

Spore yield of the microsporidium *Nosema pyrausta* (Paillot) Weiser, 1961 is influenced by the insect host rearing temperature and diet composition

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ABSTRACT: The microsporidium *Nosema pyrausta* (Paillot) Weiser, 1961 is an important mortality factor of the stem borers of the genus *Ostrinia* and may be used to control other lepidopteran pests. Rearing conditions (temperature regime and diet composition) of the laboratory host *Ostrinia furnacalis* (Guenée, 1854) (Pyraloidea: Crambidae) were compared in terms of their influence on *N. pyrausta* spore production, estimated as the mean spore number per host pupa. At lowered temperature (+20 °C), most of the experimental insects died at the larval stage, and in survived pupae, the microsporidium spore yield was below 8×10^6 spores/pupa. At the temperature of +24 °C, which is supposed to be the optimal for the insect laboratory culture exploited, *N. pyrausta* spore yield was about 50×10^6 spores/pupa. At increased temperature (+28 °C), the parasite spore yield was not higher than 28×10^6 spores/pupa. The mean spore yield values were significantly different between the variants of the three thermal regimes at 99% confidence level. The microsporidium spore production was further compared in insects fed with a standard corn flour-based diet vs modified diet (corn flour mixed with soybean flour at an equal proportion), reared at +24 °C. Insect feeding with the modified diet caused an increase of the average spore yield by 35% (70×10^6 vs 52×10^6 spores/pupa obtained using the standard diet). Similarly, the weight of the pupae reared on the modified diet was 33% higher (0.0721 g vs 0.0542 g on the standard diet). Both the spore yield and the pupal weight values were significantly different between the two diet types at 95% confidence level. This provides one logical explanation of the parasite spore production increase as conditioned by the insect host weight increment. These observations indicate that +24 °C is the optimal thermal regime for *N. pyrausta* spore production which can further be augmented by supplementing diet of infected *Ostrinia furnacalis* larvae with soybean flour.

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KEY WORDS: insect pathogen, laboratory cultivation, Lepidoptera, mass production, meridic diet, microbial control.

Споропродуктивность микроспоридии *Nosema pyrausta* (Paillot) Weiser, 1961 зависит от температуры содержания и состава корма насекомого-хозяина

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РЕЗЮМЕ: Микроспоридия *Nosema pyrausta* (Paillot) Weiser, 1961 — важный фактор смертности стеблевых мотыльков рода *Ostrinia* и может быть использована для биологической борьбы с другими чешуекрылыми вредителями. Условия выращивания (температурный режим и состав рациона) лабораторного хозяина *Ostrinia furnacalis* (Guenée, 1854) (Pyraloidea: Crambidae) сравнивали по их влиянию на спорообразование *N. pyrausta*, оцениваемое как среднее число спор на куколку хозяина. При пониженной температуре (+20 °C), большинство подопытных насекомых погибло на личиночной стадии, а у выживших куколок выход спор микроспоридий был ниже 8 млн. спор/куколку. При температуре +24 °C, которая рассматривается как оптимальная для данной лабораторной культуры насекомого, выход спор *N. pyrausta* составил около 50 млн. спор/куколку. При повышенной температуре (+28 °C) выход спор паразита не превышал 28 млн. спор/куколку. Различия между средними значениями выхода спор при разных температурных режимах были статистически достоверны при 99%-ном уровне вероятности. Далее сравнивали споропродуктивность микроспоридий в насекомых, которых выращивали при +24 °C на стандартном корме на основе кукурузной муки либо на модифицированном корме (кукурузная мука, смешанная с соевой мукой в равных пропорциях). Выращивание насекомых на модифицированном корме приводило к увеличению средней споропродуктивности на 35% (70 млн. против 52 млн. спор/куколку на стандартном корме). Аналогично, средний вес куколок на модифицированном корме был на 33% больше (0,0721 г против 0,0542 г на стандартном корме). Значения количества спор и веса куколок значительно различались для насекомых, выращенных на корме разного состава, на 95% уровне значимости. Таким образом, увеличение веса куколок служит логическим объяснением повышения продукции спор микроспоридий. Эти наблюдения показывают, что +24 °C — это оптимальный температурный режим для образования спор *N. pyrausta*, который может быть улучшен путём добавления в рацион заражённых гусениц *Ostrinia furnacalis* соевой муки.

