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RESEARCH ARTICLE

Ecological vs physiological host specificity: the case of the microsporidium *Nosema pyrausta* (Paillot) Weiser, 1961

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Abstract

The microsporidium *Nosema pyrausta* (Paillot) Weiser, 1961 plays an important role in the mortality of the European corn borer *Ostrinia nubilalis* (Hübner, 1796), and shows high virulence to the beet webworm *Loxostege sticticalis* (Linnaeus, 1761). In contrast, the greater wax moth *Galleria mellonella* (Linnaeus, 1758) and the gypsy moth *Lymantria dispar* (Linnaeus, 1758) are referred to as resistant hosts, slightly susceptible to this microparasite. The goal of the present study was to test *N. pyrausta* against a broad range of lepidopteran species with different taxonomy, physiology, and ecology. The susceptibility to *N. pyrausta* spores fluctuated greatly among members of various families and superfamilies of Lepidoptera. As many as 13 species tested were found to be refractory (not able to support the development of the microsporidium), including three species of Yponomeutoidea, four species of Papilionoidea, one species of Pyraloidea, two species of Bombycoidea, and three species of Noctuoidea. The species found to be susceptible (with a high proportion of specimens displaying developed infection) included: *Evergestis forficalis* (Linnaeus, 1758) (Crambidae), *Aglais urticae* (Linnaeus, 1758) (Nymphalidae), and *Dendrolimus sibiricus* Chetverikov, 1908 (Lasiocampidae). The species newly found to be highly susceptible (high proportion of infected insects accompanied with high levels of

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early mortality) were: *Spodoptera exigua* (Hübner, 1808) (Noctuidae) and *Aglais io* (Linnaeus, 1758). Large quantities of spores can be produced in vivo using substitute laboratory host *A. urticae*. These results confirm previous observations that physiological host range of microsporidia (observed under experimental conditions) is broader than the ecological one (observed in nature).

Keywords
Microsporidia, microbial control, bioassay, virulence, lepidopteran pest

Introduction

Microsporidia are obligate intracellular eukaryotic parasites belonging to a certain phylogenetic lineage, closely related to Fungi (Bass et al. 2018; Corsaro et al. 2019). They are ubiquitous as pathogens of animals and particular species are virulent to their insect hosts (Issi 2020) and therefore are important for natural density regulation and pest control (Franz and Huger 1970; Lipa and Madziara-Borusiewicz 1976; McManus and Solter 2003; van Frankenhuyzen et al. 2007; Hopper et al. 2016; Frolov 2019; Andreeva et al. 2021; Malysh et al. 2021). One recent example of successful application of microsporidia under field conditions is that of *Myrmecomorba nylanderiae* Plowes, Becnel, LeBrun, Oi, Valles, Jones, Gilbert, 2015 suppressing local populations of the tawny crazy ant *Paratrechina (Nylanderia) fulva* (Mayr, 1862) in North America (LeBrun et al. 2022). In Lepidoptera, several genera of microsporidian parasites are found (Canning et al. 1985; Cali and Garhy 1991; Andreadis et al. 1996; McManus and Solter 2003; Malysh et al. 2013, 2018). Among those, one of the most abundant groups is the genus of *Nosema* with the type species *Nosema bombycis* Nägeli, 1857 from the silkworm *Bombyx mori* Linnaeus, 1758 (Bombycoidea: Bombycidae), which suffers devastating epizootics of this pathogen under conditions of laboratory and industrial mass rearing (Bhat et al. 2009). One of the closest relatives of this microsporidium is *Nosema pyrausta* (Paillot) Weiser, 1961, a widespread pathogen of the European corn borer *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae). Numerous observations indicate that in North America, microsporidia infections play an important role in regulation of *O. nubilalis* population density, causing regular epizootics under field conditions (Lewis et al. 2009; Zimmermann et al. 2016). Meanwhile, epizootics in European populations of *Ostrinia* moths are less frequent and the average prevalence level typically does not exceed 10 % (Pelissie et al. 2010; Malysh et al. 2011; Grushevaya et al. 2018). This discrepancy can be explained by genetic differences of the European and North American isolates of the parasite (Tokarev et al. 2015) as well as other ecological factors differing between the primary (Europe) and the secondary (North America) areas of the pest.

Host range of a given microsporidium species is an important indicator of its ability to circulate in nature and influence insects other than the type host, as well as its potential for practical implications (Solter et al. 1997, 2010). The beet webworm

