UDC 638.153.3:638.15-092
DOI: 10.15587/2519-8025.2022.255735

FEATURES OF MICROSPORIDIA *NOSEMA APIS* AND *NOSEMA CERANAE* (*NOSEMA* SPECIES) DEVELOPMENT OF WINTER BEE (*APIS MELLIFERA L.*) GENERATION

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Temperature is one of the main abiotic factors affecting the development of causative agents of nosemosis in the bee's body.
The aim of the research. *To determine the influence of the winter and summer bee nest temperature (20–22 °C and 35–36 °C, respectively) on the duration of Nosema species development of winter bee generation isolated in hoarding cages, and to trace the life cycle of Nosema species of bees with natural infection and overwintering in natural conditions, from November to the beginning of bee brood rearing (February).*
Materials and methods. *For this, 200 bees Apis mellifera sossimai, selected from bee colony in November, were infected by syrup feeding containing Nosema species (5×10^4 spores per bee). Half of the bees were kept at 35–36 °C and half at 20–22 °C. The stages of Nosema species development were monitored daily for 13 days in midgut smears, stained according to Romanovsky-Giemsa (magnification 900x). Furthermore, with a 15 days frequency, from November to mid-February, 30 bees were selected from 20 bee colonies overwintered in natural conditions, and monitored the stages of Nosema species development at natural infection.*
Result. *It was found that the Nosema species development of winter bee generation artificially infected by Nosema species, was suspended at meronts and sporonts stages until the 13th day from the moment of infection, regardless of the temperature at which the bees were kept in the experiment. In bees selected from bee colonies naturally infected with Nosema species prevailed meronts I, II and in an insignificant amount sporonts, until the end of December, active sporulation took place from the middle of January to the beginning of February.*
Conclusion. *That is, the duration of the life cycle development of Nosema species depends little on temperature but is closely related to the life span of summer and winter bee generation and determined by the biochemistry of their relationships, which allow the parasite to save the host as its habitat*
Keywords: *Nosema, temperature, winter bee generation, life cycle duration, host-parasite relationship*

How to cite:
Odnosum, H., Yefimenko, T. (2022). Features of microsporidia *Nosema apis* and *Nosema ceranae* (*Nosema* species) development of winter bee (*Apis mellifera L.*) generation. ScienceRise: Biological Science, 1 (30), 53–56. doi: http://doi.org/10.15587/2519-8025.2022.255735

1. Introduction

Insect epizootics are often caused by microsporidiosis – the diseases caused by obligate intracellular parasites. Negative economic value for the beekeeping industry has two species of microsporidia, Nosema apis and Nosema ceranae (Nosema species), causative agents of nosemosis in bees (Apis mellifera L.). They are widespread everywhere in places of intensive beekeeping, not only in Ukraine, but also throughout the world.

Differentiation of these two species of parasites became possible with the development of molecular genetic analysis for diagnostics [1–4]. N. ceranae in many countries, including Ukraine [5, 6], predominates in distribution over N. apis already in most apiaries, but more often they are found together [7, 8], for example in Canada and central USA [9], in the Balkan countries [10], in Russia [11–13], in Japan [14], across Scotland [15], in temperate and subtropical ecoregions of Argentina [16], in Turkey [17]. Nosemosis exacerbation is one of the main causes of periodic mass death of bees in the world [18–20].

Late treatment of bees from the parasitic mite Varroa destructor, the presence in honey of sublethal concentrations of pesticides, honeydew, bee's exhaustion by late processing of sucrose syrup with a complete replacement of natural food, the presence of pathogens of other diseases in the bee's body, etc., promotes nosemosis activation [21, 22].

The presence of Nosema species in the bee's body is not always directly related to their mass death; parasites could weaken bees to the point where they are unable to resist other diseases. In particular, the parasitism of Nosema species activates exacerbation of latent viral infections in adult and larval honeybees, and most often – bee paralysis virus and sacbrood virus.

Considering the variety of host-parasite relationships – from mutualism (mutually beneficial partnerships) during the reproduction of microsporidia, to antagonism – at the stage of sporogony and sporogenesis [23, 24], as well as the fact that nosemosis exacerbation is mainly observed in late winter-early spring – during the period of high energy losses related to the beginning of brood rearing in bee colonies (in our climatic zone this is January-March), we tried to come to an understanding of the factors responsible for the pathogenesis of microsporidiosis of winter bee generation.

It is known that the life cycle duration of N. apis in summer bee generations depends on the temperature and can be completed in 48 hours. The development of microsporidia N. apis and the spore formation at temperatures below 10 °C and above 37 °C is suspended. That is, the temperatures that are optimal for the host are optimal for the parasite: 30–35 °C according to J. Weiser (1972) [25], 31 °C according to O. F. Grobov et. al. (1987) [19].

