Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 66, No 7, 2013

BIOLOGIE
Ecologie

POTENTIAL OF *ENTOMOPHAGA MAIMAIGA* HUMBER, SHIMAZU AND SOPER (ENTOMOPHTHORALES) FOR SUPPRESSING *LYMANTRIA DISPAR* (LINNAEUS) OUTBREAKS IN BULGARIA

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(*Submitted on March 26, 2013*)

Abstract

The Asian fungal entomopathogen *Entomophaga maimaiga* was first introduced in Bulgaria for biological control of gypsy moth, *Lymantria dispar*, in 1999 and the first epizootics in gypsy moth populations were observed in 2005. Six introductions of *E. maimaiga* in oak forests with heavy *L. dispar* infestations were conducted from 2008 to 2011 in different regions of the country, including Nova Zagora, Gorna Oryahovitsa, Popovo, Targovishte and Staro Oryahovo. As a result of these introductions, host density was decreased by 55.1–100% and the outbreaks were suppressed. After the introduction of the pathogen in Bulgaria, no microbiological or chemical control of *L. dispar* has been necessary and the last two gradations have been estimated to be 15–30% of the values in previous gradations.

Key words: *Lymantria dispar*, *Entomophaga maimaiga*, introductions, epizootics, biological control

The study was supported by the National Science Fund of Bulgaria, Project DO-02/282/2008.

Introduction. Gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), is native to Europe, Asia, Japan and North Africa. It is widely polyphagous species associated with more than 300 species of deciduous and coniferous trees and shrubs, but prefers a variety of oak species (*Quercus* spp.). Populations of this species periodically outbreak with peak density occurring approximately every 8–11 years, and cause a broad scale defoliation. Outbreaks are most dramatic in Southeastern Europe, especially the Balkans, where an abundance of oak trees and favourable conditions for development and survival of the pest occur. Damage leads to reduced growth and physiological damage, but defoliation for several consecutive years can cause death of infested stands.

In 1868, the gypsy moth was unintentionally introduced from Europe to North America. Due to the lack of natural enemies, the species established to become a major pest in eastern deciduous forests and spread to the Midwest and southern Appalachian states. Different control strategies have been employed, including classical biological control. Among them, the introduction of the entomopathogenic fungus *Entomophaga maimaiga* from Japan was very successful [1]. Presently, *E. maimaiga* has expanded its range and is found almost everywhere in the gypsy moth range in the United States and Canada [2, 3].

In 1999, *E. maimaiga* was successfully introduced in Bulgaria from isolates collected in the US [4]. The first strong epizootics caused by the fungus were observed in 2005 in different areas of Northern and Southern Bulgaria [5]. They showed that *E. maimaiga* is an effective regulator of gypsy moth density in Bulgaria and has great potential as a biological control agent.

In this paper, we describe the results of new introductions of *E. maimaiga* in emerging outbreaks of *L. dispar*.

Materials and methods. Studies were conducted in 2008–2012. Introductions of *E. maimaiga* were performed in six outbreaking populations of *L. dispar* in oak forests with more than 70% estimated levels of defoliation. Information about the study areas, population density of *L. dispar* and the origin of *E. maimaiga* used for introductions is provided on Table 1.

The population density of gypsy moth was based on the data from the monitoring of three generations of the pest, one in the year of introduction and two in the next two years. Newly oviposited *L. dispar* egg masses were counted in each site on 100 trees.

E. maimaiga was introduced into each site by incorporating crushed infected insect carcasses containing *E. maimaiga* resting spores into 100–150 g soil from the humus layer as described in [4]. The inoculum for the introductions was kept in soil substrate under natural conditions for more than one year. Two introductions of *E. maimaiga* were conducted during the spring months (2008 and 2011), and the remaining in the fall (Table 1).

Larvae of *L. dispar* were collected from double-layered burlap bands placed on 25 oak trees in each study site, 2–3 times a month from early May to late July.

