Short Communication

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Entomophaga aulicae (Reichardt in Bail) Humber (Entomophthorales: Entomophthoraceae), a New Entomopathogenic Fungus in the Republic of Serbia

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Abstract: A higher mortality rate of late larval instars of brown-tail moth (*Euproctis chrysorrhoea*) was reported in the forest complexes of Novi Pazar region during a culmination phase of new outbreak. Through field and laboratory studies of the mortality causes, the presence of hyphal bodies, primary conidia and resting spores of the entomopathogenic fungus *Entomophaga aulicae* were reported in the dead caterpillars. It showed to be a significant control agent of population density of *E. chrysorrhoea.* This has been the first report of this species occurrence in Serbia.

Key words: *Entomophaga aulicae*, *Euproctis chrysorrhoea*, biological control, epizootics

Introduction

Brown-tail moth, *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae), is a well-known pest of broadleaf forests of central Serbia and occurs periodically in high numbers (outbreak) on a relatively small area of a few hundred hectares.

The fungal order Entomophthorales in the class Entomophthoromycetes (Humber 2012) is mainly composed of obligate pathogens that infect arthropods. More than 300 species within the family Entomophthoraceae are well-known for their ability to cause dramatic epizootics in populations of aphids, leafhoppers and planthoppers, flies, grasshoppers, cicadas, coleopteran and lepidopteran larvae (Hajek 1999, Keller 1987, Mirchev et al. 2013, Tabakovic-Tosic et al. 2012, Tabakovic-Tosic 2014).

The entomopathogenic fungus *Entomophaga aulicae* (Reichardt in Bail) Humber (Entomophthoromycotina: Entomophthorales, Entomophthoraceae) is a widespread Holarctic species, with many host insects of the order Lepidoptera, including some of the most economically harmful, outbreaking species of forest defoliators.

Materials and methods

At the beginning of the summer in 2015 and 2016, the higher mortality rate of late larval instars of brown-tail moth was reported in oak and beech forests of south-western Serbia, in the culmination phase of the new outbreak of this pest. An intensive research of the possible causes of the mortality was conducted in the well-preserved oak [*Quercus cerris* L. and *Quercus petraea* (Matt.) Liebl.] and beech [*Fagus moesiaca* (Domin & Maly) Czecz.] coppice forest stands located in the region of Novi Pazar (Table 1).

Average values of climatic characteristics of the study area for the period 2006-2015 are presented in the Table 2 [data of the Republic Hydrometeorological Service of Serbia, Novi Pazar Meteorological Station (α: 43°08' N; δ: 20°31' E; altitude of 545 m].

A total of 480 L5-6 larval instars of brown-tail moth larvae (alive from trees, dead from trees and caterpillar litters) were sampled from 12 sites, in May and June 2015 and 2016. Alive larvae were reared 6-10 days (until their death) in the climate chamber under constant temperature 21°C and 16/8 hour (L/D) photoperiod. The larvae were fed on fresh

Table 1. The main characteristics of the plots, where samplings of mycosed caterpillars were done

Plot	Locality	Coordinates	Altitude (m a.s.l.)	Exposure
1	Blizanac – Debelica	X: 4 783 502 Y: 7 454 574	889	E - NE
2	Blizanac – Debelica	X: 4 784 189 Y: 7 454 691	863	N - NE
3	Ninaja – Koznik	X: 4 778 540 Y: 7 450 929	980	S
4	Ninaja – Koznik	X: 4 777 365 Y: 7 453 717	920	S - JE
5	Ninaja – Koznik	X: 4 774 038 Y: 7 449 982	930	JE
6	Ninaja – Koznik	X: 4 772 140 Y: 7 454 730	960	N
7	Ninaja – Koznik	X: 4 774 460 Y: 7 489 220	810	E
8	Ninaja – Koznik	X: 4 775 001 Y: 7 448 624	920	E
9	Turjak – Vršine	X: 4 774 038 Y: 7 449 982	853	W - NW
10	Turjak – Vršine	X: 4 774 133 Y: 7 451 501	850	W - NW
11	Turjak – Vršine	X: 4 773 471 Y: 7 452 257	897	SW
12	Turjak – Vršine	X: 4 775 033 Y: 7 451 039	690	S - SE