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КЛЮЧЕВЫЕ СЛОВА: энтомопатоген, лабораторное культивирование, Lepidoptera, массовое производство, искусственная питательная среда, микробиологическая защита растений.

Introduction

Insect pest control in agriculture largely relies upon synthetic insecticide application which has a broad spectrum of unwanted consequences. Global application of insecticides is a direct way to chemical pollution of the environment, negatively affecting useful entomofauna and other non-target organisms. Meanwhile, the target insect pests develop resistance to xenobiotics which makes the most widely applied chemicals ineffective relatively fast and demands for the new substances to be introduced (Mallet, 1989; Sukhoruchenko, Dolzhenko, 2008; Muntyan *et al.*, 2014; Bass *et al.*, 2015; Wang *et al.*, 2018; Benkovskaya, Dubovskiy, 2020; Hafeez *et al.*, 2022). And though modern tendencies of novel pesticide design are declared to aim at increasing specificity toward pests while decreasing toxicity to non-target organisms and persistence in the environment (Inglesfield, 1989; Abubakar *et al.*, 2020; Sukhoruchenko *et al.*, 2020; Dolzhenko *et al.*, 2021), the safety of novel classes of synthetic insecticides is extensively debated (Jeschke, 2010; Kampfraath *et al.*, 2017; Sukhoruchenko *et al.*, 2018; Khan, Ahmad, 2019; Kumar, Kumar, 2019; Rezende-Teixeira *et al.*, 2022).

Despite the fact that the modern chemical pesticides can be safe when all application regulations are respected (Dolzhenko, Laptiev, 2021), the insecticides might be applied under suboptimal conditions. The examples of such situations include unsuitable abiotic factors (Amarasekare, Edelson, 2004; Rao *et al.*, 2021) and inappropriate developmental stages of the pests (Vivan *et al.*, 2017; Stejskal *et al.*, 2021), forcing the farmers to remarkably increase dosage and frequency of insecticide application. For these reasons, alternative means of pest control are of great importance, including entomopathogenic microorganisms which do not pollute the environment yet persist in pest populations causing either acute infections providing either relatively fast killing or chronic diseases affecting pest viability with transgenerational effects (Lewis *et al.*, 2006; Solter *et al.*, 2012; Litwin *et al.*, 2020; Deka *et al.*, 2021). Facultative biotrophs can be easily produced using cell-free media (Chan-Cupul *et al.*, 2010) while obligate intracellular parasites are deeply relied on metabolic and biosynthetic pathways

and can only be propagated in cells of their hosts either in vivo or in vitro (Arif, Pavlik, 2013; Sun, 2015; Lallo *et al.*, 2016, Yadav *et al.*, 2021).

Microsporidia are unicellular parasitic eukaryotes that play an important role in population dynamics of lepidopteran insects (Solter *et al.*, 2012; Malysh *et al.*, 2013; Frolov, 2019; Issi, 2020; Andreeva *et al.*, 2021). *Nosema pyrausta* (Paillot) Weiser, 1961 is a widespread pathogen of the European corn borer *Ostrinia nubilalis* (Hübner, 1796) (Pyraloidea: Crambidae), causing increased larval mortality (especially during hibernation), retarded larval development, reduced adult fecundity, and enhanced susceptibility of the insects to chemical and microbial insecticides (Siegel *et al.*, 1988; Pierce *et al.*, 2001; Lopez *et al.*, 2010). *Nosema pyrausta* is capable of vertical transmission through generations of its host (Siegel *et al.*, 1988; Grushevaya *et al.*, 2021). In North America, which is the secondary area of *O. nubilalis*, this pathogen is considered as an important species for natural regulation of this pest and for integrated pest management implications (Lewis *et al.*, 2009; Zimmermann *et al.*, 2016). In the primary area of *Ostrinia* spp., *N. pyrausta* is also widespread (Zimmermann *et al.*, 2016). However, its prevalence usually persists at low levels for decades (Malysh *et al.*, 2011; Grushevaya *et al.*, 2018) and epizootics are not frequent (Pelissie *et al.*, 2010). Notably, the genotyping of the isolates found in France and Russia showed identity to each other but differences from the isolate reported in North America (Tokarev *et al.*, 2015).

