Годишник на департамент „Природни науки“, ISSN 2367-6302, 2020-2021
Annual of Natural Sciences Department, ISSN 2367-6302, 2020-2021
Journal homepage: https://naturalsciences.nbu.bg/bg/godishnik-na-departament-prirodni-nauki

Varia

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

Daniela Pilarska[1], Christa Schafellner[2], Gernot Hoch[3], Margarita Georgieva[4], Georgi Georgiev[4], Plamen Mirchev[4]
[1]New Bulgarian University, 21 Montevideo St., Sofia, Bulgaria
[2]BOKU University of Natural Resources and Life Sciences, Peter-Jordan-Straße 82/I, 1190 Vienna, Austria
[3]BFW Austrian Research Centre for Forests, Seckendorff-Gudent-Weg 8,1131 Vienna, Austria [4]Forest Research Institute, Bulgarian Academy of Sciences, 132, St. Kliment Ohridski Blvd., 1756 Sofia, Bulgaria
dpilarska@nbu.bg

Успешна реализация на международен проект на Департамент „Природни науки“ в Нов български университет

Даниела Пиларска[1], Криста Шафелнер[2], Гернот Хох[3], Маргарита Георгиева[4], Георги Георгиев[4], Пламен Мирчев[4]
[1]Нов български университет, департамент „Природни науки“,бул. „Монтевидео“ № 21, 1618 София, България
[2]BOKU University of Natural Resources and Life Sciences, Peter-Jordan-Straße 82/I, 1190 Vienna, Austria
[3]BFW Austrian Research Centre for Forests, Seckendorff-Gudent-Weg 8,1131 Vienna, Austria
[4]Институт за гората, Българска академия на науките, бул. „Климент Охридски“ 132, 1756 София, България
dpilarska@nbu.bg

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

https://doi.org/10.33919/ansd.20-21.6.8

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

https://doi.org/10.33919/ansd.20-21.6.8

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 showed slightly elevated population densities. Surveys of oak forests in the area of Eggenburg 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 5^{th} and 6^{th} instar larvae was recorded. Symptoms were typical of entomophtorous 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 Gabrovnitsa 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.

https://doi.org/10.33919/ansd.20-21.6.8

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.

Acknowledgments

The authors are very grateful to National Science Fund, Project DNTS/Austria 01/12/2017 and to Austrian Agency for International Cooperation in Education and Research (OeAD)/ Austrian Federal Ministry of Education, Science and Research (BMBWF), Project BG 08/2017 and to the colleagues from Forest Protection Station Plovdiv of the Executive Forestry Agency at Ministry of Agriculture and Foods.

REFERENCES

Csoka G., A. Hirka, L. Czocs, A.E. Hajek, First occurrence of the entomopathogenic fungus, *Entomophaga maimaiga* Humber, Shimazu & Soper, 1988 (Entomophtorales: Entomophtoraceae) in Hungarian gypsy moth *(Lymantria dispar)* populations. *Novenyvedelem*, 50(6), 2014, 257-262.

Georgiev G., P. Mirchev, M. Georgieva, B. Rossnev, P. Petkov, M. Matova, S. Kitanova, First Record of Entomopathogenic Fungus *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) in *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae) in Turkey. *Acta zoologica bulgarica*, 64(2), 2012, 123-127.

Georgieva M., G. Georgiev, D. Pilarska, P. Pilarski, P. Mirchev, I. Papazova-Anakieva, S. Naceski, P. Vafeidis, M. Matova, First record of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in *Lymantria dispar* populations in Greece and the Former Yugoslavian Republic of Macedonia. *Sumarski list*, 5-6, 2013, 307-311.

Hajek A.E., C. Olsen, J.S. Elkinton, Dynamics of aiirborne conidia of the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Biological Control*, 16, 1999, 111-117.

