

# ENTOMOPATHOGENIC FUNGI ISOLATED FROM SOIL IN THE VICINITY OF *Cameraria ohridella*-INFESTED HORSE CHESTNUT TREES

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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. Besides aesthetic damage (Fig. 3), 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. However, these methods also kill beneficial organisms including natural enemies of *C. ohridella*.

## Objectives

- Survey of the occurrence of entomopathogenic fungi in soil in the vicinity of horse chestnut trees.
- Building up the collection of isolated strains as a base for finding the effective biocontrol agent against *Cameraria ohridella*.

## Materials and Methods

Soil samples were collected in the neighbourhood of the horse chestnut trees heavily infested by *C. ohridella*. Four samples were taken from each of the following biotopes: (1) city park, (2) trees in gardens outside the city, (3) alley at a heavy traffic road and (4) alley at a low traffic road. All these biotopes were located in districts Písek, České Budějovice and Plzeň (Fig. 4). Totally, 48 soil samples were collected and processed.

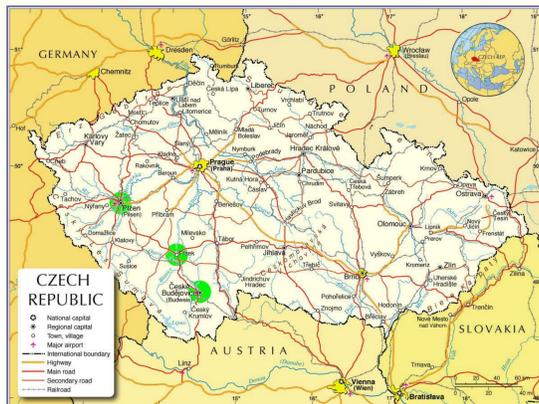


FIGURE 4: Districts where soil samples were collected (green spots).

Native entomopathogenic strains were obtained from the samples by adapted "Galleria bait method" (Zimmermann, 1986; Fig. 5).



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

## Results

Totally, 45.3% of *Galleria* larvae (n=3840) put into the soil samples were infected with entomopathogenic fungi. Four species of fungi were identified (Tables 1–4).

TABLE 1: Percentages of *G. mellonella* larvae infected by individual species of entomopathogenic fungi.

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

TABLE 2: Numbers of *G. mellonella* larvae infected by entomopathogenic fungi in Písek district.

| Species               | Biotope |        |                      |                       | Total |
|-----------------------|---------|--------|----------------------|-----------------------|-------|
|                       | Park    | Garden | Alley I <sup>a</sup> | Alley II <sup>a</sup> |       |
| <i>I. fumosorosea</i> | 102     | 113    | 109                  | 44                    | 368   |
| <i>I. farinosa</i>    | 0       | 0      | 0                    | 1                     | 1     |
| <i>B. bassiana</i>    | 50      | 16     | 14                   | 0                     | 80    |
| <i>M. anisopliae</i>  | 0       | 2      | 0                    | 0                     | 2     |
| Total                 | 152     | 131    | 123                  | 45                    | 451   |

<sup>a</sup> Alley at a heavy and a low traffic road, respectively.

TABLE 3: Numbers of *G. mellonella* larvae infected by entomopathogenic fungi in Plzeň district.

| Species               | Biotope |        |                      |                       | Total |
|-----------------------|---------|--------|----------------------|-----------------------|-------|
|                       | Park    | Garden | Alley I <sup>a</sup> | Alley II <sup>a</sup> |       |
| <i>I. fumosorosea</i> | 109     | 99     | 155                  | 65                    | 428   |
| <i>I. farinosa</i>    | 13      | 0      | 4                    | 3                     | 20    |
| <i>B. bassiana</i>    | 36      | 38     | 19                   | 45                    | 138   |
| <i>M. anisopliae</i>  | 0       | 0      | 0                    | 0                     | 0     |
| Total                 | 158     | 137    | 178                  | 113                   | 586   |

<sup>a</sup> Alley at a heavy and a low traffic road, respectively.

TABLE 4: Numbers of *G. mellonella* larvae infected by entomopathogenic fungi in České Budějovice district.

| Species               | Biotope |        |                      |                       | Total |
|-----------------------|---------|--------|----------------------|-----------------------|-------|
|                       | Park    | Garden | Alley I <sup>a</sup> | Alley II <sup>a</sup> |       |
| <i>I. fumosorosea</i> | 178     | 54     | 185                  | 138                   | 555   |
| <i>I. farinosa</i>    | 2       | 1      | 3                    | 2                     | 8     |
| <i>B. bassiana</i>    | 9       | 83     | 12                   | 37                    | 141   |
| <i>M. anisopliae</i>  | 0       | 0      | 0                    | 0                     | 0     |
| Total                 | 189     | 138    | 200                  | 177                   | 704   |

<sup>a</sup> Alley at a heavy and a low traffic road, respectively.

Statistical analysis of the data revealed:

- Highly significant effect of district on the occurrence of entomopathogenic fungi ( $\chi^2=129$ , df=8,  $P<0.0001$ ).
- Highly significant effect of biotope on occurrence of entomopathogenic fungi in district Písek ( $\chi^2=142$ , df=12,  $P<0.0001$ ), Plzeň ( $\chi^2=81.5$ , df=9,  $P<0.0001$ ) as well as České Budějovice ( $\chi^2=194$ , df=9,  $P<0.0001$ ).

## Conclusions

- Entomopathogenic fungi frequently occur in the soil collected from *C. ohridella* habitats.
- Dominant species found were *Isaria fumosorosea* and *Beauveria bassiana*.
- The isolated strains are deposited in the CCEFO (Culture Collection of Entomopathogenic Fungi Olešná) in the Czech Republic.

## Acknowledgements

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## References

- [1] Zimmermann G. 1986. The "Galleria bait method" for detection of entomopathogenic fungi in soil. *J. App. Entomol.*, 102: 213–215.