When a microsporidium is applied against insect species other than their natural host, its virulence indices may drastically change. In the case of *N. pyrausta*, though the majority of tested lepidopterans were less vulnerable as compared to *Ostrinia*, certain species turned out to be highly susceptible, including the polyphagous pests such as the beet armyworm *Spodoptera exigua* (Hübner, 1808) (Noctuoidea: Noctuidae) (Tokarev *et al.*, 2022) and the beet webworm *Loxostege sticticalis* (Linnaeus, 1761) (Pyraloidea: Crambidae). In the latter case, effective trans-generational transmission of the parasite followed by a gradual decrease of the insect fertility is also observed (Malysh *et al.*, 2021). This makes *N. pyrausta* a promising microbial control agent with capacity of long-

term impact on the pests. However, mass production of this microsporidium has not been studied in detail.

Though obligate intracellular parasites, such as baculoviruses and microsporidia, can be produced *in vitro* in cell cultures of their insect hosts for research purposes (Gisder *et al.*, 2011; Lallo *et al.*, 2016; Grzywacz, 2017), large scale propagation for practical applications can only be relied upon *in vivo* cultivation (Lacey *et al.*, 2015; Yadav *et al.*, 2021). Elucidation of conditions that affect the accumulation of infectious material of insect microsporidia is essential both for the understanding of parasite-host interactions laying in the basis of natural pest regulation (Becnel, Undeen, 1992; Goertz, Hoch, 2008) and for improvement of large-scale production of the pathogens. Studies aimed at maximization of microsporidia spore yield have been performed for the parasites of a number of invertebrate pests (Lai, Canning, 1983; Hostounsky, 1984; Maddox *et al.*, 2011; Zhang, Lecoq, 2021), including lepidopteran insects (Wilson, 1976; Pilley *et al.*, 1978; Nordin, 1981; Sedlacek *et al.*, 1985; Campbell *et al.*, 2007).

The goal of the present paper is to optimize the technology of *in vivo* *N. pyrausta* propagation in its laboratory host *Ostrinia furnacalis* (Guenée, 1854) (Pyraloidea: Crambidae) in order to increase the spore yield of the pathogen, using two approaches. The first approach is based upon assumption that insect immunity is suppressed at decreased temperatures (Kryukov *et al.*, 2018) thus facilitating the pathogen development and spore accumulation (Tokarev *et al.*, 2018). Another approach exploits a recently introduced modification of the meridic diet recommended for *Ostrinia* spp. cultivation (Frolov *et al.*, 2019). The idea of this experiment was based on the fact that the soybean is a leguminous crop rich in protein and is considered as one of the most preferred animal feeds (Asekova *et al.*, 2014). Substitution of 50% of the corn flour with the soybean flour was recommended for the corn borer (Frolov *et al.*, 2019) and changes in the dietary protein content affect fitness of both parasite and host (Ponton *et al.*, 2013; Pike *et al.*, 2019).

