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Effect of Nosema apis and N. ceranae on honey bee Apis mellifera queen development

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Nosema apis and N. ceranae are agents causing the disease called nosemosis in honey bee workers and queens. Few is known about the impacts of it on honey bee development. The royal jelly in queen cells was infected with *Nosema* spores to see whether and how it affects the development of honey bee queens. Seven groups of grafted honey bee larvae were established, and treated as follows: high and low concentrations of *N. ceranae* and *N. apis*, mixes of both species in both concentrations, and untreated control. After allowing nurse bees to fill the queen cells with royal jelly, an injection of 50 000 spores or 10 000 spores was added into the royal jelly. We found that only *N. apis* decreased the hatching rate of honey bee queens both in single and mixed treatment at high dosages, but we did not detect any morphological deviations in unhatched pupae.

Key words: honey bee *Apis mellifera*, *Nosema spp*., honey bee queen breeding, queen quality

Introduction

Honey bees (*Apis mellifera* L.) are suffering from high colony mortality rates in Europe [11]. There are several reasons for that and pathogens are considered as most important causes [3]. One of the most damaging diseases, nosemosis is caused by microsporids *N. apis Zander* [7] and *N. ceranae Fries* [8], which can also co-exist in the same colony.

Nosemosis has been under scientific interest for a long time whereas most of the work has been conducted on honey bee workers. The queens, however, can also be susceptible to nosemosis. *Nosema*-infected queens have been seen to produce a higher amount of queen mandibular pheromone, which refers to the poorer quality of the queen [1]. Nosemosis is often linked to changing vitellogenin titers in worker and

queen bees, although, the effects can be different [2]. Changes in vitellogenin amount affect the queen's hormonal balance, oxidative stress, and longevity [5]. Still, only a few studies consider the effects of nosemosis on honey bee queen development, and to our knowledge, there are none considering the developmental success due to manipulative infection. While nosemosis is detected not only the intestine but also hypopharyngeal glands of honey bees, the royal jelly produced by nurse bees can be infected with Nosema spp. spores [4].

The aim of this study was to explore i) whether *Nosema spp*. spores can be transferred from royal jelly to emerged queens and ii) whether the infection causes any morphological deviations.

Materials and Methods

The study was conducted in the summer of 2021. New queens (*A. mellifera ligustica*) were bred from one-day-old larvae originating from a single queen. Colonies used for nursing (royal jelly producing, cell building) were repeatedly checked for three weeks prior to the experiment to ensure that there was no infection present.

To obtain fresh *N. apis* and *N. ceranae* spores, infected honey bee colonies were used. From those, infected worker bees were collected and the spores were separated by homogenization. The *Nosema* species were identified by a multiplex PCR (M-PCR) assay [10]. Once the species was known, a centrifugation protocol [9] was used to purify the spores, which enabled about 85% purity. In order to separate the last precipitate, the resulting suspension was cleaned with a 10-micron filter. A flow cytometer (BD Accuri C6) was used to determine the number of spores [10].

The queen cell cups were made of organic bees wax (purchased from a local producer) to reduce the risk of pesticide contamination, which could affect the outcome of the experiment. The one-day-old larvae were grafted into the cups. A total of seven treatment groups were created with ten larvae for each: *N. apis* low (10000 spores), *N. apis* high (50.000 spores); *N. ceranae* low, *N. ceranae* high; Mix low, Mix high; Control.

After grafting the cell builder colony was checked regularly until the cups were sealed by worker bees. If a cup was sealed, an injection of 2 µL of suspension was added through the wax walls of the cup into the royal jelly (ddH_2O and nosemosis agents). Larvae were located, directing (a strong) light through the wax cell, and the suspension was injected underneath the larvae. On the tenth day after the grafting, queen cups were isolated (using Nicot's queen cages) and taken into an incubator (at 35°C and 65% humidity) until hatching.

Results and Discussion

The hatching rate of honey bee queens was affected by the treatment (**Fig. 1**). Interestingly, with *N. apis* high spore load treatment, the hatching rate was zero, while it was not affected by the same spore load of *N. ceranae*.. The dissection of sealed queen cells showed variable developmental deviations in this group (*N. apis* high), most of these queens had ended their development at the larval stage and didn't reach the metamorphosis. Only a couple of individuals started the pupation but stopped suddenly before finishing it as shown in **Fig. 2**. Similarly, a low hatching rate was observed in the mixed high concentration group. We suggest that *N. apis* caused this.

The hatching rate of other treatment groups was relatively normal. Usually, beekeepers aim to achieve a hatching rate of over 80% when breeding the queens. *N. apis* has been considered problematic for honey bees during the early season [7], but our study reveals yet another threat – honey bee breeding success can be seriously harmed when the nursing colonies are infected and not checked for this disease. There are no clear symptoms of nosemosis and only laboratory analyses of bee samples allow a correct diagnosis of this disease.

