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RESEARCH ARTICLE

Comparative analysis of changes in bioecological indicators of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: Notodontidae) for a 28-31 year period

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Abstract

In 2022, a study was conducted with experimental material from the area of Marikostinovo village, covering various aspects of species bioecology. The stability or changes that have occurred in these bioecological parameters after a 28-31 year period were analysed, with research data from this area conducted in the period 1991-1994. The fecundity of pine processionary moths in the 5 generations compared had a stable level of average values above 214 eggs. The long-term observations and reporting of this indicator gave reason to draw the conclusion that in this region of Bulgaria, the species develops stably, successfully overcoming possible occurrences of extremely high temperatures in July and August. In the egg stage, the most significant factor in reducing the number of *T. pityocampa* is the impact of parasitoids. Its effect is not a constant value but varies widely, being the difference nearly twice. The relative share of eggs destroyed by predators is insignificant. A distinctive feature of the Marikostinovo site is the high relative proportion of dead caterpillars, sterile and empty eggs, with values significantly higher than those in other areas of the species' global range. This is the warmest and driest region of Bulgaria. The high values of this indicator are also formed by the large number of unfertilised, sterile eggs, reaching in 1994 up to 1/5 of the eggs in the sample. The dominant species, with constant presence in all samples, is *Ooencyrtus pityocampae.*

Key words

Thaumetopoea pitiocampa, egg parasitoids, survival, impact, Marikostinovo, Bulgaria

Introduction

In recent decades, the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermuller, 1775) (Lepidoptera: Notodontidae) has expanded its distribution area in habitats both north of the Mediterranean belt, its traditional range, and in areas in the mountains at higher altitudes (Battisti et al., 2005, 2006).

Dynamic processes also occur in its phenology and taxonomy. Formation of a new phenological form – summer, can also be considered as the initial stage of speciation (Pimentel et al., 2006; Santos et al., 2011a, 2013; Zankov, 1960; Mirchev et al., 2019).

The assessment of its harmfulness is also multifaceted. In the field of plant protection, as a serious phyllophage (Devkota, Schmidt, 1990) it has been determined as the most significant pest of pine forests in the Mediterranean (Demolin, 1969; Schmidt et al., 1990). As a dangerous allergen (Lamy 1990; Nikolov et al., 2020) for humans and animals, medical and sanitary damage is also significant, with an indirect negative impact on the economy, causing problems with its presence in populated areas and spa centers as well.

These are valuable arguments for this species to be the subject of in-depth and multi-year research mainly in the countries of its distribution area, including Bulgaria, where they began in 1906, when it was described as a new species for the country's fauna (Drenowsky, 1923).

T. pitiocampa inhabits Southwestern Bulgaria, which is also part of the northern border of its global range, where the site near Marikostinovo village is located, a separated geographical area of Sandansko-Petrichka valley, at 180 m a. s. l., in the valley of Struma river, not far from the state border between Bulgaria and Greece.

In the period 1991-1994, a study was carried out with experimental material from this habitat, covering various aspects of its bioecology, including food plants, its fecundity, survival in egg stages, analysis of the factors responsible for the reduction of its number in this ontogenetic stage (Tsankov et al., 1996a, 1998b).

In 2022, experimental material was collected from the same site, studied according to the same methodology as in the previous studies. The purpose of this study is to analyse the sustainability or changes that have occurred in these bioecological parameters after a 28-31 year period.

Material and methods

The biometric data for the pine processionary moth, subject of the present comparison, are based on the published results (Tsankov et al., 1996a, 1998b) of the analysis of 4-year experimental material (1991-1994) collected from the area of Marikostinovo. On 12.10.2022, 40 egg batches were taken from the same site.

The collected sample was transported to the Laboratory of Entomology (Forest Research Institute, Sofia). The scales of the egg batches were removed, and the samples were analysed according to Tsankov et al. (1996a). The egg batches were placed individually in test tubes covered by cotton stoppers and kept at room temperatures

(20-22 °C). The samples were checked periodically and the emerged parasitoids were separated and identified under a stereomicroscope (40×). At the end of the experiments (10-12 months after sampling), the eggs were dissected and analysed in detail.