Loxostege sticticalis (Linnaeus, 1761), a notorious polyphagous insect belonging to the same family Crambidae as *Ostrinia*, was found to be highly vulnerable to *N. pyrausta* and demonstrates high mortality rates even at low dosages of the pathogen (Malysh et al. 2021). On the contrary, the greater wax moth *Galleria mellonella* (Linnaeus, 1758) (Pyraloidea: Pyralidae) and the gypsy moth *Lymantria dispar* (Linnaeus, 1758) (Noctuoidea: Erebidae) are considered to be the resistant hosts. In these two insect species, the mean dosage of 2 million (mln) spores per second instar larva caused infection prevalence at the level of 0-5 %, which could be further augmented only by a combination of additional immunosuppressive factors (Tokarev et al. 2018; Kononchuk et al. 2021). Information concerning susceptibility of other lepidopteran species to this pathogen could not be found, except for one study where few larvae of the common buckeye *Junonia coenia* Hübner, 1822 were tested, resulting in 50 % infection prevalence level (Hall 1952). Meanwhile, understanding of host ranges and the factors governing host specificity of insect pathogens, including microsporidia, is inevitable for exploration of patterns of their natural distribution and for prediction of interplay with other ecosystem components upon introduction into new habitats (Jeffords et al. 1989; Onstad 1993; Solter et al. 1997, 2010; Vilcinskas 2019; Issi 2020). Before such species will be considered for application as microbial control agents, their virulence against crop and forest pests, as well as interactions with non-target entomofauna, should be carefully examined to achieve desirable levels of pest management efficacy and to ensure safety for natural biodiversity.

To explain why different insect species vary in their susceptibility levels to a certain microsporidium, several reasons can be assumed. First, host specificity of the microparasite may play a certain role, when closely related insects are susceptible to a certain microsporidium while distant host taxa are less prone to this microparasite species. In fact, *L. sticticalis* belonging to the same family Crambidae and subfamily Pyraustinae as *Ostrinia* shows high susceptibility to the microsporidium from the latter host, as opposed by resistant *Galleria* and *Lymantria* from other high-level taxa (see above). Second, insect body size may be of importance, as younger (and smaller) instars are usually more susceptible to microsporidia and other microbes as compared to the older (and bigger) ones (Vogelweith et al. 2013). This negative correlation between the body size and infection susceptibility observed within the course of individual development of a certain species may also have impact when different insect species are compared. In particular, *Loxostege* is smaller than *Ostrinia* which in turn is smaller than *Galleria* and *Lymantria*; and susceptibility to *N. pyrausta* is gradually decreasing in this row. Third, feeding behaviour should define chemical composition of the insect gut juice so that the microsporidium spore activation is affected (Issi et al. 2005). A polyphagous host is expected to have a non-specific set of stimuli which potentially might activate the microsporidia spore extrusion in the midgut lumen. Hence, the polyphagous herbivores *Ostrinia* and *Loxostege* seem to be more suitable as a host for a certain species of microsporidia

as compared to the dendrophilic phyllophagous larvae of *Lymantria* and wax-consuming *Galleria*.

The goal of the present study is to test these assumptions experimentally using administration of *N. pyrausta* spores against a broad range of lepidopteran insect species with different taxonomy, physiology, and ecology.

Material and methods

Propagation of the microsporidium

Nosema pyrausta spores were propagated in *O. nubilalis* under laboratory conditions as described earlier (Grushevaya et al. 2018). Batches of spores were isolated from the host pupae, washed with distilled water by centrifugation at 1000 g for 5 min and stored prior to experimental assays as pellets in water refrigerated for 1-3 months, which should not impair significantly their infective potential (Malysh et al. 2021).

Insects

For experimental infection, second instars of lepidopteran insects were used either collected in nature and assayed directly, propagated as a temporary laboratory culture, or taken from a permanent laboratory culture.

Directly assayed insects collected in nature as the second instar larvae on their type host plants were the ermine moths *Yponomeuta evonymella* (Linnaeus, 1758) and *Yponomeuta malinellus* Zeller, 1838, the small tortoiseshell *Aglais urticae* (Linnaeus, 1758) and the European peacock *Aglais io* (Linnaeus, 1758) in St. Petersburg, the black-veined white *Aporia crataegi* (Linnaeus, 1758) in Novosibirsk and the fall webworm *Hyphantria cunea* (Drury, 1773) in Krasnodar Area. The cabbage white *Pieris brassicae* (Linnaeus, 1758) was available at all developmental stages during the vegetation season in St. Petersburg, but its larval population is constantly infested by the parasitoid, *Cotesia glomerata* (Linnaeus, 1758), at high prevalence levels. For this reason, eggs were collected in nature and hatched larvae were reared until second instar larvae under laboratory conditions. These insects were fed with fresh leaves of their type host plants or other available species: bird cherry for *Y. evonymella*, apple for *Y. malinellus*, nettle for *Aglais* spp., plum for *A. crataegi*, ash-leaved maple for *H. cunea*, and cabbage for *P. brassicae*.

Temporary cultures were also established to obtain the second instar larvae of the filial generation reared under laboratory conditions. These species included insects either caught as adults (the small white *Pieris rapae* (Linnaeus, 1758), the green-veined white *Pieris napi* (Linnaeus, 1758), and the Indian flour moth *Plodia interpunctella* (Hübner, 1813) in St. Petersburg) or sampled as the last instar larvae

(the diamondback moth *Plutella xylostella* (Linnaeus, 1758) in St. Petersburg, the cotton bollworm *Helicoverpa armigera* (Hübner, 1808) in Krasnodar Area, the cabbage moth *Mamestra brassicae* (Linnaeus, 1758) in Novosibirsk Region and the Siberian moth *Dendrolimus sibiricus* Chetverikov, 1908 in Krasnoyarsk Region). The larvae were fed either with their type host plants, such as cabbage leaves (*P. rapae*, *P. napi*, *P. xylostella*, *M. brassicae*), and fir branches (*D. sibiricus*), or meridic diet (*H. armigera*, *P. interpunctella*).

