ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 10: 1625–1645 (2024)
doi: 10.5281/zenodo.14356492
http://journal.asu.ru

RESEARCH ARTICLE

Horizontal and vertical transmission of microsporidia *Nosema pyrausta* and *Nosema bombycis* in the predatory bug *Podisus maculiventris* (Hemiptera: Pentatomidae)

Arina S. Rumiantseva[1], Anastasia N. Ignatieva[1], Inna V. Grushevaya[1], Alsu M. Utkuzova[1], Nina V. Binitskaya[1], Anastasia G. Kononchuk[1], Ekaterina G. Kozlova[1], Anastasia A. Khodzhash[1], Yuri S. Tokarev[1]

1 *All-Russian Institute of Plant Protection, 3 Podbelskogo, St. Petersburg, 196608, Russia*

Corresponding author: Inna V. Grushevaya (grushevaya_12@mail.ru)

Academic editor: R. Yakovlev | Received 12 October 2024 | Accepted 30 October 2024 | Published 13 December 2024

http://zoobank.org/F7711A5A-8926-4AE5-BDC2-8FEF585E5364

Citation: Rumiantseva AS, Ignatieva AN, Grushevaya IV, Utkuzova AM, Binitskaya NV, Kononchuk AG, Kozlova EG, Khodzhash AA, Tokarev YuS (2024) Horizontal and vertical transmission of microsporidia *Nosema pyrausta* and *Nosema bombycis* in the predatory bug *Podisus maculiventris* (Hemiptera: Pentatomidae). Acta Biologica Sibirica 10: 1625–1645. https://doi.org/10.5281/zenodo.14356492

Abstract

Predatory insects play an important role in the regulation of arthropod's numbers. When they counteract with the diseased prey, the entomopathogenic microorganisms may be disseminated mechanically or parasitize such secondary hosts. Microsporidia are wide-spread pathogens of insects with diverse host ranges, and infection of entomophagous hosts is not uncommon. In the present study, the spined soldier bug nymphs were fed with the adzuki bean borer larvae infected with *Nosema pyrausta* (A. Paillot) J. Weiser, 1961 or the silkworm larvae infected with *N. bombycis* Nägeli, 1857. Both pathogens were infective to the predator at the prevalence level of 15-30%. The former pathogen displayed a decrease of prevalence level in the filial generation to as low as 5 % and no infection in consequent generations. The latter could only be transmitted to bugs horizontally and no infections in the filial generation was found. This indicates low (or no) risk of vertical transmission of these two pathogens, making them suitable for combined application with the predatory bug in the integrated pest management programmes.

Keywords
Parasite-host interactions, infection persistence, insect pathogens, biocontrol, integrated pest management

Introduction

Pest management is important for agriculture as well as human health (Kevan et al. 2020). Extensive application of synthetic pesticides leads to environmental pollution and food contamination (Geiger et al. 2010; Tang et al. 2021; Dwivedi et al. 2022; de Azeredo Morgado et al. 2023), as well as the development of pest resistance which requires increased application rates, active compound rotation, novel delivery approaches etc. (Cross 2013; Biddinger et al. 2014; Godfray and Garnett 2014; Sparks and Nauen 2015; Kudsk et al. 2018; Möhring 2020). Even though modern chemical pest control means can be safe when all application regulations are respected (Dolzhenko and Laptiev 2021), the insecticides might be applied under suboptimal conditions. Examples of such situations include unsuitable abiotic factors (Amarasekare and Edelson 2004; Sreelakshmi 2021) and inappropriate developmental stages of the pests (Vivan et al. 2017; Stejskal et al. 2021), forcing the farmers to increase dosage and frequency of insecticide application remarkably. For these reasons, alternative means of pest control, including natural enemies, are of great importance. Entomopathogenic microorganisms are able to persist in pest populations, causing either acute infections that provide relatively quick death, or chronic diseases that affect the viability of pests with a transgenerational effect (Lewis et al. 2006; Solter et al. 2012; Litwin et al. 2020). Entomophagous arthropods are a significant component of terrestrial ecosystems. The use of predatory insects of various orders as biological means of plant protection plays an important role regulating arthropod's numbers (de Castro et al. 2015; Lin et al. 2021).

Predatory insects can act as mechanical carriers of insect pathogens. A lot of works is devoted to the study of the ability of predatory insects to spread nuclear polyhedrosis viruses through the excretion of infective particles with feces. Insect pests swallow occlusion bodies when eating infected plant parts. Predatory insects *Hippodamia convergens* Guérin-Méneville, 1842 (Pell and Vandenberg 2002), *Orius laevigatus* (Fieber, 1860) (Down et al. 2009), *Oechalia schellenbergii* (Guérin-Méneville, 1831) (Cooper 1981), *Podisus maculiventris* (Say, 1832) (Biever et al. 1982; Abbas and Boucias 1984), *P. nigrispinus* (Dallas, 1851) (Carvalho et al. 2012), *Oecanthecona furcellata* (Wolff, 1811) (Gupta et al. 2013) are considered the most effective in the spread of entomopathogenic fungi, bacteria, and viruses.

Non-target predatory insect species are often used as a targeted spread of entomopathogens directly in the phytophagous population – spread of the fungus *Pandora neoaphidis* (Remaud. & Hennebert) Humber, 1989 by the seven-spotted ladybeetle *Coccinella septempunctata* Linnaeus, 1758 entomopathogenic for *Acyrthosiphon pisum* (Harris, 1776) and *Sitobion avenae* (Fabricius, 1775) (Roy et

al. 2001), distribution conidium *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora, 2017 by the ladybeetle *H. convergens* to control the Russian wheat aphid *Diuraphis noxia* (Mordvilko, 1913) (Pell and Vandenberg 2002), transfer of the entomopathogen *Lecanicillium longisporum* (Petch) Zare & Gams, 2001 from the common ant *Lasius niger* (Linnaeus, 1758) to rosy apple aphid *Dysaphis plantaginea* (Passerini, 1860) (Bird et al. 2004), the use of predatory insects *Harmonia axyridis* (Pallas, 1773) and *Chrysoperla carnea* (Stephens, 1836) in the distribution of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin s.l., 1912 to control green peach aphid *Myzus persicae* (Sulzer, 1776) (Zhu and Kim 2012).

However, the widespread use of entomopathogens complicates the system of interactions and leads to unpredictable effects that require special studies (Haddi et al. 2020; Mansour and Biondi 2021). The assessment of possible negative effects of entomopathogens on predators is of main importance in these studies, since their antagonism impairs the effectiveness of integrated plant protection (Sedaratian et al. 2014). From the predator's perspective, many pathogens and parasites reduce the energy value of prey, reducing predator survival and reproduction (Thieltges et al. 2013; Hatcher et al. 2014; Flick et al. 2016). So, the predatory bug *P. nigrispinus* unable to live more than three generations by consuming only virus-infected of the nuclear polyhedrosis virus (NPV) of the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, 1818 (AgNPV) (de Nardo et al. 2001). Knowledge about the impact of various groups of entomopathogens on predatory insects needs to be expanded (Sedaratian-Jahromi 2021).

The spined soldier bug *P. maculiventris* is used as an effective biological pest control agent. For example, *P. maculiventris* is effective against *Leptinotarsa decemlineata* (Say, 1824) (Hare 1990) and *Pieris brassicae* (Linnaeus, 1758) (Stamopoulos and Chloridis 1994; Aldrich and Cantelo 1999), *Heliothis virescens* Fabricius, 1777 and *H. zea* (Boddie, 1850) (López et al. 1976), *Spodoptera exigua* (Hübner, 1808) (De Clercq and Degheele 1994), *Pyrrhalta viburni* (Paykull, 1799) (Desurmont and Weston 2008), and many others. So, in addition to direct predation, the *P. maculiventris* is also capable of spreading such entomopathogens as *Lacanobia oleracea granulovirus* (LoGV) and *Microsporidium necatrix* (J.P. Kramer) V. Sprague, 1977 microsporidia, infecting *Lacanobia oleracea* (L., 1758) and *S. littoralis* (Boisduval, 1833). However, the lifespan and egg production of *P. maculiventris* are significantly reduced after ingestion of caterpillars infected with the microsporidium *M. necatrix* (Down et al. 2004). When selecting infected *B. bassiana* compared to uninfected cutworm larvae *S. frugiperda* J.E. Smith, 1797 nymphs of predatory bugs more often chose uninfected individuals and avoided infection with an entomopathogen (Avery et al. 2022). However, *P. maculiventris* is not always effective and it is possible to consider methods of its effectiveness as a biological control agent (De Clercq 2000).