T a b l e 1

Main characteristics of studied areas, *L. dispar* density and origin of *E. maimaiga*

Locality	Forest (Hunting) Enterprise	Geographical coordinates	Altitude, m a.s.l.	Tree species[a]	Density of *L. dispar*[b]	Date of introduction	Origin of *E. maimaiga*
Sadievo	Nova Zagora	42°31.783'N; 026°08.901'E	151	Q.r	83	28.03.2008	Bulgaria[c]
Assenovo	G. Oryahovitsa	43°17.695'N; 026°04.051'E	401	Q.c	78	18.11.2009	USA
Slavyanovo	Popovo	43°17.090'N; 026°08.834'E	345	Q.c	89	18.11.2009	USA
Ruets	Targovishte	43°12.119'N; 026°37.950'E	312	Q.c.; C.b.	76	18.11.2010	Bulgaria[c]
Dalgach	Targovishte	43°12.966'N; 026°42.478'E	193	Q.ru.; T.p.	86	18.11.2010	Bulgaria[c]
Solnik	S. Oryahovo	42°54.268'N; 027°44.296'E	202	Q.f.; Q.c	183	05.04.2011	USA

[a] – C.b. – *Carpinus betulus* L.; Q.c. – *Quercus cerris* L.; Q.f. – *Quercus frainetto* Ten.; Q.r. – *Quercus robur* L.; Q.ru. – *Quercus rubra* L.; T.p. – *Tilia platyphyllos* Scop.

[b] – Egg masses per 100 trees

[c] – Epizootics in Bulgaria originating from material obtained from the USA

Collected larvae were returned to the laboratory and reared in groups of 10–20 on fresh oak foliage in plastic boxes (15 × 10 × 8 cm). The foliage was changed daily and dead larvae were removed for examination.

Dead gypsy moth larvae were placed individually in Petri dishes with a water-saturated filter paper disc and held at room temperature for 7 days and then refrigerated at 5 °C until microscopic evaluation was performed. Microscopic analyses were made with a Zeiss Jena NU 2 microscope at 125x. Each cadaver was dissected individually and observed for the presence of conidia or azygospores of *E. maimaiga*.

Information about *L. dispar* infestations in the forests of Bulgaria is presented graphically according to literature data [6] and information from the Bulgarian Executive Forest Agency.

Results and discussion. ***Introduction in 2008.*** In 2008, *E. maimaiga* infected and destroyed 87.5% of the fifth and sixth instar *L. dispar* larvae in Sadievo locality (Table 2).

Table 2

Mortality of late instar *L. dispar* larvae caused by *E. maimaiga*

Locality	Year	Number of studied *L. dispar* larvae	Mortality caused by *E. maimaiga*, %
Sadievo	2008	128	87.5
Assenovo	2010	39	97.4
Slavyanovo	2010	40	95.0
Ruets	2011	8	100.0
Dalgach	2011	53	100.0
Solnik	2011	107	80.4

A late epizootic, however, led to a 60–80% defoliation of the stand by the pest. The reduction of new generation number was 96.4% (Fig. 1). No egg masses of the pest were recorded in the experimental site two years later.

Introductions in 2009. In the spring of 2010, an epizootic affecting mid- and late instar larvae of the gypsy moth occurred in Assenovo and Slavyanovo localities. The mortality of mid-larvae reached 44.1–54.1%, and of late-stage larvae – 95.0–97.4% (Table 2). No defoliation was observed in the oak stands. The reduction of the new generation was 55.1–81.8%, and was 100% one year later (Fig. 1). In 2010, gypsy moth epizootics occurred in many areas in the forests of Gorna Oryahovitsa Forest Enterprise and Popovo Hunting Enterprise, and caused attenuation of pest outbreaks in the region.

