the lives of infected bees.

* Bee Research Department, Agricultural University, Al. 29 Listopada 52, 31-425 Kraków, Poland.
Correspondence: Michal Woyciechowski.

The present paper investigates the effects of different temperatures only during infection of honey bee workers with *N. apis* spores, while further parasite development takes place at the same temperature.

METHODS

The experiment was conducted in June 1997. Honey bee workers (*A. mellifera carnica*) were derived from a single inseminated queen. Workers emerging within 15 h in an incubator were individually infected with the same number of *N. apis* spores (486×10^3 spores per bee) dosed in 20 µL 50 % (w/v) sugar syrup. Each experimental group of 161 bees was placed in a wooden cage (15 × 14 × 6 cm) with mesh sides, provided with a small piece of bee comb. The cages were kept in incubators, each at one of three temperatures: 25°, 30° and 35°C. After 48 h all cages were kept at 30°C. An additional fourth cage with 161 uninfected workers (control) was kept at 30°C for the whole time of the experiment. During the experiment the cages were checked every day, the dead bees were removed and food and water were replenished in gravity feeders as necessary.

To estimate the number of *N. apis* spores in individual workers, on the 11th, 16th, 21st and 26th days after infection 30 bees were randomly chosen from each of

the cages after short treatment of each worker group with carbon dioxide. Only on the 26th day the number of remaining workers in the experimental groups was lower than 30 individuals (25°C – 15, 30°C – 12, and 35°C – 22 individuals). Altogether 439 workers were tested; their abdomens were macerated in 1 mL distilled water and the spores were counted in a haemacytometer in 0.025 mm^3 of suspension. If less than 10 spores were found in the sample they were recounted in 0.2 mm^3 of suspension. The results were given as the number of spores per 1 mL, which was treated as the number of spores per bee. To assess the significance of differences in spore numbers in infected workers at the various temperatures the Kruskal-Wallis test was used (Sokal & Rohlf, 1981).

RESULTS

There were significant differences in the numbers of *N. apis* spores between the experimental group on the 11, 21 and 26 days after infection (Fig. 1). No such a difference was found only for 16-day-old workers. These comparisons excluded the control group of uninfected workers, in which the percentage of infected individuals in all samples was not high (15.8 %) and the mean number of spores per individual did not markedly increase through time and equaled: 5,666, 2,333, 3,000 and 3,333 spores on the successive days. The biggest of these values was 1,160 times smaller than the lowest in the experimental groups (Fig. 1).

Differences in the levels of infection among the individuals in the experimental groups (Fig. 1) were due mainly to the different numbers of spores in individuals infected at 25° and 35°C. On three of the four sampling days this difference was significantly higher in individuals infected in lower temperatures (11th day: H = 7.481, n = 60, $p < 0.01$; 16th day: H = 0.079, n = 60, $p > 0.05$; 21st day: H = 8.311, n = 60, $p < 0.01$; 26th day: H = 8.819, n = 37, $p < 0.01$).

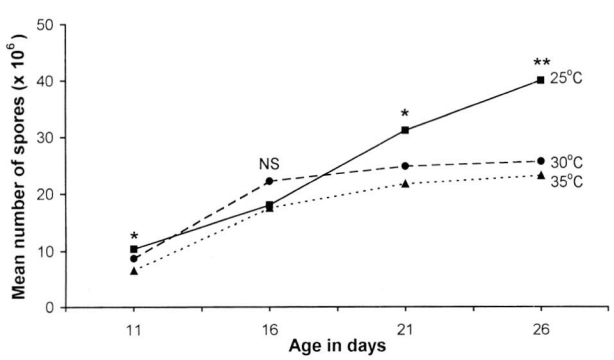

Fig. 1. – *N. apis* infection level in bees infected at various temperatures (11th day: H = 6.367, n = 90, $p < 0.05$; 16th day: H = 3.776, n = 90, $p > 0.05$; 21st day: H = 9.027, n = 90, $p < 0.05$; 26th day: H = 10.108, n = 49; * $p < 0.05$, ** $p < 0.01$, ns – $p > 0.05$).

Although on all the testing days the individuals infected at 35°C had the lowest numbers of spores, they never differed significantly from those infected at 30°C (11th day: H = 0.981, n = 60, $p > 0.05$; 16th day: H = 2.967, n = 60, $p > 0.05$; 21st day: H = 1.598, n = 60, $p > 0.05$; 26th day: H = 0.117, n = 34, $p > 0.05$). Individuals infected at 25° and 30°C differed significantly in the number of spores only on the 26th day of life (11th day: H = 1.181, n = 60, $p > 0.05$; 16th day: H = 2.597, n = 60, $p > 0.05$; 21st day: H = 3.581, n = 60, $p > 0.05$; 26th day: H = 5.952, n = 27, $p < 0.05$).