The parasitoids that had emerged before sample collection were determined by their meconia and remains, according to Schmidt, Kitt, 1994; Tanzen, Schmidt, 1995; Schmidt et al., 1997a; Tsankov et al., 1996a, 1998a; Zaemdzhikova et al., 2021.

Results

Characterisation of egg batches

The 40 egg batches collected on 12.10.2022 from the area of Marikostinovo were from a plantation of *Pinus nigra* with a single participation of *P. sylvestris*, so 80% of the sample was found on needles of the first tree species.

The average length of the egg batches was 30.1 mm but with a large variation from 10 to 37 mm, which was also observed in the distance of the ring from the needle base - with a high standard deviation (SD) value and a sample rank from 0 to 50 mm. The number of eggs in individual batches was also variable, with more than two times the difference between the minimum and maximum number (Table 1).

Table 1. Characterisation of egg batches of *T. pitiocampa* sampled in Marikostonovo

Parameters	
Date of sampling	12.10.2022
Number of egg batches	40
Tree species:	
Pinus nigra, n (%)	32 (80.0)
P. sylvestris, n (%)	8 (20.0)
Mean length of egg batches, mm M±SD (rang)	30.1±4.8 (10-37)
Distance of egg batches to base of needles, mm M±SD (rang)	6.2±8.4 (0-50)
Number of eggs per batch	147-303
Total number of eggs	10 001
Mean eggs per batch M±SD	250.0±31.7

Egg parasitoids and parasitism rate

The results obtained by analysis of parasitoids that emerged or died in their developing stages in the eggs, are presented in Table 2. Three primary egg parasitoids were established in this habitat: *Ooencyrtus pityocampae* Mercet, 1921 (Hymenoptera: Encyrtidae); *Anastatus bifasciatus* Fonscolombe, 1832, (Hymenoptera: Eupelmidae); *Baryscapus servadeii* Domenichini, 1965; and a hyperparasitoid, *B. transversalis* Graham, 1991 (Hymenoptera: Eulophidae).

Table 2. Parasitoids found in the egg batches

Parasitoids	n	%
Ooencyrtus pityocampae	946	72.6
Anastatus bifasciatus	344	26.4
Baryscapus servadeii	9	0.7
B. transversalis	4	0.3
Σ	1303	100,0

The dominant parasitoid was *O. pityocampae* (72.6%), the other representative of the polyphagous group *A. bifasciatus* had a relative share 2.75 times lower. The participation of the two species of *Baryscapus* was insignificant, below 1%, respectively for the specific parasitoid on the eggs of the pine processionary moth – *B. servadeii* – 0.7% and against the background of the total number of 1303 individuals of all parasitoids, only 4 numbers of the hyperparasitoid *B. transversalis*.

Greater survival in this host showed *A. bifasciatus*, only 1.8 % of successfully developed adults failed to imagine, while this share in *O. pityocampae* was significantly higher – 7.6 % (Table 3).

The survival rate of *T. pityocampa* in the egg stage was 73.1 %. The most significant factor for reducing the species number at this stage of its ontogenetic development was the impact of parasitoids - 20.1%. In second place were the dead unhatched eggs, with the highest proportion of dead caterpillars that died for various reasons at an early stage of their development without having the opportunity to make exit holes. Close in value to this group were the unfertilised, sterile eggs, which were 3.8% of the total number of eggs in the sample (Table 3).

Table 3. Hatching rate, mortality of caterpillars, and parasitoid species

Parameters	Number	%
Unhatched caterpillars, from which:	668	100.0
Caterpillars died without opening	341	51.1
Caterpillars died with opening	43	6.4
Undeveloped eggs with dried-up yolk	280	41.9
Eggs totally empty, without any remains	4	0.6
Total number of eggs, from which:	10 001	100.0
Unhatched caterpillars	668	6.7
Caterpillars hatches	7309	73.1
Impact of egg parasitoids	2009	20.1
Eggs destroyed by predators	15	0.1
Parasitoid species		
Ooencyrtus pityocampae	946	100.0
Emerged	874	92.4
Dead adults	72	7.6
Anastatus bifasciatus	344	100.0
Emerged	338	98.2
Dead adults	6	1.8

Baryscapus servadeii	9	100.0
Emerged	9	100.0
B. transversalis	4	100.0
Emerged	4 (3♀♀; 1♂)	
Non identified parasitoid larvae	706	7.1

The analysis (Table 4) showed that the presence of parasitoids was found in 90% of the egg batches. In 35% of them, the parasitism was below the calculated average value and 45% above it, i.e. according to this indicator, the sample was not homogeneous enough.

