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Full Length Research Paper

Entomophaga maimaiga – New entomopathogenic fungus in the Republic of Serbia

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The higher mortality rate of the older gypsy moth larval instars was reported in the forest complexes of Belgrade and Valjevo region, in the culmination phase of the new outbreak of the gypsy moth in Serbia. By field and laboratory studies of the causes of their death, the presence of conidia and resting spores of the entomopathogenic fungus *Entomophaga maimaiga* was reported in the dead caterpillars. This has been the first report of occurrence of this species in Serbia, that is, Serbia is the third European country in which this fungus has been reported. It showed to be a powerful reducer of the population size of the gypsy moth, and in both regions it caused the collapse of the outbreak in 2011.

Key words: *Entomophaga maimaiga, Lymantria dispar*, gypsy moth, biological control, epizootics.

INTRODUCTION

Classical biological control is simply a special case of a general pattern in which populations are regulated by density-dependent processes, a major class of which involves predator-prey and parasitoid-host interactions.

Naturally occuring entomopathogens are important regulatory factors in insect population. Entomopathogenic organisms, various types of viruses, microsporidia, bacteria, protozoa, fungi, nematodes, which can under favourable conditions cause massive insect mortality and are of great breeding capacity, normally live in nature. Epizootics caused by naturally occurring viral and fungal pathogens are often responsible for spectacular crashes of insect pest populations (Evans, 1986; McCoy et al., 1988).

Although there are numerous entomopathogenic microorganisms which cause insect mortality and in this way have one of the dominant roles in the regulation of their number in nature, relatively small number of them has so far being practically used in the harmful insect control (Tabakovic-Tosic, 2008).

Fungal entomopathogens have been used more frequently than other types of pathogens for classical biological control. Among 136 programs using different groups of arthropod pathogens, in 49.3% the fungal pathogens were introduced. The most comonly introduced species was *Metarhizium anisopliae* (Metschnikoff) Sorokin, which was released 13 times, followed by *Entomophaga maimaiga* Humber, Shimazu and Soper, which was released seven times (Hajek and Delalibera, 2010).

The fungal order Entomophthorales in the class Zygomycetes is mainly composed of obligate pathogens that infect arthropods. More than 300 species infect a wide variety of hosts, and nearly every fungal species or strain is quite host specific. Species within the Entomophthorales are well-known for their ability to cause dramatic epizootics in populations of aphids, leafhoppers and planthoppers, flies, grasshoppers, cicadas, and coleopteren and lepidopteren larvae (Hajek, 1999).

The entomopathogenic fungus *E. maimaiga* (Entomophtorales: Entomophtoraceae) was isolated and described as the natural enemy of the gypsy moth in Japan, where it causes periodical epizootics. It is also spread in some parts of China and the Russian Far East (Hajek et al., 2005). In spite of the fact that it was

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introduced in North America in 1910-1911 (Speare and Colley, 1912), its presence in the natural populations of gypsy moth was determined only in 1989 (Hajek et al., 1996), when the pathogen caused pandemic in several countries (Andreadis and Veseloh, 1990; Hajek et al., 1990b; Smitley et al., 1996; Reardon and Hajek, 1998). Today *E. maimaiga* is a very significant pathogen of gypsy moth in North America and in Canada (Balser and Baumgand, 2001; Hajek, 1997; Hajek et al., 2005; Howse, 2002; Hoover, 2000).

Bulgaria has been the third country in the world and the first one in Europe in which *E. maimaiga* was introduced successfully. The first epizootic of it occurred in 2005, and the latest ones were reported in the very vicinity of the Bulgarian borders with Serbia, Greece and Turkey (Pilarska et al., 2000, 2006; Georgiev et al., 2007, 2010). It is assumed that this species is also present in oak forests in some Serbian regions. This hypothesis can be supported by the fact that the increased population size of gypsy moth during the latest outbreak (2003-2005) was not reported only in the oak stands of the border, Pirot region (Tabakovic-Tosic et al., 2011).

We believed it was of vital importance to start the research program on the presence and spread of *E. maimaiga* in the Republic of Serbia, so that in this paper we present the first results of this program.

