Preliminary Study of a Georgian Isolate of *Isaria fumosorosea* Wize against *Lymantria monacha* and *L. dispar*

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**Abstract:**

We studied the susceptibility of *Lymantria monacha* and *L. dispar* to the isolate of *Isaria fumosorosea* from *Hyphantria cunea* and re-isolates from *L. dispar* and *L. monacha*. It was found that larvae of both species were tolerant to the mycosis induced by *I. fumosorosea*. Corrected efficacy of *I. fumosorosea* for *L. dispar* was 30.0% when $1.10^9$ conidia/ml of the isolate from *H. cunea* was applied on filter paper. Similar efficacy of 28.8% was recorded for *L. monacha* when $4.10^8$ conidia/ml of re-isolate from *L. dispar* were applied on larch needles.

**Key Terms:** *Isaria fumosorosea*, *Lymantria monacha*, *Lymantria dispar*, bioassays.

**Introduction:**

*Isaria fumosorosea* Wize is a well-known entomopathogenic fungus. It has a worldwide distribution and a relatively wide host range which makes it an interesting agent for the development of biocontrol methods (Zimmermann 2008). 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). Isolates of *I. fumosorosea* species complex have been isolated from many arthropods, mainly Lepidoptera, from air, water, plants, often from soil. In the catalogue of the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF) (Humber and Hansen 2005) strains of *I. fumosorosea* are listed from 27 different countries, comprising North America, Central America, South America, Europe, Africa, Australia, and Asia. *I. fumosorosea* is regarded as a species complex, and various strains are successfully used for biocontrol of several pest insects. Its application for whitefly control started in about 1990, when one strain was isolated in 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).

Because of the high interest for *I. fumosorosea* its biology, ecology, natural occurrence and geographical distribution, host range, production of metabolites and effect of abiotic and biotic factors are well studied. Furthermore, the use of this species in biocontrol experiments in the laboratory and field including their effects on non-target organisms were investigated by different authors and discussed by Zimmermann (2008).

The aim of our study was to determine the efficacy of an isolate of *I. fumosorosea*, isolated from pupae of *Hyphantria 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 pest insects *L. monacha* and *L. dispar* under laboratory conditions.

**Materials and Methods:**

*Lymantria monacha* and *L. dispar* larvae were used for the experiment. 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 University of...
Applied Sciences at Eberswalde. *L. dispar* larvae originated from a laboratory strain of the insect (New Jersey standard-strain from USA).

Four isolates of *I. fumosorosea* were used in bioassay: *I. fumosorosea* isolated from *Hyphantria cunea* in Georgia (ARSEF access no. 10244), re-isolates from *D. pini*, *L. monacha* and *L. dispar*. They 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 of conidia in a Thoma chamber. Insects were treated by several methods: direct method - *L. dispar* larvae were dipped into the suspension and indirect methods - by surface contact of larvae with 1 ml of conidial suspensions (1.10^8, 1.10^7, 3.10^7, 3.10^8, 10^9 conidia/ml) placed on filter paper discs (90 mm in diameter) in Petri dishes (Draganova and Staneva 1990) or by contact with oak leaves or larch needles dipped in 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 treatments were performed (Table 1). All larvae were kept under laboratory conditions at 25 ± 2 °C, 60 ± 5% R.H and 12:12 h L: D. Mortality of larvae was checked daily for 20 days and efficacy of the fungus corrected with mortality in the control treatments was calculated according to Schneider-Orelli's formula (Püntener 1981).

### Table 1. Bioassay with *I. fumosorosea* isolates against larvae of *Lymnantria monacha* and *Lymnantria dispar*

<table>
<thead>
<tr>
<th>Variant</th>
<th>Treated insect</th>
<th>Larval stages</th>
<th>Concentration (conidia/ml)</th>
<th>Mode of treatment</th>
<th>Insect host used to obtain isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lm-v-1</td>
<td><em>L. monacha</em></td>
<td>3rd instar</td>
<td>1.10^8</td>
<td>filter paper disk</td>
<td><em>H. cunea</em></td>
</tr>
<tr>
<td>Lm-v-2</td>
<td><em>L. monacha</em></td>
<td>3rd instar</td>
<td>1.10^7</td>
<td>filter paper disk</td>
<td><em>L. monacha</em></td>
</tr>
<tr>
<td>Lm-v-3</td>
<td><em>L. monacha</em></td>
<td>3rd instar</td>
<td>4.10^8</td>
<td>Larch</td>
<td><em>L. dispar</em></td>
</tr>
<tr>
<td>Ld-v-1</td>
<td><em>L. dispar</em></td>
<td>2nd instar</td>
<td>1.10^8</td>
<td>filter paper disk</td>
<td><em>H. cunea</em></td>
</tr>
<tr>
<td>Ld-v-2</td>
<td><em>L. dispar</em></td>
<td>3rd instar</td>
<td>1.10^7</td>
<td>filter paper disk</td>
<td><em>H. cunea</em></td>
</tr>
<tr>
<td>Ld-v-3</td>
<td><em>L. dispar</em></td>
<td>3rd instar</td>
<td>1.10^8</td>
<td>dip in suspension</td>
<td><em>H. cunea</em></td>
</tr>
<tr>
<td>Ld-v-4</td>
<td><em>L. dispar</em></td>
<td>3rd instar</td>
<td>1.10^7</td>
<td>filter paper disk</td>
<td><em>L. dispar</em></td>
</tr>
<tr>
<td>Ld-v-5</td>
<td><em>L. dispar</em></td>
<td>2nd instar</td>
<td>4.10^8</td>
<td>Oak leaves</td>
<td><em>L. dispar</em></td>
</tr>
<tr>
<td>Ld-v-6</td>
<td><em>L. dispar</em></td>
<td>3rd instar</td>
<td>3.10^7</td>
<td>filter paper disk</td>
<td><em>D. pini</em></td>
</tr>
<tr>
<td>Ld-v-7</td>
<td><em>L. dispar</em></td>
<td>3rd instar</td>
<td>1.10^8</td>
<td>filter paper disk</td>
<td><em>D. pini</em></td>
</tr>
<tr>
<td>Ld-v-8</td>
<td><em>L. dispar</em></td>
<td>4th instar</td>
<td>3.10^8</td>
<td>filter paper disk</td>
<td><em>D. pini</em></td>
</tr>
</tbody>
</table>

