



ISSN 2320-7078

JEZS 2014; 2 (5): 262-266

© 2014 JEZS

Received: 25-08-2014

Accepted: 26-09-2014

Mara Tabaković-Tošić

Institute of Forestry, Kneza
Višeslava 311000 Belgrade, Serbia

Margarita Georgieva

Forest Research Institute, Sofia,
Bulgarian Academy of Sciences,
Bulgaria.

Zdravko Hubenov

National Museum of Natural
History, Sofia, Bulgarian
Academy of Sciences, Bulgaria.

Georgi Georgiev

Forest Research Institute, Sofia,
Bulgarian Academy of Sciences,
Bulgaria.

Impact of tachinid parasitoids of gypsy moth (*Lymantria dispar*) after the natural spreading and introduction of fungal pathogen *Entomophaga maimaiga* in Serbia

Mara Tabaković-Tošić*, Margarita Georgieva, Zdravko Hubenov, Georgi Georgiev

Abstract

In 2013, parasitoids of Tachinidae family of *Lymantria dispar* (L.) were studied in 12 forest stands in four regions in Serbia. Biological material for the investigations was collected in mixed oak and beech stands in which gypsy moth increased in number and where entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & Soper was established or introduced. A total of 1117 larvae and 814 pupae of *L. dispar* were collected and analyzed. Host mortality caused by tachinids in different localities varied between 0.7% and 37.1%. A total of 274 tachinid larvae and pupae were reared from gypsy moth larvae and pupae. Out of these, 266 died as pupae, resulting in an extremely high overall mortality of 97.1%. Only eight tachinid adult specimens of 3 species (*Compsilura concinnata*, *Exorista larvarum* and *Carcelia gnava*) emerged from tachinid pupae. *E. maimaiga* azygospores were observed on the surface of 69.5% but not in internal tissues of the dead pupae. The results of this study support the hypothesis of competition between natural enemies of the pest.

Keywords: *Entomophaga maimaiga*, impact, *Lymantria dispar*, mortality, Tachinidae.

1. Introduction

Gypsy moth, *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), is one of the major serious defoliators of broadleaved forests and orchards throughout Northern Hemisphere. It is characterised by high reproductive capacity, considerable ecological plasticity and polyphagia. Gypsy moth populations undergo periodic outbreaks to extremely high densities. Although it is found on four continents (North Africa, Asia, Europe, North America), the pest causes the greatest damage to forests of Balkan Peninsula, which have all the favourable environmental conditions for gypsy moth development, and it often increases its number. In the central part of Serbia there were five outbreaks of *L. dispar* during the period of seventy years, and the sixth outbreak started in the period 2009-2010 [1]. The outbreaks do not occur in regular time intervals. Generally, pest outbreaks in Serbia lasted for an average time period of 4.3 years (minimum two to three, and maximum five to six years). Also, certain phases of outbreak do not happen at the same time due to variety of biotic and abiotic influence on gypsy moth dispersal and occupation of new territories. Regular occurrence and characteristics of all outbreaks is their spatial expansion.

The population density of *L. dispar* is regulated by many biotic factors – parasitoids, predators and pathogens. Based on the literature data, a total of 102 species have been reported in Central Serbia as natural enemies of gypsy moth, i.e. 24 predators, 60 parasitic insects, 10 saprophagous insects and 8 pathogens [2-3]. Regarding the number of the species, the representatives of Diptera and Hymenoptera orders are the most frequent and most important ones [2].

Entomopathogenic fungus *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) was isolated and described as a natural enemy of the gypsy moth in Japan, where it causes the periodical epizootics. It is also spread in some parts of China and Russian Far East. Despite of the fact that it was introduced in North America in 1910-1911, its presence in natural populations of gypsy moth was determined only in 1989,

Correspondence:

Mara Tabaković-Tošić

Institute of Forestry, Kneza
Višeslava 311000 Belgrade,
Serbia.

when the pathogen caused pandemic in several countries of USA [4]. Today *E. maimaiga* is a very significant pathogen of gypsy moth there and in Canada.

