

SUSCEPTIBILITY OF *Lymantria monacha* AND *L. dispar* TO THE ENTOMOPATHOGENIC FUNGUS *Isaria fumosorosea* WIZE

PODLOŽNOST *Lymantria monacha* I *L. dispar* NA ENTOMOPATOGENU GLJIVU *Isaria fumosorosea* WIZE

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Summary:

Isaria fumosorosea is a cosmopolitan fungal species with a large host range including insects which are economically important pests in agriculture and forestry. In the current study the susceptibility of two forest pests *Lymantria monacha* and *L. dispar* to an isolate of the fungus *Isaria fumosorosea* obtained from *Hyphantria cunea* and re-isolates from *L. dispar*, *L. monacha* and *Dendrolimus pini* was investigated under laboratory conditions. Newly molted third instar larvae of *L. monacha* and newly molted second, third and fourth instar larvae of *L. dispar* were inoculated with fungal conidia by various methods: Larvae of *L. dispar* were either dipped directly into the conidia suspension (1×10^8 conidia/ml), or indirect methods were applied – by surface contact of larvae with conidial suspensions (1×10^8 , 1×10^9 , 3×10^7 , 3×10^8 , or 4×10^8 conidia/ml) placed on filter paper discs in Petri dishes or by contact with oak leaves or larch needles dipped in conidia suspension. Larvae in control variants were treated with water. Mortality of larvae was checked daily for 20 days and the efficacy of the fungus was corrected with mortality in the control treatments. It was found that larvae of both *Lymantria* – species can be infected experimentally with *Isaria fumosorosea*. Similar corrected efficacy of *Isaria fumosorosea* for the third instars larvae of *L. dispar* (12.37 %) and for *L. monacha* (12.66 %) was found when 1×10^8 conidia/ml of the isolate from *H. cunea* was applied on filter paper. The highest corrected efficacy of *Isaria fumosorosea* for *L. dispar* larvae was 60.0 % when 1×10^9 conidia/ml of the isolate from *H. cunea* was applied on filter paper. A corrected efficacy of 27.85 % was recorded for *L. monacha* when 4×10^8 conidia/ml of re-isolate from *L. dispar* were applied on larch needles. Our results show that *L. dispar* and *L. monacha* larvae are within the psihological host range of the used *Isaria fumosorosea* isolate from *H. cunea* and re-isolates obtained from infected larvae of *D. pini*, *L. monacha* and *L. dispar*, however their susceptibility is low. Indirect treatment by surface contact of host larvae with fungal conidia caused higher efficacy of mycosis than dipping into the suspension.

KEY WORDS: *Isaria fumosorosea*, *Lymantria monacha*, *Lymantria dispar*, bioassays

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Introduction

Uvod

Isaria fumosorosea Wize is a well-known entomopathogenic fungus with a worldwide distribution and a relatively wide host range which makes it an interesting agent for the development of biocontrol methods (Zimmermann 2008; Hunter et al. 2011). For more than 30 years, it was named *Paecilomyces fumosoroseus* and recently transferred to the genus *Isaria* (Samson 1974; Luangsa-ard et al. 2004, 2005; Gams et al. 2005; Hodge et al. 2005; Sung et al. 2007). *Isaria fumosorosea* has been isolated from many arthropods, mainly Lepidoptera, from air, water, plants, and often from soil samples (Meyer et al. 2008; Zimmermann 2008; Tkaczuk et al. 2011). In the catalogue of the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF) (Humber and Hansen 2005) strains of *Isaria fumosorosea* are listed from 27 different countries, comprising North America, Central America, South America, Europe, Africa, Australia, and Asia. *Isaria fumosorosea* is regarded as a species complex, and various strains are successfully used for biocontrol of several pest insect species, for example whiteflies, thrips, aphids, and spider mites. It's application for whitefly control, for example, started in 1990 with an isolate from Apopka, Florida (later named PFR 97, Apopka strain). This strain was also highly virulent to sweet potato whitefly and other pests (Osborne and Landa 1992, 1994; Landa et al. 1994; Stauderman et al. 2012). De Faria and Wraight (2007) assembled information about mycopesticides and disclosed that the most common among the presented 171 products were mycoinsecticides and mycoacaricides based on *Beauveria bassiana* (33.9 %), *Metarhizium anisopliae* (33.9 %), *Isaria fumosorosea* (5.8 %), and *B. brongniartii* (4.1 %).