Predatory insects, when spreading pathogens such as microsporidia in phytophagous prey's populations, contribute to the persistence of pathogens and further reduce the number of pests. The ability for many species of microsporidia to infect

insects of various families and orders is described (Tokarev et al. 2016; Malysh et al. 2018). This is especially pronounced in close and constant contacts, which are observed, in particular, in parasitic insects that infect Coleoptera (Saito and Bjørnson 2013), Diptera (Futerman et al. 2006), Hymenoptera (Schuld 1999) and Lepidoptera (Simões 2015). Microsporidia spores are spread both from an infected host to a susceptible host, with faeces, through decomposing tissues, through cannibalistic feeding (horizontal transmission) (Brooks 1988; Becnel and Andreadis 1999; Campbell et al. 2007; Saito and Bjørnson 2006; Goertz and Hoch 2008; Goertz and Hoch 2011; Wang-Peng et al. 2018) and from parental to filial generation (vertical transmission) (Becnel and Andreadis 1999; van Frankenhuyzen et al. 2007; Han and Watanabe 1998; Grushevaya et al. 2021). So, Wang-Peng et al. (2018) found that healthy individuals of the migratory locust *Locusta migratoria* (Linnaeus, 1758) can become infected with *Antonospora locustae* (E.U. Canning) C.H. Slamovits, B.A.P. Williams & P.J. Keeling, 2004 microsporidia by eating food contaminated with the predatory insect's feces. Moreover, microsporidia *A. locustae* is able to survive among migratory locusts' populations for several years. Predators of migratory locusts are able to acquire large numbers of spores while consuming the infected specimens. Marti & Hamm (1985) showed that spores of an unidentified *Vairimorpha* sp. remained intact in the intestines of the big-eyed bug *Geocoris punctipes* (Say, 1832) after ingesting infected tissues from *S. frugiperda* larvae. It has been determined that *G. punctipes* can be a potential vector for spreading pathogenic spores of *Vairimorpha* sp. Goertz and Hoch studied the influence of the forest caterpillar hunter *Calosoma sycophanta* (Linnaeus, 1758) on the interaction between its prey *Lymantria dispar* Linnaeus, 1758 and two microsporidia species *Vairimorpha lymantriae* (J. Weiser) Kunimi, 1993 and *V. disparis* Vávra J., Hyliš M., Vossbrinck C.R., Pilarska D.K., Linde A., Weiser J., McManus M.L., Hoch G. & Solter L.F., 2006 that infect the phytophagous insect. *V. lymantriae* and *V. disparis* spores were spread by predatory insects by consuming the infected caterpillars. When spreading spores by *C. sycophanta*, 45% to 69% of the caterpillars tested were infected (Goertz and Hoch 2013). Spores of microsporidia *V. necatrix* and *Pleistophora* sp. (extracted from *S. frugiperda* and the *Pleistophora* sp. from *Dasychira basiflava* Packard, 1864, respectively) are able to remain infective after passing through the digestive tract of the bug *Zelus exsanguis* Stål, 1862. However, none of the tested bugs was infected with microsporidia (Kaya 1979).

The present work aimed to study the possibility of microsporidia commonly found in lepidopteran hosts to infect a predatory bug and to persist in a row of subsequent generations under lab conditions. Our main hypothesis is that the broad host range of microsporidia of the genus *Nosema* allows for successful infection of a predator belonging to another insect order, such as the spined soldier bug *P. maculiventris*, feeding on heavily infected prey (horizontal transmission). Another question under investigation is whether this infection can persist through the generations of the new host (vertical transmission).

Materials and methods

Insect cultures and their pathogens

The adzuki bean borer and *Nosema pyrausta*

Diapausing last instar larvae of the adzuki bean borer *Ostrinia scapulalis* (Walker, 1859) were collected from the stalks of the common cocklebur of *Xanthium strumarium* L., 1753 in Slavyansky District of Krasnodar Area in April 2021 and were transported to St. Petersburg. The whole life cycle was maintained at +24 °C using a meridic diet for larval feeding (Frolov et al. 2019).

The culture of the adzuki bean borer was maintained in the same facility and manipulated by the same operator as the European corn borer *O. nubilalis* Hübner, 1796 culture. The latter originated from a field population described elsewhere (Grushevaya et al. 2021) which turned out to be naturally infected with the microsporidium *N. pyrausta* (Paillot) Weiser, 1961. As a result, the *O. scapulalis* culture became occasionally contaminated with the microsporidium and the next generation of the insect was subsequently infected at the rate of about 30 %. Thus, the *N. pyrausta*-infected larvae were utilized for feeding and respective infection of *P. maculiventris* (see below).

The silkworm and *Nosema bombycis*

The silkworm eggs were acquired from the Stavropol sericulture station (Pyatigorsk) and propagated in the laboratory. The mulberry leaves grown at the facilities of All-Russian Institute of Plant Protection were used to feed the larvae *Bombyx mori* (Linnaeus, 1758). The *N. bombycis* Nägeli, 1857 spores were obtained from the Scientific Research Institute of Sericulture (Tashkent, Uzbekistan) and kept as a refrigerated water suspension for one month prior to the experiments. The experimental infection was performed by feeding *N. bombycis* spores to the third instar *B. mori* larvae at the dosage of 10^4 spores/larva, spore suspension smeared against the mulberry leaflet fed to the insects. After complete consumption of the infective dosage, the larvae were maintained at the same conditions for three weeks, fed with fresh mulberry leaves. The presumably infected larvae, as well as the control ones, were exposed to *P. maculiventris* (see below).

The spined soldier bug

The spined soldier bug is maintained in a permanent laboratory culture at the facilities of All-Russian Institute of Plant Protection in a separate building where experimental handling of the pathogens is excluded. The last instar larvae and newly emerged pupae of the mealworm *Tenebrio molitor* Linnaeus, 1758 were used as the feed source for nymphs and adults (De Clercq et al. 1998).

Experimental infection of the spined soldier bug

Horizontal transmission test

Either fifth instar larvae of *O. scapulalis* or third instar larvae of *B. mori* were placed individually in glass Petri dishes, 20 cm in diameter. As many as five one-day old feed-deprived second instar nymphs of *P. maculiventris* were placed per dish. The bugs attacked the larvae and collective feeding of nymphs was observed (Fig. 1). After 48 hours, the cadavers of prey larvae were removed for analysis (see below, section 2.3). A moistened piece of cotton and fresh feed insects as above were provided to the bugs for the rest of the experiment. The bugs were reared until molting to the adult stage and used for vertical transmission test (section 2.2.1). To test for horizontal transmission, presence of microsporidia was examined in larval and adult cadavers (section 2.3).

Vertical transmission test

After molting to adult, the pairs of bugs (one male and one female) obtained within each variant, referred to as parental generation (P_0), were placed into separate glass Petri dishes for mating and oviposition. For vertical transmission test, their offspring, referred to as filial generation (F_1) was routinely maintained in glass Petri dishes until the adult stage. Then the new adult bug pairs were formed to obtain the consequent second filial generation (F_2) in the variants where the microsporidian infection tended to persist. Those bugs initially reared on microsporidia-free larvae of both *O. scapulalis* and *B. mori* (in the assays using *N. pyrausta* and *N. bombycis*, respectively) were utilized as the control in the parental generation, as well as in consequent filial generations to ensure absence of cross-contamination during the whole experiment.

