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First Record of Entomopathogenic Fungus *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) in *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) in Turkey

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Abstract: The entomopathogenic fungus *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) was detected for first time in the European part of Turkey. During the period 21-26 May 2011, 43 larvae of *Lymantria dispar* (Lepidoptera: Lymantriidae) were collected in Kiyiköy, Vize and Yaliköy on different oak trees in the Turkish part of Strandzha Mountains and transferred to the laboratory. Larvae were fed on fresh oak leaves and dead larvae were analysed under a light microscope for presence of entomopathogens. *E. maimaiga* azygospores were found in the bodies of 9 larvae – 8 from the region of Vize and 1 from Yaliköy. The higher mortality of the host in the region of Vize (53.3%) compared with the region of Yaliköy (11.1%) is due to the differences in age structure of the larvae in both localities. In the future it is desirable to monitor the distribution of *E. maimaiga* in Turkey and to study the impact of the pathogen on the population density of the pest.

Key words: *Entomophaga maimaiga*, first record, *Lymantria dispar*, Turkey

Introduction

The gypsy moth, *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Lymantriidae) is the most dangerous insect pest in the deciduous forests of the Palaearctic region. It is polyphagous, and mainly connected with different oak (*Quercus* spp.) species. In Bulgaria, the pest gradually increases its number and every 8-12 years causes significant defoliations of large areas of forests. The attacks are heaviest in South Eastern Europe and especially on Balkan Peninsula, because of the abundance of oak forests and favourable conditions for the development and survival of gypsy moth – high temperatures and humidity deficiency, which contribute to weakening of the plantations and causing them to become more vulnerable. The damages usually lead to growth loss and physiological weakness of food plants. Defoliations during several consecutive years can cause dieback or even death of the infested stands.

In the late 1860's, *L. dispar* expanded it's range accidentally from Europe to North America (McManus, McIntyre 1981). Due to the lack of natural enemies in the newly conquered territories the species increased it's number fast and turned into the main pest of the deciduous forests. Annual defoliation exceeding 500 thousand ha occurred during the period 1970-1995, and over 5.2 million ha were defoliated in 1981 (McManus, Csóka 2007). Now the species is widely distributed in North-East regions of the USA and Canada. The danger of range extension of *L. dispar* in North America lead to the development of programmes for control of the pest, incl. classic forms of biological control via introducing natural enemies (parasitoids and pathogens) taken from different places of its natural habitats (Reardon 1981; Coulson 1981; Pilarska *et al.* 2010, etc.). Among them, the introduction of the entomophthorous fungus *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) has proven the greatest effect.

Entomophaga maimaiga was discovered in Japan and described as a pathogen of *L. dispar.* It periodically causes heavy fungal epizootics on the host in its native range. The natural area of distribution includes Japan, the Pacific parts of China and the Far East of Russia (Hajek *et al.* 2005). The first attempts for the introduction the fungus pathogen were made in USA (Massachusetts) with material from Japan (Tokyo) in the period of 1910-1911. (Speare, Colley 1912). A second introduction of *E. maimaiga* in USA was made in 1985-1986 in New York and Virginia with an isolate from the region of Ishikawa (Hajek *et al.* 1995). The pathogen was discovered in 1989 during a fungal epizootic of gypsy moth in many areas of the North-Eastern United States (Andreadis, Weseloh 1990). So far the species has been found in 17 USA states (Smitley *et al.* 1995, Hajek *et al.* 2005, etc.) and one province of Canada (Ontario) (Howse, Scarr 2002). The results from the investigations in North America showed that *E. maimaiga* is a specific pathogen of gypsy moth, it is spreading naturally and capable of limiting the pest population density.

Bulgaria is the first country in Europe where a successful introduction of *E. maimaiga* was made in 1999 with biological material from USA (Pilarska *et al.* 2000). In 2005 the first heavy epizootic of *E. maimaiga* in gypsy moth was reported from forests in a distance of 30-90 km from the places of fungal introduction (Pilarska *et al.* 2006). Monitoring of the distribution of the fungus now showed that it can be found all over the country (Georgiev *et al.* 2010). The proximity of three localities – Elovitsa, Kremen and Zvezdets – to the borders with neighbouring countries gave us a reason to suppose that the fungus penetrated the territory of Serbia, Greece and European part of Turkey.

This note reports information about the finding of *E. maimaiga* in Turkey.