Because of the high interest for *Isaria fumosorosea*, it's biology, ecology, natural occurrence and geographical distribution, host range, production of metabolites and effects of abi-

otic and biotic factors on the fungus are well studied (Avery et al. 2010). Furthermore, the use of this species in biocontrol in laboratory and field experiments (Feng et al. 2004; Pineda et al. 2007; Daniel and Wyss 2009), including their effects on non-target organisms (Tounou et al. 2003), were investigated by different authors and discussed by Zimmermann (2008).

The aim of our study was to determine the efficacy of an isolate of *Isaria fumosorosea*, isolated from pupae of *Hypphantria cunea* Drury in Georgia and re-isolates from *Dendrolimus pini* L., *Lymantria monacha* L. and *L. dispar* L. and to evaluate their potential as biological control agents of the forest pest insects *L. monacha* and *L. dispar* under laboratory conditions.

Materials and Methods

Materijali i metode

Lymantria monacha and *L. dispar* larvae were used for the experiments. First instar *L. monacha* larvae were collected in May 2013 from pine trees in the vicinity of Biebersdorf (region of Forest District Lieberose, Southern Brandenburg) and second and third instar larvae were collected in June in the vicinity of Staakow (region of Forest District Lieberose, Southern Brandenburg). Material was transferred to the laboratory of the University for Sustainable Development at Eberswalde. *L. dispar* larvae originated from a laboratory strain of the insect (New Jersey standard-strain from USA).

Four isolates of *Isaria fumosorosea* were used in the bioassays: One original isolate of *Isaria fumosorosea* from *Hypphantria cunea* found in Georgia (ARSEF access no. 10244), and three re-isolates from *D. pini*, *L. monacha* and *L. dispar*. Re-isolates were obtained after contamination of *D. pini*, *L. monacha* and *L. dispar* larvae with conidia of the original isolate of *Isaria fumosorosea* in the laboratory and isolation of new fungal isolates from dead hosts into pure cultures

Table 1. Bioassays with *I. fumosorosea* isolates against larvae of *Lymantria monacha* and *Lymantria dispar*

Tablica 1. Biološka ispitivanja izolata *I. fumosorosea* na ličinkama *Lymantria monacha* i *Lymantria dispar*

Variant Varijanta	Inoculated insect Inokulirani insekt	Larval stage Larvalni stadij	Concentration (conidia/ml) Koncentracija (konidija/ml)	Mode of infection Način infekcije	source of isolate Izvor izolata
Lm-v-1	<i>L. monacha</i>	3 rd instar	1x10 ⁸	Filter paper disk	<i>H. cunea</i>
Lm-v-2	<i>L. monacha</i>	3 rd instar	1x10 ⁹	Larch needles	<i>L. monacha</i>
Lm-v-3	<i>L. monacha</i>	3 rd instar	4x10 ⁸	Larch needles	<i>L. dispar</i>
Ld-v-1	<i>L. dispar</i>	2 nd instar	1x10 ⁸	Filter paper disk	<i>H. cunea</i>
Ld-v-2	<i>L. dispar</i>	3 rd instar	1x10 ⁸	Filter paper disk	<i>H. cunea</i>
Ld-v-3	<i>L. dispar</i>	3 rd instar	1x10 ⁹	Filter paper disk	<i>H. cunea</i>
Ld-v-4	<i>L. dispar</i>	3 rd instar	1x10 ⁸	Dipping	<i>H. cunea</i>
Ld-v-5	<i>L. dispar</i>	2 nd instar	1x10 ⁸	Filter paper disk	<i>L. dispar</i>
Ld-v-6	<i>L. dispar</i>	3 rd instar	4x10 ⁸	Oak leaves	<i>L. dispar</i>
Ld-v-7	<i>L. dispar</i>	3 rd instar	3x10 ⁷	Filter paper disk	<i>D. pini</i>
Ld-v-8	<i>L. dispar</i>	4 th instar	3x10 ⁸	Filter paper disk	<i>D. pini</i>