Diagnostics of microsporidia in insects

Prior to the experiments, the specimens from the stock cultures of Lepidoptera and the spined soldier bug were checked using light microscopy to ensure they are free from the microsporidia infections. After the larvae of Lepidoptera were exposed for bug feeding, they were dissected and smears were prepared from the inner tissues. Similarly, the cadavers of the adult bugs were dissected for light microscopy. Smears of inner tissues were examined by bright field light microscopy using Carl Zeiss Axio 10 Imager M1 at 400x magnification. The intensity of infection was estimated basing upon the number of spores per microscope field at 400× magnification, counted at 10 different spots of the smear: light (1-10 spores per field), mediocre (10–100 spores) and heavy (over 100 spores). The remaining parts of microsporidia-positive specimens were frozen for consequent molecular detection.

For genomic DNA extraction we used a simplified protocol of Sambrook et al. (1998) without addition of phenol. Insect tissues were homogenized with a plastic pestle in 1.5 mL microcentrifuge tubes in 100 µl lysis buffer CTAB. After homogenization, 500 µl lysis buffer with 0.2 % β-mercaptoethanol was added to the samples and incubated for 2 hrs at + 65°C. DNA was further extracted with chloroform and isoamyl alcohol (24:1), precipitated with isopropanol and washed with 70 % and 90% ethanol. Dried DNA pellets were resuspended in 50 µl of ultra-purified water. The primers 18f/1047r specific to the locus of SSU rRNA were used to obtain fragments ~900 bp. The PCR mix consisted of 4 µl of DNA, 5 µl of DreamTaq Green PCR Master Mix, and 0.5 µl of primers (forward and reverse). The PCR conditions: initial denaturation (95°C for 5 min), 35 amplification cycles (denaturation at 95°C for 1min; annealing at 54°C for 1 min, elongation at 72°C for 1 min) and a final extension step (72°C for 5 min). The amplicons were visualized using electrophoresis in 1 % agarose gels with GeneRuler Ladder Mix molecular weight marker (Thermo Fisher Scientific). The cut sections of the gel were melted in a 3 M solution of guanidine isothiocyanate, and the amplicons were purified by the silica sorption method (Vogelstein and Gillespie 1979).

The purified amplicons were sequenced in forward direction according to Sanger (Sanger et al. 1977) using an ABI Prism sequencer by Evrogen, Moscow, corrected manually in BioEdit and compared with Genbank entries using BLAST utility. Genbank (http://www.ncbi.nlm.nih.gov.nuccore/) was used to extract nucleotide sequence data for SSU rRNA gene of *N. bombycis* and *N. pyrausta*. The nucleotide sequences were aligned in BioEdit (Hall 1999).

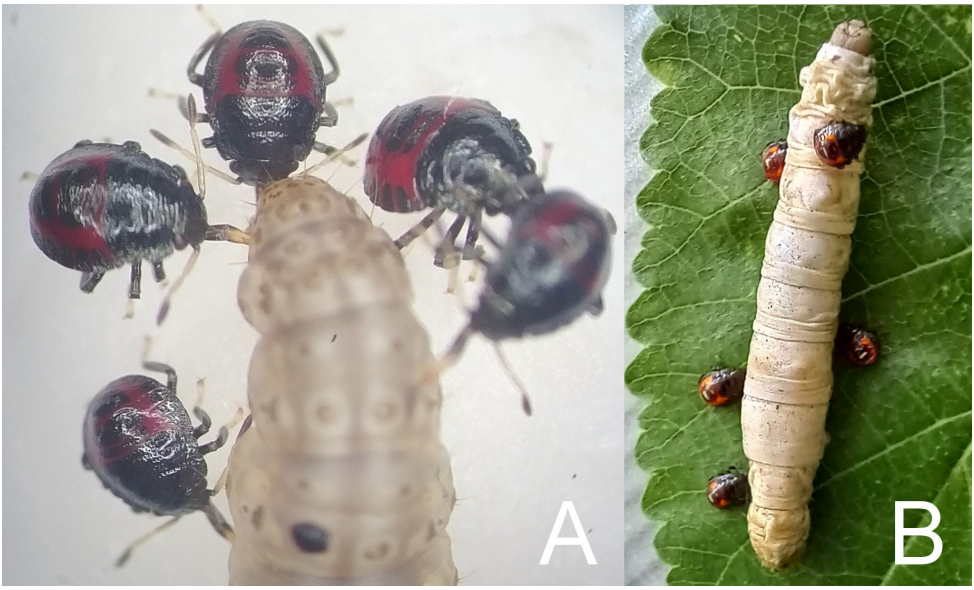

Figure 1. Collective feeding of *Podisus maculiventris* second instar larvae utilizing lepidopteran larvae: (A) fifth instar larva of *Ostrinia scapulalis* infected with *Nosema pyrausta*; (B) third instar larva of *Bombyx mori* infected with *Nosema bombycis.*

Statistical analysis

The exact Fisher test (Fisher 1992) to compare the infection prevalence levels between the variants. The main goal was to evaluate the differences between the variants of the parental bugs fed with the control and microsporidia-infected prey larvae, as well as between the parental and the filial generations within each variant.

Results

Experiments with *Nosema pyrausta*

Cultivation of the microsporidia-free *O. scapulalis* culture in one facility with the temporary *N. pyrausta*-infected culture of *O. nubilalis* resulted in contamination of the healthy culture. Out of 34 *O. scapulalis* larvae assayed, 8 specimens turned out to be heavily infected with microsporidia spore masses, corresponding to the 23.5 ± 7.3 % infection. When the infected insects were exposed to the groups of 5 second instar nymphs of *P. maculiventris*, they were eagerly attacked within the first 12 hrs and perished within 48 hrs since exposure. The groups of nymphs which fed on the infected adzuki bean borer larvae were chosen for further rearing until the adult stage. The 25 adult bugs that survived in the parental generation (P_0) were subjected to post-mortem analysis. Microsporidia spores of shape and size characteristic of *N. pyrausta* were observed in adipose tissue and silk glands of 4 specimens, corresponding to 16.0 ± 7.3 % mean prevalence level. This value was significantly different from the uninfected control group, as inferrect from the results of the exact Fisher test (Table 1). To determine species allocation of the microsporidium observed in the predator tissues, SSU rRNA gene fragment was sequenced, showing 100 % identity to the homologous sequence of *N. pyrausta* available under Genbank accession # HM566196.

The intensity of infection was high, comparable to that of the primary insect host (Fig. 2). The progeny of these infected females was further reared as the first filial generation (F_1) using only microsporidia-free feed insects. Consequently, the second (F_2) and the third (F_3) filial generations were acquired. Among 116 F_1 bugs reared till adult stage, as many as 6 specimens were microsporidia-positive, showing multiple parasite spores in the smears prepared from the whole body homogenates or separate samples of fat body, muscles, Malpighian tubules and salivary glands. This corresponds to 5,17 ± 2,06 % prevalence level, which is about three times as low as in the parental generation, though these values are not significantly different. The intensity of infection varied from mediocre to heavy. However, F_2 and F_3 bugs turned out to be uninfected. As for the control, no microsporidia infection was detected both in the parental generation (Table 1) and subsequent filial generations (not shown).

