Entomophaga maimaiga, an introduced pathogen of the gypsy moth, *Lymantria dispar*, in Europe: A joint study in Bulgaria and Austria

Daniela Pilarska [1], Christa Schafellner [2], Georgi Georgiev [3], Margarita Georgieva [3], Plamen Mirchev [3], Peter Zelinka [2], Katrin Grollnigg [2], Dörte Goertz [2], Andreas Linde [4] & Gernot Hoch [5]

[1] New Bulgarian University, Sofia, Bulgaria;
[2] BOKU University of Natural Resources and Life Sciences, Vienna, Austria;
[3] Forest Research Institute, Bulgarian Academy of Science, Sofia, Bulgaria;
[4] Eberswalde University for Sustainable Development, Germany;
[5] BFW Austrian Research Centre for Forests, Vienna, Austria

Zusammenfassung: Der aus Ostasien stammende entomopathogene Pilz *Entomophaga maimaiga* (Entomophthoromycota: Entomophthorales) hat in Nordamerika sein Potential als wirksamer Antagonist des Schwammspinners *Lymantria dispar* (Lepidoptera, Erebidae) gezeigt. In den Jahren 1999 und 2000 wurde der Pilz als Maßnahme der biologischen Bekämpfung von *L. dispar* in Bulgarien freigesetzt. Seither hat sich *E. maimaiga* rasch auf der Balkanhalbinsel und in Südosteuropa ausgebreitet. Untersuchungen an zahlreichen blattfressenden Insekten bestätigten die hohe Wirtsspezifität. In einem Kooperationsprojekt wurde der Status von *E. maimaiga* in Bulgarien und Österreich untersucht. Achtzehn Jahre nach der Einbürgerung ist der Pilz in Bulgarien etabliert, und hohe Mortalitäten bei *L. dispar* werden seither regelmäßig dokumentiert. In Österreich wurde *E. maimaiga* in Screenings in den Jahren 2014 und 2018 in insgesamt 15 Eichenwäldern mit bekannten *L. dispar*-Vorkommen nicht nachgewiesen. Im Sommer 2019 wurden in zwei Eichenwäldern in Niederösterreich tote *L. dispar* Larven gefunden, die mit *E. maimaiga* infiziert waren.

Key Words: *Entomophaga maimaiga*, entomopathogenic fungi, *Lymantria dispar*, biological control

Gernot Hoch: BFW Austrian Research Centre for Forests, Seckendorff-Gudent-Weg 8, 1131 Vienna, Austria; E-Mail: gernot.hoch@bfw.gv.at

Entomophaga maimaiga (Entomophthorales: Entomophthoraceae) is a fungal pathogen that demonstrated efficacy against the gypsy moth, *Lymantria dispar* (Lepidoptera, Erebidae), a serious pest of deciduous forests. It was introduced from Japan to North America as classical biological control agent against *L. dispar* in the early 20th century but was not recovered until the 1980s. However, since 1989 epizootics of *E. maimaiga* have been regularly observed and the pathogen now plays an important role in population dynamics of *L. dispar* in North America (Hajek 1999). Laboratory and field studies showed high host specificity of the pathogen. In order to enhance the natural biological control of gypsy moth in Bulgaria successful introductions of the pathogen were conducted at two sites in 1999 and 2000; more introductions followed. The pathogen quickly spread on the Balkan Peninsula and in southeastern Europe. The potential of *E. maimaiga* to regulate *L. dispar* populations in Europe is reviewed by Zubrik & al. (2016). In a joint study, we analyzed the current status of the pathogen in Bulgaria and Austria.

Establishment in Bulgaria and spread in Europe, impact and host specificity

The first successful introductions of *E. maimaiga* in Bulgaria were realized in the region of Karlovo (Central Bulgaria) in 1999 and Svoge (West Bulgaria) in 2000. In 2005, the first strong epizootics caused

by the fungus were noticed in four *L. dispar* outbreak populations located 30-70 km from the introduction sites (Pilarska & al. 2016). More introductions were realized in different areas all over Bulgaria from 2001 to 2014. Surveys showed that *E. maimaiga* has become well established (Fig. 1); further epizootics occurred and suppressed several strong outbreaks of *L. dispar* (Pilarska & al. 2016).