Material and Methods

The study was conducted in Strandzha Mountains in the European part of Turkey. Biological material – larvae of *L. dispar* – were collected during the period 21-26 May 2011 from leaves in the lower parts of oak tree crowns. Different oak stands were visited and studied in Vize and Çatalca districts (Kiyiköy, Kizilagaç, Kömürköy, Sergen, Vize, Yaliköy and Celepköy).

The larvae collected were sorted into groups, by localities, in plastic boxes and transferred to the laboratory, where they were fed on oak leaves. The food was changed every 1-2 days. Each of the pupae (and parasitized specimens) were kept in separate plastic containers and checked every day until host and/or parasitoids emerged. Dead larvae were separated into Petri dishes and stored for one month in a refrigerator at 4 to 6 °C, and later for 5 to 6 days in a humid chamber at room temperature (18 to 20 °C). Microscopical analyses were made with a microscope NU 2. The evaluation of *E. maimaiga* infections was recorded as positive when azygospores were detected in the bodies of dead larvae. The average number of azygospores in the samples was calculated on the base of three visual fields at magnification × 125.

Results

The results from the field collections showed that the number of *L. dispar* in the European part of Strandzha Mountains was very low in 2011. *L. dispar* was found and collected in three localities only – Kiyiköy, Vize and Yaliköy (Fig. 1). In Kiyiköy and Yaliköy mixed oak stands were formed by *Quercus cerris* L., *Quercus frainetto* Ten., *Quercus hartwissiana* Stev. and *Quercus pubescens* Willd., whereas in Vize predominantly *Q. pubescens* in a karst habitat was found In total, only small numbers of larvae were found (n= 43, Table 1). An indication of the *L. dispar* low population density in these and other studied Strandzha Mountain locations was also the lack of egg batches of the pest on tree stems in the forest stands.

The collected *L. dispar* larvae were in II to IV instar. The youngest larvae were observed in Kiyiköy (89.5% of the larvae were in II instar), and the oldest larvae in Vize (73.3% – IV instar) (Fig. 2).

During the observation period in the laboratory 19 larvae of *L. dispar* died (6 from the region of

