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Impact of Entomophages on Density of *Thaumetopoea pityocampa* in Egg Stage near Ivaylovgrad, Bulgaria

***Plamen Mirchev*[1]*, Georgi Georgiev*[1]*, Peter Boyadzhiev*[2]*, Maria Matova*[1]**

[1] Forest Research Institute -Bulgarian Academy of Sciences, 132 St. K. Ochridski Blvd., 1756 Sofia, Bulgaria;
E-mail: plmirchev@hotmail.com; ggeorgiev.fri@gmail.com; mimilota22@abv.bg
[2] Department of Zoology, University of Plovdiv, 24 Tsar Assen Str., BG-4000, Plovdiv, Bulgaria; E-mail: boyadz@mail.bg

Abstact: In Bulgaria, near Ivaylovgrad, the Eastern Rhodopes, in a forest of *Pinus nigra* Arn. 85 egg batches were sampled over three generations (2009, 2010 and 2011 – time of deposit of eggs) of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep., Thaumetopoeidae). Directly after collection the batches were singled in test tubes, closed with cotton stoppers and stored under laboratory conditions at 20-22 °C. After removal of scales, the numbers of caterpillars hatched were counted. The final analysis was made after termination of parasitoid emergence. For that all eggs were opened carefully. Average number of eggs per egg batches was the most of generation 2011 – 279.5 and in the previous two generations is about 10% lower: 2010 – 250.1; 2009 – 256, 8. Survival of pine processionary moth in egg stages, expressed in percentage of hatched caterpillars was between 60 and 75%. The most significant factor for reducing of density was entomophages (parasitoids and predators). Parasitized eggs amount to 30%. Destroyed eggs of predators were below 1%. Five species of parasitoids were identified: *Ooencyrtus pityocampae* (Mercet 1921) (Encyrtidae), *Baryscapus servadeii* (Domenichini 1965) (Eulophidae), *Baryscapus transversalis* Graham 1991 (Eulophidae), *Pediobius bruchicida* (Rondani 1872) (Eulophidae) and *Anastatus bifasciatus* (Fonscolombe 1832) (Eupelmidae). All *A. bifasciatus*, which emerged after collection, were male, only 1 of generation 2010 was female and 4 of generation 2011, all *O. pityocampae* and *B. servadeii* – were female; *P. bruchicida* and *B. transversalis* – both sex. The most abundance was the first two parasitoids, whose number is about 80% of all parasitoids.

Key words: *Thaumetopoea pityocampa*, egg parasitoids, predators, Eastern Rhodopes, Bulgaria

Introduction

For Bulgaria, pine processionary moth *Thaumetopoea pityocampa* (Denis & Schiffermüller 1775) is a grave insect pest: most significant needle-nibbling in coniferous forests of the country (Mirchev *et al.* 2003); and, on the other hand, through its direct harmful impact on humans and animals (Lamy 1990; Fuentes *et al.* 2004) it reduces the recreational functions of forests mainly around population centers and resorts.

Among natural factors, most essential regulator of pest numbers are egg parasitoids. A study on them has been conducted in the Eastern Rhodopes on a site (Janino) at a distance of 70 km to the south-west of the current site (Mirchev *et al.* 1998).

Among the parasitoids on pine processionary moth's eggs reported (Mirchev, Tsankov 2005), the predominating part are polyphagous parasitizing various numbers of alternative hosts (Battisti *et al.* 1988; Noyes 2012). What is unique about the site near Ivaylovgrad, where the material collected for the analysis in the current study comes from, is that two of the total of three species of pine processionary moths found in Bulgaria occur here – *T. pityocampa* and *T. solitaria* (Freyer 1838), a potential host of some of egg parasitoids on pine processionary moth. Both species of processionary moths had high population numbers in the years of the study.

The aim of this paper is to examine, under these specific conditions, the species composition of egg parasitoids on pine processionary moth, their relative share and their regulating effect on the numbers of the host.