In 1999, *E. maimaiga* was introduced into Bulgaria from the USA [5] and thereafter, in 2005, caused epizootics and mortality in four gypsy moth outbreak populations, located 30-70 km from the introduction sites [6]. From 2008 to 2011, six introductions of *E. maimaiga* were performed in outbreak populations of *L. dispar* in oak forests in different parts of the country [7]. This entomopathogenic species has slowly spread along Balkan Peninsula and Southeast Europe, and so far its presence has been reported also in European part of Turkey [8], Serbia [9-10], Greece, FYR Macedonia [11], Croatia [12], Hungary [13] and Slovakia [14]. Regarding the situation in Serbia, *E. maimaiga* was first established to spread naturally in two regions in central part of the country, Belgrade and Valjevo [9]. Then, the fungus was introduced into *L. dispar* population situated in mountain Avala in Belgrade region and was also introduced or found in many places of the country [15-16].

E. maimaiga is a host specific pathogen of gypsy moth in its natural range. In USA, the fungus causes low mortality in only 3 closely related species of Erebidae and single representative species from 2 other lepidopteran families [17]. In Bulgaria, a total of 1499 larvae of 38 non-target phylophagous insect species from 10 lepidopteran and 1 hymenopteran families were studied but *E. maimaiga* was not found in any of them [18]. The high level of host specificity of *E. maimaiga* limits the negative impact on non-target species but in biological control programs it is also important to evaluate impact on other biological agents of the target host. This is especially true for

the parasitoids in the family Tachinidae, which are strongly associated with late instar gypsy moth larvae where *E. maimaiga* also develops but insufficient information in entomological literature occurs. Currently there is only one more precise study on potential interactions between *E. maimaiga* and tachinid parasitoids during development in the gypsy moth larvae [19].

This paper reports results from the study on impact of tachinids of gypsy moth after natural spreading and introduction of *E. maimaiga* in Serbia and mortality of the parasitoids in pupal stage.

2. Material and Methods

The studies were conducted during the growing season in 2013, in some forest areas of Central Serbia (Kruševac, Donji Milanovac, Krupanj and Leskovac regions) in which *L. dispar* increases in number and *E. maimaiga* was found in the host populations.

Gypsy moth larvae were collected in oak or mixed forest stands dominated by different oak species, *Quercus cerris* L., *Q. petraea* (Matt.) Liebl., *Q. frainetto* Ten., and beech, *Fagus sylvatica* L. (Table -1). Each study plot was 100×100 m (1 ha) in size and included a minimum of 25 oak trees: five groups of five trees each, one chosen as the centre of the plot, and four additional trees located approximately 50 m from the centre tree at approximately 90°. During the spring and early summer, gypsy moth larvae were collected two to three times in each of the study sites. Larvae and pupae were collected manually from foliage in the lower parts of tree crowns and tree branches.

Table 1. Main characteristics of study localities.