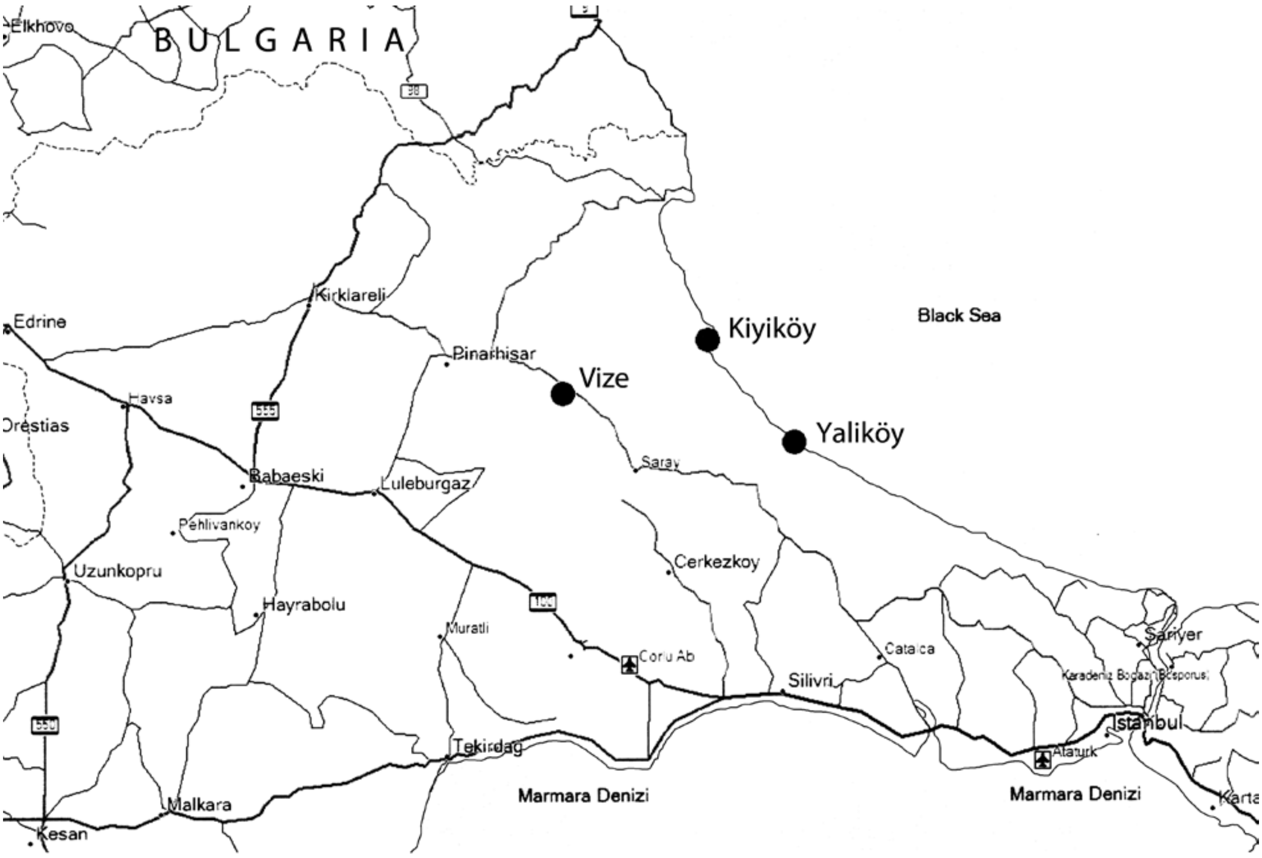

Fig. 1. Localities where *Lymantria dispar* was found in the European part of Turkey.

Table 1. Main characteristics of studied areas and mortality of *Lymantria dispar* caused by *Entomophaga maimaiga.*

Locality	Geographical coordinates		Altitude, m a.s.l.	Date of collection of *L. dispar* larvae	Studied larvae of *L. dispar*	Mortality of *L. dispar* caused by *E. maimaiga*, %
	N	E				
Kiyiköy	41°39’26.2”	28°05’08.6”	26	22 May 2011	19	0
Yaliköy	41°29’26.6”	28°16’35.8”	8	23 May 2011	9	11.1
Vize	41°35’02.1”	27°47’41.0”	338	25 May 2011	15	53.3

Kiyiköy, 9 from Vize and 4 from Yaliköy). Another 2 larvae from the region of Kiyiköy were parasitized by *Cotesia melanoscela* (Ratzeburg, 1984) (Hymenoptera: Braconidae). The rest of the larvae (n = 24) developed successfully to adults.

The microscopic analyses showed azygospores and mycelia of *E. maimaiga* in the dead bodies of 9 larvae – 8 from the region of Vize and 1 from Yaliköy. The mortality caused by the pathogen was 11.1% in larvae from the region of Yaliköy and 53.3% in larvae from the region of Vize (Table 1). The average number of azygospores in the dead larvae from Vize (1.71 ± 1.25 for a visual field) was nearly the same as the number of azygospores in the dead larvae from Yaliköy (1.67). *E. maimaiga* or other (bacterial or viral) pathogens were not found in the bodies of the remaining dead larvae and we conclude that this mortality was most likely caused by physiological disturbances.

Discussion

We were able to show that *E. maimaiga* was detected in two localities in the European part of Turkey. It is possible for the species to be widely distributed but the low number and mainly the young age of *L. dispar* larvae studied is the cause for lack or low pest infestation. *E. maimaiga* is able to infest all larval in-

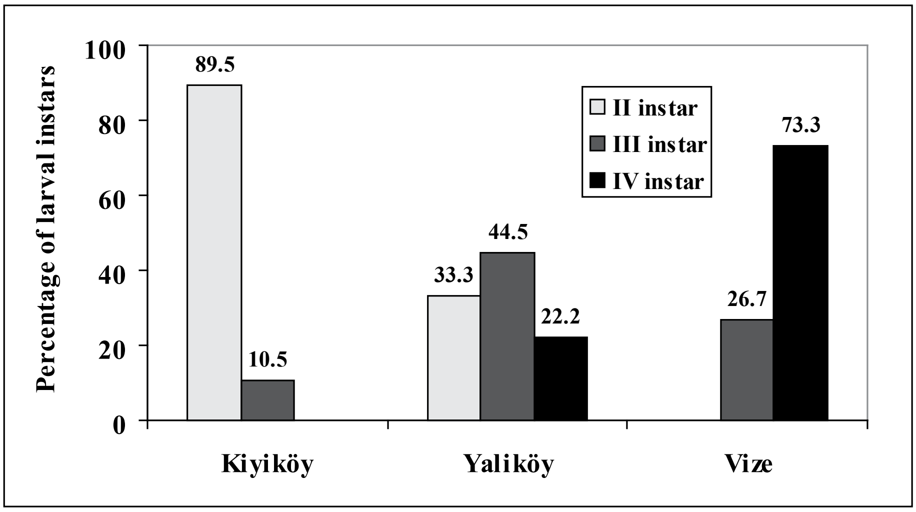

Fig. 2. Larval instar structure of *Lymantria dispar* at the moment of collection.