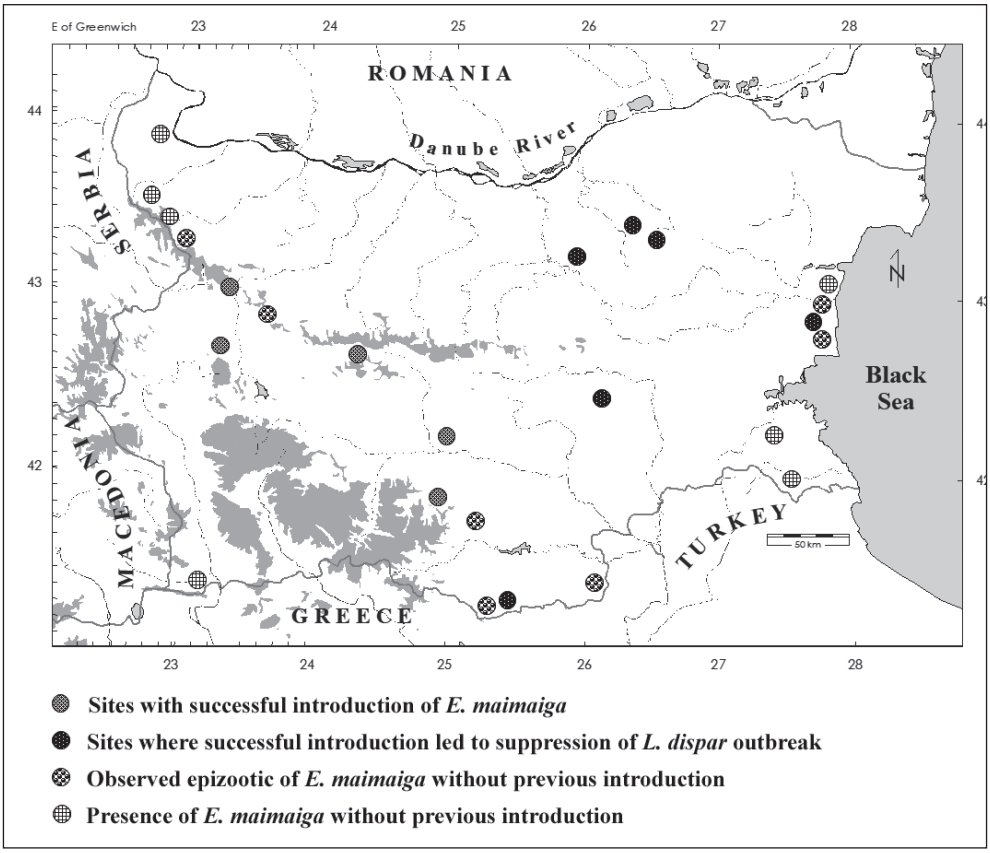

Fig. 1: Status of *E. maimaiga* in Bulgaria

After the introductions in Bulgaria, *E. maimaiga* quickly spread on the Balkan Peninsula and in southeastern Europe. In 2011, the fungus was detected in Serbia and the European part of Turkey and in 2012 in Greece and North Macedonia. The pathogen further expanded its range to the north and west; it was detected in Croatia, Bosnia and Herzegovina, Hungary, and Slovakia in 2013 (Pilarska & al. 2016). Moreover, *E. maimaiga* was found in Georgia in 2005 (Kereselidze & al. 2011). The inoculative releases of *E. maimaiga* between 1999 and 2014 in Bulgaria frequently led to epizootics in *L. dispar* populations: in 14 out of 18 populations where the pathogen had been released, mortality of *L. dispar* reached 80 – 100 % (Pilarska & al. 2016) and areas affected by outbreaks of *L. dispar* significantly decreased to 2 – 18 % compared to the decades before the introduction of *E. maimaiga*.

Studies on non-target phyllophagous insects in Bulgaria indicate high host specificity of the fungus. In 2009 – 2011, microscopic examination of a total of 1499 larvae (38 Lepidoptera species and 2 Hymenoptera species) collected from the sites where *E. maimaiga* occurred indicated no evidence of *E. maimaiga* life stages in any of the larvae (Georgieva & al. 2014). In 2017, 4045 larvae (104 species of Lepidoptera, Hymenoptera and Coleoptera) collected from six oak forests in Bulgaria, Hungary and Slovakia, where *E. maimaiga* had previously been reported, were investigated for the presence of the pathogen.

The microscopic examination and DNA analyses of these insect specimens revealed no infection caused by *E. maimaiga* (Zubrik & al. 2018). This corroborates the high ecological host specificity of the fungus concluded from studies in North America (Hajek 1999; Hajek & al. 2004).

Screening for *E. maimaiga* in Bulgaria and Austria

Bulgaria. In 2018, *E. maimaiga* was confirmed again in a low density gypsy moth population in Gabrovnitsa (Svoge Region), where an introduction was conducted in 2000. A sample of larvae collected near Asenovgrad in spring 2018 was negative for the fungus. No introduction had been done on this site.

Austria. An initial screening for *E. maimaiga* was carried out in 2014. Soil samples were collected in 5 mixed oak forests, where *L. dispar* was present. In 2018, ten mixed oak forests with known *L. dispar* populations (including two at outbreak level) were analyzed (Fig. 2).

Fig. 2: Mixed oak forests in Austria surveyed for *E. maimaiga* by soil sampling in 2014 (grey dots) and 2018 (black dots) as well as by collection of larvae during an outbreak in 2018 (black diamonds). No infection with *E. maimaiga* was detected in both years. Nearest sites where the fungus was recorded in Hungary and Slovakia (Zubrik & al. 2016) (black stars) are shown in the small map in the grey insert.