Table 4. Parasitism of individual egg batches

Parasitism of individual egg batches, %	Egg batches, n	%
0	4	10.0
0.1 – 10.0	10	25.0
10.1 – 20.0	8	20.0
20.1 –30.0	7	17.5
30.1 – 40.0	4	10.0
40.1 –50.0	5	12.5
< 50.0	2	5.0
Σ	40	100.0

Emergence dynamics

Among the parasitoids, clearly distinguishable periods were observed in their imaginal dynamics, distinguishing from each other (Table 5).

Table 5. Emergence dynamics of parasitoids in the laboratory from eggs collected on 12.10. 2022

Parasitoids		*O. pityocampae*	*A. bifasciatus*	*B. servadeii*	*B. transversalis*
Number of emergence parasitoids		874	338	9	4
-before collection egg batches, n (%)		510 (58.4)	(12.9)	-	-
-after collection egg batches in.		364	288	9	4
Month	**A ten-day**	**Number of emergence parasitoids**			
October 2022	2 3	2 6	17 2	- -	- -
November 2022	2 3	1 -	228 20	- -	- 2♀♀
December 2022	1 2	- -	23 7	- -	1♂ -
January 2023	1 2 1	- 4 3	1 - -	- - -	- 1♀ -
February 2023	2 3 1	14 6 8	- 1 -	- - -	- - -

March 2023	2 3 1	15 50 15	- - -	- - -	- - -
April 2023	2 3 1	29 31 42	- - -	1 2 -	- - -
May 2023	2 3 1	53 34 15	- - -	- - -	- - -
June 2023	2 3	13 8	- -	1 3	- -
July 2023	1 3	15 -	- -	- 2	- -

A. *bifasciatus* completed their development before the middle of October, i.e. before its hibernation, they had completed their development and only 12.9% of them had imagined. In laboratory conditions, the emergence of adults practically occurred in the next 2,5 months. This is an indication that this parasitoid, as a polyphage, does not have an established synchronisation with the pine processionary moth phenology.

The main part (58.4 %) of the individuals, in the other polyphagous and dominant species *O. pityocampae*, imagined before the winter diapause. Its mass flight was after the end of March, i.e. weather was associated with a greater likelihood of some of its alternative hosts being present. Although these results were obtained under laboratory conditions, this is a sign of its higher adaptability compared to the first species. It is a strong argument in support of the hypothesis that this is one of the leading reasons for its nearly 3 times higher number in this biotope.

In the case of the specific parasitoid on pine processionary moth eggs *B. servadeii*, although the number was too low, which did not allow drawing reasonable conclusions, the tendency towards synchronization of its time of imagining and the appearance of the eggs of its only host can be seen.

Discussion

Climatic characterisation of the studied area

The village of Marikostinovo is located in the southernmost part of the Bulgarian part of the Sandansko-Petrichka valley. This geographical area occupies an area of 550 km^2 along the Struma River, surrounded by Pirin, Maleshevska, Ograzhden, Belasitsa, Sengelska Planina and Slavyanka mountains. It belongs to the Continental-Mediterranean climate zone (Sabev, Stanev, 1959). This is the warmest and driest part of Bulgaria. The maximum daytime temperatures in July and August sometimes reach 42 ÷ 43 ^{o}C, i.e. exceeding those fatal to newly hatched caterpillars, according to Santos et al. (2011a,b) above 32°C or 40°C (Robinet et al., 2013).

Fecundity of the pine processionary moth

Female moths of *T. pityocampa* usually lay only one egg batch (Douma-Petridou, 1990), so the average number of eggs in egg batches corresponds to the true average fecundity of the species in the given area.