Considering that temperature is one of the main abiotic factors affecting the development of causative agents of nosemosis in the bee's body, the aim of our study was to determine the influence of the winter and summer bee nest temperature on the duration of Nosema species development of winter bee generation isolated in hoarding cages and to trace the life cycle of Nosema species of bees naturally infected and overwintering in natural conditions to the beginning of their bee brood rearing (February).

2. Materials and methods

The experiments were performed in 2020–2021 on the honeybee *Apis mellifera sossimai* at the NSC "Institute of Beekeeping named after P. I. Prokopovich" in the Laboratory of technological and special preventive measures of bee diseases, which consisted of 25 bee colonies for the period of the experiment.

To find out how temperature effects on the *Nosema* species development in winter bee generation, we modeled conditions of a summer and winter bee nest temperature (35–36 °C and 20–22 °C, respectively) in laboratory experiments for bees selected in hoarding cages at the end of November. For this, 400 bees were selected from the bee colony and some (200 pcs.) were infected by 50 % sucrose syrup feeding containing spores of *Nosema* species at the rate of 5×10^4 spores per bee. Half of the bees (100 pcs.) were kept in a thermostat at 35–36 °C and the other half (100 pcs.) in a thermostat at 20–22 °C. At the same temperatures, uninfected bees (control) were kept in the same amount (50 pcs. per cage).

For bee's infection in laboratory conditions spores of *Nosema* species were isolated from the bee corpses that had a high infection level with two species of microsporidia (*Nosema apis* and *Nosema ceranae*) in approximately equal proportions. For this, abdomens from infected by *Nosema* species bees were separated and homogenized with distilled water. The homogenate was filtered. The filtrate was purified from associated microflora by centrifugation at 2000 rpm for 20 minutes. The supernatant was decanted. The sediment was resuspended with distilled water. The procedure was repeated three times. Then, the titer of spores was calculated in the purified suspension using a Goryaev chamber [26]. The required spore concentration was obtained by dilution method. Bees were infected in a group manner.

The stages of parasite development were monitored daily in artificially infected bees for 13 days in midgut smears, stained according to Romanovsky-Giemsa, using light microscopy (at a magnification of 900x) [27].

The development cycle of *Nosema* species were also monitored in winter bee generation naturally infected mainly with a low and high level of infection by *Nosema* species, which overwintered in hives in natural conditions. For this, with a 15 day frequency, from November to mid-February – the beginning of brood rearing, 30 bees were selected from 20 bee colonies and monitored the stages of parasite development using light microscopy.

All experiments on bees were conducted in accordance with Directive 2010/63/EU as amended by Regulation (EU) 2019/1010.

3. Results

Determination of the influence of winter and summer bee nest temperature (20-22 °C and 35–36 °C, respectively) on the duration of the development cycle of the parasite in winter bee generation, infected with *Nosema* species at the rate of 5×10^4 spores per bee, showed that temperature is not a determining factor in the development of parasites. In the hindgut and midgut of bees, after 48 hours from the moment of infection, at 20–22 °C temperature, first-generation meronts (meronts-I) rarely shot spores and sporoplasm were mainly observed. At 35–36 °C currently, along with meronts-I, there were second-generation meronts (meronts-II). Thus, in the early stages of *Nosema* species life cycle the temperature accelerated the passage of their stages. Subsequently, the temperature did not affect the rate of parasites development. This could be seen from the fact that on the 7th day after infection in bees of both variants (at the summer and winter nest temperature – 35–36 °C and 20–22 °C, respectively) *Nosema* species was detected at the stages of meronts-II and sporonts, that is, the development of microsporidia was suspended. Regardless of the temperature conditions, spores were not formed in both variants within 13 days from the moment of bee's infection (the period over which laboratory observations were conducted) (Fig. 1).