Method

Soil samples (100 – 200 g) were collected next to 40 trees (in 2014) or 5 trees (in 2018) per locality and combined to one mixed sample. In the laboratory, sub-samples (6 in 2014, 3 in 2018) were drawn from each mixed sample; laboratory-reared fourth instar *L. dispar* larvae (5 in 2014, 10 in 2018) were incubated per soil sub-sample (i.e., a total of 30 larvae per locality) at 15°C for 3 days without food in a container with the soil sample. The larvae were then reared individually on meridic wheat germ diet for 14 days at 24 °C / 18°C (16hL / 8hD) in 2014. All larvae were dissected after this period and microscopically inspected for the presence of the fungus. Test larvae were reared for 10 days at 20 °C (16hL / 8hD) in 2018. Larvae that died during this period were placed in a humid chamber at 20 °C for 7 days to allow formation of azygospores and then inspected under light microscopy.

Results

No infection with *E. maimaiga* was detected in 2014. In 2018, twenty six out of 300 *L. dispar* test larvae died during the soil incubation experiment and were further analyzed. *E. maimaiga* was not detected. Secondary fungal infections were observed in three and soil-borne nematodes in two of the analyzed larvae. Moreover, 217 *L. dispar* larvae, pupae and adults were collected at two locations where an outbreak of the insect occurred in 2018. Of these, six larvae died from nuclear polyhedrosis virus (NPV) and 87 larvae died from parasitism by tachinids or braconids. *E. maimaiga* was not detected.

Conclusions

The presence of *E. maimaiga* was confirmed in a low density population of *L. dispar* in Bulgaria 18 years after the release of the fungus on this site. *E. maimaiga* is established in Bulgaria and has spread remarkably fast in Europe. The surveys in mixed oak forests with known *L. dispar* populations including two at outbreak level in Austria did not show the presence of *E. maimaiga.* The nearest locations where the fungus has been confirmed are approximately 100 km east of the Austrian border (Zubrik & al. 2016). In Summer 2019, dead *L. dispar* larvae infected with *E. maimaiga* were detected in the province of Lower Austria, which were negative in the 2018 survey (Hoch & al. 2020).

Acknowledgements

Funding for this cooperation is provided by the Austrian Agency for International Cooperation in Education and Research (OeAD) / Austrian Federal Ministry of Education, Science and Research (BMBWF) (Project BG 08/2017) and National Science Fund (Project DNTS/Austria 01/12/2017). Assistance by the colleagues from Plovdiv Forest Protection Station is highly appreciated.

References

Georgieva, M., Takov. D., Georgiev, G., Pilarska, D., Pilarski, P., Mirchev, P. & Humber, R. (2014): Studies on non-target phyllophagous insects in oak forests as potential hosts of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Bulgaria. – Acta Zool. Bulg. **66**: 115-120.

Hajek, A.E. (1999): Pathology and epizootiology of *Entomophaga maimaiga i*nfections in forest Lepidoptera. – Microbiol. Molec. Biol. Rev. **63**: 814-835.

Hajek, A.E., Strazanac, J.S., Wheeler, M.M., Vermeylen, F.M. & Butler, L. (2004): Persistence of the fungal pathogen *Entomophaga maimaiga* and its impact on native Lymantriidae. – Biol. Contr. **30**: 466-473.

Hoch, G., Pilarska, D., Georgieva, M., Georgiev, G., Mirchev, P. & Schafellner, C. (2020). Erstnachweis des insektenpathogenen Pilzes *Entomophaga maimaiga* in Populationen des Schwammspinners in Österreich. Forstschutz Aktuell **66**: https://bfw.ac.at/rz/bfwcms.web?dok=10710.

Kereselidze, M., Pilarska, D., Hajek, A.E., Jensen, A.B. & Linde, A. (2011): First record of *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) in Georgia. – Biocontrol Sci. Technol. **21**: 1375-1380.

Pilarska, D., Georgiev, G., Golemansky, V., Pilarski, P., Mirchev, P., Georgieva, M., Tabakovic-Tošic, M., Todorov, M., Takov, D., Pernek, M., Hrasovec, B., Milotic, M., Dautbasic, M., Mujezinovic, O., Naceski, S., Papazova-Anakieva, I., Matova, M. & Vafeidis, P. (2016): *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Balkan Peninsula – an overview. Silva balcanica **17**: 31-40.

Zubrik, M., Hajek, A., Pilarska, D., Špilda, I., Georgiev, G., Hrašovec, B., Hirka, A., Goertz, D., Hoch, G., Barta, M., Saniga, M., Kunca, A., Nikolov, C., Vakula, J., Galko, J., Pilarski, P. & Csóka, G. (2016): The potential for *Entomophaga maimaiga* to regulate gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Erebidae) in Europe. – J. Appl. Entomol. **140**: 565-579.

Zubrik, M., Pilarska, D., Kulfan, J., Barta, M., Hajek, A.E., Bittner, T.D., Zach, P., Takov, D., Kunca, A., Rell, S., Hirka, A., & Csóka, G. (2018): Phytophagous larvae occurring in Central and Southeastern European oak forests as a potential host of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) – A field study. J. Invertebr. Pathol. **155**: 52-54.