Table 1. Prevalence levels of *Nosema pyrausta* in parental generation (P_0) of *Podisus maculiventris* primarily fed with the infected *Ostrinia scapulalis* larvae and their respective progeny of first (F_1), second (F_2) and third (F_3) filial generations

Variants	Infective dosage, spores/larva	Generation	Sample size, pcs	Samples identified as infected ± SE	
				Pcs	%
Control	–	P_0	75	0	0^{aA}
Nosema pyrausta	Spontaneous infection	P_0	25	4	17.39 ± 7.90^{bB}
		F_1	116	6	5.17 ± 2.06^{b}
		F_2	43	0	0^{a}
		F_3	42	0	0^{a}

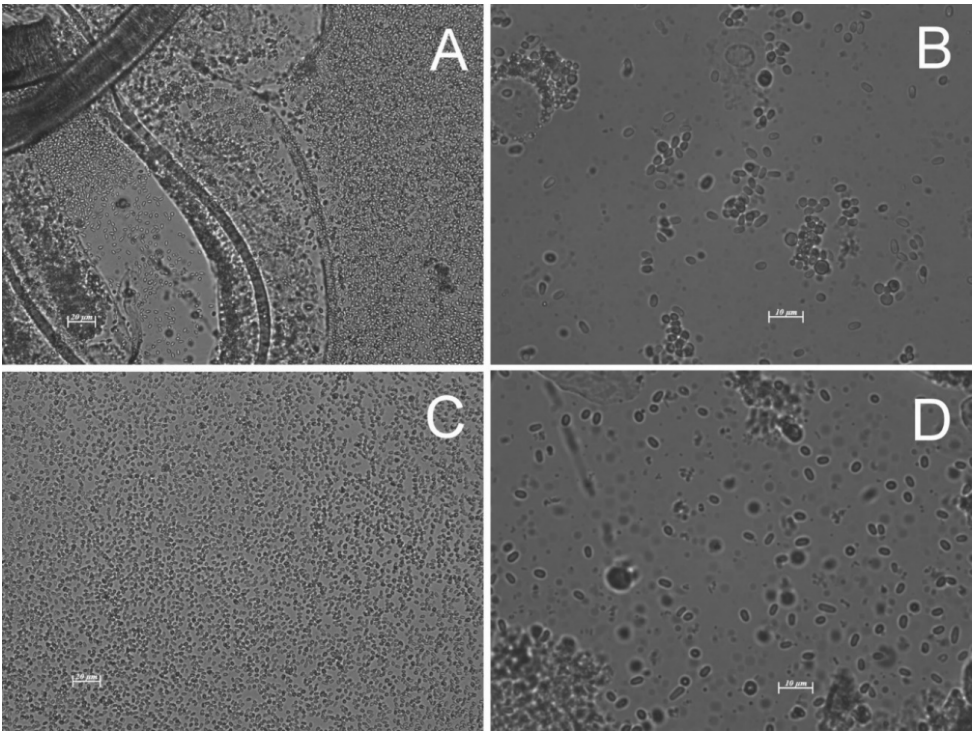

Figure 2. Spores of *Nosema pyrausta* on smears prepared from infected insect tissues: (A) *Ostrinia scapulalis*, magnification ×400, scale bar = 20 μm; (B) *Ostrinia scapulalis*, magnification ×1000, scale bar = 10 μm; (C) *Podisus maculiventris*, magnification ×400, scale bar = 20 μm; (D) *Podisus maculiventris*, magnification×1000, scale bar = 10 μm.

Experiments with *Nosema bombycis*

Out of 10 silkworm larvae fed with *N. bombycis* at the dosage of 10^4 spores/larva, only one turned out to be free from the infection. The other nine specimens had their inner tissues heavily loaded with the spores. Only those bugs fed with the infected larvae were considered for the experiment. Among those 43 bugs reared till adulthood, there were 12 specimens showing microsporidian spores (Fig. 3) with light to heavy infection intensity, thus constituting 27,91 ± 6,84 % prevalence level, statistically significant as compared to the control. However, among 83 bugs of the filial generation, none was infected.

Similarly, all the 15 silkworm larvae fed with *N. bombycis* at the dosage of 10^4 spores/larva became infected and among 73 P_0 bugs, the infection with mediocre to heavy intensity was found in 12 bugs, i.e. 15,07 ± 4,19 %, again significantly different from the control. Just like in case of *N. pyrausta*, the infected tissues were the adipose tissue and salivary glands. Their filial generation was also not infected, just like the control (Table 2). Sequencing the diagnostic fragment of SSU rRNA gene in microsporidia-positive bugs showed 100 % identity with the homologous sequence of *N. bombycis* (#D85503).

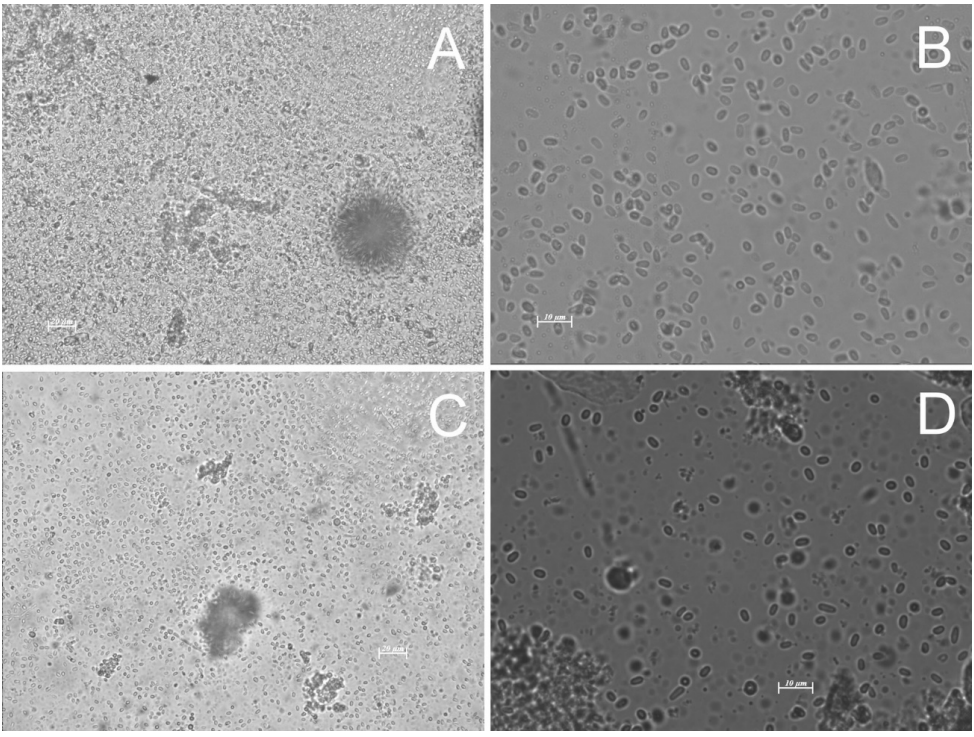

Figure 3. Spores of *Nosema bombycis* on smears prepared from infected insect tissues: (A) *Bombyx mori*, magnification ×400, scale bar = 20 μm; (B) *Bombyx mori*, magnification ×1000, scale bar = 10 μm; (C) *Podisus maculiventris*, magnification ×400, scale bar = 20 μm; (D) *Podisus maculiventris*, magnification ×1000, scale bar = 10 μm.

Table 2. Prevalence levels of *Nosema bombycis* in parental generation (P_0) of *Podisus maculiventris* primarily fed with the infected *Ostrinia nubilalis* larvae and their respective progeny of first (F_1), second (F_2) and third (F_3) filial generations

Variants	Infective dosage, spores/larva	Generation	Sample size, pcs	Samples identified as infected ± SE	
				Pcs	%
Control	–	P_0	75	0	0^A
Nosema bombycis	10^4	P_0	43	12	27.91 ± 6.84^B
		F_1	84	0	0^A
	10^5	P_0	73	11	15.07 ± 4.19^B
		F_1	175	0	0^A

Discussion

Many species of microsporidia are highly contagious to their insect hosts and cause intensive infections both in nature and during artificial cultivation under conditions of laboratory and industrial rearing (van Frankenhuyzen et al. 2004; Babin et al. 2022). *N. pyrausta* is not an exception. In field, the infection prevalence rates typically average at the level of 5-10 % in Europe (Malysh et al. 2011; Grushevaya et al. 2018) but is higher by an order of magnitude in North America (Lewis et al. 2006). In the laboratory, experimental infection even with low dosages, such as 10^4 spores/larva, results in infection levels exceeding 90 % in both type host (Grushevaya et al. 2020) and other vulnerable pyraloid moths, such as the beet webworm *Loxostege sticticalis* Linnaeus, 1761 (Malysh et al. 2018).

The same applies to *N. bombycis*, which is widespread in nature, infecting wild populations of various butterfly species (Tokarev et al. 2020), not to mention of extensive epizootics occurring in captive industrial cultures of the main host *B. mori* (Chakrabarty et al. 2013). It is therefore expected that predatory insects may encounter high loads of microsporidian spores while feeding on lepidopteran larvae. Infections of entomophagous insects with the host-derived microsporidia have been documented repeatedly (see Introduction).