Locality	Region	Altitude (m a.s.l.)	Geographic coordinates		Tree species
			N	E	
Srndałjska reka-1	Kruševac	493	43° 27' 18.73"	21° 26' 47.64"	<i>Quercus cerris</i> , <i>Quercus frainetto</i> , <i>Quercus petraea</i> , <i>Carpinus betulus</i>
Srndałjska reka-2	Kruševac	762	43° 26' 05.24"	21° 27' 42.25"	<i>Fagus sylvatica</i>
Srndałjska reka-3	Kruševac	500	43° 27' 08.71"	21° 29' 18.86"	<i>Quercus cerris</i> , <i>Quercus frainetto</i> , <i>Quercus petraea</i> , <i>Carpinus betulus</i>
Srndałjska reka-4	Kruševac	488	43° 27' 41.91"	21° 28' 38.07"	<i>Quercus cerris</i> , <i>Quercus frainetto</i> , <i>Quercus petraea</i> , <i>Carpinus betulus</i>
Monte Miroč	Donji Milanovac	365	44° 27' 28.10"	22° 12' 39.30"	<i>Quercus cerris</i> , <i>Quercus frainetto</i> , <i>Quercus petraea</i>
East Boranja-1	Krupanj	365	44° 22' 16.94"	19° 20' 31.77"	<i>Fagus sylvatica</i>
East Boranja-2	Krupanj	423	44° 22' 41.28"	19° 21' 40.19"	<i>Fagus sylvatica</i>
Village Miroševce-1	Leskovac	334	42° 52' 14.77"	21° 49' 30.44"	<i>Fagus sylvatica</i>
Village Miroševce-2	Leskovac	292	42° 51' 22.21"	21° 50' 06.19"	<i>Fagus sylvatica</i>
Monte Vučje-1	Leskovac	546	42° 50' 26.63"	21° 53' 19.99"	<i>Fagus sylvatica</i>
Monte Vučje-2	Leskovac	935	42° 50' 47.80"	21° 56' 05.97"	<i>Fagus sylvatica</i>
Monte Vučje-3	Leskovac	618	42° 51' 27.40"	21° 56' 01.10"	<i>Fagus sylvatica</i>

Collected larvae were transported to the laboratory of Institute of Forestry (Belgrade) and reared in 15×10×8 cm plastic boxes, ten larvae per box. The larvae were grown in a climate chamber under the laboratory conditions. During the

experiment, temperature and light conditions were constant (temperature 21 °C, light regime - 8 h dark, 16 h light). The larvae were fed on daily basis on fresh oak leaves, brought from the sample plots.

Larvae that were parasitized with braconid (Hymenoptera) puparia and those from which tachinid larvae emerged were separated and held individually in Petri dishes for observation and microscopic examination. Individual gypsy moth pupae were placed in 50 cm³ plastic containers, one pupa per container, and mortality caused by parasitoids was recorded. Tachinid pupae were maintained in sterile Petri dishes containing sand moistened with sterile water. Relative humidity of 75-80% was maintained in order to prevent drying of pupae.

Microscopic analyses of dead tachinid pupae were conducted two or more months after pupation using the protocol described in another publication [28].

3. Results

A total of 1117 larvae and 814 pupae of *L. dispar* were collected and analyzed in 2013 (Table -2). Host mortality due to tachinid parasitism in different localities varied from 0.7% to 37.1%; the overall average parasitism by tachinids was

14.2%.

Mortality occurred in late larval stage (\geq IV instar) but much more in the pupal stage of gypsy moth. Mortality due to the hymenopteran species *Protapanteles liparidis* (Bouché, 1834) and *Cotesia melanoscela* (Ratzeburg, 1844) (Hymenoptera: Braconidae) was recorded in earlier stage larvae (\leq III instar) (unpublished data). *E. maimaiga* was not observed in early instar larvae from which hymenopteran parasitoids emerged. No pathogens (*Lymantria dispar* multicapsid nuclear polyhedrosis virus - LdMNPV or microsporidia) were observed in the parasitized larvae.

A total of 274 tachinid larvae were reared from gypsy moth larvae and pupae. Of these, 266 died as pupae, resulting in an extremely high overall mortality of 97.1% (Table -2). No *E. maimaiga* azygospores were observed in internal tissues of dead pupae, but were detected on the surface of 69.5% of the dead pupae. No other pathogens were established in the tissues of the dead parasitoids.

Table 2: Impact of tachinids on gypsy moth and mortality of parasitoids in pupal stage in 2013.