Material and Methods

The egg batches of pine processionary moth, which are subject of this study, were collected from the forest park in the Dupkata protected area near Ivaylovgrad, with coordinates N41°31'41,7"; E26°06'57,1", altitude of 320 m a.s.l. of the hilly sub-belt of South Arda, the Eastern Rhodopes. The terrain is karst, occupied by a 50-year-old plantation of European black pine, *Pinus nigra* Arnold 1785., mixed primarily with oriental hornbeam (*Carpinus orientalis* Miller, 1768), manna ash (*Fraxinus ornus* Linnaeus, 1753), and the meadows and barren areas – with turpentine tree (*Pistacia terebinthus* Linnaeus 1753).

The material analysed is from three generations of pine processionary moth. Eggs laid in 2009 – from which generation the egg batches were collected on 27.03.2010; and, respectively, 2010 – on 17.11.2010; and 2011 – on 27.03.2012. A total of 85 egg batches with 22 349 eggs in them were collected and processed.

After collection, batches were transported directly to the laboratory of the Forest Research Institute of Sofia. The scales of the egg batches were removed, and were singled in test tubes with cotton stoppers. The unscaled eggs, from which the parasitoids emerged, were marked and the date of emergence was quoted in a table for each egg batch separately, presented by Tsankov *et al.* (1996a). All laboratory studies were carried out at room temperature (20-22 ºC). The emerged parasitoids were periodically reported and taken out of the test tubes.

Under a stereomicroscope (x 40 magnification), the final analysis of the eggs was carried out, the eggs without emergence openings being cut. Parasitoids emerged before collections were determined by their meconia and remains after Schmidt, Kit (1994); Tanzen, Schmidt (1995); Schmidt *et al.* (1997a); Tsankov *et al.* (1996a) and Tsankov *et al.* (1998a).

Results

Viability of pine processionary moth in egg stage

The viability of pine processionary moth at egg stage, expressed by the percentage of the caterpillars successfully hatched in the egg batches analysed, is between 60 and 75% (Table 1). The last sample was the most homogeneous, with the highest average number of eggs in the individual egg batch. At this stage of the ontogenetic development of the species, significant regulator of its number are parasitoids and predators. The eggs parasitized and destroyed by predators are close to 30%. The number of eggs with dead caterpillars is 3 times lower as an average. Two groups are set apart in this category: caterpillars, which had made an opening in the egg shell but still died for one reason or another, and caterpillars, which died at different stages of their embryonic development before the moment of emergence, i.e. the making of an emergence opening in the egg (Table 2). Mushroom spawns were observed on a number of caterpillars, the pathogen being impossible to identify and also not being possible to conclude if the pathogen is responsible for the lethal outcome with these individuals. The number of unfertilized eggs is relatively significant, such eggs being found, although sometimes just a few odd ones, in almost all egg batches. In some individual batches they can reach substantial numbers. For instance, in the sample from 17. 11. 2010 a batch with 19.5% sterile eggs was registered, whereas in the last sample from 27. 03. 2012 they were as much as 31.7%. They were just registered, it being impossible to explain the existence of empty eggs, eggs formed of chorion only, without any content inside whatsoever or any visible signs of the impact of predators.

Egg parasitoid spectrum

Five parasitoids were determined in analysed samples (Table 3). The relative numbers of established species is stable and varies within narrow limits in the 3 years of the study. The most numerous is *Baryscapus servadei,* followed by *Ooencyrtus pityocampae* at 7.5 – 13.3 points lower number, and *Anastatus bifasciatus*, whose share is 3-4 times lower compared to the former two. The numbers of the hyper-parasitoid *Baryscapus transversalis* is more variable, its percentage participation in the first sample being almost 2 times higher compared to the last one. *Pediobius bruchicida* has been registered in all samples but its participation is sporadic.

In this study, with all three primary parasitoids on the pine processionary moth's eggs, *B. servadeii*, *O. pityocampae* and *A. bifasciatus*, a

Table 1. Hatching rates and mortality of caterpillars of *Thaumetopoea pityocampa*