The data from the present comparative analysis showed that fecundity is not a constant value for the species in the given area (Table 6). Masutti, Battisti (1990) believe that it depends on the host plant, climatic conditions, and the population cycle of the species. When comparing multi-year data from the same object, a constant value is only the first factor.

Table 6. Changes in bioecological indicators of *T. pityocampa* over a 28-31 year period

Parameters Generations	**1991**	**1992**	**1993**	**1994**	**2022**
Fecundity					
M±SD	242.6±45.0	242.7±47.3	214.5±22.5	224.3±41.2	250.0±31.7
range	68-310	69-316	171-243	108-303	147-303
Caterpillars hatches, %	53.2	41.4	54.1	40.1	73.1
Impact of egg parasitoids, %	38.2	24.4	19.3	32.4	20.1
Eggs destroyed by predators, %	-	-	-	0.3	0.1
* Unhatched caterpillars, %	8.6	34.2	26.6	27.2	6.7
Undeveloped eggs with dried-up yolk, %	3.1	17.7	9.4	20.7	2.8
Parasitoid species					
Total number	**6418**	**3 225**	**456**	**2673**	**1303**
Ooencyrtus pityocampae, %	87.9	100.0	99.8	97.8	72.6
Anastatus bifasciatus, %	10.9	-	-	0.7	26.4
Baryscapus servadeii, %	0.4	-	0.2	1.2	0.7
B. transversalis, %	0.8	-	-	0.3	0.3
Σ %	100.0	100.0	100.0	100.0	100.0

* - Eggs from which no caterpillars without influence of parasitoids and predators

The fertility of the pine processionary moth in the compared 5 generations had a stable level of average values above 214 pcs., the highest being in the last sample from 2022 – 250 eggs. These were consistent results typical of the species in this geographic area. When studying 7 generations in the period 1991-2017 from the Sandanski region, the average number of eggs was between 224 and 247 eggs (Georgieva et al., 2018). As the crow flies, the distance between Sandanski and the current research site is 15 km.

The values of this indicator from the region of Sandansko-Petrichka valley significantly exceed those from other regions of its range. In Israel, Halperin (1970) found an average of 191, Kitt, Schmidt (1993) - 178 for *T. wilkisoni*, and the species is closely related to *T. pityocampa*, in Algeria – 154, (Tsankov et al., 1995), Morocco – 175 (Schmidt et al., 1997b), Portugal – 183 (Mirchev, Tsankov, 2000).

The long-term observations and reporting of the fertility indicator of *T. pityocampa* gave reason to draw the conclusion that in this region of Bulgaria, the species develops stably, successfully overcoming the possible occurrences in July and August of extremely high temperatures.

Survival of *T. pityocampa* in egg stage

The data on 28 pine processionary moth habitats in 5 Balkan countries showed that the percentage of hatched caterpillars varied between 40.1 and 95.8, the major part being grouped around 60% (Mirchev, 2005). This shows that it has experienced serious fluctuations in its number over the years, which should be considered as a cumulative effect of species survival during the individual stages of its ontogenetic development. This finding was confirmed by the data on the areas attacked in Bulgaria (Fig. 1). The difference between the maximum affected ones in 1998 and the minimum presence of the pest in 2018 is 3.3 times.