As many as three insect species were available as the permanent laboratory cultures. The silkworm *B. mori* first instar larvae were purchased from Research Station of Sericulture (Stavropol Area) and maintained at the facilities of Slavyansk Experimental Station of Plant Protection (Krasnodar Area) on mulberry leaves. The tobacco hornworm *Manduca sexta* Linnaeus, 1763 eggs were purchased from the group of companies "T-RexFoods" (Moscow) and reared on a commercial meridic diet ("T-RexFoods"). The beet armyworm *Spodoptera exigua* (Hübner, 1808) was propagated at the facilities of the University of Silesia on a meridic diet.

Experimental infection

The second instars were used for all experimental treatments. The groups of 21-30 larvae of each insect species were maintained for 4-24 hours without feed. Then the starved insects were provided with *N. pyrausta*-contaminated feed. The plant leaves (or the fir needles) were evenly covered with the spore suspension on both sides and left for air-drying for several minutes. When necessary, the leaf cuticle was scratched by a pin to facilitate moistening of the leaf surface. For the insects maintained on the meridic diets, a diet portion was mixed with the spore suspension. In all cases, the amount of spores was adjusted to provide the mean dosage of 1 mln of spores per larva, taken that 90-100 % of the contaminated feed portion is consumed. After contaminated feed consumption, the groups of larvae were split into three equal groups to represent the repetitions, or maintained as a single repetition. The pathogen-free feed was provided to the insects for the rest of the experiment. Control insects were treated similarly but without addition of the microsporidium spores. Mortality was screened on a daily basis, cadavers were dissected, and inner tissues examined using light microscopy. In *A. urticae*, an additional experiment was performed using the dosage lower by an order of magnitude (0.1 mln spores/larva). In *A. crataegi*, two dosages were also used, 0.1 mln and 1 mln spores/larva, respectively. In this experiment, ten larvae in each variant, including control, were dissected at 30th day post treatment (d.p.t.). The rest of the insects were transferred to +4°C for hibernation for four months and then dissected. For *M. sexta*, *P. brassicae* and *P. rapae*, additional series of experiments were performed using insects fed with the diet (*M. sexta*) with addition of 1 % phenylthiourea (PTU) or the cabbage leaves (*P. brassicae*, *P. rapae*) sprayed with 1 % PTU one day prior to experimental infection with microsporidia. This was done to increase insect susceptibility to the non-specific microsporidium infection (Tokarev et al. 2018). After one day feeding

on the PTU-treated fodder, the insects were treated with the parasite's spores as above. To calculate the spore loads in infected insects, the homogenates were prepared from the individual specimens and amount of spores was quantified using a haemocytometer.

Molecular genetic diagnosis

Samples of infected tissues or isolated spores were subjected for DNA extraction, PCR with microsporidia-specific primers 18f:1047r and sequencing to confirm the species diagnosis of the parasite (Weiss and Vossbrinck 1999; Malysh et al. 2019). When necessary, the primers LepF1:LepR1 specific for mitochondrial cytochrome oxidase subunit I (COI) were used for barcoding of Lepidoptera (Hebert et al. 2004). Sequences were analyzed and compared using BioEdit software (Hall 1999) and BLAST utility at the NCBI server (Altschul et al. 1990).

Statistical analysis

Survival analysis, including estimation of median survival time (LT_{50}), was estimated using Kaplan-Meier procedure followed by log rank test in SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, 2011).

Results

Among the three species assayed in Yponomeutoidea, neither Yponomeutidae (the two species of the ermine moths) nor Plutellidae (the diamondback moth) became infected with *N. pyrausta* after feeding with the spores at early larval stage and maintainance until pupation or adult emergence.

Among Papilionoidea, four species of Pieridae and two species of Nymphalidae were tested. None of the *P. rapae*, *P. brassicae*, and *P. napi*, including those pretreated with PTU for immunosuppression (*P. rapae* and *P. brassicae*) became infected. However, in the group of *P. rapae*, one larva out of 30 turned out to possess morphology (Fig. 1) drastically distinct from that of other Pieridae as the insects grew up. This specimen did not pupate for as long as three months after successful pupation of all other insects in both experimental and control groups. Obviously, another species was assayed accidentally alongside with *P. rapae* larvae. The adipose tissue of this larva was loaded with *Nosema*-like spores. The tissue sample was used for both parasite and host identification using molecular genetic tools. As a result, the parasite was confirmed as *N. pyrausta* basing upon 100 % identity of the SSU rRNA gene sequence (852 bp) to Genbank accession # HM566196. Meanwhile, the host showed 100 % identity of 640 bp long COI sequence to the respective nucleotide sequence (# GU828662) of the garden pebble moth, *Evergestis forficalis* (Linnaeus, 1758) (Lepidoptera: Crambidae).