Hajek A.E., D.C. Steinkraus, L.A. Castrillo, Sleeping beauties: horizontal transmission via resting spores of species in the Entomophthoromycotina. *Insects*, 9(3), 2018, pii: E102. doi: 10.3390/insects9030102

Hoch G., D. Pilarska, M. Georgieva, G. Georgiev, P. Mirchev, C. Schafellner, Erstnachweis des insektenpathogenen Pilzes *Entomophaga maimaiga* in Populationen des Schwammspinners in Österreich. *Forstschutz Aktuell*, 66 (2), 2019, 1-5.

Milotic M., O. Mujezinovic, M. Trestic, D. Pilarska, D. Diminic, First record of gypsy moth entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & Soper in Bosnia and Herzegovina. *Sumarski list*, 1-2, 2015, 59-67.

Netoiu C., R. Tomescu, O. Iliescu, A. Buzatu, *Entomophaga maimaiga* in Romania and future possibilities in biological control of *Lymantria dispar* populations. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, 46(2), 2016, 646-655.

Pilarska, D., M. McManus, A. E. Hajek, F. Herard, F. Vega, P. Pilarski, G. Markova, Introduction of the entomopathogenic fungus *Entomophaga maimaiga* Hum., Shim. & Sop. (Zygomycetes:Entomophtorales) to a *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) population in Bulgaria. *Journal of Pest Science (Anzeiger fur Schädlingskunde)*, 73(5), 2000, 125-126.

Pilarska D., G. Georgiev, M. McManus, P. Mirchev, P. Pilarski, A. Linde, *Entomophaga maimaiga* – an effective introduced pathogen of the gypsy moth (*Lymantria dispar* L.) in Bulgaria. *Proceedings of the International Conference* "*Alien Arthropods in South East Europe – Crossroad of three continents",* 19-21.09.2007, Sofia, Bulgaria, 2007, 37-43.

Pilarska D., G. Georgiev, V. Golemansky, P. Pilarski, P. Mirchev, M. Georgieva, M. Tabakovic-Tosic, M. Todorov, D. Takov, M. Pernek, B. Hrasovec, M. Milotoc, M. Dautabasic, O. Mujezinovic, S. Naceski, I. Anakieva-Papazova, M. Matova, P. Vafeidis, *Entomophaga maimaiga* (Entomophtorales, Entomophtoraceae) in Balkan peninsula – an overview. *Silva Balcanica*, 17(1), 2016, 31-40.

Pilarska D., G. Georgiev, M. Dobreva, D. Takov, P. Mirchev, D. Doychev, M. Georgieva, R. Nachev, P. Dermendzhiev, S. Draganova, A. Linde, A.E. Hajek, Pathogens and parasitoids of forest pest insects in the region of the Forest Protection Station Plovdiv (Bulgaria), during the period 1990-2017. *Silva Balcanica*, 19(3), 2018, 49-59.

Pilarska D., C. Schafellner, G. Georgiev, M. Georgieva, P. Mirchev, P. Zelinka, K. Grollnig, D. Goertz, A. Linde, G. Hoch, *Entomophaga maimaiga*, an introduced pathogen of the gypsy moth, *Lymantria dispar*, in Europe: A joint study in Bulgaria and Austria. *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie*, 22 (1), 2020, 285-288.

Tabaković-Tošić M., G. Georgiev, P. Mirchev, D. Tošić, V. Golubović-Ćurguz, *Entomophaga maimaiga* – new entomopathogenic fungus in the Republic of Serbia. *African Journal of Biotechnology*, 11(34), 2012, 8571-8577.

Zúbrik M., M. Barta, D. Pilarska, D. Goertz, M. Úradník, J. Galko, J. Vakula, A. Gubka, S. Rell, A. Kunca, First record of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Slovakia. *Biocontrol Science and Technology*, 24, 2014, 710-714.

Zúbrik M., I. Špilda, D. Pilarska, A. Hajek, D. Takov, Ch. Nikolov, A. Kunca, J. Pajtík, K. Lukasova, J. Holusa, Distribution of the entomopathogenic fungus *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) at the northern edge of its range in Europe. *Annals of Applied Biology*, 173, 2018, 35-41.

https://doi.org/10.33919/ansd.20-21.6.8