Locality	<i>Lymantria dispar</i> L.				Mortality caused by tachinids (%)	Tachinid pupae		
	Larvae		Pupae			N	Mortality (%)	With <i>E. maimaiga</i> azygospores (%)
	Collection dates	N	Collection dates	N				
Srdaljska reka-1	11 May-1 June	60	18 June	75	15.6	21	100.0	72.2
Srdaljska reka-2		71		42	11.5	13	92.3	36.6
Srdaljska reka-3		34		51	10.6	9	100.0	77.8
Srdaljska reka-4		53		30	4.8	4	100.0	50.0
Monte Miroč	9 May	342	13 June	209	19.1	105	98.1	83.3
East Boranja-1	5 May-5 June	176	11 June	55	5.6	15	80.0	33.3
East Boranja-2		93		46	0.7	1	0.0	0
Miroševce-1	16 May	74	3 July	50	8.1	10	90.0	70.0
Miroševce-2	20 June	56		73	10.1	13	100.0	69.2
Monte Vučje-1	11 May	60	3 July	64	37.1	46	93.5	62.8
Monte Vučje-2		41		37	17.9	14	100.0	64.3
Monte Vučje-3		57		82	16.5	23	100.0	52.2
Total		1117		814	14.2	274	97.1	69.5

Only eight tachinid adult specimens emerged from the pupae and three species were identified: *Compsilura concinnata* (Meigen, 1824) – 37.5%, *Exorista larvarum* (Linnaeus, 1758) – 37.5%, and *Carcelia gnava* (Meigen, 1824) – 25.0% (Table -

3). The high tachinid mortality in the pupal stage did not allow identification of predominate part of specimens or evaluation of the role of different species in the regulation of gypsy moth density.

Table 3. Parasitoid adults reared from *Lymantria dispar*.

Parasitoid species	Locality	Parasitoid number	Date of emergence
<i>Compsilura concinnata</i> (Meigen, 1824)	East Boranja-1	2 ♂, 1 ♀	4-6 July 2013
	East Boranja-2	1 ♂	6 July 2013
<i>Exorista larvarum</i> (Linnaeus, 1758)	Miroševce-1	1 ♂	19 July 2013
	Srdaljska reka-2	1 ♂	3 July 2013
<i>Carcelia gnava</i> (Meigen, 1824)	Monte Miroč	1 ♂, 1 ♀	8-10 July 2013

4. Discussion

Family Tachinidae (Diptera) occupies an important place among the parasitoids, not only by the richness of species and wide geographical distribution but also because they parasitize a large number of economically important insect pests. For

Serbia, Hubenov [3] reported 288 tachinid species from which 21 (*Exorista larvarum*, *E. segregata*, *E. sorbillans*, *Parasetigena silvestris*, *Phorocera assimilis*, *Blondelia inclusa*, *B. nigripes*, *Compsilura concinnata*, *Drino inconspicua*, *Carcelia gnava*, *C. lucorum*, *Senometopia*

confudens, *S. excisa*, *S. separata*, *S. susurrans*, *Zenillia libatrix*, *Pales pavidata*, *Blepharipa pratensis*, *B. schineri*, *Baumhaueria goniaeformis*, *Tachina fera*) are connected with *L. dispar* [1, 3, 9].

Previous studies in Serbia showed that some tachinids are important in gypsy moth population dynamics with relatively high host mortality: *C. conncinata*, 60.0% [20]; *E. larvarum*, most frequent species [21]; *B. pratensis*, 4.7-92.5% [22]; and *P. silvestris*, 3.0-61.0% [20]. The four species are one of the most effective gypsy moth tachinid parasitoids in Europe, and were introduced into North America. These are polyphagous on a variety of phytophagous host species [23-24]. They are typically multivoltine with two or more generations per year, the first parasitizing gypsy moth. In Serbia, the degree of parasitism of gypsy moth caused by polyphagous tachinid species is the largest in the progressive stage of gradation, as well as the stage of latency.

The lack of azygospores inside dead tachinid pupae is an indication of the absence of a direct effect of fungal pathogen on parasitoids. Tachinids evidently do not become infected by *E. maimaiga* while parasitizing the infected host but the presence of azygospores on the surface of 69.5% of tachinid puparia is an evidence of development of entomophthoraceous fungus in parasitized gypsy moth larvae.