Materials and Methods

Insect source and rearing

Paper sheets with egg masses of *O. furnacalis* from a continuous laboratory culture were a kind gift of Prof. Jiang Xingfu (Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China). This culture has been routinely maintained in the Chinese laboratory at +24 °C (Zhou *et al.*, 1992) for more than 200 generations prior to shipping to St. Petersburg. In the beginning of October 2019, paper sheets with freshly laid eggs, packed in a carton box, were acquired from Chinese colleagues and transported by air as luggage (implying chilling for several hours in a luggage department of a passenger plane). The paper spots containing the eggs were excised and placed into 0.5 L glass jars with semi-artificial diet. The neonate larvae hatched within 3–4 days after delivery, corresponding to 7–8 days from the egg laying date. The delay in hatching, as compared to 3–5 days under normal conditions (Nafus, Schreiner, 1991; Chang *et al.*, 1998; Zhengyue, Mei-rong, 1998), was obviously due to chilling during transportation. The whole life cycle was maintained at +24 °C (Frolov *et al.*, 2019). The diet was composed of 13% corn flour, 4.8% yeast extract, 3% wheat bran, 1.6% agar-agar, 0.5% ascorbic acid, 0.2% benzoic acid, 0.2% Vanderzandt insect vitamin mixture (Sigma-Aldrich), 0.02% amoxiclav, 0.01% nystatin, and 0.01% kanamycin (Frolov *et al.*, 2019). The thickness of the diet layer equaled to 5 and 10 mm for younger and elder instar larvae, respectively. The jars were turned upside down due to negative geotaxis of larvae, so that the latter moved up to find the source of feed. The larvae were reared until pupation and the pupae were transferred to plastic tubes capped with moistened cotton plugs. The newly emerged adults in groups of 6–8 were kept for mating and oviposition in 0.5 L glass jars with paper inserts and cloth covers. A piece of cotton moistened with 2% sugar syrup was placed on the cover for additional feeding of the moths. The paper inserts with eggs were removed to initiate rearing of the next insect generation as above. Prior to the experiments, the adults and larvae from the stock culture were checked using light microscopy to ensure they are free from the microsporidia infections. Smears of inner tissues were examined using Carl Zeiss Axio 10 Imager M1 at 400× magnification.

Microsporidium source, rearing and identification

Spores of *N. pyrausta* were obtained from infected diapausing *O. nubilalis* larvae collected during the autumn of the previous year (2018) in the maize fields in Gulkevichi District of Krasnodar Area and perished during hibernation under laboratory condi-

tions. The spores were isolated from homogenized cadavers which proved to be microsporidia-positive using light microscopy as above. The homogenates were centrifuged at 1000 g for 5 min to pellet the spores. Then the pellets were repeatedly washed by resuspending in distilled water with a consequent round of centrifugation as above. The upper layer of cell debris was removed by a pipette and the lower part of the pellets contained the purified spores which are the heaviest fraction of the homogenate. The pelleted spore samples were stored under water layer at +4 °C. This “natural” isolate was propagated in *O. furnacalis* laboratory culture using alimentary infection of 2nd–3rd instar larvae followed by spore isolation from fully-grown larvae and pupae (Grushkevaya *et al.*, 2018). Prior to further experiments, a spore aliquot was used for DNA extraction and PCR using NbHKfor1 and NpyrHKrev1 primers specific to *N. pyrausta* hexokinase (Tokarev *et al.*, 2019) to confirm the species diagnosis. Total DNA was extracted using a simplified protocol of Sambrook *et al.* (1989) without addition of phenol, with adjustments in the volumes of DNA washing agents (Malysch *et al.*, 2019). Samples were homogenized in 100 µl of CTAB (Cetyl Trimethyl Ammonium Bromide). Then, 500 µl of CTAB + β-mercaptoethanol (final concentration 0.2%) were added and incubated at +65 °C for 2 hours, consequently washed with a mixture of chloroform and isoamyl alcohol (24:1), precipitated with ethanol and resuspended in 50 µl of ultra-purified water. The DNA solution was used for PCR analysis. The PCR mix consisted of 4 µl of 10- and 100-fold diluted DNA sample, 5 µl of DreamTaq Green PCR Master Mix (2X) (DreamTaq DNA polymerase, 2X DreamTaq Green buffer, dATP, dCTP, dGTP and dTTP (0.4 mM each), and 4 mM MgCl₂), and 1 µl of each of the primers (forward and reverse).