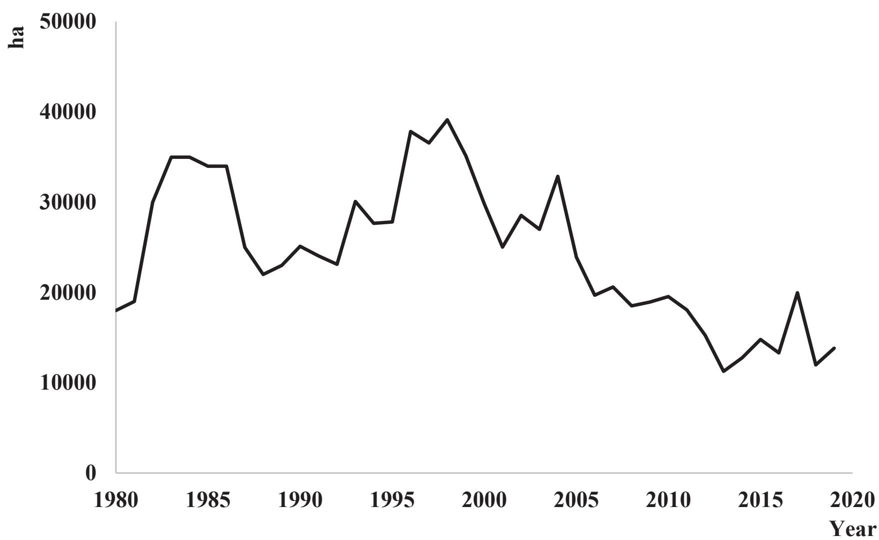

Figure 1. Areas attacked by *T. pityocampa* in over the years in Bulgaria

Fluctuations in the relative share of hatched caterpillars were also observed in neighbouring sites from Sandansko-Petrichka basin: Sandanski between 73.1 and 75.6% (Mirchev, Tsankov 2003), in the land of Ploski village – 42.4 -74.2% (Tsankov et al., 1996a; 1998a).

Fluctuations in the level of survival in the egg stage in individual generations in the Marikostinovo area should be considered as a natural course of a normally functioning population of the pest in this habitat.

Impact of egg parasitoids and predators

In the egg stage, the most significant factor for reducing the number of *T. pityocampa* in all compared generations from Marikostinovo was the impact of parasitoids. Its effect was not a constant value but varied widely, reaching a difference of almost twice: in the sample from 1991, the parasitized eggs were 38.2%, against 19.3% in 1993 (Table 6).

The leading role of parasitoids is typical of the pine processionary moth, and is not a specific feature of the genera now being compared. The conclusion that parasitoids are the most serious natural regulator of the pine processionary moth number in egg stage was made by a number of authors, in areas of the world range (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; El-Yousfi, 1989; Kitt, Schmidt, 1993; Tiberi, Roversi, 1987; Tsankov, 1990; Szeczepaňski, Tzankov, 1967, etc.).

Parasitism is dynamic and varies widely over the years. The values of parasitised eggs reported from some Mediterranean countries are heterogeneous: Portugal – 9.9 % (Mirchev, Tsankov, 2000); Spain – 11.3-31.7 % (Schmidt et al., 1999); Morocco – 21.4 % (Schmidt et al., 1997b), Algeria – 33.7 % (Tsankov et al., 1995), in various regions of Italy - from 6.0 % to 72.0 % (Tiberi, 1990); Israel in *T. wilkinsoni* – 38.6 % (Kitt, Schmidt, 1993) and 22.0 % (Halperin, 1970).

The share of eggs destroyed by predators was insignificant, and in the 1991-1993 generations there was none at all (Table 6). In a survey of 28 sites in some countries of the Balkan Peninsula, only half of the samples were found to be damaged, and only 3 of them (10.7%) had more than 2.0% damaged eggs (Mirchev, 2005).

Only two predators, the tetigonids *Ephippiger ephippiger* (Fieb.) and *Rhacocleuis germanica* (H.-S.) have been established and reported in Bulgaria (Tsankov et al., 1996b).

The conclusions from the analysed generations of the pine processionary moth from Marikostinovo region are: parasitoids are the most significant regulator of the species' abundance in the egg stage; and the role of predators is negligible.

These data fit the population of *T. pityocampa* from this site into its typical developmental pattern.

Unhatched caterpillars

In terms of the relative proportion of dead caterpillars, sterile and empty eggs of the pine processionary moth, Marikostinovo differs significantly from previously published studies for other areas of the species' world range. These differences are mainly based on the results obtained in the 1992-94 generations, when their share was 27.2 and 34.2% of the total number of eggs in the samples, while in the studies of 28 sites from some countries of the Balkan Peninsula, the main part of them were below 5%. With the highest value of 7.7% is Sandanski, a site from the same climatic region as

Marikostinovo, followed by Dupnitsa (Bulgaria) – 6.1% and Gönen (Turkey) – 6.0% (Mirchev, 2005).

The causes of mortality in eggs and young caterpillars can be traced to the specific climatic conditions. As mentioned above, Marikostinovo falls in the warmest and driest region of Bulgaria. In addition, the temperature in the annulus can rise by 14 °C compared to the air when it is exposed to sunlight (Milani, 1990).