on SDAY. The isolates were cultured for 15 days on slopes of SDAY in tubes and on Petri dishes at 22°C, and obtained conidia were washed down with sterilized water. The concentrations of conidia were determined by enumeration in a Thoma chamber. Insects were inoculated with conidia using several methods (Table 1). Larvae of *L. dispar* were either dipped directly into the conidia suspension (1×10^8 conidia/ml), or indirect methods were applied: a) surface contact of larvae with 1 ml of conidial suspensions (1×10^8 , 1×10^9 , 3×10^7 , 3×10^8 , or 4×10^8 conidia/ml) placed on filter paper discs (90 mm in diameter) in Petri dishes (Draganova and Staneva 1990) or b) contact with oak leaves or larch needles dipped in conidia suspension. Larvae in control variants were treated with water. Second, third and fourth instar larvae of *L. monacha* and *L. dispar* were used in the experiments. In total, 11 variants were performed with 20 larvae per repetition, with 4 repetitions in each variant. The acronyms for variants are presented in Table 1. All larvae were kept under laboratory conditions at 25 ± 2 °C, 60 ± 5 % R.H and 12:12 h L: D in a climate chamber (Percival Inc.). *Lymantria monacha* larvae were fed with larch needles. Larvae of *Lymantria dispar* were reared on artificial diet of Bell et al. (1981) or fed with oak leaves in case of treatment of oak leaves. Mortality of larvae was checked daily for 20 days and the efficacy of the fungus was corrected with mortality in the control treatments and calculated according to Schneider-Orelli's formula (Püntener 1981).

Results and Discussion

Rezultati i rasprava

The results of the conducted studies are shown in Fig. 1, 2, 3 and 4.

It is evident that *L. dispar* and *L. monacha* larvae are within the host range of the used *Isaria fumosorosea* isolate from *H. cunea* and the re-isolates obtained from infected larvae of *D. pini*, *L. monacha* and *L. dispar*, but their susceptibility is rather low. The mortality due to mycoses showed a slow increase in all variants and was below 20 % with the exception of the mortality in the variant Ld-v-3 (Fig.1). The cumulative daily mortality due to mycosis in this variant increased to 41.25 ± 12.26 on the 4th day and to 60.00 % ± 12.26 on the 11th day after the inoculation with 1×10^9 conidia/ml of the Georgian isolate of *Isaria fumosorosea*.

Although insects were inoculated with conidial suspensions with very high concentrations (1×10^9 conidia/ml), the mortality 20 dpi didn't exceed 60 % (Fig. 1, 2). In experiments with third instar *L. dispar* larvae an increase of the concentration of the conidial suspensions of the same isolate (Georgian isolate of *Isaria fumosorosea* isolated from *H. cunea*) from 1×10^8 to 1×10^9 conidia/ml resulted in higher efficacy – from 12.37 ± 4.40 in the variant Ld-v-2 to 60.00 % ± 12.26 in the variant Ld-v-3 (Fig. 2).

According to Keller and Zimmermann (1989) the concentration of infective material necessary to initiate infection depends largely on the host and the pathogen. In our study we observed that larvae still living after 20 days post inoculation in all variants were not infected. They successfully completed their metamorphosis and turned into pupae. This is an evidence of low susceptibility of both *Lymantria* species to the Georgian isolate and three re-isolates of *Isaria fumosorosea*.

