Varia

Successful Completion of an International Project of the Department of Natural Sciences at New Bulgarian University

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Abstract: The paper presents the results of a successfully completed international project of the Department of Natural Sciences titled “Monitoring and identification of the entomopathogenic fungus Entomophaga maimaiga in Lymantria dispar populations” and funded by the National Research Fund in 2017-2019. Investigating the distribution of E. maimaiga in Austria and Bulgaria in 2018-2019 shows that the pathogen has expanded its range to the west and reached Lower Austria. E. maimaiga has been identified in Bulgaria as well and has become part of the natural enemy complex of L. dispar.

Key words: Entomophaga maimaiga, Lymantria dispar, Austria, Bulgaria

Резюме: Представени са резултатите от успешно реализиран международен проект на Департамент „Природни науки“ “Мониторинг и идентификация на ентомопатогенната гъба Entomophaga maimaiga в популации на Lymantria dispar“, финансиран от ФНИ, МОН в периода 2017 – 2019 г. Проучването на разпространението на E. maimaiga в Австрия и България през 2018 – 2019 г. показа, че патогенът е разширил своя ареал на запад и е
достигнал Долна Австрия. *E. maimaiga* е установена и в България и е част от естествения комплекс неприятели на *L. dispar*.

**Ключови думи:** *Entomophaga maimaiga, Lymantria dispar*, Австрия, България.

The gypsy moth (*Lymantria dispar*, Lepidoptera) is one of the most dangerous pests of deciduous tree species in Bulgaria. Calamities of this pest occur every 8-10 years, causing enormous damage, mainly in oak and poplar forests. Huge financial resources are spent to control the pest.

*E. maimaiga* is a fungal pathogen of the gypsy moth that demonstrated efficacy against this pest in the USA as well as in Europe. It forms two types of spores after the death of its host, the gypsy moth: conidia and resting spores or azygospores. Conidia are only alive for a short period of time. They are formed externally on the dead larvae of gypsy moth. Under favorable conditions, after they reach the cuticle of another larva, they immediately germinate and infect new larvae of the host during the same season. Azygospores have thick double envelopes and are resistant to adverse external influences [Hajek, 1999]. They are formed inside the larvae. In the fall, the azygospores accumulate in the soil around the stems of the attacked trees. They can survive adverse conditions and maintain their viability in the soil for more than 12 years [Hajek et al. 2018].

Bulgaria is the first country in Europe where a successful introduction of *E. maimaiga* was performed. The fungus was imported from the United States in 1999 and 2000 and was introduced into two gypsy moth populations. In 2005, strong epizootics of *E. maimaiga* were observed in four regions of Bulgaria, located 30-70 km from the sites of introduction. During the period 2005 - 2016, *E. maimaiga* expanded its distribution in Bulgaria, both through new introductions and by natural dispersal. The pathogen is currently common throughout the country [Pilarska et al. 2016]. After the introduction of the pathogen in the country, almost no other control measures against the gypsy moth had to be performed [Pilarska et al. 2018].

In 2011, *E. maimaiga* was detected in the European part of Turkey [Georgiev et al. 2012] and Central Serbia [Tabaković-Tošić et al. 2012], and in 2012 in Greece and the Republic of Macedonia [Georgieva et al. 2013]. In 2013, the pathogen caused epizootics in Croatia, Slovakia, Hungary, Bosnia and Herzegovina [Zubrik et al. 2014; Csoka et al. 2014; Milotic et al. 2015], and in 2014 in Romania [Netoiu et al. 2016]. A study from 2014-2016 showed that *E. maimaiga* was already widespread in Slovakia and Hungary, reaching the border of the Czech Republic and Austria [Zubrik et al. 2018].

Studies on the impact of *E. maimaiga* on gypsy moth larvae and other lepidopteran species inhabiting oak forests in Slovakia, Hungary, and Bulgaria confirm the host specificity of the pathogen [Zubrik et al. 2018].