Table 2. Climatic conditions of the study area during the period 2006-2015

Mean monthly values	Year	Month											
		1	2	3	4	5	6	7	8	9	10	11	12
Air temperature (°C)	2015	1.0	2.6	5.2	8.9	15.0	17.7	22.4	22.0	17.6	10.5	6.0	1.4
	2014	3.2	5.5	7.8	10.7	14.4	18.1	20.2	20,5	15.6	11.8	7.9	2.6
	2006-2015	0.5	2.1	6.1	10.9	15.1	18.7	21.0	21.1	16.4	11.1	6.2	1.2
Relative humidity (%)	2015	90	87	85	77	76	76	69	71	78	85	82	91
	2014	93	86	83	83	80	78	75	76	81	82	85	90
	2006-2015	87	84	79	73	74	73	70	70	76	81	83	87
Precipitation (mm)	2015	42	59	67	63	44	67	3	50	53	69	54	4
	2014	22	4	59	127	113	69	95	43	134	57	58	57
	2006-2015	42	40	62	48	77	67	53	34	55	65	51	51

Table 3. Microscopic analysis of the brown-tail moth dead caterpillars

Plot*	Sample size	Caterpillars infected by *E. aulicae*		Microstructures of *E. aulicae* detected in caterpillars**					
				Hyphal bodies		Primary conidia		Resting spores	
	N	N	%	N (%)	Intensity	N (%)	Intensity	N (%)	Intensity
1	40	40	100	2 (5.0)	+	19 (47.5)	++	40 (100)	+++
2	40	40	100	4 (10.0)	+	27 (67.5)	++	40 (100)	+++
3	40	40	100	10 (25.0)	+	21 (52.5)	+	40 (100)	+++
4	40	36	90.0	3 (7.5)	+	10 (25.0)	+	36 (90.0)	++
5	40	40	100	6 (15.0)	+	29 (72.5)	+++	40 (100)	+++
6	40	40	100	7 (17.5)	+	5 (12.5)	+	40 (100)	++
7	40	31	77.5	3 (7.5)	+	31 (77.5)	+	31 (77.5)	++
8	40	40	100	5 (12.5)	+	22 (55.0)	+	40 (100)	++
9	40	40	100	0	-	11 (27.5)	+	40 (100)	+++
10	40	37	92.5	11 (27.5)	+	37 (92.5)	++	37 (92.5)	+
11	40	40	100	0	-	40 (100)	+++	40 (100)	+++
12	40	40	100	1 (2.5)	+	40 (100)	+++	40 (100)	+++

* The main characteristics of the plots are given in Table 1.
** Number of caterpillars (N) with particular fungal microstructures detected and their intensity in the cadavers.

oak leaves on daily basis. Dead larvae were placed in Petri dishes with wet filter paper. They were kept for seven days in the laboratory and then stored in the refrigerator for three months. Detailed microscopic examination of the dead brown-tail moth caterpillars was carried out using a MOTIC optical Trinocular, equipped with a camera MOTICAM 10.0. Measurements of hyphal bodies, primary conidia and resting spores were processed using Motic Images Plus 2.0 ML software.

The species identification was based on the size, shape and structural characteristics of different microstructures of the fungus (Keller 1987, Keller & Petrini 2005).

Fig. 1. Browntail moth larvae (A) and larval litters (B).