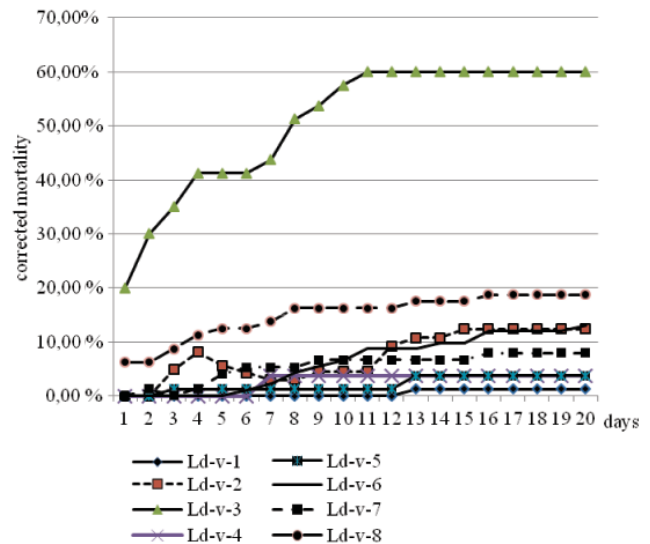


Figure 1 Cumulative daily mortality of *Lymantria dispar* larvae due to mycosis (in percent, corrected with control treatment mortality) (SD-Ld-v-1 = ± 0.63 ; SD-Ld-v-2 = ± 4.40 ; SD-Ld-v-3 = ± 12.26 ; SD-Ld-v-4 = ± 1.76 ; SD-Ld-v-5 = ± 1.41 ; SD-Ld-v-6 = ± 4.93 ; SD-Ld-v-7 = ± 2.67 ; SD-Ld-v-8 = ± 4.08)

Slika 1. Kumulativni dnevni mortalitet vrste *Lymantria dispar* uzrokovanih mikozom (u postotku, korigiran s mortalitetom kontrolnog tretmana) (SD-Ld-v-1 = ± 0.63 ; SD-Ld-v-2 = ± 4.40 ; SD-Ld-v-3 = ± 12.26 ; SD-Ld-v-4 = ± 1.76 ; SD-Ld-v-5 = ± 1.41 ; SD-Ld-v-6 = ± 4.93 ; SD-Ld-v-7 = ± 2.67 ; SD-Ld-v-8 = ± 4.08)

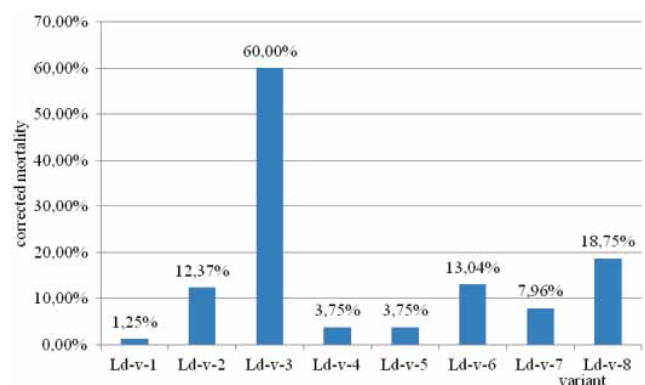


Figure 2 Corrected efficacy of *Isaria fumosorosea* isolates against larvae of *L. dispar* 20 dpi (SD-Ld-v-1 = ± 0.63 ; SD-Ld-v-2 = ± 4.40 ; SD-Ld-v-3 = ± 12.26 ; SD-Ld-v-4 = ± 1.76 ; SD-Ld-v-5 = ± 1.41 ; SD-Ld-v-6 = ± 4.93 ; SD-Ld-v-7 = ± 2.67 ; SD-Ld-v-8 = ± 4.08)

Slika 2. Korigirana uspešnost izolata vrste *Isaria fumosorosea* na ličinkama *L. dispar* 20 dpi (SD-Ld-v-1 = ± 0.63 ; SD-Ld-v-2 = ± 4.40 ; SD-Ld-v-3 = ± 12.26 ; SD-Ld-v-4 = ± 1.76 ; SD-Ld-v-5 = ± 1.41 ; SD-Ld-v-6 = ± 4.93 ; SD-Ld-v-7 = ± 2.67 ; SD-Ld-v-8 = ± 4.08)