Based on the distribution of the pathogen on the Balkan Peninsula and in Central Europe we supposed that the fungus has spread also in Austria. To check this hypothesis, we submitted a cooperative research project to the Bulgarian National Science Fund (NSF) and the Austrian Agency for International Cooperation in Education and Research (OeAD) together with colleagues from the University of Natural Resources and Life Sciences (BOKU) and the Austrian Research Centre for Forests (BFW) in Vienna, Austria, and the Forest Research Institute, Bulgarian Academy of Sciences in Sofia, Bulgaria. The project entitled “Monitoring and identification of the entomopathogenic fungus *Entomophaga maimaiga* in *Lymantria dispar* populations” was accepted by NSF and OeAD and received funding for 2 years; it started in October 2017. The project coordinators were Assoc. Prof. Dr. Christa Schafellner from BOKU University of Natural Resources and Life Sciences on the Austrian side and Prof. Dr. Daniela
Pilarska from the Department of Natural Sciences at New Bulgarian University on the Bulgarian side. The purpose of the project was to study L. dispar populations in Austria for the presence of E. maimaiga and to monitor the distribution of the fungus in Bulgaria.

Monitoring of Entomophaga maimaiga in Austria in 2018 and 2019

In 2018, an analysis of soil samples for the presence of E. maimaiga was conducted. Samples were collected in ten mixed oak forests with known L. dispar populations. Two of the sites in the area of Eggenburg had gypsy moth populations at outbreak levels and the site in the area of Ebergassing ascertained that the population density of the gypsy moth was very high with 800-1300 egg masses per 100 trees. As a result, the trees were completely defoliated by the pest. Laboratory analyses of gypsy moth larvae, pupae, and adults collected from both localities near Eggenburg did not show the presence of E. maimaiga [Pilarska et al. 2020]. However 18% of the older larvae were parasitized by braconids – (e.g. Glyptapanteles liparidis (Hymenoptera: Braconidae), and tachinids (e.g. Blepharipa pratensis, Drino incospicua (Diptera: Tachinidae). Additionally, another pathogen, nucleopolyhedrosis virus (Ld NPV) was detected in 1.3 % of all collected larvae.

In 2019, L. dispar population density in the Eggenburg area remained very high (400-500 egg masses/100 trees). At the time of the survey defoliation in the forests reached 90-100 %. No signs of E. maimaiga infections were detected in the field. However, laboratory analyses showed that 33% of the collected larvae and 65 % of the dead larvae contained spores (conidia or azygospores) of E. maimaiga [Hoch et al. 2019]. Parasitoids were found in 2 % and Ld NPV in 18 % of the collected larvae.

In the second monitored site in Ebergassing slight defoliation of mixed oak-hornbeam forest was observed. In 2019, massive mortality of 5th and 6th instar larvae was recorded. Symptoms were typical of entomoptorous infection - dead larvae hang upside down, clamped to the stems of the tree with legs spread. Microscopic examinations revealed the presence of E. maimaiga in 99.4% of all collected dead and live specimens of L. dispar. All the dead larvae collected contained conidia and azygospores.

In 2019, climatic conditions were favorable for the development of the entomopathogenic fungus. May 2019 was cool and rainy [Hoch et al. 2019].

Monitoring of Entomophaga maimaiga in Bulgaria

The monitoring conducted in six gypsy moth habitats in Bulgaria in 2018 and 2019 confirmed the presence of E. maimaiga in three of the six studied sites, despite the low pest population density in the country. E. maimaiga was detected in 50 % of larvae collected in the village of Gabrovitsa in 2018, where the second successful introduction of the pathogen was made in 2000. In 2019, in the village of Gorni Lom, the pathogen was observed in 100 % of the collected larvae.

The third site where the fungus was found in 2019 was a site near Petrich, in Belasitsa Mountain; 65 % of the investigated larvae were infected. Microscopic inspections of larvae, pupae, and moths collected in the village of Topolovo did not reveal the presence of E. maimaiga. However, the microsporidium Nosema lymantriae (Microsporidia, Nosematidae) was detected in 12 % of the collected larvae.

No pathogens were observed in larvae from the area of Kremen and Chakalarovo villages in 2018 and 2019.

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CONCLUSION

The survey of *E. maimaiga* distribution in Austria and Bulgaria showed that the pathogen has expanded its range in Europe to the west and reached Lower Austria. It was the first record of the fungus in Austria. The border to Slovakia, where the fungus was detected in 2014 [Zúbrik et al. 2014], is approximately 35 km from the easternmost location where the fungus was found.

The frequent finding of *E. maimaiga* despite the very low population density of gypsy moth shows that the pathogen has been well established in many regions in Bulgaria. It has become an effective part of the natural enemy complex that controls the population densities of this pest.

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