DISCUSSION

Many authors investigating the number of *N. apis* spores in the digestive system of honey bee workers have observed great variation in the levels of infection among individuals from the same colony (El-Shemy & Pickard, 1989; Woyciechowski *et al.*, 1996). Attention has been given to different genetic-dependent levels of resistance in individuals (Malone *et al.*, 1995) as well as to the significance of their age (El-Shemy & Pickard, 1989). The effects of various temperature conditions have also been observed, but only in cage experiments (Karmo & Morgenthaler, 1939; Lotmar, 1943). Approximately the same levels of infection have been found in infected bees irrespective of the spore infection dose (Fries, 1988).

Our experiment points to another factor that seems to have a significant influence on the parasite development. It is the temperature of the bees' environment during infection. Here the results suggest that the optimal temperature during infection differs from the optimal temperature for multiplication of the spores already present in the cells of the worker's digestive system. The level of infection in bees kept for 48 h at 25°C and fed *N. apis* spores was higher than in those kept at 35°C. This result is interesting because temperatures between 30°-35°C are considered optimal for development of *N. apis* (Karmo & Morgenthaler, 1939; Lotmar, 1943). It appeared that the results were not influenced by the low level of uncontrolled infection noted in the uninfected control group of workers. This conclusion confirms the fact that the biggest mean number of spores observed in control group was more than thousand times lower than the lowest value in any infected group of workers.

Without more experiments it is difficult to determine whether the differences we observed in the number of spores are the result of the host's reaction (for example, turnover of the epithelial cells of the midgut, as suggested by Lotmar in 1943) or the parasite's reac-

tion (for example, different possibilities of spore germination depending on temperature conditions or spore dimorphism observed by De Graaf *et al.*, 1994 during developmental cycle). Nor do we know whether bees can exploit temperature as a defense against parasite development, as do *Bombus terrestris* parasitized with conopid fly larvae (Müller & Schmid-Hempel, 1993). Observations that *N. apis* infected bees tend to congregate in the warmer parts of the hive (Moeller, 1956) support this supposition.

ACKNOWLEDGEMENTS

We thank Magda Góral for technical assistance with this project, and Stefan Fuchs, Michael Jacobs and an anonymous referee for helpful comments on the manuscript.

REFERENCES

BÜDEL A. Bienenphysik. *In* Büdel A., Herold E. (eds) *Biene und Bienenzucht,* Ehrenwirth; München, Germany, 1960, pp 115-170.

DE GRAAF D.C., RAES H. & JACOBS F.J. Spore dimorphism in *Nosema apis* (Microsporida, Nosematidae) developmental cycle. *Journal of Invertebrate Pathology,* 1994, *63*, 92-94.

EL-SHEMY A.A.M. & PICKARD R.S. *Nosema apis* Zander infection levels in honeybees of known age. *Journal of Apicultural Research,* 1989, *28*, 101-106.

FRIES I. Infectivity and multiplication of *Nosema apis* Z. in the ventriculus of the honey bee. *Apidologie,* 1988, *19*, 319-328.

KARMO E. & MORGENTHALER O. The development of *Nosema apis* at various temperatures. *Bee World*, 1939, *20*, 57-58.

LOTMAR R. Über den Einfluss der Temperatur auf den Parasiten *Nosema apis. Beihefte zur Schweizerichen Bienen-Zeitung*, 1943, *1*, 261-284.

MALONE L.A., GIACON H.A. & NEWTON N.R. Comparison of the responses of some New Zealand and Australian honey bees (*Apis mellifera* L.) to *Nosema apis* Z. *Apidologie*, 1995, *26*, 495-502.

MOLLER F.E. The behaviour of nosema-infected bees affecting their position in the winter cluster. *Journal of Economic Entomology*, 1956, *49*, 743-745.

MÜLLER C.B. & SCHMID-HEMPEL P. Exploitation of cold temperature as defence against parasitoids in bumblebees. *Nature*, 1993, *363*, 65-67.

SOKAL R.R. & ROHLF F.J. Biometry. W.H.Freemam and company, N. York, USA, 1981, 859 pp.

VOGT F.D. & HEINRICH B. The response of workers to thermal stress in honeybee colony thermoregulation. *American Zoologist*, 1985, *25*, 65.

WANG I. & MOELLER F.E. The division of labor and queen attendance behavior of nosema-infected worker honey bees. *Journal of Economic Entomology*, 1970, *63*, 1539-1541.

WOYCIECHOWSKI M. & LOMNICKI A. Honey bee workers parasitized by *Nosema apis* choose riskier foraging strategies. Pszczelnicze Zeszyty Naukowe, 1995, *39*, 209-210.

WOYCIECHOWSKI M., KOZLOWSKI J. & GRZANKA G. Life expectancy and risk taking in foraging honey bee workers. Proceedings XX International Congress of Entomology, Firenze, Italy, 1996, 394.

WOYCIECHOWSKI M. & KOZLOWSKII J. Division of labor by division of risk according to worker life expectancy in the honey bee (*Apis mellifera* L.). *Apidologie*, 1998, *29*, 191-205.

Reçu le 4 janvier 1999
Accepté le 11 mars 1999