Some papers indicate that nosemosis agents are able to transfer through the metamorphosis process in worker bees [2, 6, 12], although, we could not confirm this in queen bees – we did not detect any infection from the adult newborn queens.

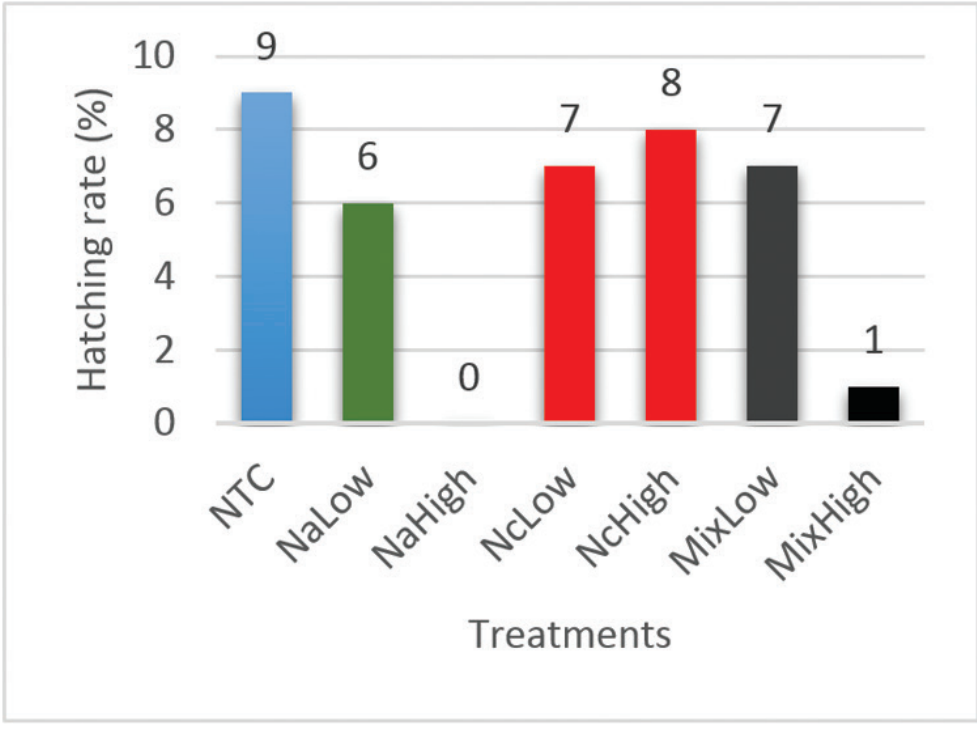

Fig. 1. The hatching rate of the treatment groups. NTC-no treatment control. Na –*N. apis*, Nc –*N. ceranae*, Mix – Na + Nc, low – 10 000 spores, high – 50 000 spores injected intoroyal jelly in queen cells.

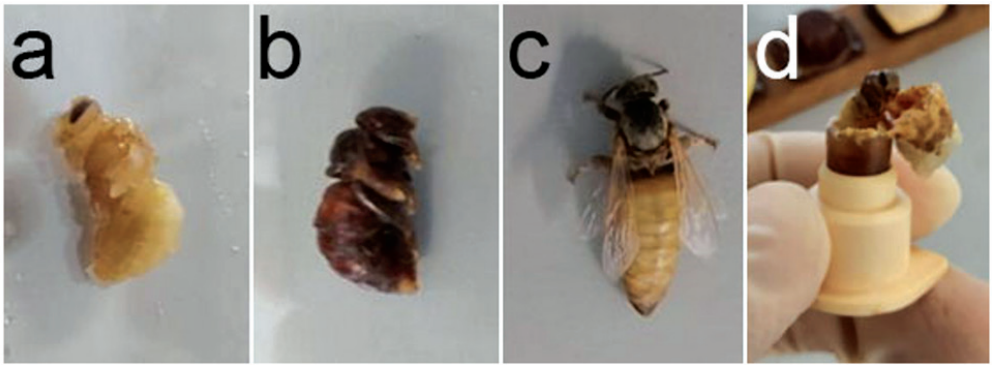

Fig. 2. High doses of *N. apis* both as pure treatment (a and b) or in mix with *N. ceranae* (c and d) caused disruption of metamorphosis at different developmental timepoints (Photos by S. Naudi)

Conclusions

Our study showed that royal jelly contamination with high concentrations of *N. apis* can cause honey bee queen mortality during breeding. This first study on *Nosema* affecting queen development through contamination of royal jelly is indicating the need for more targeted research on this topic and is really important for the honey bee queen breeding system.

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