The dosages of *N. bombycis* spores utilized for experimental infection of silkworm larvae are variable. In the study of transovarial transmission of *N. bombycis*, 50 third instar larvae were fed a diet containing 1.8×10^4 spores/cm^3 and only 19 specimens survived to moth stage with 15 % infection level, while higher dosages caused negligible or zero moth survival (Han and Watanabe 1998). In the studies of the effects of gut commensal bacteria supplementation on larval susceptibility to the microsporidium, the dosages used ranged between 25 and 2.5×10^7 spores per larva (Suraporn and Terenius 2021; Zhang et al. 2022). Similarly, to study the midgut response of silkworm larvae to infection at the level of differentially expressed

genes, the dosages of 2×10^3 (Li et al. 2018) and 2×10^4 spores/larva (Ma et al. 2013) were applied. Thus, the parasite load exploited in the present work is in the range of dosages routinely applied in experimental studies to induce massive infection of inner tissues of the host.

To our mind, the exposure of the heavily infected phytophagous insects to the predator is an appropriate model of possible natural interactions to test susceptibility of the predator to microsporidia in their prey at the maximally available level. It perfectly shows that even high loads of the pathogens are not causing epizootics in the predator under conditions of artificial rearing and same should be expected in nature.

Interestingly, even though *N. bombycis* and *N. pyrausta* display different host ranges when assayed against a series of lepidopteran species (Tokarev et al. 2020), both were able to infect the spined soldier bug at similar levels via the horizontal transmission route. This serves as another example of host switching of microsporidia (Malysh et al. 2018; Saito and Bjørnson 2013; Futerman et al. 2006; Schuld 1999), explaining the broad distribution of these parasites in insects and other invertebrate, as well as vertebrate hosts.

Infection of entomophagous arthropods can be beneficial for the pathogen dissemination in the primary phytophagous insect host populations and transmission to the new pest species, but possible adverse effects cannot be ruled out when intensive infections are developed in the predatory and parasitic insects.

In another study, *N. pyrausta* did not affect the fertility and lifespan of the common green lacewing *C. carnea*. Throughout the development of this host, swallowed spores remained in the intestinal lumen of the predatory insect and were excreted with feces. As a result, these spores did not infect the predator's tissues, but remained infective for the corn borer larvae (Sajap and Lewis 1989). Similarly, other species of microsporidia from phytophagous prey did not adversely affect predatory insects, as the infection of their inner tissues was not established (Kaya 1979; Smirnoff and Eichhorn 1970; Young and Hamm 1985). The predators therefore only participated in spreading of the entomopathogens through defecation (Cooper 1981; Kaya 1979; Young and Hamm 1985; Capinera and Barbosa 1975). Interestingly, similar observations were made when an insect microsporidium *N. ceranae* Fries I., Feng F., da Silva A., Slemenda S.B. & Pieniazek N.J., 1996 was exposed to an insectivorous vertebrate, the bee eater *Merops apiaster* Linnaeus, 1758. The feces of this bird, which feeds on honey bees, contained spores of the microsporidium originating from, and retaining infectivity to, this insect host (Valera et al. 2011). Participation of the Lepidoptera-associated microsporidia in natural trophic webs and their virulence to the predators under the conditions of horizontal and vertical transmission is of great importance both for ecosystem balance and application of biocontrol agents. In the present study, horizontal transmission was observed for the two microsporidia species when the predator fed on prey intensively infected with the microparasites. The prevalence range of infections developed in the horizontally infected bugs was be-

low 30 %. Presence of the spore masses in infected bugs suggests possibility of further horizontal dissemination of the infection because cannibalism is widespread among the representatives of the genus *Podisus* (De Clercq and Degheele 1992). Yet, low levels of horizontally transmitted infection from the primary host to the predator suggests that further spreading within the predator's populations will be even less intensive.

Another principal route of dissemination of microsporidia is via vertical transmission from the parental generation to the filial one, ensuring and seasonal progress of infection (Zimmermann et al. 2016) and long-term persistence in populations of particular species over time (Siegel et al. 1988). In case of *P. maculiventris*, however, it can be concluded that the microsporidia from the lepidopteran insects are not well suited for efficient vertical transmission in the non-specific heteropteran host. This is well supported by the in diminishing prevalence rates of *N. pyrausta* in the row of consequent generations in the present study. Moreover, absence of *N. bombycis* infection in the filial generation of the bug ensures safety of this pathogen from the standpoint. This indicates low risk of vertical transmission of these two pathogens, making them suitable for combined application with the predatory bug in the biological and integrated pest management programmes.

Conclusion

It is vitally important to understand how biocontrol agents of different groups counteract when applied simultaneously. In the present study, two microsporidia from lepidopteran insects showed low levels of persistence in the predatory bug. *N. pyrausta* was able to infect the bug directly and their progeny at a diminishing rate. Meanwhile, *N. bombycis* could only infect the bug directly with no transmission to the progeny. Thus, these microsporidian species pose little risk to the spined soldier bug which is widely utilized for plant protection.

Acknowledgments

The authors are indebted to Bakhtyar Mirzakhodzhaev (Scientific Research Institute of Sericulture, Tashkent, Uzbekistan) for *N. bombycis* spore load and to Vadim Krukov (Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia) for consultations on statistical analysis, as well as to Anna Felgenhauer, Master of Arts in Applied Linguistics (Wisconsin, USA) for grammar correction. The research is supported by Russian Science Foundation grant #22-76-00032, https://rscf.ru/project/22-76-00032/.

References

Abbas MST, Boucias DG (1984) Interaction between nuclear polyhedrosis virus-infected *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) larvae and predator *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Environmental Entomology 13(2): 599–602. https://doi.org/10.1093/ee/13.2.599

Aldrich JR, Cantelo WW (1999) Suppression of Colorado potato beetle infestation by pheromone-mediated augmentation of the predatory spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Agricultural and Forest Entomology 1: 209–217. https://doi.org/10.1046/j.1461-9563.1999.00026.x

Amarasekare KG, Edelson JV (2004) Effect of temperature on efficacy of insecticides to differential grasshopper (Orthoptera: Acrididae). Journal of Economic Entomology 97(5): 1595–1602. https://doi.org/10.1603/0022-0493-97.5.1595

Avery PB, George J, Markle L, Martini X, Rowley AL, Meagher RL, Barger RE, Duren EB, Dawson JS, Cave RD (2022) Choice behavior of the generalist pentatomid predator *Podisus maculiventris* when offered lepidopteran larvae infected with an entomopathogenic fungus. Biological Control 67: 201–211. https://doi.org/10.1007/s10526-021-10124-4

Babin A, Schurr F, Rivière MP, Chauzat MP, Dubois E (2022) Specific detection and quantification of three microsporidia infecting bees, *Nosema apis*, *Nosema ceranae*, and *Nosema bombi*, using probe-based real-time PCR. European Journal of Protistology 86: 125935. https://doi.org/10.1016/j.ejop.2022.125935

Becnel JJ, Andreadis TG (1999) Microsporidia in insects. In: Wittner M, Weiss LM (Eds) The Microsporidia and Microsporidiosis. American Society for Microbiology Press, Washington, DC, 447e501.

Biddinger DJ, Leslie TW, Joshi NK (2014) Reduced-risk pest management programs for Eastern US peach orchards: effects on arthropod predators, parasitoids, and select pests. Journal of Economic Entomology 107(3): 1084–1091. https://doi.org/10.1603/EC13441

Biever KD, Andrews PL, Andrews PA (1982) Use of a predator, *Podisus maculiventris*, to distribute virus and initiate epizootics. Journal of Economic Entomology 75(1): 150–152. https://doi.org/10.1093/jee/75.1.150

Bird AE, Hesketh H, Cross JV, Copland M (2004) The common black ant, *Lasius niger* (Hymenoptera: Formicidae), as a vector of the entomopathogen *Lecanicillium longisporum* to rosy apple aphid, *Dysaphis plantaginea* (Homoptera: Aphididae). Biocontrol Science and Technology 14: 757–767. https://doi.org/10.1080/09583150410001720716

Brooks WM (1988) Entomogenous protozoa. In: Ignoffo CM (Ed) Handbook of Natural Pesticides. Vol. V. Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi. CRC Press, Inc., Boca Raton, 1–149.