In this study in Serbia, higher mortality of tachinid pupae (97.1%) was established during their development in gypsy moth larvae infected with *E. maimaiga*, in comparison to a similar study in Bulgaria (86.5%). Georgiev *et al.* [19] hypothesized that, during fungal development, nutritional resources available to the parasitoids decrease, limiting available energy for successful pupation and eclosion.

The causes of tachinid mortality during their development in gypsy moth larvae which have been parasitized by *E. maimaiga* are not clarified but it is likely a result of the competition between the fungus and tachinids. *E. maimaiga* has become the primary biological control agent in gypsy moth populations in USA [25], Bulgaria [7, 19] and Serbia [15] but its impact on other natural enemies of gypsy moth is still not well known. Additional studies are needed to clarify the specific causes of mortality of tachinid parasitoids in order to obtain an objective assessment of the impact of *E. maimaiga* on gypsy moth and role of the pathogen in programs for biological control of the pest.

5. Acknowledgments

The study was partly financed by the Ministry of Science of the Republic of Serbia, the Project 31070 - SUBPROJECT: New technological methods in the integral protection of forests with the focus on the entomopathogenic fungus *Entomophaga maimaiga*, as the possible solution to the problem regarding the frequent occurrences of the outbreak of gypsy moth in the forest ecosystems of Serbia.

6. References

1. Tabaković-Tošić M. Gypsy moth, *Lymantria dispar* (L.) outbreak in the central part of Republic of Serbia in the period 2010-2013. *Sustainable Forestry* 2013; 67-68:141-150.
2. Tabaković-Tošić M. Gypsy moth, *Lymantria dispar* (L.), and its natural enemies in the forests of central Serbia. *Sustainable Forestry* 2012; 65-66:133-148.
3. Hubenov Z. Composition and zoogeographical characteristics of the family Tachinidae (Diptera: Insecta) in Serbia and Bulgaria. *Advances in Arachnology and Developmental Biology. Papers* dedicated to Prof. Dr. Božidar Čurčić, S. E. Makarov & R. N. Dimitrijević (eds). Inst. Zool., Belgrade; BAS, Sofia; Fac. Life Sci., Vienna; SASA, Belgrade & UNESCO MAB Serbia. Vienna – Belgrade – Sofia, 2008, Monographs 2008; 12:375-394.
4. Andreadis TG, Weseloh RM. Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. *Proceedings of the National Academy of Sciences of the USA* 1990; 87:2461-2465.
5. Pilarska D, McManus M, Hajek A, Herard F, Vega F, Pilarski P *et al.* Introduction of the entomopathogenic fungus *Entomophaga maimaiga* Hum, Shim. & Soper (Zygomycetes: Entomophthorales) to a *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) population in Bulgaria. *Journal of Pest Science* 2000; 73:125-126.
6. Pilarska D, McManus M, Pilarski P, Georgiev G, Mirchev P, Linde A. Monitoring the establishment and prevalence of the fungal entomopathogen *Entomophaga maimaiga* in two *Lymantria dispar* L. populations in Bulgaria. *Journal of Pest Science* godina 2005; 79:63-67.
7. Mirchev P, Linde A, Pilarska D, Pilarski P, Georgieva M, Georgiev G. Impact of *Entomophaga maimaiga* on gypsy moth populations in Bulgaria. *IOBC-WPRS Bulletin* 2006; 90:359-363.
8. Georgiev G, Mirchev P, Georgieva M, Rossnev B, Petkov P, Matova M *et al.* First Record of Entomopathogenic Fungus *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) in *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae) in Turkey. *Acta Zoologica Bulgarica* 2012; 64:123-127.
9. Tabaković-Tošić M, Georgiev G, Mirchev P, Tošić D, Golubović-Čurguz V. *Entomophaga maimaiga* – new entomopathogenic fungus in the Republic of Serbia. *African Journal of Biotechnology* 2012; 11: 8571-8577.
10. Tabaković-Tošić M, Georgiev G, Mirchev P, Tošić D, Golubović-Čurguz V. Gypsy Moth in Central Serbia over the Previous Fifty Years. *Acta Zoologica Bulgarica* 2013; 65(2):165-171.
11. Georgieva M, Georgiev G, Pilarska D, Pilarski P, Mirchev P, Papazova-Anakieva I *et al.* First record of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in *Lymantria dispar* populations in Greece and the Former Yugoslavian Republic of Macedonia. *Forestry Journal (Croatia)* 2013; 5-6: 307-311.
12. Hrašovec B, Pernek M, Lukić I, Milić M, Diminić D, Franjević M *et al.* First record of the pathogenic fungus *Entomophaga maimaiga* Humber, Shimazu, and Soper (Entomophthorales: Entomophthoraceae) within an outbreak populations of *Lymantria dispar* (Lepidoptera: Erebididae) in Croatia. *Periodicum Biologorum* 2013; 115(3):379-384.
13. Csóka G, Hirka A, Szócs L, Hajek AE. A rovarpatogén *Entomophaga maimaiga* Humber, Shimazu & Soper, 1988 (Entomophthorales: Entomophthoraceae) gomba megjelenése magyarországi gyapjaslepke (*Lymantria dispar*) populációkban. *Növényvédelem* 2014; 50(6):257-262.
14. Zúbrík M, Barta M, Pilarska D, Goertz D, Úradník M, Galko J *et al.* First record of *Entomophaga*