Introductions in 2010. Frequent and heavy rainfall occurred in May and June 2011 in the region of Targovishte and a strong *E. maimaiga* epizootic destroyed mid- and late instar *L. dispar* larvae in Ruets and Dalgach localities (Table 2) (Fig. 2). Defoliation in the experimental sites was not observed and

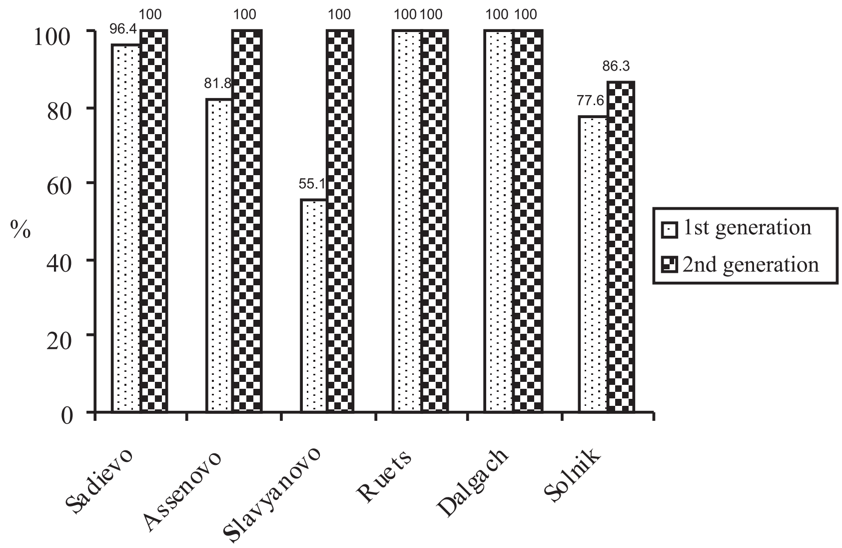

Fig. 1. Reduction of population density of *L. dispar* after *E. maimaiga* introduction

no egg masses were recorded for the next *L. dispar* generations (Fig. 1). Conidial infections were recorded in many areas near Targovishte, which undoubtedly caused the rapid suppression of the outbreak in Northeastern Bulgaria, where most oak forests and strongest gypsy moth infestations in the country typically occur. *E. maimaiga* is known to spread to more than 100 km in one season [7].

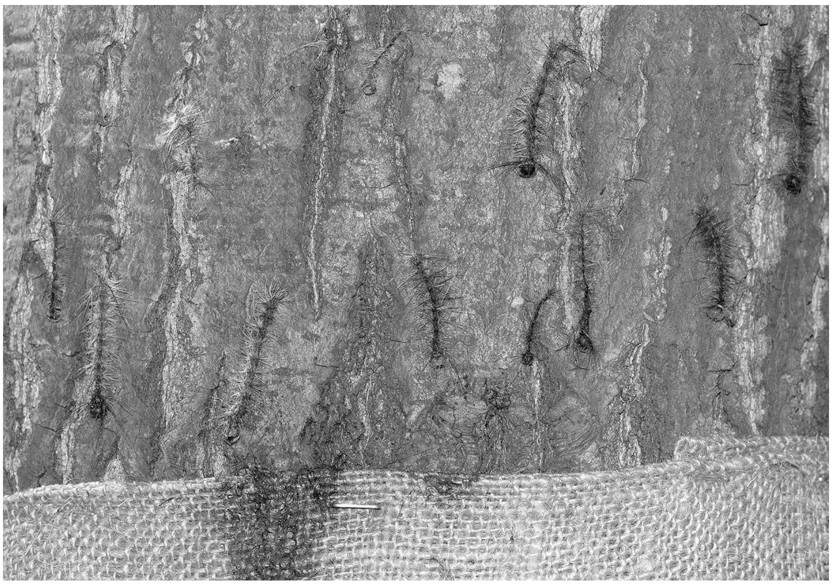

Fig. 2. Mortality of *L. dispar* larvae in Dalgach locality caused by *E. maimaiga*