Generation / Date of collection	**2009/27. 03. 2010**		**2010/17. 11. 2010**		**2011/27. 03. 2012**	
Parameters	Number	%	Number	%	Number	%
Total number of egg batches	22		31		32	
Total number of eggs	5651	100.0	7 754	100.0	8 944	100.0
Mean of eggs per batch ±SD (range)	256.8±32.3 (199-324)		250.1±32.8 (180-303)		279.5±28.7 (208-333)	
Caterpillars hatched	**4267**	**75.5**	**4 651**	**60.0**	**5 861**	**65.5**
Impact of egg parasitoids and predators	**1 133**	**20.1**	**2 259**	**29.1**	**2 351**	**26.3**
Unhatched caterpillar	**251**	**4.4**	**844**	**10.9**	**732**	**8.2**

Table 2. Structure of unhatched caterpillar without influence of entomophages

Date of collection	**27. 03. 2010**		**17. 11. 2010**		**27. 03. 2012**	
Parameters	Number	%	Number	%	Number	%
Total number	**251**	**100.0**	**844**	**100.0**	**732**	**100.0**
Caterpillars died, of them	127	50.6	259	30.7	245	33.5
- without opening (of them with mushroom spawn)	118 (7)		237 (3)		198 (1)	
- with opening	9		22		47	
Undeveloped eggs with dried-up yolk (of them with mushroom spawn)	72 (6)	28.7	335 (39)	39.7	271 (12)	37.0
Eggs totally empty, without any remains	52	20.7	250	29.6	216	29.5

Table 3. Persentage of the parasitioids

Parasitoids	**Date of collection**					
	27. 03. 2010		**17. 11. 2010**		**27. 03. 2012**	
	N	**%**	**N**	**%**	**N**	**%**
Ooencyrtus pityocampae (Mercet 1921)	370	34.3	696	35.1	760	38.4
Baryscapus servadeii (Domenichini 1965)	495	46.0	959	48.4	910	45.9
Baryscapus transversalis Graham 1991	96	8.9	147	7.4	90	4.5
Anastatus bifasciatus (Geoffroy 1785)	114	10.6	179	9.0	215	10.8
Pediobius bruchicida (Rondani 1872)	2	0.2	1	0.1	7	0.4
Total	1 077	100.0	1 982	100.0	1 982	100.0

remnant from the host's epicranium was found, i.e. the parasitisation happened after the completed development of the caterpillars (Table 4). With *O. pityocampae* and *A. bifasciatus,* this phenomenon can be accepted as an exception – it was registered only in isolated cases and only in the last sample, whereas with *B. servadeii* this is the rule and comes up to 17.2%.

The adults if the primary parasitoids *O. pityocampae* and *B. servadeii* emerged after the date of the sample collection are only female individuals, whereas with *A. bifasciatus* they are exclusively male – out of 354 adults only 5 are females. Both genders were established with *B. transversalis* and *P. bruchicida*.

For *O. pityocampae* there are wide variations in the time of emergence. In the first sample ¾ emerged after hibernation, whereas in the next two years of study this percentage is respectively 34.9 and 49.2.

The imagination with *B. servadeii* comes primarily (80.1-86.1%) after the winter diapause, the exception being the sample from 17.11.2010, when