stars of gypsy moth after they start the day-and-night migrations. Infections of the resting phases of larvae occur on the ground. During this phase it is most likely to come into contact with the pathogen since the azygospores are in the upper layers of soil, and their concentration around the tree stems is the highest (Hajek 1999). This explains the higher level of infection in Vize, where the predominant part of *L. dispar* larvae were in IV instar, in comparison with Yaliköy, where the larvae were mainly in III and II instar, as younger larvae do not rest on the ground so often. The pathogen was not found at the third studied locality (Kiyiköy) although it might be possible that infections occurred later in the older instars of the host after the collection of the samples.

The cool spring of 2011 in Bulgaria led to delay in the development of *L. dispar* of 3 to 4 weeks (unpublished data). A similar delay in the gypsy moth development was also observed during the collections in the European part of Turkey. No doubt the more advanced developmental stage of gypsy moth larvae in the region of Vize is due to the warmer habitat on the karst terrain. Also and despite its higher altitude, compared to the other two localities, the cool Black sea in spring is too far away to have an effect on larval development (Fig. 1).

Entomophaga maimaiga is an extremely efficient pathogen. It controls the number of *L. dispar* at both high and low levels of population density. Since 1989, the pest is under the economic thresholds of damage at all sites with fungal epizootics in the USA. The main reason for this is considered to be the impact of *E. maimaiga* (Schneeberger 1996). During peak devastation years before 2000 in Bulgaria, gypsy moth defoliated up to 110 000 to 370 000 ha forest stands (Georgiev *et al.* 2007). After the introduction of *E. maimaiga* in 1999, the last gradation (2001-2009) of *L. dispar* damaged 108 000 ha only, which is about 10-20% of the typical damages in the previous 5 known gradations of the pest since 1953, which accounted for 492 000 to 1 028 000 ha (Georgiev *et al.* 2011). The data on the effectiveness of the pathogen are extremely impressive having in mind that after the introduction of *E. maimaiga* ten years ago no insecticides were used in Bulgaria against the *L. dispar*, as compared to previous periods when large-scale application of both, bacterial preparations on the base of *Bacillus thuringiensis* var. *kurstaki* (Dipel, Foray, etc.), synthetic hormones, and insect growth regulators (Dimilin, Mimic, etc.) were used.

The data from this study do not allow an assessment of the impact of *E. maimaiga* on the population of *L. dispar* in Turkey. However, it is possible that the low population density of the pest in Strandzha Mountains in 2011 is a result of both, a regular gradation decrease and the limiting role of the pathogen. We recommend that monitoring should be carried out at more sites via regular collection of gypsy moth larvae in burlap bands, located on the tree stems. Such collections provide abundant biological material (Pilarska *et al.* 2006). The monitoring should cover the Asiatic part of Turkey, too, as *E. maimaiga* was recently found in Georgia, east of Turkey (Kereselidze *et al.* 2010).

In conclusion, *E. maimaiga* proves to be a preferred alternative to the use of microbial and chemical insecticides to control *L. dispar*. In case a fungal epizootic occurs in Turkey, it is suggested to augmentatively expand the range of pathogen in oak forests newly attacked by the pest. The introduction of *E. maimaiga* into the local complex of antagonistic species will increase the stability within deciduous forest ecosystems and decrease the frequency and intensity of *L. dispar* outbreaks.

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References

Andreadis T. G., R. M. Weseloh. 1990. Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. – *Proceedings of the National Academy of Sciences of the USA*, **87**: 2461-2465.

Georgiev G., P. Mirchev, D. Pilarska, V. Golemanski, P. Pilarski, H. Tomovski, N. Bochev. 2007. The gypsy moth will be neutralized. – *Gora*, **5**: 8-10. (In Bulgarian).