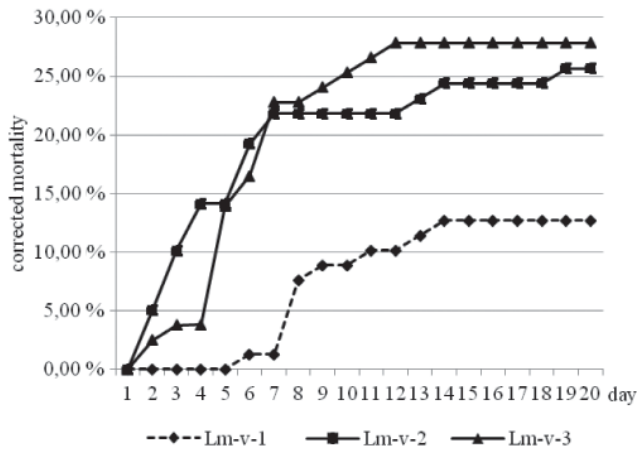


Figure 3 Cumulative daily mortality of *Lymantria monacha* larvae due to mycosis (in percent, corrected with control treatment mortality) (SD-Lm-v-1 = ± 5.52 ; SD-Lm-v-2 = ± 7.16 ; SD-Lm-v-3 = ± 10.04)

Slika 3. Kumulativni dnevni mortalitet ličinki vrste *Lymantria monacha* uzrokovanih mikozom (u postotku, korigiran s mortalitetom kontrolnog tretmana) (SD-Lm-v-1 = ± 5.52 ; SD-Lm-v-2 = ± 7.16 ; SD-Lm-v-3 = ± 10.04)

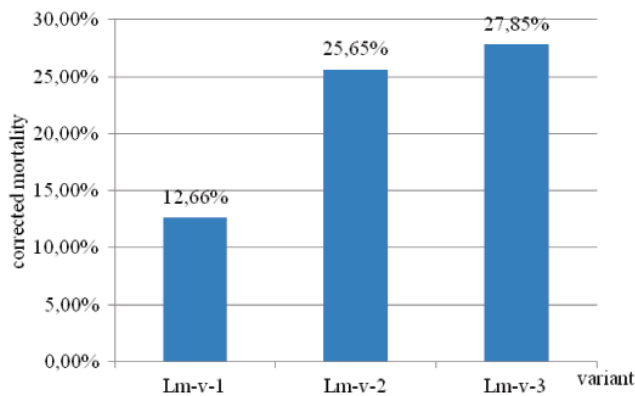


Figure 4 Efficacy of *Isaria fumosorosea* isolates against the third instar larvae of *L. monacha* (corrected with control treatment mortality) (SD-Lm-v-1 = ± 5.52 ; SD-Lm-v-2 = ± 7.16 ; SD-Lm-v-3 = ± 10.04)

Slika 4. Uspješnost izolata vrste *Isaria fumosorosea* na ličinkama III. stadija vrste *L. monacha* (korigiran s mortalitetom kontrolnog tretmana) (SD-Lm-v-1 = ± 5.52 ; SD-Lm-v-2 = ± 7.16 ; SD-Lm-v-3 = ± 10.04)

Like the other entomopathogenic fungi, *Isaria fumosorosea* infects its host mainly through the external cuticle penetrating by mechanic pressure of germ tubes and releasing hydrolytic enzymes (St. Leger et al. 1986; Draganova 1988; Hajek and St. Leger 1994; Ali et al. 2010; Augustyniuk-Kram 2011; Augustyniuk-Kram and Kram 2012).

As the aim of this part of the study was to investigate how different modes of exposure of host larvae to fungal conidia affected the mortality caused by the mycosis, larvae were dipped into the suspension, or they were made to walk on filter paper discs in Petri dishes soaking with conidial suspension, or they were made to walk on oak leaves or larch needles sprayed with suspension. The dipping of *L. dispar* larvae into the suspension (variant Ld-v-4) resulted in the lowest efficacy of 3.75 % ± 1.76 in comparison to the vari-

ants with the other modes of inoculation where efficacy was 12.37 % ± 4.40 in the variant Ld-v-2 (indirect treatment by contact with conidia on filter paper discs) and 13.04 % ± 4.93 in the variant Ld-v-6 (indirect treatment by contact with conidia on oak leaves), respectively (Fig. 2). We conclude that indirect exposure of larvae through surface contact of host with conidia caused higher efficacy of mycosis.