MATERIALS AND METHODS

During the growing season in 2011, in some forest areas of central Serbia, where the increased mortality rate of the gypsy moth larvae was reported and where there was no significant damage of the foliage caused by the feeding of them, the intensive research of the possible causes of this condition was done. The intensive research was conducted in the forest complexes located in Belgrade and Valjevo regions: Management Unit Košutnjačke šume (Public Enterprise Srbijašume, Forest Estate Belgrade, Forest Administration Lipovica) – Borački gaj (α: 44° 32' N, δ: 20° 21' E; from 120 to 170 m above sea level): Even-aged (about 65-year-old), well-preserved *Quercus cerris* L. (80%) and *Quercus frainetto* Tenore (20%) coppice stands; Management Unit Bogovadja (Diocese of Valjevo) – Monastery forests (α: 44° 19' N, δ: 20° 11' E; from 140 to 190 m above sea level): Approximately even-aged (up to 82-year-old), mixed coppice *Quercus cerris* L. (60%), *Carpinus betulus* L. (30%), and *Quercus frainetto* Tenore or *Tilia argentea* Desf. (10%) (Figure 1). Average values of climatic elements of the study area [data of the Republic Hydrometeorological Service of Serbia, Belgrade Meteorological Station (α: 44°48´ N; δ: 20°28´ E; the altitude of 132 m] are presented in the Figure 2.

From both sites, the dead gypsy moth larvae of the L_{4-6} larval instars were sampled from the oak tree, in the summer and autumn 2011. Every seven days the caterpillars (alive and dead) were collected in order to grow under laboratory conditions and to determine the cause of the high mortality rate of them that is, to verify the presence of the permanent spores of the entomopathogenic fungus *E. maimaiga* on gypsy moth cadavers.

The larvae were grown in the climate chamber under the laboratory conditions. During the experiment, temperature and light conditions were constant (temperature 21°C, light regime - 8 h a night, 16 h a day). The larvae were on daily basis fed on the fresh leaves of the oak, brought from the sample plots.

The dead larvae were placed in Petri dishes with wet filter paper. They were kept 7 days in the laboratory and then stored in the refrigerator. After the storage in the refrigerator for 3 months, in October 2011 the detailed microscope survey of the dead gypsy moth caterpillars was done. The evaluation of *E. maimaiga* infections was recorded as positive when azygospores and conidiospores were detected in the cadavers of dead gypsy moth larvae. The species identification was based on the size, shape and structural characteristics of different life forms of the fungus – azygospores, conidiospores and mycelia.

RESULTS AND DISCUSSION

The Palearctic, economically harmful insect species *Lymantria dispar* is one of the major pests of broadleaf forests in Serbia, where the forest complexes cover an area of 2.4 million hectares, with the wood volume which is 235 million m^3 (Bankovic et al., 2009). The outbreak of gypsy moth, over the 150-year-period occurred 17 times (Tabakovic-Tosic and Jovanovic, 2007), and the 18th outbreak started in 2009-2010.

In spring 2011, the selected forest areas in Belgrade and Valjevo regions where the great increase of the population size of the gypsy moth was reported (magnitude of attack in autumn 2010: 5,000 and 3,200 egg masses/ha) were observed in a great detail. It was first observed that there was no considerable damage of the foliage, which would normally be clearly visible. Even at the sample plots where the intensity of the attack was equal to several hundred egg masses per a hectare, which implies that the next instar can cause 100% defoliation, the trees looked as if the gypsy moth was in the latency phase. In addition, at some sample plots the increased mortality rate of the older larval instars in comparison with the expected one was reported. Then, the following question was posed: What prevent the larvae from the intensive feeding and doing harm and what has been killing them? In order to get the answer to this question, the detailed analysis of the possible causes was conducted.

The following results were obtained from the weekly field studies conducted in May, June and July 2011, when the gypsy moth is in L_{4-6} instars: a huge amount of dead larvae was found on the trees. By the detailed study of the dead gypsy moth larvae, two groups of the characteristic symptoms were reported:

The first group: The bodies tend to be stiff and straight, and the legs extend stiffly from the body (Figure 3A). Some of the dead larvae had tiny white conidia attached to the hairs on the body, characteristic symptoms caused by the entomopathogenic fungus *E. maimaiga*.

The second group: The bodies of dead larvae are soft, filled with a brown liquid and disintegrate rapidly. Usually, they hang limply in an inverted "V" position, characteristic symptoms caused by the entomopatogenic baculovirus *Lymantria dispar nuclear polyhedrosis virus* – (LdNPV) (Table 1).