The amplification program included initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min, elongation at 72 °C for 1 min, and final elongation step of 72 °C for 5 min. The amplicons were visualized using electrophoresis in 1% agarose gels with GeneRuler Ladder Mix molecular weight marker, 75–20000 bp (Thermo Fisher Scientific). Amplicons in the gel of about 700 bp, were excised with a scalpel and frozen until further purification. 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, Gillepsie, 1979; Malferrari *et al.*, 2002). The purified amplicons were sequenced at the Core Centrum “Genomic Technologies, Proteomics and Cell Biology” of the All-Russian Institute of Agricultural Microbiology in forward direction by a standard method of chain termination (Sanger *et al.*, 1977) using an ABI Prism 3500 genetic analyzer. The

obtained sequencing chromatograms were analyzed using the BioEdit software (Hall, 1999). The search for homologous sequences in GenBank was performed on the NCBI server using the builtin BLAST utility using the megablast and blast algorithms (Altschul *et al.*, 1990).

Experimental infection

For the experimental infection, a pooled sample of spores adjusted to 10⁵ spores/larva (counted using a haemocytometer) was mixed with a portion of the standard diet and exposed to the groups, each containing 30 individuals of 2nd instar larvae in a tightly closed vial to prevent the larvae from escaping. The diet portion size was adjusted to ensure complete consumption by the larvae within 24 hrs. After consumption of the contaminated diet, every experimental group of insects (containing 26–28 larvae survived during the inoculation procedure) was split into three replicates of 8–10 specimens, each maintained in a glass Petri dish (11 cm in diameter) and fed with the meridic diet *ad libitum*. For the control group, insects were treated similarly using the same number of replicates and specimens per replicate but without addition of the microsporidium spores.

In the first series of the experiments, the microsporidium-treated and untreated (control) variants (each consisting of three replicates as above) were reared on standard diet at three temperature regimes: +20 °C, +24 °C, and +28 °C, respectively. In the second series of the experiments, there were two variants of the microsporidium-treated insects, one reared on standard diet and another reared on a modified diet where 50% of the corn flour was substituted by the respective amount of the soybean flour (i.e., 6.5% corn flour and 6.5% soybean flour in the diet recipe). The insects in these two variants were reared at +24 °C. In both assays, after 20–30 days of the experiment, the pupae were collected and counted, individual insect samples were homogenized using a plastic pestle adjusted to an Eppendorf tube in 1 ml of distilled water and spore counts were done with a haemocytometer at appropriate dilution.

Additionally, a group of neonate larvae hatched from an egg mass (20 larvae in total) laid by a single female was split in two subgroups of ten larvae each, reared at +24 °C. One subgroup was fed the standard and another the soybean modified diet. The pupae were collected and weighted using OHAUS PX224.

Statistical analysis

Data were analyzed using PAST 3 (Hammer *et al.*, 2001). As the data were normally distributed (Shapiro-Wilk W test, $P > 0.05$) but showed unequal homogeneity of variances (Levene’s test $P < 0.05$), two-tailed Welch’s *t*-test followed by Bonferroni adjustment (in case of multiple comparison) was

Table 1. Abbott's corrected survival levels of *Ostrinia furnacalis* infected with *Nosema pyrausta*, reared at three thermal regimes.

Таблица 1. Скорректированные по Эбботу уровни выживаемости *Ostrinia furnacalis*, инфицированных *Nosema pyrausta*, выращенных при трех температурных режимах.

Maintenance temperature	Sample size	Survival level, %	Chi-square (left-lower cells), p-value & Bonferroni's correction (right-upper cells); df = 1		
			+20 °C	+24 °C	+28 °C
+20 °C	30	33.3	–	0.0007 & 0.0021	0.0003 & 0.009
+24 °C	30	76.6	11.4	–	0.754 & 2.262
+28 °C	30	80.0	13.3	0.1	–

applied. The survival data were corrected to the control using the Abbott's formula (Abbott, 1925).