Figure 1. Full-grown larva of *Evergestis forficalis* heavily infected with *Nosema pyrausta*. Ruler division = 1 mm.

In *A. crataegi* larvae, specimens positive for *Nosema*-like spores were found in all treatment groups dissected both before and after the hibernation, as well as in the control, presumably indicating either successful infection with *N. pyrausta*, including contamination of the control group, or natural infection with a microsporidium. These spores were elongated oval with blunt ends (Fig. 2A). Their length was in the range of 3.3-4.5 (mean 3.8) μm, the width was 1.7-2.2 (mean 1.9) μm (number of measured spores N=20). Meanwhile, the spores of the *N. pyrausta* isolate used for the bioassays in the present study were oval with more tapered ends (Fig. 2B). The *N. pyrausta* spore length was 3.0-3.9 (mean 3.4) μm, the width was 1.4-1.8 (mean 1.7) μm (N=20 spores). The prevalence level ranged between 20 and 40 % with an average of 27.7±3.3 % (mean±SE, number of dissected insects N=67). In order to identify the parasite, SSU rRNA gene fragment was sequenced, which showed 100 % identity (617 bp) to the respective sequence of *Nosema* sp. CmM2 (# KC836092) from *Cnaphalocrocis medinalis* Guenée, 1854 (Lepidoptera: Crambidae). Other most similar entries found in Genbank with 99.7 % sequence identity belonged to *N. furnacalis* (# U26532), *N. granulosis* (# FN434087), and two isolates of unidentified *Nosema* species from *Spodoptera litura* (Fabricius, 1775) (# LC422335) and *Operophtera bruceata* Hulst, 1886 (# MG456600). On the other hand, identity to *N. pyrausta* (# HM566196) and *N. bombycis* (# D85503) was below 98 %.

In *A. urticae*, mortality in control did not exceed 13 % during 25 days of the experiment, and similar dynamics was observed in insects treated with 0.1 mln spores/larva. On the contrary, the larvae treated with 1 mln spores/larva displayed mortality of 30 % and 48 % at 10th and 25 d.p.t., respectively. These values were significantly different from both the lower dosage and the control groups (Fig. 3). The survived larvae both in control and treatment groups successfully pupated on 15-20th d.p.t. Notably, 100 % of perished larvae and survived pupae dissected at 10-30th d.p.i. showed presence of spores in treatment groups, as opposed to the control group. Multiple infection loci filled with prespore stages and mature spores were found in salivary glands and adipose tissue (Fig. 4). The sequencing of SSU rRNA of selected specimens confirmed the diagnosis of *N. pyrausta* infection as above. The pupae from the experimental group infected with 1 mln spores/larva contained from 4 to 625 mln spores, with an average of 246±54.3 spores/pupa (N=12). When *A. io* was

assayed, this species also showed 100 % infection with *N. pyrausta* spore masses (N=23), though exact mortality and spore load data were not collected. It can only be noted, that on 7th d.p.t., as many as 12 out of 30 larvae perished (40.0±5.77 %) with zero mortality in control. Among those perished larvae, microsporidia prevalence level reached 100 % and the spore load averaged 5.9±0.14 spores/larva.

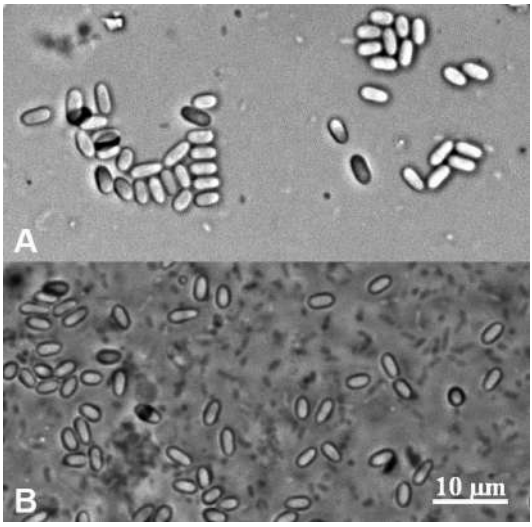

Figure 2. Bright field light microscopy of the spores of *Nosema* sp. from *Aporia crataegi* (**A**) and *Nosema pyrausta* from *Ostrinia nubilalis* (**B**).