Table 4. Parasitism of egg-batches of *T. pityocampa*

Date of collection	**27. 03. 2010**		**17. 11. 2010**		**27. 03. 2012**	
Parasitoids	**Number**	**%**	**Number**	**%**	**Number**	**%**
Total number of *O. pityocampae*	**370**		696		760	
Number of emerged *O. pityocampae*, of them	345	93.2	565	81.2	706	92.9
♦ opening on top of egg shell	260	75.4	474	83.9	605	85.7
♦ opening on side of egg shell	85	25.6	91	13.1	101	14.3
• emerged before collection of egg-batches	88	*25.5*	368	*65.1*	359	*50.8*
• emerged after collection of egg-batches	257	*74.5*	197	*34.9*	347	*49.2*
With remains of the caterpillars	-	-	-	-	7	0.9
Adults died in eggs without opening	25	6.8	131	18.8	54	7.1
Total number of *B. servadeii*	**495**		959		910	
Number of emerged *B. servadeii*, of them	491	99.2	947	98.7	908	99.8
♦ opening on top of egg shell	441	89.8	885	93.5	873	96.1
♦ opening on side of egg shell	50	10.2	62	6.5	35	3.9
• emerged before collection of egg-batches	94	*19.1*	487	*51.4*	126	*13.9*
• emerged after collection of egg-batches	397	*80.1*	460	*48.6*	782	*86.1*
With remains of the caterpillars	85	17.2	144	15.1	87	9.6
Adults died in eggs without opening	4	0.8	12	1.3	2	0.2
Total number of *B. transversalis*	**96**		147		90	
Number of emerged *B. transversalis*, of them	91	94.8	130	88.4	75	83.3
♦ opening on top of egg shell	88	96.7	113	86.9	70	93.3
♦ opening on side of egg shell	3	3.3	17	13.1	5	6.7
• emerged before collection of egg-batches	6	*6.6*	21	*16.2*	3	4.0
• emerged after collection of egg-batches	85	*93.4*	109	*83.8*	72	96.0
Adults died in eggs without opening	5	5.2	17	11.6	15	16.7
Total number of *A. bifasciatus*	**114**		179		215	
Number of emerged *A. bifasciatus*, of them	98	86.0	160	89.4	195	90.7
♦ opening on top of egg shell	65	66.3	148	92.5	156	80.0
♦ opening on side of egg shell	33	33.7	12	7.5	39	20.0
• emerged before collection of egg-batches	15	*15.3*	45	*28.1*	39	20.0
• emerged after collection of egg-batches	83	*84.7*	115 (1♀)	*71.9*	156 (4♀♀)	80.0
With remains of the caterpillars	-	-	-	-	1	0.5
Adults died in eggs without opening	16	14.0	19	10.6	20	9.3
Total number of *P. bruchicida*	**2**		1		7	
Number of emerged ***P. bruchicida***, of them	2		1		5	
♦ opening on top of egg shell	2		1		5	
• emerged after collection of egg-batches	2(1♂;1♀)		1♀		5(3♂♂;2♀♀)	
Adults died in eggs without opening					2(1♂;1♀)	
Undetermined parasitoids	49		219		279	
Eggs destroyed of predators	7		58		91	
Impact of egg parasitoids and predators	1 133	20.1	2 259	29.1	2 351	26.3

the number of those emerged before and after the winter period is almost equal. The dynamics of the emergence of the other 3 parasitoids is of the same order: *A. bifasciatus, B. transversalis* and *P. bruchicida* emerged mainly after hibernation.

Out of the four more numerous parasitoids – *B. servadeii*, *O. pityocampae, B. transversalis* and *A. bifasciatus*, the highest viability is observed with *B. servadeii,* where the percentage of dead, unemerged individuals is around 1%, whereas with *O. pityocampae* it reaches 18.8%, and with *B. transversalis* and *A. bifasciatus* – 11.6 and 10.6%, respectively.

A high percentage of the cases where the emergence opening of the parasitoid is at the side of the host's egg, is observed with O. *pityocampae* and *A. bifasciatus*. With the former species they reach up to ¼, with the latter species – ⅓, whereas with the two representatives of the genus *Baryscapus* these cases are around 10% on the average.

Distribution of the egg parasitoids in different parts of the egg batches

The distribution of the parasitized eggs on different parts of the batches can be accepted as an indication of the adaptability of the particular parasitoid to this host, insofar as it is capable of overcoming the protective functions of the covering scales. The ends of the batches offer better opportunities for parasitisation, insofar as in these parts there is a greater probability of the absence of covering scales. With all four mass parasitoids, a higher relative share of their location at the ends of the batches is observed, whereas in the central part it is about 2 times lower (Table 5).

Discussion

Host egg mortality and sterile host eggs

For some categories of eggs, where no caterpillars emerge, the causes have not been established. These are the dead caterpillars, the empty and sterile eggs. The presence of dead, unemerged caterpillars is reported in similar studies conducted in different parts of the pine processionary moth area, their share being about 2.0-3.0% (Mirchev 2005), but there are also cases on sites in Turkey, where they reach up to 10.0% (Mirchev *et al.* 2004). In samples of *Thaumetopoea pinivora* (Tr.) from the Baltic Sea region, almost 100% of dead caterpillars have been found (Tsankov *et al.* 1993). It can be surmised that, in addition to some disease, the reasons for the demise of part of the pine processionary moth larvae can also be abiotic, such as high temperature, low air humidity, etc. The temperature in the batch increases by 14 °C compared to the air temperature, when the batch is subjected to sunshine (Milani 1990). Egg batches with empty eggs, in insignificant shares, have been found in samples from different countries: Greece (Bellin *at al.* 1990), Morocco (Schmidt *at al.* 1997b) and Portugal (Mirchev, Tsankov 2000). The shares of the sterile, unfertilized eggs are substantially higher. There is a report about the region of Marikostinovo, Bulgaria, where in samples from various years they reach up to 30.0%, and in individual batches up to 100% (Tsankov *et al.* 1998b). Most likely, particularly where batches with a high share of such eggs are concerned, we should look for the reason for this phenomenon in the quantity or quality of the semen fluid of male individuals which have copulated.