Results

The microsporidium used for the purposes of the present study showed 100% sequence identity to the respective Genbank record # MH669406 corresponding to *N. pyrausta* hexokinase (Tokarev *et al.*, 2019). This verifies the diagnosis of the parasite as *N. pyrausta*.

Survival levels of untreated *Ostrinia* larvae ranged between 60.0±5.78% (mean±standard error) and 83.3±3.33% when reared at the three thermal regimes, the values being not statistically significant. As for the microsporidia-treated variants, at +20 °C, the survival reached 20.0± 5.78%. This was significantly lower ($p < 0.01$) as compared to the survival levels of 60.0±6.67% at +24 °C and 63.3±8.82% at +28 °C. When survival levels were corrected with respect to the control, significant differences ($p < 0.001$) were found when the value observed at +20 °C was compared to the other two variants (Table 1).

Because most of the experimental insects died during the larval stage at +20 °C, only a few pupae ($N = 6$) could be analyzed to determine spore yield of *N. pyrausta* in this variant. The spore yield ranged between 2.2 and 17.5 × 10⁶ with the mean of 7.8±5.12 × 10⁶ and the median of 6.1 × 10⁶ spores/pupa, which was the lowest level observed in the study (Welch's test, $P < 0.003$ as compared to the other thermal regimes, Fig. 1). At +24 °C, the pathogen's spore yield ranged between 21.7 and 84.2 × 10⁶ spores/pupa, with the mean of 49.6±4.45 × 10⁶ and the median of 46.3 × 10⁶ spores/pupa ($N = 18$), which was the maximal value in this exper-

iment. At +28 °C, the parasite spore yield was in the range of 3.25–62.5 × 10⁶ spores/pupa with the mean of 27.9±4.53 × 10⁶ and the median of 25.8 × 10⁶ spores/pupa ($N = 17$), that was

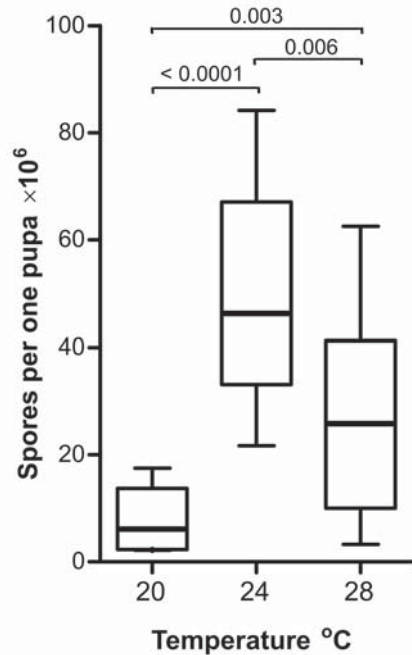


Fig. 1. *Nosema pyrausta* spore production in *Ostrinia furnacalis* pupae reared at different temperature regimes. Data presented as median, 25–75% quartiles and min and max values. Bonferroni corrected *P*-values of Welch's test indicated above the boxes.

Рис. 1. Спородуктивность *Nosema pyrausta* в куколках *Ostrinia furnacalis* при различных температурных режимах. Данные представлены медианной, 25–75% квартильными отклонениями, минимальным и максимальным значениями. *P* значения теста Уэлча с поправкой Бонферрони представлены сверху.