Campbell C, van Frankenhuyzen K, Smith S (2007) Incubation period, spore egestion and horizontal transmission of *Nosema fumiferanae* (Microsporidia: Nosematidae) in spruce budworm (*Choristoneura* sp., Lepidoptera: Tortricidae): The role of temperature and dose. Journal of Invertebrate Pathology 94(3): 204–210. https://doi.org/10.1016/j.jip.2006.11.005

Capinera JL, Barbosa P (1975) Transmission of nuclear-polyhedrosis virus to gypsy moth larvae by *Calosoma sycophanta*. Annals of the Entomological Society of America 68(3): 593–594. https://doi.org/10.1093/aesa/68.3.593

Carvalho VFP, Vacari AM, Pomari AF, De Bortoli CP, Ramalho DG, De Bortoli SA (2012) Interaction between the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae) and the entomopathogenic bacteria *Bacillus thuringiensis*. Environmental Entomology 41(6): 1454–1461. https://doi.org/10.1603/EN12060

Chakrabarty S, Saha AK, Manna B, Kumar SN (2013) Secondary contamination is the main source for spread of *Nosema bombycis* resulting in outbreak of pebrine disease in *Bombyx mori* L. International Journal of Industrial Entomology 27(2): 282–288. https://doi.org/10.1002/9780470015902.a0022555

Cooper DJ (1981) The role of predatory Hemiptera in disseminating a nuclear polyhedrosis virus of *Heliothis punctiger*. Australian Journal of Entomology 20(2): 145–150. https://doi.org/10.1111/j.1440-6055.1981.tb01017.x

Cross P (2013) Pesticide hazard trends in orchard fruit production in Great Britain from 1992 to 2008: a time-series analysis. Pest Management Science 69(6): 768–774. https://doi.org/10.1002/ps.3436

de Azeredo Morgado MG, Passos CJS, Garnier J, De Lima LA, de Alcântara Mendes R, Samson-Brais É, Lucotte M (2023) Large-scale agriculture and environmental pollution of ground and surface water and sediment by pesticides in the Brazilian Amazon: the case of the santarém region. Water, Air, & Soil Pollution 234(3): 150. https://doi.org/10.1007/s11270-023-06152-8

de Castro AA, Poderoso JCM, Ribeiro RC, Legaspi JC, Serrão JE, Zanuncio JC (2015) Demographic parameters of the insecticide-exposed predator *Podisus nigrispinus*: implications for IPM. Biological Control 60: 231–239. https://doi.org/10.1007/s10526-014-9639-y

De Clercq P (2000) Predaceous stinkbugs (Pentatomidae: Asopinae). In: Schaefer CW, Panizzi AR (Eds) Heteroptera of economic importance. CRC Press, Boca Raton, Florida, 759–812. https://doi.org/10.1201/9781420041859

De Clercq P, Degheele D (1992) A meat-based diet for rearing the predatory stink bugs *Podisus maculiventris* and *Podisus sagitta* [Heteroptera: Pentatomidae]. Entomophaga 37: 149–157.

De Clercq P, Degheele D (1994) Laboratory measurement of predation by *Podisus maculiventris* and *P. sagitta* (Hemiptera: Pentatomidae) on beet armyworm (Lepidoptera: Noctuidae). Journal of Economic Entomology 87(1): 76–83. https://doi.org/10.1093/jee/87.1.76

De Clercq P, Merlevede F, Tirry L (1998) Unnatural Prey and Artificial Diets for Rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). Biological Control 12(2): 137–142. https://doi.org/10.1006/bcon.1998.0611

de Nardo EAB, Maia AHN, Watanabe MA (2001) Effect of a formulation of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus on the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae: Asopinae), using the fertility life table

parameters. Environmental Entomology 30: 1164–1173. https://doi.org/10.1603/0046-225X-30.6.1164

Desurmont G, Weston PA (2008) Predation by *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) on viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), under laboratory and field conditions. Environmental Entomology 37(5): 1241–1251. https://doi.org/10.1093/ee/37.5.1241

Dolzhenko VI, Laptiev AB (2021) Modern range of plant protection means: biological efficiency and safety. Plodorodie 3(120): 71–75. https://doi.org/10.25680/S19948603.2021.120.13 [In Russian with English summary]

Down RE, Cuthbertson AG, Mathers JJ, Walters KF (2009) Dissemination of the entomopathogenic fungi, *Lecanicillium longisporum* and *L. muscarium*, by the predatory bug, *Orius laevigatus*, to provide concurrent control of *Myzus persicae*, *Frankliniella occidentalis* and *Bemisia tabaci*. Biological Control 50(2): 172–178. https://doi.org/10.1016/j.biocontrol.2009.03.010

Down RE, Bell HA, Matthews HJ, Kirkbride-Smith AE, Edwards JP (2004) Dissemination of the biocontrol agent *Vairimorpha necatrix* by the spined soldier bug, *Podisus maculiventris*. Entomologia Experimentalis et Applicata 110(2): 103–114. https://doi.org/10.1111/j.0013-8703.2004.00122.x

Dwivedi SA, Sonawane VK, Pandit TR (2022) Review on the impact of insecticides utilization in crop ecosystem: Their prosperity and threats. In: Ranz RER (Ed.) Insecticides-Impact and Benefits of Its Use for Humanity. IntechOpen, London, UK. https://doi.org/10.5772/intechopen.100385

Fisher RA (1992) Statistical Methods for Research Workers. In: Kotz S, Johnson NL (Eds) Breakthroughs in Statistics. Springer Series in Statistics. Springer, New York, NY, USA, 66–70. https://doi.org/10.1007/978-1-4612-4380-9_6

Flick AJ, Acevedo MA, Elderd BD (2016) The negative effects of pathogen-infected prey on predators: a meta-analysis. Oikos 125: 1554–1560. https://doi.org/10.1111/oik.03458

Frolov AN, Berim MN, Grushevaya IV (2019) Rearing of trilobed male uncus *Ostrinia* species in laboratory for experimental purposes. Vestnik zashchity rasteniy 3(101): 58–62. https://doi.org/10.31993/2308-6459-2019-3(101)-58-62

Futerman PH, Layen SJ, Kotzen ML, Franzen C, Kraaijeveld AR, Godfray HCJ (2006) Fitness effects and transmission routes of a microsporidian parasite infecting *Drosophila* and its parasitoids. Parasitology 132(4): 479–492. https://doi.org/10.1017/S0031182005009339

Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, Ceryngier P, Liira J, Tscharntke T, Winqvist C, Eggers S, Bommarco R, Pärt T, Bretagnolle V, Plantegenest M, Clement LW, Dennis C, Palmer C, Oñate JJ, Guerrero I, Inchausti P (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic and Applied Ecology 11(2): 97–105. https://doi.org/10.1016/j.baae.2009.12.001

Godfray HCJ, Garnett T (2014) Food security and sustainable intensification. Philosophical transactions of the Royal Society B: biological sciences 369: 20120273. http://dx.doi.org/10.1098/rstb.2012.0273

Goertz D, Hoch G (2013) Influence of the forest caterpillar hunter *Calosoma sycophanta* on the transmission of microsporidia in larvae of the gypsy moth *Lymantria dispar*. Agricultural and Forest Entomology 15(2): 178. https://doi.org/10.1111/afe.12000

Goertz D, Hoch G (2008) Horizontal transmission pathways of terrestrial microsporidia: A quantitative comparison of three pathogens infecting different organs in *Lymantria dispar* L. (Lep.: Lymantriidae) larvae. Biological Control 44(2): 196–206. https://doi.org/10.1016/j.biocontrol.2007.07.014