- maimaiga* (Entomophthorales: Entomophthoraceae) in Slovakia. *Biocontrol Science and Technology* 2014; 24(6):710-714.
15. Tabaković-Tošić M. Suppression of gypsy moth population in Mountain Avala (Republic of Serbia) by introduction of entomopathogenic fungus *Entomophaga maimaiga*. *Comptes rendus de l'Académie bulgare des Sciences* 2014; 67(1):61-66.
 16. Tabaković-Tošić M. Distribution of *Entomophaga maimaiga* in Central part of Serbia in the period 2011-2013. *Silva Balcanica* 2014; 15(1):110-115.
 17. Hajek AE, Butler L, Walsh SRA, Silver JC, Hain FP, Hastings FL *et al.* Host range of the gypsy moth (Lepidoptera: Lymantriidae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in the field versus laboratory. *Environmental Entomology* 1996; 25:709-721.
 18. Georgieva M, Takov D, Georgiev G, Pilarska D, Pilarski P, Mirchev P *et al.* Studies on non-target phyllophagous insects in oak forests as potential hosts of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Bulgaria. *Acta Zoologica Bulgarica* 2014; 66(1):115-120.
 19. Georgiev G, Hubenov Z, Georgieva M, Mirchev P, Matova M, Solter LF *et al.* Interactions between the introduced fungal pathogen *Entomophaga maimaiga* and indigenous tachnid parasitoids of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae) in Bulgaria. *Phytoparasitica* 2013; 41:125-131.
 20. Sisojević P. Interactions in the host-parasite system, with special reference to the gypsy moth-tachinids (*Lymantria dispar* L.–Tachinidae). 6th Interbalkan Plant protection Conference, Izmir, Turkey, 1979, 108-114.
 21. Sisojević P. Contribution to the knowledge of the parasitic Diptera of the gypsy moth in Yugoslavia. *Plant Protection (Serbia)* 1955; 28:3-10.
 22. Vasić K, Sisojević P. Parasites of pronymphs and pupae of the gypsy moth in Yugoslavia in 1957. *Plant Protection (Serbia)* 1958; 41-42:49-52.
 23. Cerretti P, Tschorsnig HP. Annotated host catalogue for the Tachinidae (Diptera) of Italy. *Stuttgarter Beiträge zur Naturkunde A, Neue Serie* 2010; 3:305-340.
 24. Shima H. A host-parasite catalog of Tachinidae (Diptera) of Japan. *Makunagi Acta Dipterologica* 2006; 2:1-171.
 25. Kereselidze M, Pilarska D, Hajek A, Jensen AB, Linde A. First record of *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) in Georgia. *Biocontrol Science and Technology* 2011; 21:1375-1380.