According to Dunlap et al. (2007) susceptible insects exposed to blastospores and conidia of *Isaria fumosorosea* showed declined growth and high levels of mortality. We found that the two lepidopteran hosts species were not susceptible to the examined *Isaria fumosorosea* isolates. Furthermore, although the larvae were inoculated with highly concentrated suspensions placed on filter papers, the efficacy was rather low.

Bioassays with *Beauveria bassiana* (Bals. – Criv.) Vuill. and larvae of different insect pests (*Ostrinia nubilalis* Hb, Lepidoptera; *Leptinotarsa decemlineata* Say, Coleoptera) showed that the age of the inoculated larvae is of importance for the efficacy (Feng et al. 1985; Draganova 2000). Contrary to the expectation that the susceptibility of larvae will decrease with age as described by Keller and Zimmermann (1989), in our experiments younger instars of *L. dispar* were more tolerant to mycosis caused by *Isaria fumosorosea* isolates (variants Ld-v-1 vs Ld-v-2 and Ld-v-5 vs Ld-v-6). Similar observations were made by Ferron (1967) who showed that larvae of *Melolontha melolontha* F. were more sensitive to *Beauveria brongniartii* (Saccardo) Petch with increased age.

The bioassays with *L. monacha* show that 3rd instar larvae have a low susceptibility to mycosis caused by *Isaria fumosorosea* isolated from *H. cunea* and to two other re-isolates of the fungus (from *L. monacha* and from *L. dispar* (Fig. 3)). The development of the infection was slow in the variant Lm-v-1 with a small increase in efficacy on dpi 7. In contrast, the mortality in the variants Lm-v-2 and Lm-v-3 increased from 14.12 % ± 7.16 and 13.92 % ± 10.04 on dpi 5 to 21.80 % ± 7.16 and 22.78 % ± 10.04 on dpi 7, respectively.

According to Goettel et al. (1990) and Lecheva and Draganova (1998), fungal isolates are more virulent to their initial hosts. In our experiments, discrepant results concerning the initial host and the virulence of the isolates to *L. dispar* and *L. monacha* larvae were obtained (Fig. 2, 4). A comparison of the variants Lm-v-2 (inoculation with *Isaria fumosorosea* isolated from *L. monacha* larvae) vs Lm-v-1 (inoculation with *Isaria fumosorosea* isolated from *H. cunea*) (Fig. 4) and the variants Ld-v-5 (inoculation with *Isaria fumosorosea* isolated from *L. dispar* larvae) vs Ld-v-1 (inoculation with *Isaria fumosorosea* isolated from *H. cunea*) (Fig. 2) confirms the findings of the cited authors. The efficacy in the variants reached values of 25.65 % ± 7.16 vs 12.66 % ± 5.52 and 3.75 % ± 1.41 vs 1.25 % ± 0.63 , respectively. However, when comparing the efficacy in the vari-

ants Lm-v-2 vs Lm-v-3, the results are contrary. The efficacy was minor in inoculation of *L. monacha* larvae with conidia of a re-isolate obtained from the initial host ($25.65\% \pm 7.16$ vs $27.85\% \pm 10.04$).

In bioassays with different isolates of *B. bassiana* against *L. dispar* larvae, Draganova et al. (2013) describe that host larvae were tolerant to mycosis caused by the tested isolates, which is in accordance with the findings reported here.

We found that the mycoses caused by *Isaria fumosorosea* or *Isaria farinosa* show a remarkably lower lethal effect to forest pests compared to mycoses caused by *B. bassiana* or *M. anisopliae*, as has been shown in laboratory studies of Nedveckyte et al. (2011) with larvae of *Bupalus piniaria* L. (Lepidoptera: Geometridae) and the entomopathogenic fungi *Isaria farinosa*, *B. bassiana* and *M. anisopliae*. Although some hyphomycete species were found in natural populations of *L. dispar*, and *Paecilomyces farinosus* was the most common (however in very low infection levels of 4.6 % to 12.2 %), the fungal pathogen with the highest virulence for *L. dispar* larvae is the entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu et Soper (Hajek et al. 1997). *E. maimaiga* is a pathogen with high specificity, and its life cycle is perfectly synchronized with the life cycle of its insect host (Hajek 1999).