Parasitoid impact

The data in this study show that parasitoids are the most serious natural regulator of pine processionary moth numbers at the egg stage. This can be defined as a rule with this insect species, as a number of authors have reached this conclusion in their studies covering a large part of the species area and carried out in a wide time scope (Battisti 1989; Biliotti 1958; Biliotti *et al.* 1962; Cabral *et al.* 1965; Cadahia, Cuevas 1964; Cadahia *et al.* 1967; Ceballos 1969; Demolin, Delmos 1967; Harapin 1986; Jamaâ *et al.* 1996; Kitt, Schmidt 1993; Tiberi, Roversi 1987; Tsankov 1990; Szeczepański, Tzankov 1967, etc.). The percentage of parasitisation varies in wide limits, reaching almost half the eggs in some samples – Ploski, Bulgaria – 44.7% (Tsankov *et al.* 1998a).

In this study, the role of predators as a regulator of pine processionary moth is insignificant, which has also been reported about other regions (Mirchev 2005). The cause of the destruction of eggs has not been established. Two species destroying egg batches have been found in Bulgaria: *Ephippiger ephippiger* (Fiebig 1784) (Orthoptera: Bradyporidae) and *Pterolepis* (=*Rhacocleuis*) *germanica* (Herrich-Schäffer 1840) (Orthoptera: Tettigoniidae) (Tsankov *et al.* 1996b).

The parasitoid *P. bruchicida* established in this study has also been found, as a parasitoid on pine processionary moth, in other regions (Mirchev *et al.* 2011). The species is reported as hyper-parasitoid (Temerak *et al.* 1984). Palmeri, Pulvirenti (2004) have found that this species is a hyperparasitoid on *O. pityocampae*.

The percentage of parasitisation and the ratio between the particular parasitoids is determined of some ecological factors. Masutti (1964) points out that temperature is the limiting factor, the representatives of Eulophidae are more plastic and develop suc-

Table 5. Distribution of egg parasitoids in the egg batches of *T. pityocampa*

Species of parasitoids	Date of sampling	Part of batches					Total
		base 1/5	2/5	3/5	4/5	5/5 top	
Baryscapus servadeii	27. 03. 2010 *n* (%)	144 (29.1)	92 (18.6)	65 (13.1)	83 (13.1)	111 (22.4)	495 (100.0)
	11. 11. 2010 *n* (%)	223 (23.3)	193 (20.1)	180 (18.8)	191 (19.9)	172 (17.9)	959 (100.0)
	27. 03. 2012 *n* (%)	244 (26.8)	194 (21.3)	160 (17.6)	158 (17.4)	154 (16.9)	910 (100.0)
Ooencyrtus pityocampae	27. 03. 2010 *n* (%)	78 (21.1)	36 (9.7)	49 (13.2)	73 (19.7)	134 (36.3)	370 (100.0)
	11. 11. 2010 *n* (%)	165 (23.7)	95 (13.6)	113 (16.2)	135 (19.4)	188 (27.1)	696 (100.0)
	27. 03. 2012 *n* (%)	223 (29.4)	96 (12.6)	88 (11.6)	147 (19.3)	206 (27.1)	760 (100.0)
Anastatus bifasciatus	27. 03. 2010 *n* (%)	24 (21.0)	18 (15.8)	14 (12.3)	19 (16.7)	39 (34.2)	114 (100.0)
	11. 11. 2010 *n* (%)	41 (22.9)	26 (14.5)	18 (10.1)	38 (21.2)	56 (31.3)	179 (100.0)
	27. 03. 2012 *n* (%)	62 (28.8)	40 (18.6)	29 (13.5)	42 (19.5)	42 (19.6)	215 (100.0)
Baryscapus transversalis	27. 03. 2010 *n* (%)	23 (24.0)	4 (4.2)	7 (7.3)	27 (28.1)	35 (36.1)	96 (100.0)
	11. 11. 2010 *n* (%)	29 (18.4)	27 (16.3)	24 (17.7)	26 (15.6)	23 (32.0)	147 (100.0)
	27. 03. 2012 *n* (%)	16 (17.8)	12 (13.3)	12 (13.3)	25 (27.8)	25 (27.8)	90 (100.0)