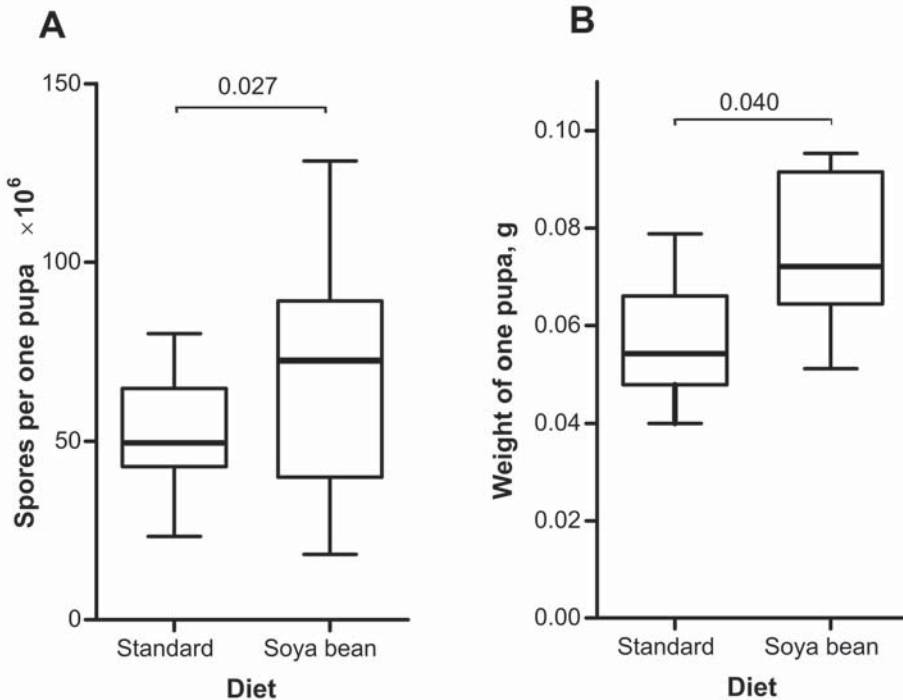


Fig. 2. *Nosema pyrausta* spore production in *Ostrinia furnacalis* pupae (A) and weight of pupae (B) reared on different diets. Data presented as median, 25–75% quartiles and min and max values. *P*-values of Welch's test indicated above the boxes.

Рис. 2. Споропродуктивность *Nosema pyrausta* в куколках *Ostrinia furnacalis* (A) и вес куколок (B) при выращивании на разном корме. Данные представлены медианной, 25–75% квартильными отклонениями, минимальным и максимальным значениями. *P* значения теста Уэлча представлены сверху.

significantly lower as compared to +24 °C (Welch's test, $P = 0.006$).

Nosema pyrausta spore production was further compared in microsporidia-treated insects fed with the standard corn flour-based diet vs modified diet, reared at +24 °C. In this experimental series, the spore yield ranged between 23.3×10^6 and 80×10^6 (mean — $52.0 \pm 3.51 \times 10^6$, median — 50.0×10^6) spores/pupa ($N = 20$) on the standard diet (Fig. 2). Meanwhile, the spore yield under rearing on the modified diet ($N = 19$) increased significantly (Welch's test, $P = 0.027$) and achieved the mean of $69.9 \pm 6.79 \times 10^6$ and the median of 72.5×10^6 spores/pupa.

To understand how addition of soybean flour affected fitness of premature *Ostrinia* stages, two groups of sibling larvae were reared either on standard or on modified diet to compare the

pupal weight. In the first subgroup (standard diet), the mean weight was 0.0571 ± 0.00481 g (median — 0.0542 g) while in the second (soybean modified diet), it reached 0.0752 ± 0.00612 g (median — 0.0721 g) ($N = 7$ for each group). In spite of the small sample size, 33% increase in weight of the pupae fed with the soybean diet was statistically significant (Welch's test, $P = 0.04$).

Discussion

Attempts to propagate *N. pyrausta* in substitute laboratory hosts, such as the greater wax moth, *Galleria mellonella* (Linnaeus, 1758) (Pyraloidea: Pyralidae) (Tokarev *et al.*, 2018), and the gypsy moth, *Lymantria dispar* (Linnaeus, 1758) (Noctuoidea: Erebidae) (Kononchuk *et al.*, 2021), were reported. Those, however,