The detailed microscope survey of the sampled dead

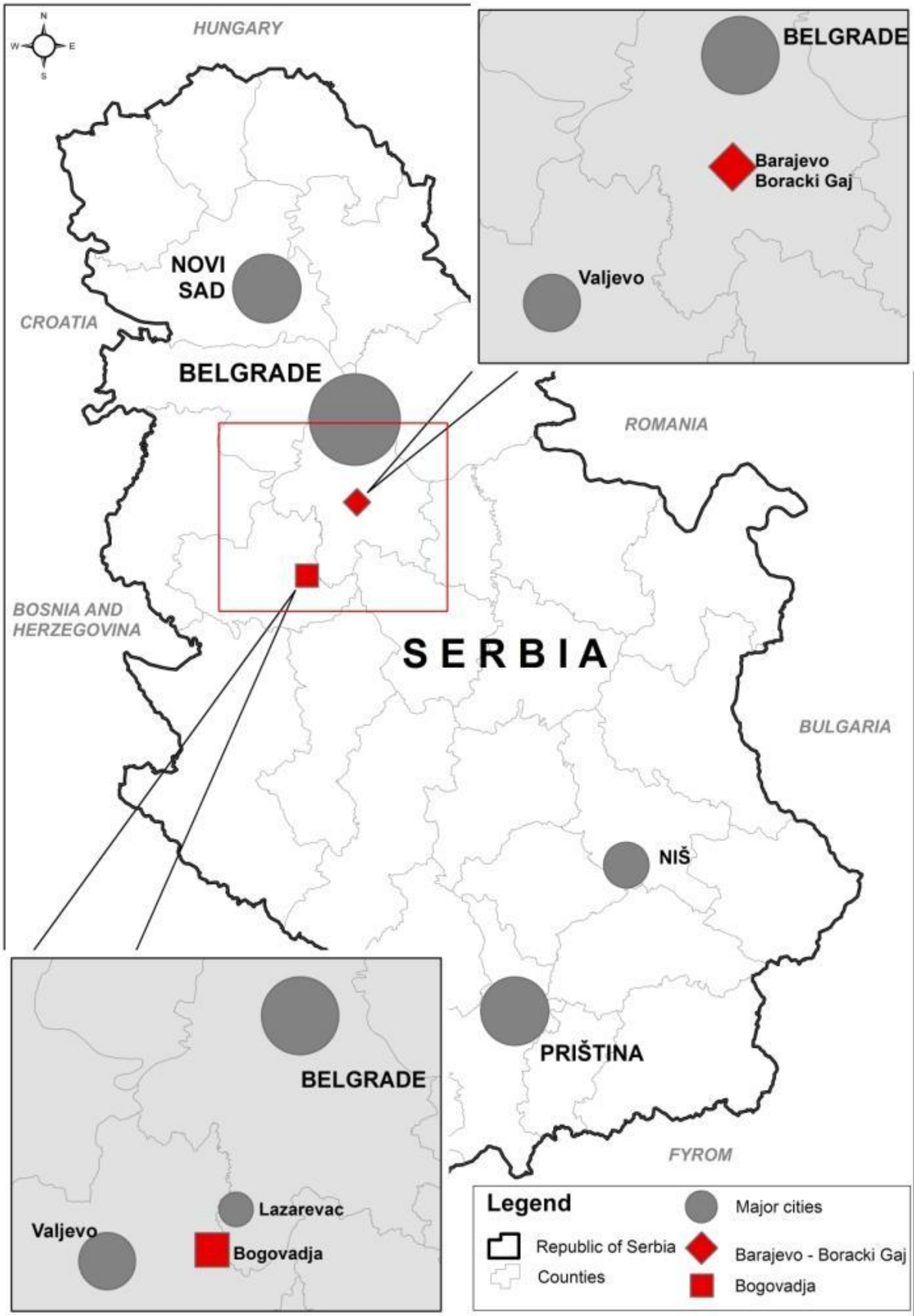

Figure 1. Geographical location of the investigated forest areas.