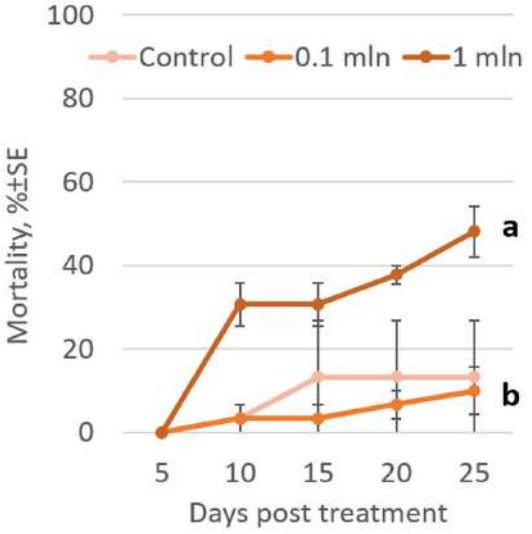

Figure 3. Mortality dynamics in *Aglais urticae* after feeding with *Nosema pyrausta* at the dosages of 0.1 or 1 million spores per second instar larva. Different letters indicate mortality curves with significantly different median lethal time at $p<0.01$. For raw data, see supplementary material, Table 1.

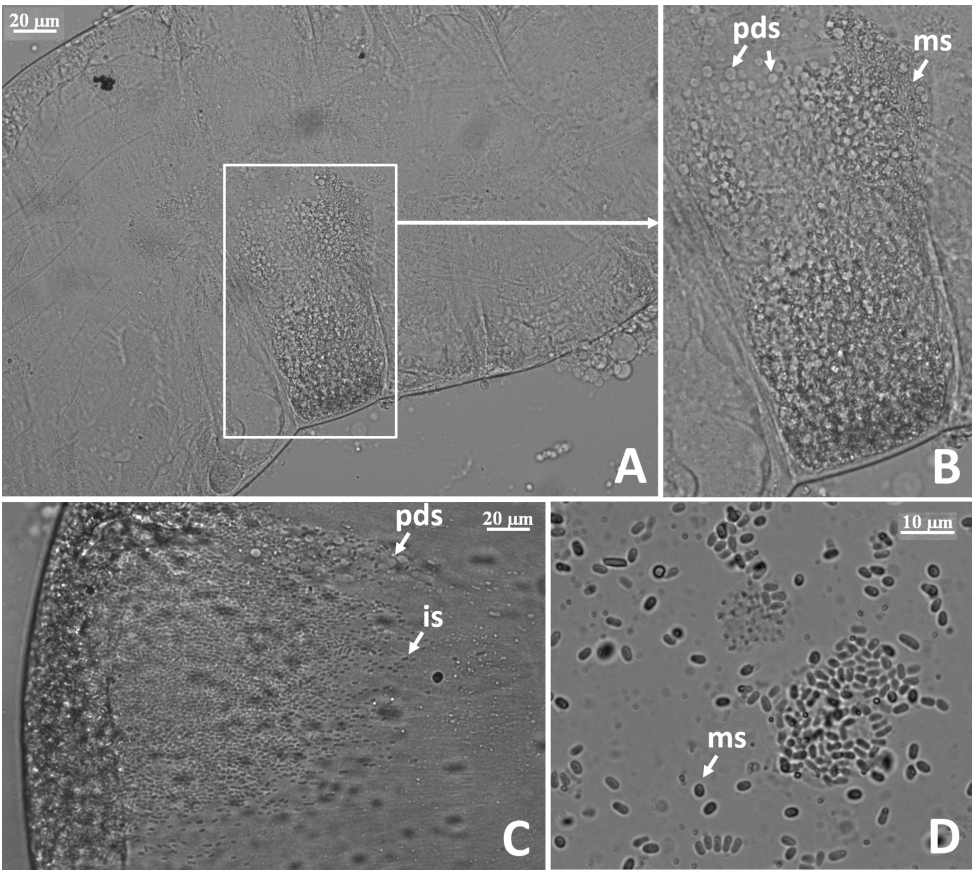

Figure 4. Bright field light microscopy of *Nosema pyrausta* infection in salivary glands (**A-C**) and adipose tissue (**D**) of the small tortoiseshell *Aglais urticae* with clearly seen prespore developmental stages (pds), immature (is) and mature spores (ms).

In Pyraloidea, the only species assayed within the frames of the present study was *P. interpunctella*, while data for other Pyralidae and Crambidae have been retrieved from published literature. In this stored grain pest, only a limited sample of 13 larvae was available for *N. pyrausta* treatment assay, and 12 in control. Early larval mortality within 10-14 d.p.t. reached as much as 25-33 % in both groups, followed by pupation of the survivors within 15-45 d.p.t. In one pupa from the *N. pyrausta* treatment group, formed by 34th d.p.t., few oval *Nosema*-like spores were detected. In another pupa from 43rd d.p.t., both few *Nosema*-like spores and multiple oval spores of smaller size in packets by 8 were observed, the latter referred to as the octospores (Fig. 5). The *Nosema*-like spores measured 3.5 × 1.7 µm, length/width ratio of 2.1 (N=18) while the octospores measured 2.5 × 1.5 µm, length/width ratio of 1.7 (N=18). None of the control insects were infected (N=12). Sequencing of SSU rRNA of both the *Nosema*-like spores and the octospores resulted in the identical reads, up to 802 bp long (#ON256647), with 99.4 % identity to the reference sequence of *Vairimorpha carpocapsae* (Paillot, 1938) (# AF426104).