**Results and Discussion:**

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

![Fig. 1. Efficacy of *I. fumosorosea* isolates against larvae of *L. dispar*](image_url)

Our results show that *L. dispar* and *L. monacha* larvae are tolerant to mycosis caused by the Georgian isolate of *I. fumosorosea* and re-isolates obtained from infected larvae of *D. pini*, *L. monacha* and *L. dispar*. Although insects were treated with conidial suspensions with a very high concentration (1.10^9 conidia/ml), the lethal effect didn’t exceed 30% (Fig. 1, 2). In experiments with third instar *L. dispar* larvae it was observed that the increase of the concentration of the conidial suspensions of the same isolate (Georgian isolate of *I. fumosorosea* isolated from *H. cunea*) from 1.10^8 to 1.10^9 conidia/ml resulted in higher efficacy – from 9.00% in the variant Ld-v-2 to 30.00% in the variant Ld-v-3 (Fig. 1). According to Keller and Zimmermann (1989) the density of infective material necessary to initiate
infection depends largely on the host and the pathogen.

Like most entomopathogenic fungi, *I. fumosorosea* infects its host by breaching the cuticle (St. Leger et al. 1986; Draganova 1988; Hajek and St. Leger 1994). Therefore, we tested different modes for the application of the suspension: a) suspension placed on filter paper discs in Petri dishes, b) suspension application on oak leaves or larch needles and c) dipping larvae into the suspension. Dipping of *L. dispar* larvae into the suspension showed the lowest effect, but there was almost no difference of pest mortality using these three different designs of application (Fig. 1, 2). It should be noted that although the experimental larvae were treated by a surface contact with high concentration suspensions placed on filter papers the effects of the mycosis induced was rather low. According to Dunlap et al. (2007) susceptible insects exposed to blastospores and conidia of *I. fumosorosea* showed declined growth and high levels of mortality.

According to Goettel et al. (1990) and Lecheva and Draganova (1998), fungal isolates were more virulent to their initial hosts. In our experiments, discrepant results about initial host and virulence of the isolates to *L. dispar* and *L. monacha* larvae were obtained (Fig. 1, 2). Comparison between the variants Lm-v-2 (treatment with *I. fumosorosea* isolated from *L. monacha* larvae) vs Lm-v-1 (treatment with *I. fumosorosea* isolated from *H. cunea*) (Fig. 2) and the variants Ld-v-5 (treatment with *I. fumosorosea* isolated from *L. dispar* larvae) vs Ld-v-1 (treatment with *I. fumosorosea* isolated from *H. cunea*) (Fig. 1) confirmed the findings of the cited authors. The efficacy in the variants reached values of 28.80% vs 1.71% and 9.00% vs 1.25%, respectively. However comparing the efficacy in the variants Lm-v-2 vs Lm-v-3 showed that the difference between the values of the efficacy was minor (26.00% vs 28.80%).

In bioassays with different isolates of *B. bassiana* against *L. dispar* larvae Draganova et al. (2013) established that the host larvae were tolerant to mycosis caused by the tested isolates, which is in accordance with the findings reported here. Although some hyphomycete species were found in natural populations of gypsy moth and *Paecilomyces farinosus* was the most common (but in very low infection levels of 4.6% to 12.2%), the fungal pathogen with the highest virulence for *L. dispar* larvae is the entomophthoralean 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), *I. fumosorosea* should be applied in combination with other entomopathogenic fungi, such as *Lecanicillium* and *Beauvaeria*. This suggestion will be considered in our further experiments. Due to the capacity of *I. 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:

Our investigations on the susceptibility of *L. monacha* and *L. dispar* larvae to the isolate of *I. fumosorosea* from *H. cunea* and the re-isolates from *L. dispar* and *L. monacha* showed that larvae of both species were tolerant to the mycosis induced by *I. fumosorosea*

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