Goertz D, Hoch G (2011) Modeling horizontal transmission of microsporidia infecting gypsy moth, *Lymantria dispar* (L.), larvae. Biological Control 56(3): 263–270. https://doi.org/10.1016/j.biocontrol.2010.11.013

Grushevaya I, Ignatieva A, Tokarev Y (2020) Susceptibility of three species of the genus *Ostrinia* (Lepidoptera: Crambidae) to *Nosema pyrausta* (Microsporidia: Nosematida). BIO Web of Conf EDP Sciences 21: 00040. https://doi.org/10.1051/bioconf/20202100040

Grushevaya IV, Ignatieva AN, Malysh SM, Senderskiy IV, Zubarev IV, Kononchuk AG (2018) Spore dimorphism in *Nosema pyrausta* (Microsporidia, Nosematidae): From morphological evidence to molecular genetic verification. Acta Protozoologica 57: 49–52. https://doi.org/10.1017/S0031182018001737

Grushevaya IV, Senderskiy IV, Zubarev IV, Tokarev YS (2021) Transovarial transmission of *Nosema pyrausta* in three generations of *Ostrinia nubilalis* in laboratory tests. Entomologia Experimentalis et Applicata 169(11): 1057–1060. https://doi.org/10. 1111/eea.13100

Gupta RK, Gani M, Jasrotia P, Srivastava K (2013) Development of the predator *Eocanthecona furcellata* on different proportions of nucleopolyhedrovirus infected *Spodoptera litura* larvae and potential for predator dissemination of virus in the field. Biological Control 58: 543–552. https://doi.org/10.1007/s10526-013-9515-1

Haddi K, Turchen LM, Viteri Jumbo LO, Guedes RN, Pereira EJ, Aguiar RW, Oliveira EE (2020) Rethinking biorational insecticides for pest management: Unintended effects and consequences. Pest Management Science 76(7): 2286–2293. https://doi.org/10.1002/ps.5837

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Han M-S, Watanabe H (1998) Transovarian transmission of two microsporidia in the silkworm, *Bombyx mori* and disease occurrence in progeny population. Journal of Invertebrate Pathology 51(1): 41–45. https://doi.org/10.1016/0022-2011(88)90086-9

Hare JD (1990) Ecology and management of the Colorado potato beetle. Annual Review of Entomology 35: 81–100. https://doi.org/10.1146/annurev.en.35.010190.000501

Hatcher MJ, Dick JTA, Dunn AM (2014) Parasites that change predator or prey behavior can have keystone effects on community composition. Biology Letters 10: 20130879. https://doi.org/10.1098/rsbl.2013.0879

He X, Deng H, Hwang HM (2019) The current application of nanotechnology in food and agriculture. Journal of Food and Drug Analysis 27(1): 1–21. https://doi.org/10.1016/j.jfda.2018.12.002

Kaya HK (1979) Microsporidan spores: retention of infectivity after passage through the gut of the assassin bug, *Zelus exsanguis* (Stal). In: Proceedings of the Hawaiian Entomological Society 13(1): 91–94.

Kevan PG, Shipp L, Smagghe G (2020) Ecological intensification: Managing biocomplexity and biodiversity in agriculture through pollinators, pollination and deploying biocontrol agents against crop and pollinator diseases, pests and parasites. In: Smagghe G, Boecking O, Maccagnani B, Mänd M, Kevan PG (Eds) Entomovectoring for Precision Biocontrol and Enhanced Pollination of Crops. Springer Switzerland, 19–51. https://doi.org/10.1007/978-3-030-18917-4_2

Kudsk P, Jørgensen LN, Ørum JE (2018) Pesticide Load – A new Danish pesticide risk indicator with multiple applications. Land Use Policy 70: 384–393. https://doi.org/10.1016/j.landusepol.2017.11.010

Lewis LC, Sumerford DV, Bing LA, Gunnarson RD (2006) Dynamics of *Nosema pyrausta* in natural populations of the European corn borer, *Ostrinia nubilalis*: A six-year study. Biological Control 51: 627–642. https://doi.org/10.1007/s10526-005-2937-7

Li Z, Wang Y, Wang L, Zhou Z (2018) Molecular and biochemical responses in the midgut of the silkworm, *Bombyx mori*, infected with *Nosema bombycis*. Parasites & Vectors 11: 1–10. https://doi.org/10.1186/s13071-018-2755-2

Lin T, Zeng Z, Chen Y, You Y, Hu J, Yang F, Wei H (2021) Compatibility of six reduced-risk insecticides with *Orius strigicollis* (Heteroptera: Anthocoridae) predators for controlling *Thrips hawaiiensis* (Thysanoptera: Thripidae) pests. Ecotoxicology and Environmental Safety 226: 112812. https://doi.org/10.1016/j.ecoenv.2021.112812

Litwin A, Nowak M, Różalska S (2020) Entomopathogenic fungi: unconventional applications. Reviews in Environmental Science and Bio/Technology 19(1): 23–42. https://doi.org/10.1007/s11157-020-09525-1

López Jr JD, Ridgway RL, Pinnell RE (1976) Comparative efficacy of four insect predators of the bollworm and tobacco budworm. Environmental Entomology 5(6): 1160–1164. https://doi.org/10.1093/ee/5.6.1160

Ma Z, Li C, Pan G, Li Z, Han B, Xu J, Lan X, Chen J, Yang D, Chen Q, Sang Q, Ji X, Li T, Long M, Zhou Z (2013) Genome-Wide Transcriptional Response of Silkworm (*Bombyx mori*) to Infection by the Microsporidian *Nosema bombycis*. PLoS ONE 8(12): e84137. https://doi.org/10.1371/journal.pone.0084137

Malysh JM, Ignatieva AN, Artokhin KS, Frolov AN, Tokarev YS (2018) Natural infection of the beet webworm *Loxostege sticticalis* L. (Lepidoptera: Crambidae) with three Microsporidia and host switching in *Nosema ceranae*. Parasitology Research 117(9): 3039–3044. https://doi.org/10.1007/s00436-018-5987-3

Malysh YuM, Tokarev YuS, Sitnikova NV, Kononchuk AG, Grushetskaya TA, Frolov AN (2011) Incidence of microsporidian infection of stem borers of the genus *Ostrinia* (Lepidoptera: Crambidae) in the Krasnodar territory. Parazitologiya 45(3): 234–244. [In Russian with English summary]

Mansour R, Biondi A (2021) Releasing natural enemies and applying microbial and botanical pesticides for managing *Tuta absoluta* in the MENA region. Phytoparasitica 49(2): 179–194. https://doi.org/10.1007/s12600-020-00849-w

Marti Jr OG, Hamm JJ (1985) Effect of *Vairimorpha* sp. on survival of *Geocoris punctipes* in the laboratory. Journal of Entomological Science 20(3): 354–358. https://doi.org/10.18474/0749-8004-20.3.354

Möhring N, Ingold K, Kudsk P, Martin-Laurent F, Niggli U, Siegrist M, Studer B, Walter A, Finger R (2020) Pathways for advancing pesticide policies. Nature food 1(9): 535–540. https://doi.org/10.1038/s43016-020-00141-4

Pell JK, Vandenberg JD (2002) Interactions among the aphid *Diuraphis noxia*, the entomopathogenic fungus *Paecilomyces fumosoroseus* and the coccinellid *Hippodamia convergens*. Biocontrol Science and Technology 12(2): 217–224. https://doi.org/10.1080/09583150120124478

Roy HE, Pell JK, Alderson PG (2001) Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the aphid predator *Coccinella septempunctata*. Biocontrol Science and Technology 11(1): 99–110. https://doi.org/10.1080/09583150020029781

Saito T, Bjørnson S (2006) Horizontal transmission of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), to three coccinellid species of Nova Scotia. Biological Control 39(3): 427–433. https://doi.org/10.1016/j.biocontrol.2006.06.012

Saito T, Bjørnson S (2013) The convergent lady beetle, *Hippodamia convergens* Guérin-Méneville and its endoparasitoid *Dinocampus coccinellae* (Schrank): the effect of a microsporidium on parasitoid development and host preference. Journal of Invertebrate Pathology 113(1): 18–25. https://doi.org/10.1016/j.jip.2013.01.003

Sajap AS, Lewis LC (1989) Impact of *Nosema pyrausta* (Microsporida: Nosematidae) on a predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environmental Entomology 18(1): 172–176. https://doi.org/10.1093/ee/18.1.172

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences 74(12): 5463–5467.

