Evaluation of entomopathogenic fungi as a biological control agent of *Cameraria ohridella*, an invasive pest of *Aesculus hippocastanum* in Europe

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Introduction

Invasive pest

The horse chestnut, *Aesculus hippocastanum* L. (Fig. 1), is an important ornamental tree in Europe. It is attacked by the horse chestnut leaf-miner, *Cameraria ohridella* Deschka et Dimic (Fig. 2), an important invasive lepidopteran pest.

FIGURE 1: A horse chestnut tree in a city park.

FIGURE 2: Adult of the horse chestnut leaf-miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae).

Since its first record in Macedonia in 1985 the pest rapidly spread and colonized major parts of Europe including Denmark, south of Sweden, Belorussia and Ukraine where it encounters favourable conditions for its development.

Damage

The larvae feed on leaf parenchyma (Fig. 3). Besides aesthetic damage, injury inflicted to the leaves can result in weakening of infested trees and reduction of their growth.

FIGURE 3: Horse chestnut leaves damaged by *Cameraria ohridella* larvae.

Pest control

Present methods of *C. ohridella* control are based on the application of non-selective insecticides to *Aesculus* trees and composting or burning of leaf litter in which *C. ohridella* overwinters as pupa (Fig. 4). However, these methods also kill beneficial organisms including natural enemies of *C. ohridella*.

FIGURE 4: Diapausing pupa of *Cameraria ohridella* in a chamber.

Objectives

- Potential for using entomopathogenic fungi to control *C. ohridella* is evaluated.
- The research includes the following steps:
  1. Survey of the occurrence of entomopathogenic fungi in soil samples collected in the vicinity of horse chestnut trees.
  2. Searching for a spontaneous infection of *C. ohridella* hibernating pupae by entomopathogenic fungi.
  3. The laboratory bioassays of isolated strains.

Materials and Methods

FIGURE 5: *Isaria fumosorosea* on *Galleria mellonella* larva.

The occurrence of entomopathogenic fungi in 48 soil samples collected in the vicinity of horse chestnut trees heavily infested by *C. ohridella* was surveyed using the adapted *Galleria*-bait method (Fig. 5).

Spontaneous infection of *C. ohridella* by entomopathogenic fungi was investigated in samples of hibernating *C. ohridella* pupae collected in autumn. The strains of the fungus were isolated from individual infected pupae using growth medium (Sabourau's agar).

FIGURE 6: Petri dish with treated pupae of *Cameraria ohridella*.

The bioassays were carried out using a new strain of *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) CCM 8367 discovered in the Czech Republic. Virulence of both blastospores and conidiospores was tested using a dip assay. Diapausing pupae of *C. ohridella* were treated with a concentration of 6×10^7 spores/ml and incubated in Petri dishes (Fig. 6) at 23±1°C and R.H. approx. 95%. Pupae treated with destilled water served as a control.

Results

Totally, 45.3% of *Galleria* larvae (n=3840) put into the soil samples were infected with entomopathogenic fungi (Deuteromycetes). The following species of fungi were found:

Species	%
Isaria fumosorosea (Wize) Brown et Smith	77.6
Beauveria bassiana (Balsamo) Vuillemin	20.6
Isaria farinosa (Holm ex S.F. Gray) Brown et Smith	1.7
Metarhizium anisopliae (Metschnikoff) Sorokin	0.1

We discovered spontaneous infection of *C. ohridella* hibernating pupae by two species of entomopathogenous fungi: *I. fumosorosea* and *B. bassiana* (Fig. 7). Both species were successfully isolated from the host, cultivated on artificial medium and deposited in the CCEFO (Culture Collection of Entomopathogenic Fungi Olešná) in the Czech Republic.

FIGURE 7: Hibernating pupae of *Cameraria ohridella* infected by *Isaria fumosorosea* (left) and *Beauveria bassiana* (right).

Results of bioassays with *I. fumosorosea* revealed faster virulence of blastospores compared to conidiospores (Figs. 8–9).

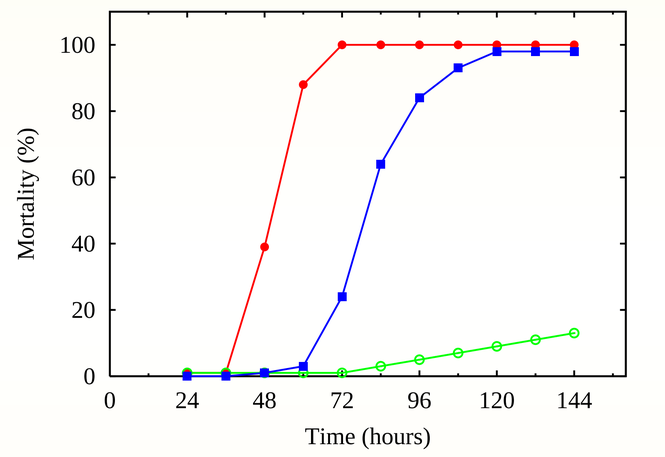

FIGURE 8: Cumulative mortality of *Cameraria ohridella* pupae treated with *Isaria fumosorosea* blastospores (red line), conidiospores (blue line) and destilled water only (green line); n=100.

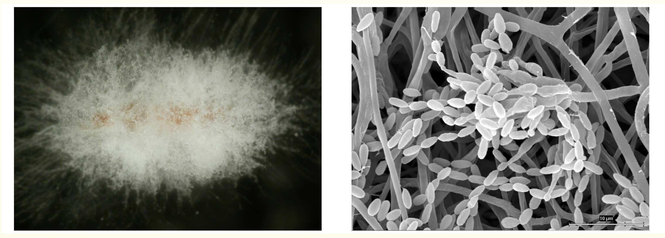

FIGURE 9: *Cameraria ohridella* pupae 120 hours after the application of *Isaria fumosorosea* (left) and scanning electron micrograph of conidial chains (right).

Conclusions

- **Four species of entomopathogenic fungi were found in soil samples and two species in hibernating *C. ohridella* pupae.**
- **Bioassays revealed a high insecticidal activity of *I. fumosorosea*; blastospores kill the host faster than conidiospores.**
- **Entomopathogenic fungi are promising biocontrol agents against the horse chestnut leaf-miner.**

Acknowledgements

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