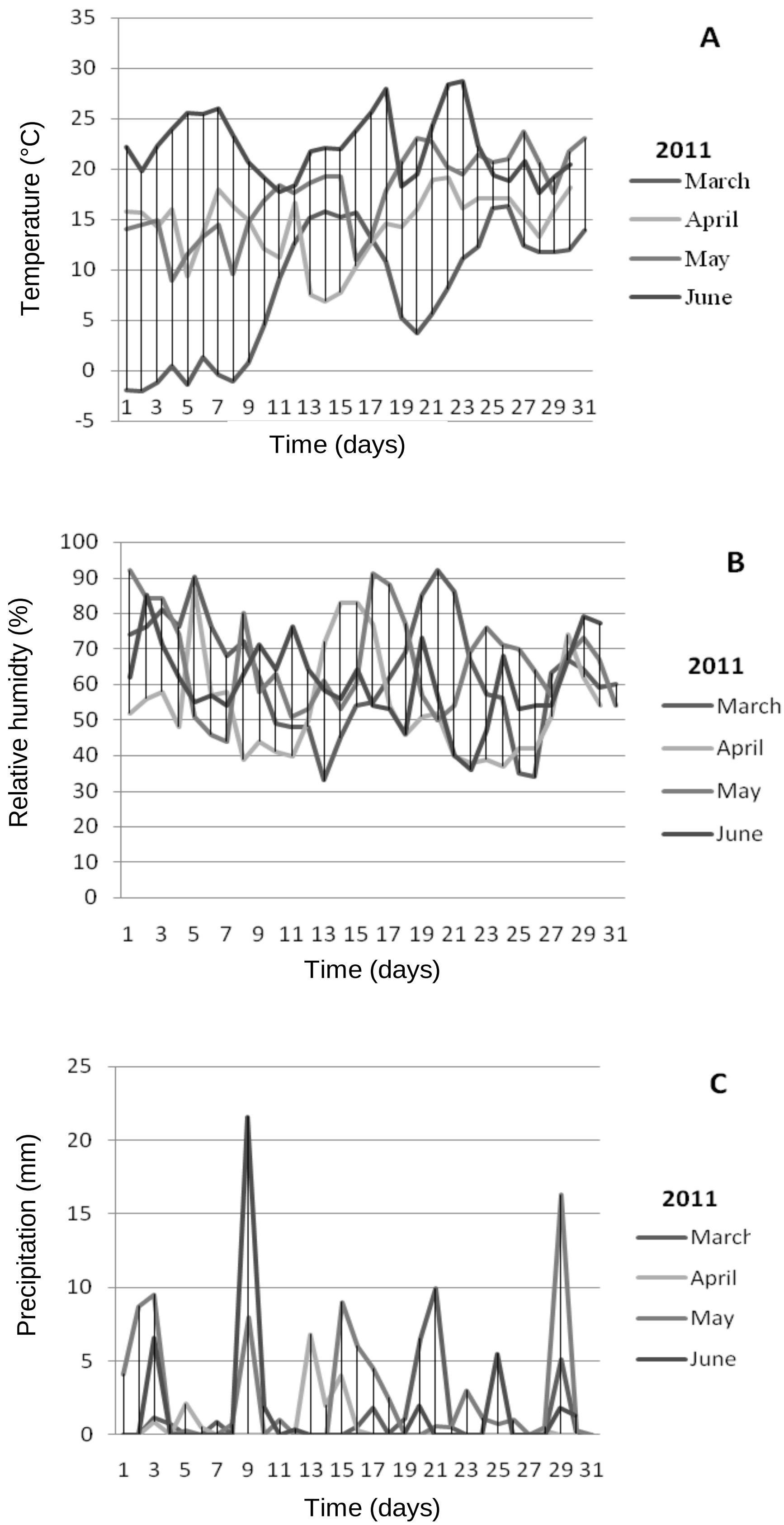

Figure 2. Climatic conditions of the study area during the experimental period. (A) Mean daily air temperature; (B) Mean daily relative humidity; (C) Precipitation.