Table 1. Raw data on the mortality of the small tortoiseshell, *Aglais urticae* after treatment of II instar larvae with *Nosema pyrausta* spores at two dosages

Treatment, spores/larva	Repetion #	Sample size	Number of insects perished on days 5-25				
			5	10	15	20	25
Control	1	10	0	1	1	0	0
	2	10	0	0	0	0	0
	3	10	0	0	2	0	0
0.1 mln	1	7	0	0	0	0	0
	2	7	0	0	1	0	0
	3	6	0	1	0	0	0
1 mln	1	10	0	3	0	1	2
	2	10	0	4	0	0	0
	3	9	0	2	0	1	1

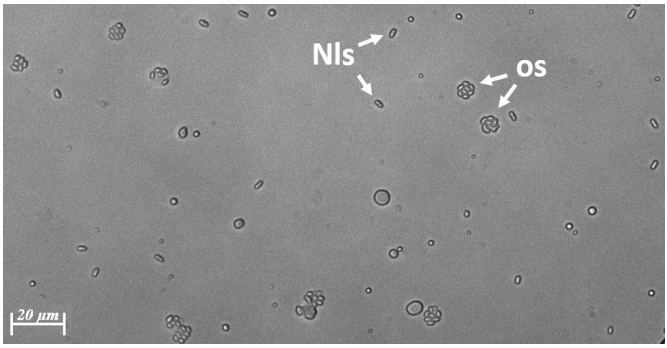

Figure 5. Bright field light microscopy of the *Nosema*-like spores (Nls) and the octospores (os) of *Vairimorpha* cf *carpocapsae* detected in *Plodia interpunctella*.

In Lasiocampoidea, *D. sibiricus* was the only species assayed. In control, as many as 6.7±4.55 % larvae perished by 3rd d.p.t., and mortality remained at this level throughout the 60 days of the experiment. When challenged with *N. pyrausta* spores (2 mln spores/larva), larval mortality steadily increased, reaching 33-71 % within 10th-50th d.p.t. (Fig. 6). LT50 was 30.0±13.73 days. Both perished (starting from 7th d.p.t.) and survived larvae were dissected, showing prevalence of the microsporidium at 92 % (N=26). Sequencing of amplicons obtained from five selected specimens (collected at 10th, 20th, 30th, 40th, and 50th d.p.t.) using microsporidia-specific primers confirmed the diagnosis of *N. pyrausta*.

In Bombycoidea, two representatives of two respective families, *B. mori* (Bombycidae) and *M. sexta* (Sphingidae) were assayed, both showing no changes in mortality levels and pupation speed as compared to control, and no infection with microsporidia.

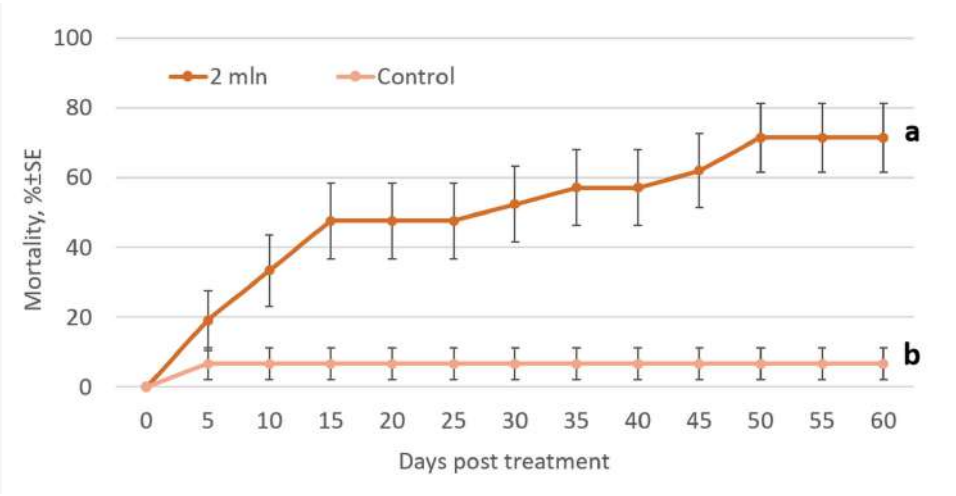

Figure 6. Mortality dynamics in *Dendrolimus sibiricus* after feeding with *Nosema pyrausta* at the dosage of 2 million spores per second instar larva. Different letters indicate mortality curves with significantly different median lethal time at $p<0.01$. For raw data, see supplementary material, Table 2.