According to Zimmerman (2008), *Isaria fumosorosea* should be applied in combination with other entomopathogenic fungi, such as *Lecanicillium* and *Beauveria*. This suggestion will be considered in our future experiments. Due to the capacity of *Isaria fumosorosea* to cause natural epizootics and the rising commercial demand for bioproducts based on this fungus (Zimmermann 2008), further experiments should be directed to the development of effective laboratory trials.

Conclusions

Zaključak

Our results show that *L. dispar* and *L. monacha* larvae are within the physiological host range of the used *Isaria fumosorosea* isolate from *H. cunea* and re-isolates obtained from infected larvae of *D. pini*, *L. monacha* and *L. dispar*, however their susceptibility is low. Indirect exposure through surface contact of host larvae with fungal conidia caused higher efficacy of mycosis.

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Zahvale

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Sažetak:

Isaria fumosorosea je kozmopolitska vrsta gljive s velikim brojem domaćina, a među njima se nalaze insekti koji imaju veliko ekonomsko značenje kao važni štetnici za poljoprivredu i šumarstvo. U ovome istraživanju podložnost prema izolatima gljive *Isaria fumosorosea* istraživana je pod laboratorijskim uvjetima na dvije vrste šumskih štetnika, *Lymantria monacha* i *L. dispar*, a izolati su dobiveni iz vrste *Hyphantria cunea* te iz reizolata od *L. dispar*, *L. monacha* i *Dendrolimus pini*. Zaraza je obavljena na tek presvučenom III. larvalnom stadiju vrste *L. monacha* i na tek presvučenim II., III., i IV. larvalnom stadiju vrste *L. dispar*. Inokulacija s konidijama gljive odradena je različitim metodama: larve vrste *L. dispar* su izravno umočene u suspenziju konidija (1×10^8 konidija/ml) ili su korištene neizravne metode – površinski kontakt larvi sa suspenzijom konidija (1×10^8 , 1×10^9 , 3×10^7 , 3×10^8 ili 4×10^8 konidija/ml) stavljenih na filter papir u Petrijeve posude, ili kontakt s lišćem hrasta ili iglica ariša umočenih u suspenziju konidija. Larve u kontrolnom tretmanu tretirane su s običnom vodom. Mortalitet larvi je svaki dan provjeravan u razdoblju od 20 dana, a uspješnost gljive korigirana je s mortalitetom u kontrolnom tretmanu. Pokusi zaraze vrstom *Isaria fumosorosea* na obje vrste iz roda *Lymantria*, utvrdili su da postoji mogućnost zaraze ovom gljivom. Slična korigirana uspješnost vrste *Isaria fumosorosea* pronađena je kada su III. larvalni stadiji vrsta *L. dispar* (12,37 %) i *L. monacha* (12,66 %) bili stavljeni na filter papir sa suspenzijom vrijednosti 1×10^8 konidija/ml iz vrste *H. cunea*. Najveća korigirana uspješnost (60 %) bila je kada je izolat iz *H. cunea* na filter papiru bio korišten za larve *L. dispar* u suspenziji od 1×10^9 konidija/ml. Za vrstu *L. monacha* korigirana uspješnost od 27,85 % zabilježena je kada je korištena iglica ariša umočena u suspenziju od 4×10^8 konidija/ml iz reizolata *L. dispar*. Rezultati ovoga istraživanja pokazuju da larve vrsti *L. dispar* i *L. monacha* pripadaju među moguće domaćine izolata gljive *Isaria fumosorosea* dobivenih iz *H. cunea* i reizolata dobivenih iz zaraženih larvi vrsta *D. pini*, *L. monacha* i *L. dispar*, iako je njihova podložnost vrlo niska. Neizravni tretmani površinskim kontaktom larvi s konidijama gljive, uzrokovoli su veću uspješnost razvoja mikoze, za razliku od umakanja u suspenziju.

KLJUČNE RIJEČI: *Isaria fumosorosea*, *Lymantria monacha*, *Lymantria dispar*, biološka ispitivanja