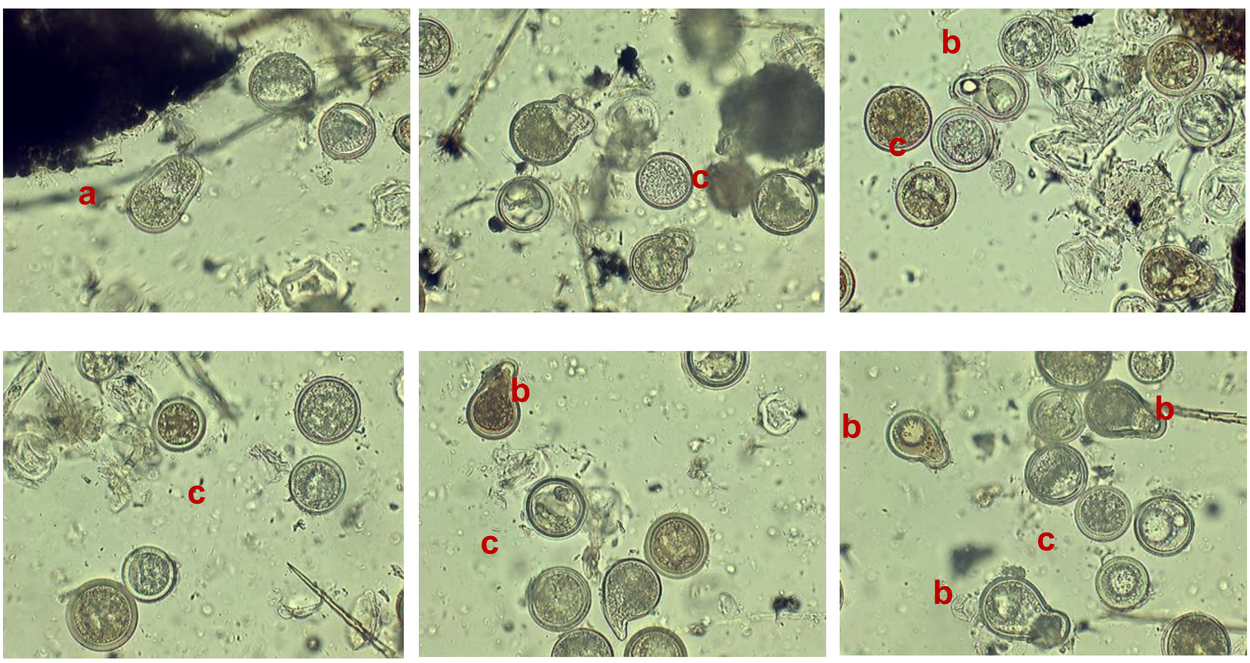

Fig. 2. Hyphal body (a), primary conidia (b) and resting spores (c) isolated from dead larvae.

Results

In the spring (May and June) of 2015 and 2016 in the area of Novi Pazar (oak and beech coppice forest stands), a great increase of the population size of the brown-tail moth on an area covering 613 ha was reported. A huge amount of alive and dead L4-6 instars larvae was found on the trees. In late June, in the sampled 40 newly litters from 12 sites, there were an average of 5.7 dead developed caterpillars.

The detailed microscope survey of the dead brown-tail moth larvae showed that in most of them numerous resting spores of the entomopathogenic fungus *E. aulicae* were present. In addition, the presence of hyphal bodies and primary conidia of this pathogen species was reported but their number was

Fig. 3. Climatic conditions of the study area during the period 2010-2015. (A) Mean monthly air temperature; (B) Mean monthly relative humidity; (C) Precipitation.

considerably smaller (Table 3). Hyphal bodies were spherical or subspherical, resting spores – globose, while primary conidia were pyriform to obovate with a broad and rounded papilla. The mean dimensions of resting spores were 44.1 µm (32.4–48.5 µm; n=317), primary conidia are 34.1 (26.7–38.6; n=94) × 29.3 (21.0-43.1; n=94) µm. Hyphal bodies were not measured. The morphological data correspond to descriptions given by MacLeod & Müller-Kögler (1973), Hamm (1980), Pilarska et al. (2001) and Kalkar & Carner (2005).