Table 2. Raw data on the mortality of the Siberian moth, *Dendrolimus superans* after treatment of II instar larvae with *Nosema pyrausta* at the dosage of 2 mln spores/larva

Treatment	Repetition #	Sample size	Number of insects perished on days 5-60											
			5	10	15	20	25	30	35	40	45	50	55	60
Control	1	10	1	0	0	0	0	0	0	0	0	0	0	0
	2	10	1	0	0	0	0	0	0	0	0	0	0	0
	3	10	0	0	0	0	0	0	0	0	0	0	0	0
N. pyrausta	1	21	4	3	3	0	0	1	1	0	1	2	0	0

In Noctuoidea, representatives of two families were tested. Development of *H. cunea* from Erebidae was not affected by *N. pyrausta* treatment challenging and no infection was observed. Among Noctuidae, two species, *H. armigera* and *M. brassicae*, were not affected by *N. pyrausta* challenging. On the contrary, *S. exigua* displayed 100 % mortality of larvae challenged both with 1 and 0.1 mln spores/larva within 10-12 d.p.t., which was by an order of magnitude higher as compared to control. Tissues of perished larvae were filled with *N. pyrausta* spores, as shown by light microscopy and confirmed by PCR and sequencing as above.

Discussion

Susceptibility to *N. pyrausta* varied greatly across families and higher rank taxa of Lepidoptera. As many as 13 species assayed in the present study were found to be refractory, i.e. not able to support development of the microsporidium, including the representatives of Yponomeutoidea (3 species), Papilionoidea (4 species of Pieridae), Bombycoidea (4 species), and Noctuoidea (2 species). Resistant hosts, which could become infected only at low prevalence levels, were found in our previous studies among Pyralidae, namely *G. mellonella* (Tokarev et al. 2018), and Erebidae, represented by *L. dispar* (Kononchuk et al. 2021). Susceptible species with high proportion of specimens displaying developed infection include the type host *O. nubilalis* and its congeners (Grushevaya et al. 2020). In the present study, new cases of susceptible hosts are *A. urticae* and *D. sibiricus*. Moreover, in spite of occasional observation and the limited sample size (N=1), it can also be indicated that *E. forficalis* is susceptible to infection with *N. pyrausta*. Finally, highly susceptible species, displaying high proportion of insects with developed infection accompanied with high levels of early mortality, are also found across higher rank taxa. The new cases, in addition to the previously reported *L. sticticalis* (Malysh et al. 2021), include *A. io* and *S. exigua*.

From the presented dataset (Table 3), this can be judged that the susceptibility to a given species of a microsporidian parasite (exemplified here by *N. pyrausta*) does not follow a strict high-rank taxonomy-driven pattern in Lepidoptera. Indeed, susceptible hosts are found in four different superfamilies. Moreover, both the refractory and susceptible, as well as highly susceptible species could be found within a given superfamily (Papilionoidea) and family (Noctuidae).

Table 3. Summary of susceptibility data of lepidopteran insect hosts to *Nosema pyrausta* infection under experimental conditions

Insect host			Trophic preferences	Susceptibility to *Nosema pyrausta**	Reference
Higher-rank taxonomy	**Species**	**Wingspan, mm**			
Yponomeutoidea					
Yponomeutidae	*Yponomeuta evonymella*	16-25	Oligophagous: *Prunus*, *Sorbus*	-	This paper
	Yponomeuta malinellus	16-20	Oligophagous: *Malus*	-	This paper
Plutellidae	*Plutella xylostella*	15	Oligophagous: Brassicaceae	-	This paper

Insect host			Trophic preferences	Susceptibility to *Nosema pyrausta**	Reference
Higher-rank taxonomy	**Species**	**Wingspan, mm**			
Papilionoidea					
Pieridae	*Pieris brassicae*	45-60	Oligophagous: Brassicaceae	-	This paper
	Pieris rapae	45-50	Oligophagous: Brassicaceae	-	This paper
	Pieris napi	45-55	Oligophagous: Brassicaceae	-	This paper
	Aporia crataegi	75	Oligophagous: Rosaceae, *Salix*, *Betula*	-**	This paper
Nymphalidae	*Aglais urticae*	45-62	Monophagous: *Urtica*	+	This paper
	Aglais io	50-55	Oligophagous: *Urtica*, *Humulus*	++	This paper
	Junonia coenia	50-65	Oligophagous: *Plantaginaceae* etc.	+	Hall 1952
Pyraloidea					
Pyralidae	*Plodia interpunctella*	18-28	Grain-feeding	-**	This paper
	Galleria mellonella	30-41	Wax-feeding	±	Tokarev et al. 2018
Crambidae	*Ostrinia nubilalis*	26-30	Polyphagous, herbivorous	+	Grushevaya et al. 2020
	Ostrinia scapulalis	26-30	Polyphagous, herbivorous	+	Grushevaya et al. 2020
	Ostrinia furnacalis	26-30	Polyphagous, herbivorous	+	Grushevaya et al. 2020
	Loxostege sticticalis	24-29	Polyphagous, herbivorous	++	Malysh et al. 2021
	Evergestis forficalis	25-28	Oligophagous: Brassicaceae	+	This paper
Lasiocampoidea					
Lasiocampidae	*Dendrolimus sibiricus*	60-120	Oligophagous: *Abies sibirica* Ledebur, 1833, *Larix*	+	This paper
Bombycoidea					
Bombycidae	*Bombyx mori*	40-50	Monophagous: *Morus*	-	This paper
Sphingidae	*Manduca sexta*	95-120	Oligophagous: Solanaceae	-	This paper