Georgiev G., D. Pilarska, P. Mirchev, B. Rossnev, P. Petkov, P. Pilarski, V. Golemansky, M. Todorov, D. Takov, Z. Hubenov, M. Georgieva, M. Matova, S. Kitanova. 2010. *Entomophaga maimaiga* – a factor for increasing stability and enhancing biodiversity in oak forests on the Balkan Peninsula. – In: Rakonjac L. (Ed.): Proceedings of International Scientific Conference 'Forest Ecosystems and Climate Changes', Vol. 1, Belgrade, 181-185.

Georgiev G., P. Mirchev, B. Rossnev, P. Petkov, M. Georgieva, M. Matova, S. Kitanova, D. Pilarska, P. Pilarski, V. Golemansky, M. Todorov, Z. Hubenov, D. Takov. 2011. Introduction of *Entomophaga maimaiga* and control of *Lymantria dispar* calamities in Bulgaria. – In: Kitanova S. (Ed.): Proceedings of Scientific Conference 'Sustainable management of oak forests in Bulgaria', Primorsko, 72-79. (In Bulgarian, English summary).

Coulson J. R. 1981. Parasites, Foreign Explorations, Recent History: 1961-77. – In: Doane C., McManus M. (Eds.): The Gypsy Moth: Research Toward Integrated Pest Management, U. S. Department of Agriculture, Washington D. C., 302-310.

Hajek A. E. 1999. Pathology and Epizootiology of *Entomophaga maimaiga* Infections in Forest Lepidoptera. – *Microbiology and Molecular Biology Reviews*, 814-835.

Hajek A. E., E. L. Butler, M. M. Wheeler. 1995. Laboratory bioassays testing the host range of the gypsy moth fungal pathogen *Entomophaga maimaiga*. – *Biological Control*, **5**: 530-544.

Hajek A. E., M. McManus, I. Jr. Delalibera. 2005. Catalogue of Introductions of Pathogens and Nematodes for Classical Biological Control of Insects and Mites. FHTET, USDA Forest Service, Morgantown, 59 p.

Howse G. M., T. Scarr. 2002. Status of important forest insects in Ontario in 2002, Report prepared for the annual Forest Pest Management Forum, Ottawa, 24 p.

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

McManus M., T. McIntyre. 1981. Introduction. – In: Doane C., M. McManus (Eds.): The Gypsy Moth: Research Toward Integrated Pest Management, U. S. Department of Agriculture, Washington D. C., 1-7.

McManus M., G. Csóka. 2007. History and Impact of Gypsy Moth in North America and Comparison to Recent Outbreaks in Europe. – *Acta Silvatica et Lignaria Hungarica*, **3**: 47-64.

Pilarska D., M. McManus, A. Hajek, F. Herard, F. Vega, P. Pilarski, G. Markova. 2000. 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*, **73**: 125-126.

Pilarska D., M. McManus, P. Pilarski, G. Georgiev, P. Mirchev, A. Linde. 2006. Monitoring the establishment and prevalence of the fungal entomopathogen Entomophaga maimaiga in two *Lymantria dispar* L. populations in Bulgaria. – *Journal of Pest Science*, **79**: 63-67.

Pilarska D., A. Linde, P. Pilarski, G. Georgiev, D. Takov, L. Solter. 2010. Release of *Nosema lymantriae, Vairimorpha disparis* and *Entomophaga maimaiga* for classical and augmentative biological control of gypsy moth in Bulgaria and the United States. – In: Proceedings of 43rd Annual Meeting of the Society for Invertebrate Pathology, Trabzon, CD, 1-6.

Reardon R. C. 1981. Parasites, Foreign Explorations, Past History: 1900-60. – In: Doane C., M. McManus (Eds.): The Gypsy Moth: Research Toward Integrated Pest Management, U. S. Department of Agriculture, Washington D. C., 301-302.

Smitley D. R., L. S. Bauer, A. E. Hajek, F. J. Sapio, R. A. Humber. 1995. Introduction and establishment of *Entomophaga maimaiga*, a fungal pathogen of Gypsy moth (Lepidoptera: Lymantriidae) in Michigan. – *Environmental Entomology*, **24** (6): 1685-1695.

Speare A. T., R. H. Colley. 1912. The artificial use of the brown-tail fungus in Massachusetts with practical suggestions for private experiments, and a brief note on a fungous disease of the gypsy caterpillar. Wright & Potter, Boston, 31 p.

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