Weather plays an important role for the effectiveness of *E. aulicae*. Our results indicated that May (in both years 2014 and 2015) was the favourable month for the germination of the resting spores and the infection of the brown-tail moth larvae (Table 2). The frequency of the rainy days and an average monthly air temperature around 20ºC, supported the epizootics in the observed area in the second half of the month. The reduction of the intensity of the brown-tail moth attack (three times smaller dimension of newly litters with mostly mycosed dead caterpillars and pupae/tree) in 2016 was caused by the activity of the entomopathogenic fungus *E. aulicae* in the previous two years.

Discussion

Using field and laboratory studies of the causes of the mortality of the older brown-tail moth larval instars, the presence of hyphal bodies, primary conidia and resting spores of the entomopathogenic fungus *E. aulicae* were confirmed. It has been the first discovery of this species is Serbia. It proved to be a significant control agent of population size of this economically important insect species. The biggest advantage of using *E. maimaiga* is that it can be easily introduced in the host population, small amounts of the pathogen are needed and inexpensive field equipment is used. In addition, the results of the effective suppression of the outbreak of *Lymantria dispar* (Linnaeus) in Serbia by the related species *E. maimaiga* Humber, Shimazu & R. S. Soper (Tabakovic-Tosic 2014) justifies the continuation of the above studies.

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References

Hajek A. E. 1999. Pathology and Epizootiology of *Entomophaga maimaiga* Infections in Forest Lepidoptera. Microbiology and Molecular Biology Reviews 63 (4): 814-835.

Humber R. A. 1984. The identity of *Entomophaga* species (Entomophthorales: Entomophthoraceae) attacking Lepidoptera. Mycotaxon 21: 265-272.

Humber R. A. 2012. Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. Mycotaxon 120: 477-492.

Kalkar Ö. & Carner G. R. 2005. Characterization of the Fungal Pathogen, *Entomophaga aulicae* (Zygomycetes: Entomophthorales) in Larval Populations of the Green Cloverworm, *Plathypena scabra* (Lepidoptera: Noctuidae). Turkish Journal of Biology 29: 243-248.

Keller S. 1987. Arthropod pathogenic Entomophthorales of Switzerland I. Conidiobolus, Entomophaga, and Entomophthora. Sydowia 40: 122-167.

Keller S. & Petrini O. 2005. Keys to the identification of the arthropod pathogenic genera of the families Entomophthoraceae and Neozygitaceae (Zygomycetes) with descriptions of three new subfamilies and new genus. Sydowia 57 (1): 23-53.

MacLeod D. M. & Müller-Kögler E. 1973. Entomopathogenous fungi: *Entomophthora* species with pear-shaped to almost spherical conidia (Entomophthorales: Entomophthoraceae). Mycologia 65: 823-893.

Mirchev P., Linde A., Pilarska D., Pilarski P., Georgieva M. & Georgiev G. 2013. Impact of *Entomophaga maimaiga* on gypsy moth populations in Bulgaria. IOBC-WPRS Bulletin 90: 359-363.

Pilarska, D., Zimmermann G., Linde A., McManus M., Pilarski P. & Takov D. 2001. On the occurrence of *Entomophaga aulicae* in high density brown-tail moth *(Euproctis chrysorrhoea L.)* populations in Bulgaria. In: Naydenova T. et al. (Eds.). Proceedings of Third Balkan Scientific Conference 'Study, Conservation and Utilisation of forest Resources', 2-6 October 2001, Sofia, Bulgaria, Vol. 3: 139-143.

Tabakovic-Tosic M. 2014. 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 67 (1): 61-66.

Tabakovic-Tosic M., Georgiev G., Mirchev P., Tosic D. & Golubovic-Curguz V. 2012. *Entomophaga maimaiga* – new entomopathogenic fungus in the Republic of Serbia. African Journal of Biotechnology 11 (34): 8571-8577.

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