Insect host			Trophic preferences	Susceptibility to *Nosema pyrausta**	Reference
Higher-rank taxonomy	**Species**	**Wingspan, mm**			
Noctuoidea					
Erebidae	*Hyphantria cunea*	25-42	Polyphagous, dendrophilic phyllophagous	-	This paper
	Lymantria dispar	37-62	Polyphagous, dendrophilic phyllophagous	±	Kononchuk et al. 2021
Noctuidae	*Helicoverpa armigera*	30-40	Polyphagous, herbivorous	-	This paper
	Spodoptera exigua	26-32	Polyphagous, herbivorous	++	This paper
	Mamestra brassicae	34-50	Polyphagous, herbivorous	-	This paper

* The insect species is referred to as a) refractory (-) when it develops no infection; b) resistant (±) when low proportion of insects with developed infection or low intensity of infection is observed; c) susceptible (+) when high proportion of specimens display developed infection; and d) highly susceptible (++) when high proportion of insects with developed infection is accompanied with high mortality level.

** The data should be treated as preliminary due to presence of naturally occurring microsporidia which might have influenced interactions of the test insects with the experimentally applied pathogen.

Our idea that the polyphagous lepidopteran larvae may be more susceptible to infection with microsporidia as compared to the oligophagous and the monophagous ones, does not find a confirmation as well. In particular, among polyphagous herbivorous noctuids, only one out of three tested species turned out to be (highly) susceptible, while the others two were refractory. On the other hand, monophagous *A. urticae*, as well as oligophagous *J. coenia*, *D. sibiricus*, and *E. forficalis*, were susceptible while oligophagous *A. io* was highly susceptible.

The size of the insect host species also does not seem to directly define levels of larval susceptibility to *N. pyrausta*, as can be seen from comparison of the smallest (refractory, *P. xylostella*) vs one of the largest (highly susceptible, *D. sibiricus*) of the tested insects. On the other hand, *P. xylostella* is known to be a very fast developing species (Andreeva et al. 2021) while *D. sibiricus* development is very slow (Kirichenko et al. 2009) and this might have been a clue to understanding why the former species is refractory, and the latter is highly susceptible. This assumption, however, can be opposed by the case of the fast developing *L. sticticalis* which is highly susceptible.

Results of microsporidia testing against insects originating from nature should always be treated with care and verified using appropriate methods of pathogen species identification to exclude the cases of natural infection, especially in isolates

and species with similar morphology, as shown for *A. crataegi* and *P. interpunctella*. Presence of a natural infection might have influenced interactions of the test insects with the experimentally applied microsporidium. On the one hand, inherent microsporidia may prevent from infection with an externally applied pathogen species due to competition. On the other hand, application of a non-specific microsporidium, which cannot infect the insect itself, may however induce activation of other microsporidia which are present in the covert form (Issi 1986). Anyway, such results should be considered cautiously and require additional studies using microsporidia-free colonies of the test insects.

In spite of observed infectivity of *N. pyrausta* to a broad range of Lepidoptera, it has not been reported in those or similar host species under natural conditions when extensive surveys were performed. For example, in the Illinois Natural History Survey (INHS) Collection, none of the 30 microsporidia samples from Crambidae, Pyralidae, Erebidae, Lasiocampidae, Noctuidae, and other families, showed reliable identity with *N. pyrausta* (Tokarev et al. 2020). Similarly, among 161 microsporidia-positive specimens of *S. litura* in Japan, none displayed infection with *N. pyrausta* (Shigano et al. 2015). Additionally, the survey of the Gebank database indicated absence of entries which can be identified as *N. pyrausta* from hosts other than *O. nubilalis*. The obtained data are in good agreement with the previous observations that physiological host range of microsporidia, observed under experimental conditions, is broader than the ecological one, observed in nature (Solter and Maddox 1998). In case of *N. pyrausta*, one logical explanation is the cryptic lifestyle of the host larvae, preventing them from excessive exchange of parasite burden with other Lepidoptera.

Conclusion

Experimental evidence of microsporidian host range is of great importance both for elucidation of factors which define insect interactions with eukaryotic microparasites and for evaluation of microsporidia potential for microbial pest management, including susceptibility of possible target pest species, suitability of certain laboratory models for large-scale propagation of the entomopathogens and their side effects on the non-target entomofauna. In this study, the ability of *N. pyrausta* to infect lepidopteran larvae was found to vary depending upon the insect species. Susceptible hosts are found within different taxonomic and ecological groups. High mortality levels induced in *S. exigua* suggest *N. pyrausta* could be a promising agent to control the pest. Meanwhile, spore yield indices observed in *A. urticae* indicate the prospects of in vivo mass production of the pathogen in this host species. By this research, we encourage other scientific groups to gather as much information as possible concerning host ranges of microsporidian species they work with.

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