Table 6. Percentage of parasitoids of *T. pityocampa* by different site in the Eastern Rhodopes

Site	Janino*		Ivailovgrad		
Generation Parasitoids	1994 %	1995 %	2009 %	2010 %	2011 %
Ooencyrtus pityocampae	2.6	6.5	34.3	35.1	38.4
Baryscapus servadeii	92.5	81.9	46.0	48.4	45.9
Baryscapus transversalis	3.2	2.1	8.9	7.4	4.5
Anastatus bifasciatus	0.3	9.4	10.6	9.0	10.8
Pediobius bruchicida**	0.0	0.1	0.2	0.1	0.4
Trichogramma embryophagum	1.4	0.0	0.0	0.0	0.0
Total number of parasitoids	1 456	2 025	1 077	1 982	1 982
Impact of parasitoids	24.3	27.0	19.9	28.4	25.3

* According to Mirchev *et al* (1998)

** In the publication of Mirchev *et al* (1998) is reported as *Pediobius* sp.

cessfully even in regions with temperatures of over 30 °C – conditions which make the development of *O. pityocampae* more difficult. That is why in Italy, *B. servadeii* has the highest numbers in warmer regions of the central and southern parts of the country, but it has not been found in Sicily and in the pine forests of Abruzzo at low altitudes (Tiberi 1990). It is our opinion that the numbers of polyphagous parasitoids are seriously influenced by other factors, the decisive one being the presence of a richer entomofauna ensuring the availability of alternative hosts, since the phenology of *O. pityocampae* and *A. bifasciatus*, unlike that of *B. servadei,* is not synchronized with that of the pine processionary moth (Mirchev 2005). The data in Table 6 support this conclusion – these data compare the numbers of *O. pityocampae* in the current site, Ivailovgrad, and another site in the region of the Eastern Rhodopes, where it can quite reasonably be accepted that the climatic conditions of the two sites are comparable but the numbers of *O. pityocampae* are drastically different.

Comparing *B. servadeii* with the other parasitoids found in the study by the indicator of viability (% of the dead, unemerged parasitoids of the particular species found), the data distinctly show several times lower mortality with this parasitoid. At least two reasons for this can be cited: *B. servadeii*, as a specific parasitoid on the pine processionary moth eggs, is the best adapted and, secondly, as it has already been mentioned above, *O. pityocampae* is sensitive to the increase in air temperature above certain limits.

Sites of egg-batches attacked by parasitoids

According to researchers of this process, the location of the eggs parasitized by the various parasitoids is a sign of their adaptability to that host. *B. servadeii*, which is the best adapted, has, according to MASSUTTI (1964), an unusual behaviour of egg deposition – sliding between the scales, it reaches the eggs and attacks them. BILIOTTI (1958) considers that the presence of scales is an obstacle for *O. pityocampae*. The perception exists that most frequently the scales are absent at the ends of the batches. MIRCHEV (2005) notes that if, for some reason, covering scales are absent, it happens primarily in the top part, this probably being due to some disturbances with the female butterfly leading to the presence of fewer covering scales or insufficient conglutination.

In literature, there are contradictory reports about the location of the eggs parasitized by the various parasitoids: TIBERI (1978) has found that *O. pityocampae* is more often in the base part of the batches, whereas *B. servadeii* prefers the top parts, but the results obtained by KIT, SCHMIDT (1993) show that *O. pityocampae* parasitises primarily the top part of the batch, whereas *B. servadeii* shows preference for the base part.

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