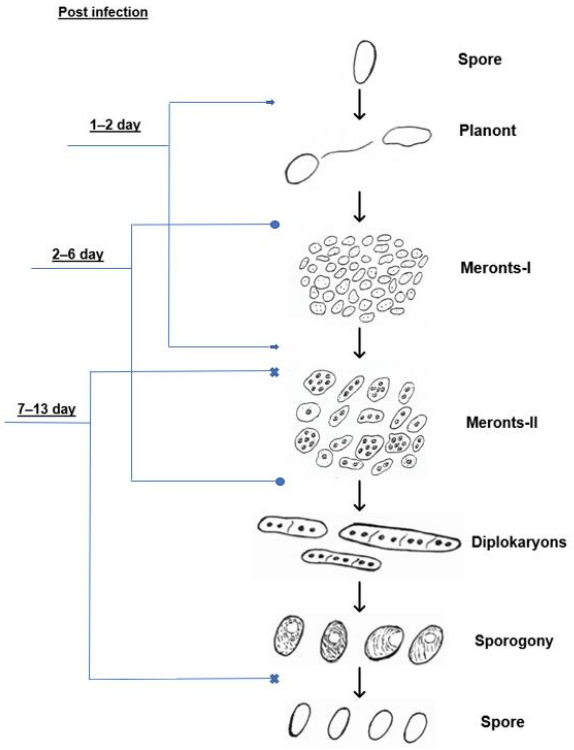

Fig. 1. *Nosema* species development cycle in winter bee generation at 20–22 °C, 35–36 °C in November – December (stages according to light microscopy data)

Parallel microscopic analysis of bees from colonies (30 pcs. from 20 colonies every 15 days), overwintered in hives in natural conditions, showed that vegetative stages of reproduction – meronts-I and II, and sporonts in a small amount, prevailed over other stages of *Nosema* species development in the bee guts until the end of December, while intensive sporulation took place from mid-January to early February.

That is, the obtained data allow us to conclude that the duration of the *Nosema* species development cycle little depends on temperature but is closely related to the lifespan of winter bee generation.

4. Discussion

Considering that the development of *N. apis* in summer bee generations at summer bee nest temperatures (30–35 °C) is completed in 3–5 days [19, 25, 28], and in winter bee generations at the same temperatures the development cycle of *Nosema* species is suspended mainly at the stages of merogony, while active sporulation (according to microscopic analysis of bees taken from colonies) begins only from mid-January to early February, we consider that winter bee generation have mechanisms that inhibit and trigger the sporulation of the parasite. This could be explained by the different lifespan of summer and winter bee generations. It is known that the lifespan of summer bee generations at least three to five times shorter (depending on their physiological state, which is determined by vital functions in the colony, quality of food, exhaustion by the parasitic mite *Varroa destructor* etc.) compared to winter bee generation, which determines the biochemistry of host-parasite relationship between bees and *Nosema* species.

It is pertinent to note that the duration of the development cycle of *Nosema* species differs even among bees of the same generation, depending on their age. Some authors consider that summer bee generations do not become infected until the 12th day from the moment of hatching [18], while other researchers succeeded to infect newly hatched bees with single spores of the parasite [29]. Our data indicate that infection occurs (3 and 8-day old bees were infected), but the development of *Nosema* species slows down and sporulation begins only on the 17th and 12th day from the moment of infection, respectively [30, 31].

Suspension of microsporidia development is known in the overwintering stages of many insects from the *Lepidoptera* and *Diptera* orders [24]. Parasite spores are not detected in the eggs of many butterflies through sexual transmission (except for oak silkworm eggs, which spore formation begins in spring) [32].

Thus, to date there is no clear understanding of the mechanisms regulating the insect's microsporidia development. The biochemistry of the intimate relationships between insects and microsporidia is poorly studied. Perhaps the above facts are associated with the hormonal balance of the insects themselves, as well as with the presence of an analog of the juvenile hormone in microsporidia [33]. Furthermore, lysozyme, humoral immunity factor of insects, was found in microsporidia, including *N. apis*. The discovered enzyme is associated both with the spore itself and with its internal contents [34].

Therefore, these data indicate that such coexistence mechanisms have developed between insects and microsporidia that allow the parasite to preserve the host as its habitat at least until they complete their development cycle [35]. However, even in the most stable "host-parasite" systems, partnerships are built on the principle of an unstable equilibrium, the violation of which leads to the disintegration of the system and the death of one of the partners [36]. The last is confirmed by the constant presence of *Nosema* species in bee colonies and only a seasonal manifestation of the disease. The parasite, deprived of its own mitochondria and using the energy of the host, is dangerous in the spring for overwintered bees, which expended enormous energy to maintain an optimal microclimate in the colony in winter and brood feeding in spring [37].

Prospects for further research. The prospect for further research is to continue the study of the features of the parasite-host relationship at different levels of organization of the parasite and the host.

5. Conclusions

Based on the obtained data, it is possible to propose recovery approaches for bees at nosemosis. Considering that microsporidia spores are more resistant to environmental factors and medicines than the vegetative reproduction stages, preventive bee health improvement (for the prevention of spring nosemosis exacerbation) is advisable to provide primarily in autumn (when parasite vegetative reproduction stages prevail), and therapeutic, if necessary, in the spring (during the sporulation of the parasite).

Conflict of interests

The authors declare that they have no conflicts of interest.

Financing

The study was performed without financial support.

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Received date 20.01.2022
Accepted date 17.02.2022
Published date 31.03.2022

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