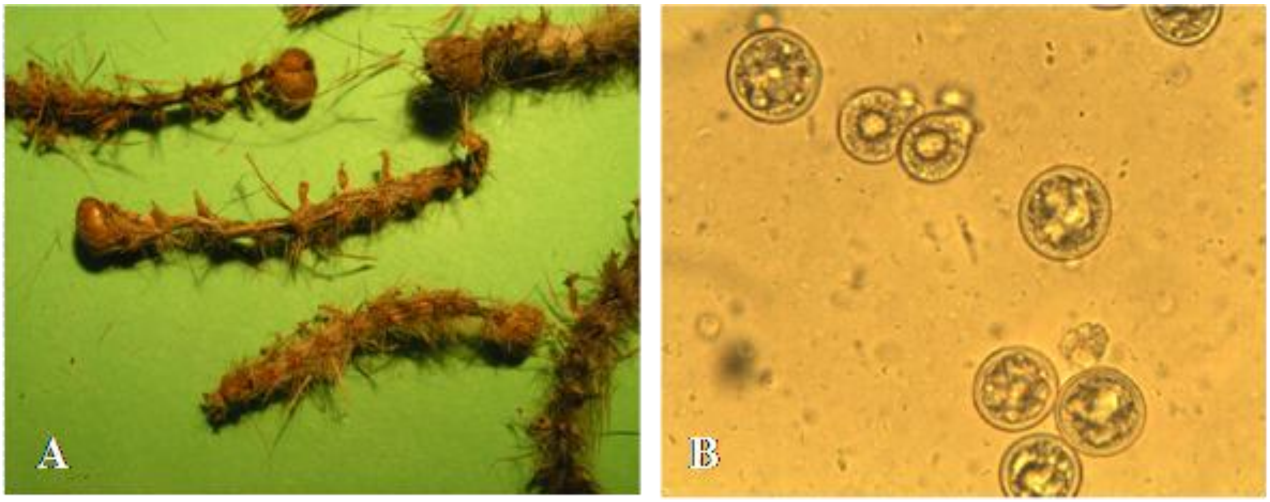

Figure 3. Pathogenic activity of *E. maimaiga* for larvae of *Lymantria dispar*. (A) Infected dead gypsy moth larvae; (B) Conidia and resting spores isolated from dead larvae.

Table 1. Larvae of *Lymantria dispar* with symptons of death caused by *Entomophaga maimaiga* and *Lymantria dispar nuclear polyhedrosis virus* in regions of Serbia.

Serbia region	Place of death							
	Entomophaga maimaiga symptoms				Baculovirus LdNPV symptoms			
	Laboratory		Forest		Laboratory		Forest	
	N	%	N	%	N	%	N	%
Management Unit Košutnjačke šume – Borački gaj	212	91.8	436	85.0	19	8.2	77	15.0
Management Unit Bogovadja – Monastery forests	98	94.2	269	92.4	6	5.8	22	7.6

gypsy moth larvae showed in all of them, without any exception, the presence of the numerous resting spores of the entomopathogenic fungus *E. maimaiga.* In addition, the presence of the conidia of this pathogen species was reported, but the number of them was considerably smaller (Figure 3B). Regardless of the fact that during the field research the clear symptoms of the disease, resulted in death (15% of the total number of the dead larvae), caused by the activity of LdNPV, were reported, during the laboratory microscope analysis the presence of them was not confirmed, which was expected, since the dead larvae in the decomposition phase were not sampled.

The weather plays an important role in the anticipation of the effectiveness of *E. maimaiga*. Like most fungi, its spores need moisture and high humidity to germinate (Hajek et al., 1990a; Hajek and Soper, 1992). Frequent rainfall during May and June contributes to the start and spread of *E. maimaiga* through a gypsy moth population (Weseloh and Andreadis, 1992; Weseloh et al., 1993; Smitley et al., 1996; Hajek et al., 1996). The temperature around 20°C greatly enhances fungal growth (Hajek, 1999).

It is clearly visible in the Chart 3 that May was the favourable month to the germination of the resting spores and to the infection of the gypsy moth larvae. The frequency of the rainy days and the average daily air temperature around 21°C (Chart 1) in the second half of the month, caused the massive epizootics in the observed area.

It is clearly visible in Figure 2B and C that May was the favourable month to the germination of the resting spores and to the infection of the gypsy moth larvae. The frequency of the rainy days and the average daily air temperature around 21°C (Figure 2A) in the second half of the month, caused the massive epizootics in the observed area.

In September 2011, the number of the newly laid egg masses of the gypsy moth was determined. In the oak stand located in Borački gaj, the number of the newly laid egg masses of the gypsy moth is 15 per a hectare (333 times less in comparison with 2010 when more than 5,000 egg masses/hectare was reported), whereas the presence of them was not reported in Bogovadja (the intensity of the attack in 2010 was 3,200 egg masses/ hectare). The reduction of the intensity of the attack and the complete collapse of the outbreak of the gypsy moth are caused by the activity of the entomopathogenic fungus *E. maimaiga*.

Conclusions

By field and laboratory studies of the causes of the mortality of the older gypsy moth larval instars the presence of the conidia and resting spores of the entomopathogenic fungus *E. maimaiga* was confirmed in the dead larvae. It has been the first discovery of this kind is Serbia, that is, Serbia is the third European country in which this fungus has been reported. It proved to be a powerful reducer of the population size of the gypsy moth since the population size was reduced from 5,000 and 3,200 egg masses/hectare in 2010 to only 15, and zero in 2011 at Borački gaj and Bogovadja, respectively.

By field and laboratory studies, the mortality of the older larval instars of *L. dispar* was confirmed throught the presence in the dead larvae of conidia and resting spores of the entomopathogenic fungus *E. maimaiga*. It has been the first record of this kind in Serbia, that is, Serbia is the third European country in which this fungus has been reported. It showed to be a powerful reducer of the population size of the gypsy moth since the population size was drastically reduced in Borački gaj and Bogovadja regions.

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