Introduction in 2011. In the spring of 2011, 80.4% mortality of the late instar gypsy moth larvae was recorded in the experimental site at Solnik (Table 2). The reduction of *L. dispar* in the next generation was 77.6%, and it was 86.3% one year later (Fig. 1). Strong defoliation of oak forests of *L. dispar* was observed in 2011 on the Black Sea coast in the region of Nessebar Hunting Enterprise, approximately 30–50 km from Solnik. *E. maimaiga* was not introduced in the area, but because it was found in many areas along the coast and in Strandzha Mt. (unpublished data), it is possible that it was naturally dispersed in the region. The lack of rainfall from mid-May to mid-July could be the reason that no epizootic occurred. *E. maimaiga* is not dependent on *L. dispar* population density due to long survival of resting spores in the soil [8] but successful epizootics depend largely on the availability of rainfall during the period of larval development of the host. In 2012, the conditions for development of *E. maimaiga* in the area were more favourable and the outbreaks that occurred in the central Black Sea coast area were suppressed by massive epizootics caused by the pathogen (unpublished data).

Attacks of L. dispar in the forests of Bulgaria. Until the introduction of *E. maimaiga* in Bulgaria, *L. dispar* was the most deleterious pest of deciduous forests in Bulgaria. In the years of massive outbreaks, the pest defoliated between 150 000 and 370 000 ha of forest (Fig. 3). After the introduction of the pathogen (1999), outbreaks were not observed and annual infestations did not exceed 25 thousand ha.

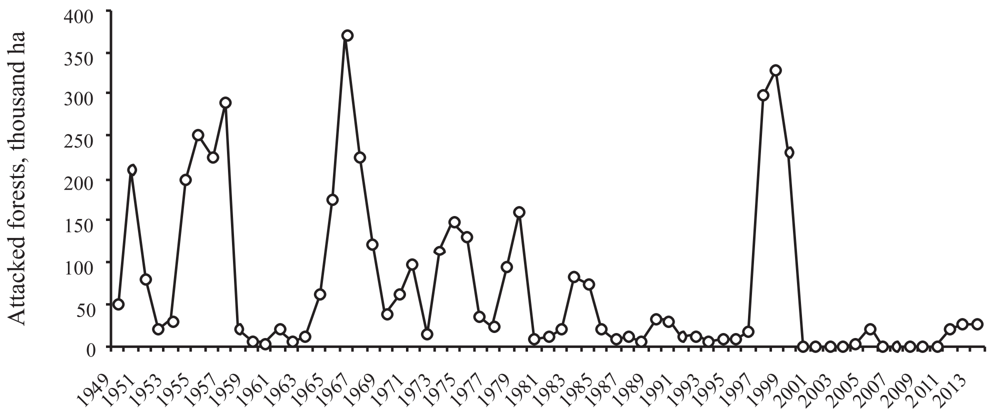

Fig. 3. *Lymantria dispar* outbreaks in Bulgarian forests

Outbreaks in different gradations of *L. dispar* until 2000 covered 492 to 1028 thousand ha of forests [9]. In the first graduation after introduction of *E. maimaiga* (2000–2009), outbreaks were recorded in only 23 thousand ha (Fig. 3), which accounted for only 2–5% of normal levels of the infestations until the introductions. For the first four years of the last graduation (2010–2013), outbreaks were recorded in 71 000 ha, 7–15% of the values recorded before the introduction of the pathogen. Forecasting by extrapolation shows that by the end of the current gradation (about 2017–2018), infested forests should not exceed 150 000 ha or 15–30% of outbreaks before 2000.

Conclusions. Low levels of *L. dispar* outbreaks after the introduction of *E. maimaiga* are strong evidence for the effectiveness of the pathogen as regulating factor of the pest. After introduction of *E. maimaiga* in Bulgaria, control measures were significantly reduced.

E. maimaiga is expanding its range in the Balkan countries [10] and in the near future it should be expected to occur in other countries in Southeastern Europe. Specific characteristics of *E. maimaiga*, including high virulence, species specificity and the ability to regulate *L. dispar* density at both high and low host population density, suggest that this classical biological control agent will have enormous positive economic, social and environmental impacts as an alternative to the use of bacterial and chemical insecticides.

Acknowledgements. We are very grateful to Dr Leellen Solter from Illinois Natural History Survey for her valuable comments and editing.

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