were not successful as the tested species were only slightly vulnerable to the infection. Another substitute host, *L. sticticalis* is small-sized and has shown high susceptibility to the microsporidium which killed this host fast, being not able to accumulate spores in large quantities (Malysh *et al.*, 2021). Unless other suitable laboratory hosts are found, in vivo mass production of *N. pyrausta* can be relied upon the type host species, *O. nubilalis* and its congeners. In particular, the trilobed uncus *Ostrinia* are very closely related (Frolov *et al.*, 2012) and *N. pyrausta* is infective to *O. furnacalis* (Grushkevaya *et al.*, 2018). As opposed to the temporary culture of *O. nubilalis* originating from insects collected in field and maintained in the lab for three generations only (Grushkevaya *et al.*, 2018), *O. furnacalis* is being stably reproduced over numerous generations as the laboratory culture. This suggests better adaptation of the latter culture to laboratory rearing which uses the adjusted parameters of the thermal regime and artificial diet (Zhou *et al.*, 1992). Therefore, the *O. furnacalis* culture was chosen for the purposes of the present study.

Decreased temperature causes suppression of insect immunity to pathogens and in particular, facilitates infection of resistant hosts with microsporidia, as can be exemplified by *N. pyrausta* development in *G. mellonella* (Tokarev *et al.*, 2018). However, in a host being fairly susceptible to the infection, which is *O. furnacalis* in the present study, rearing at lowered temperature decreased both the host survival level and the parasite spore production in the survived pupae. The exploited laboratory culture seems to be adapted to the constant rearing at +24 °C (see Materials and Methods) and a decrease by 4 degrees may incur a significant stress. It can be assumed that under such unfavorable conditions the most heavily infected or the most susceptible individuals are killed by the pathogen at the larval stage and only insects receiving a lower dosage or those less prone to the infection could survive until pupation. Rearing at elevated temperatures is known to reduce spore loads of the insect microsporidia (Jouvenaz, Lofgren, 1984; Boohene *et al.*, 2003) and in the case of *O. furnacalis*, the spore yield was also notably decreased. These findings are in good agreement with previous observations in different parasite-host systems involving mi-

crosporidia and insects (Issi, 2020). For example, when *Vairimorpha necatrix* (Kramer, 1965) was propagated in two lepidopteran hosts (*Trichoplusia ni* (Hübner, 1800–1803) (Noctuoidea: Noctuidae) and *Helicoverpa (Heliothis) zea* (Boddie, 1850) (Noctuoidea: Noctuidae)), spore loads were decreased at both lowered and elevated temperatures as compared to the thermal regimes corresponding to the optimal range (Fowler, Reeves, 1975).

Modification of the meridic diet by addition of soybean allowed to substantially increase the microsporidium spore yield. Similar observations were made for *Vairimorpha apis* (Zander, 1909) (Rinderer, Elliott, 1977) and *Vairimorpha ceranae* (Fries, da Silva, Slemenda, Pieniazek, 1996) (Jack *et al.*, 2016) which showed increased spore loads in honey bees supplied with additional dietary protein source. Dietary protein may positively affect insect fitness in different ways and have multiple outcomes (Broadway, Duffey, 1986; Lee *et al.*, 2008; Orpet *et al.*, 2015; Barragan-Fonseca *et al.*, 2019; Wilson *et al.*, 2019). Within the frames of the present study, one simple explanation of the parasite spore load increase is the corresponding host pupal body weight increment in the insects fed with soybean modified diet as compared to the standard diet. In fact, microsporidia tend to inhabit the most metabolically active tissues of their insect hosts (Vávra, Lukeš, 2013) and *N. pyrausta* is not an exception, occupying the fat body and many other tissues. Hence, the simultaneous increase of the insect body weight and the microsporidium spore load is quite expected.

Thus, suboptimal thermal regimes for insect rearing are also unfavorable for *N. pyrausta* multiplication and should be avoided in mass production of its spores. Meanwhile, admixture of a protein-rich component (the soybean flour) caused 35% increase in the spore production and this approach should be exploited for large scale in vivo propagation of *N. pyrausta* in *Ostrinia*.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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