Schuld M, Madel G, Schmuck R (1999) Impact of *Vairimorpha* sp. (Microsporidia: Burnellidae) on *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae), a hymenopteran parasitoid of the cabbage moth, *Plutella xylostella* (Lepidoptera, Yponomeutidae). Journal of Invertebrate Pathology 74(2): 120–126. https://doi.org/10.1006/jipa.1999.4865

Sedaratian A, Fathipour Y, Talaei-Hassanloui R (2014) Deleterious effects of *Bacillus thuringiensis* on biological parameters of *Habrobracon hebetor* parasitizing *Helicoverpa armigera*. Biological Control 59: 89–98. https://doi.org/10.1007/s10526-013-9531-1

Sedaratian-Jahromi A (2021) Effects of entomopathogens on insect predators and parasitoids. In: Khan MA, Ahmad W (Eds) Microbes for Sustainable Insect Pest Management: Hydrolytic Enzyme & Secondary Metabolite. Springer, Cham, Switzerland, 183–231. https://doi.org/10.1007/978-3-030-67231-7_9

Siegel JP, Maddox JV, Ruesink WG (1988) Seasonal progress of *Nosema pyrausta* in the European corn borer, *Ostrinia nubilalis*. Journal of Invertebrate Pathology 52: 130–136. https://doi.org/10.1016/0022-2011(88)90111-5

Simões RA, Feliciano JR, Solter LF, Delalibera JrI (2015) Impacts of *Nosema* sp. (Microsporidia: Nosematidae) on the sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Crambidae). Journal of Invertebrate Pathology 129: 7–12. https://doi.org/10.1016/j.jip.2015.05.006

Smirnoff WA, Eichhorn O (1970) Diseases affecting predators of *Adelges* spp. on fir trees in Germany, Switzerland, and Turkey. Journal of Invertebrate Pathology 15(1): 6–9. https://doi.org/10.1016/0022-2011(70)90091-1

Solter LF, Becnel JJ, Vávra J (2012) Research methods for entomopathogenic microsporidia and other protists. In: Lacey LA (Ed) Manual of Techniques in Invertebrate Pathology 12: 329–371. https://doi.org/10.1016/B978-0-12-386899-2.00011-7

Sparks TC, Nauen R (2015) IRAC: Mode of action classification and insecticide resistance management. Pesticide Biochemistry and Physiology 121: 122–128. https://doi.org/10.1016/j.pestbp.2014.11.014

Sreelakshmi B, Induja S, Adarsh PP, Rahul HL, Arya SM, Aswana S, Haripriya R, Aswathy BR, Manoj PK, Vishnudasan D (2021) Drought stress amelioration in plants using green synthesised iron oxide nanoparticles. Materials Today: Proceedings 41: 723–727. https://doi.org/10.1016/j.matpr.2020.05.801

Stamopoulos DC, Chloridis A (1994) Predation rates, survivorship and development of *Podisus maculiventris* (Het.: Pentatomidae) on larvae of *Leptinotarsa decemlineata* (Col.: Chrysomelidae) and *Pieris brassicae* (Lep.: Pieridae), under field conditions. Entomophaga 39: 3–9. https://doi.org/10.1007/BF02373489

Stejskal V, Vendl T, Aulicky R, Athanassiou C (2021) Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food-industry pest control. Insects 12(7): 590. https://doi.org/10.3390/insects12070590

Suraporn S, Terenius O (2021) Supplementation of *Lactobacillus casei* reduces the mortality of *Bombyx mori* larvae challenged by *Nosema bombycis*. BMC Research Notes 14: 398. https://doi.org/10.1186/s13104-021-05807-1

Tang FHM, Lenzen M, McBratney A, Maggi F (2021) Risk of pesticide pollution at the global scale. Nature Geoscience 14: 206–210. https://doi.org/10.1038/s41561-021-00712-5

Thieltges DW, Amundsen PA, Hechinger RF, Johnson PT, Lafferty KD, Mouritsen KN, Preston DL, Reise K, Zander CD, Poulin R (2013) Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. Oikos 122(10): 1473–1482. https://doi.org/10.1111/j.1600-0706.2013.00243.x

Tokarev YS, Simakova AV, Timofeev SA, Malysh JM, Sokolova OI, Issi IV (2016) Host specificity in microsporidia. Parasitologia 50(6): 446–459. [In Russian with English summary]

Tokarev YS, Huang WF, Solter LF, Malysh JM, Becnel JJ, Vossbrinck CR (2020) A formal redefinition of the genera *Nosema* and *Vairimorpha* (Microsporidia: Nosematidae) and reassignment of species based on molecular phylogenetics. Journal of Invertebrate Pathology 169: 107279. https://doi.org/10.1016/j.jip.2019.107279

Valera F, Martín-Hernandez R, Higes M (2011) Evaluation of large-scale dissemination of *Nosema ceranae* spores by European beeeaters *Merops apiaster*. Environmental Microbiology Reports 3: 47–53. https://doi.org/ 10.1111/j.1758-2229.2010.00186.x

van Frankenhuyzen K, Ebling P, McCron B, Ladd T, Gauthier D, Vossbrinck C (2004) Occurrence of *Cystosporogenes* sp. (Protozoa, Microsporidia) in a multi-species insect production facility and its elimination from a colony of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Journal of Invertebrate Pathology 87(1): 16–28. https://doi.org/10.1016/j.jip.2004.06.001

van Frankenhuyzen K, Nystrom C, Liu Y (2007) Vertical transmission of *Nosema fumiferanae* (Microsporidia: Nosematidae) and consequences for distribution, post-diapause emergence and dispersal of second-instar larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Journal of Invertebrate Pathology 96(2): 173–182. https://doi.org/10.1016/j.jip.2007.03.017

Vivan LM, Torres JB, Fernandes PLS (2017) Activity of selected formulated biorational and synthetic insecticides against larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae). Journal of Economic Entomology 110(1): 118–126. https://doi.org/10.1093/jee/tow244

Vogelstein B, Gillespie D (1979) Preparative and analytical purification of DNA from agarose. The Proceedings of the National Academy of Sciences 76(2): 615–619.

Wang-Peng S, Zheng X, Jia WT, Li AM, Camara I, Chen HX, Tan SQ, Liu YQ, Ji R (2018) Horizontal transmission of *Paranosema locustae* (Microsporidia) in grasshopper populations via predatory natural enemies. Pest Management Science 74(11): 2589–2593. https://doi.org/10.1002/ps.5047

Young OP, Hamm JJ (1985) Compatibility of two fall armyworm pathogens with the predaceous beetle, *Calosoma sayi* (Coleoptera: Carabidae). Journal of Entomological Science 20(2): 212–218. https://doi.org/10.18474/0749-8004-20.2.212

Zhang X, Feng H, He J, Liang X, Zhang N, Shao Y, Zhang F, Lu X (2022) The gut commensal bacterium *Enterococcus faecalis* LX10 contributes to defending against *Nosema bombycis* infection in *Bombyx mori*. Pest Management Science 78(6): 2215–2227. https://doi.org/10.1002/ps.6846

Zhu H, Kim JJ (2012) Target-oriented dissemination of *Beauveria bassiana* conidia by the predators, *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae) for biocontrol of *Myzus persicae*. Biocontrol Science and Technology 22(4): 393–406. https://doi.org/10.1080/09583157.2012.661843

Zimmermann G, Huger AM, Langenbruch GA, Kleespies RG (2016) Pathogens of the European corn borer, *Ostrinia nubilalis*, with special regard to the microsporidium *Nosema pyrausta*. Journal of Pest Science 89(2): 329–346. https://doi.org/10.1007/s10340-016-0749-4