High summer temperatures are crucial for PPM's survival. Temperatures above 32 °C are extremely dangerous for its egg incubation (Huchon, Demolin, 1970). Robinet et al. (2013) suggest that the upper temperature threshold for PPM's survival is much higher than 32 °C. According to their study, egg masses could survive at temperatures of 40 °C for several days, assuming that it could be a result of species adaptation to climate warming.

In the 1992-94 generations, the high percentage of unhatched eggs was also formed by the large number of unfertilised, sterile eggs, reaching in 1994 up to 1/5 of the eggs in the sample. Such eggs were found in all 49 analysed samples from 28 sites from countries of the Balkan Peninsula, but in the majority their relative proportion was around or below 2%. Exceptions are samples from Ploski with 6.7%, which is from the same climatic region as Marikostinovo, Patras (Greece) – 6.0% and Iskenderum (Turkey) – 5.4 but these values are far from their registered relative share of 20.7% in 1994 in Marikostinovo. The reasons leading to a high number of unfertilised eggs are not clear. In *Lymantria dispar* (L.) (Lepidoptera: Erebidae) Tobin et al. (2014) found that with the increasing age of copulating individuals, the number of unfertilised eggs increased as well.

Egg parasitoids

Among the 3 primary parasitoids and 1 hyperparasitoid found in the samples from Marikostinovo, only *O. pityocampae* was found in all 5 generations. Its dominant presence was evident in the samples from 1992-1994, and relative share nearly reached 100% (Table 6). The two representatives of the genus *Baryscapus* had a modest presence of less than 1%, if found at all.

As it is already said, the research site is in the warmest and driest climatic region of Bulgaria, and in the summer months it is common for the air temperature to exceed 40 °C. The present results do not at all support the theory of Massuti (1964) that temperature is the limiting factor, the representatives of Eulophidae are more plastic and develop successfully also in areas with temperatures above 30 °C conditions that hinder the development of *O. pityocampae*. Therefore, in Italy *B. servadeii* had the highest number in the warmer regions of the central and southern part of the country.

It can be supposed that the presence and abundance of *O. pityocampae* as a polyphage depends not so much on the temperature conditions, but on the floristic richness of the given biotope providing an environment for the abundance of its alternative hosts. Supporting the thesis that climatic conditions are not the main factor was the study of parasitoids in some Balkan countries (Mirchev, 2005). *O. pityocampae* was the dominant species in 46.4% of the sites located from almost sea level, such as the island of Hydra (Greece) (10 m) and Athens (Greece) (50 m) to Iğdecik (Turkey) (870 m), Atabey (Turkey) (1050 m), Qarr (Albania) (1050 m) and Isparta (Turkey) (1150 m).

The factor floristic diversity, providing an environment for the development of entomofauna, is decisive for the presence and abundance of the other polyphagous *A. bifasciatus*. From the eggs of *T. pityocampa*, only males imaginate (Bellin et al., 1990), i.e. its existence in a given biotope is determined by the availability of alternative hosts.

The low abundance of the specific parasitoid *B. servadeii* was characteristic of the new habitats of *T. pityocampa* when expanding its distribution area (Belilov et al., 2023; Mirchev et al., 2017, 2021), but the case of Marikosinovo is not like that. It has been present in this area for decades, i.e. the factors limiting its presence are other and unclear for now.

Conclusion

The village of Marikostinovo is located along the Struma river, in the southernmost part of the Bulgarian part of the Sandansko-Petrichka valley. It falls into the Continental-Mediterranean climate zone. The maximum daytime temperatures in July and August sometimes reach 42 ÷ 43 ºC, i.e. exceeding those fatal to newly hatched caterpillars.

The fecundity of the pine processionary moth in the 5 generations compared had a stable level of average values above 214 eggs . The long-term observations and reporting of this indicator gave reason to draw the conclusion that in this region of Bulgaria, the species develops stably, successfully overcoming possible occurrences in July and August of extremely high temperatures.

Fluctuations in the egg survival rate between generations is considered a natural course of a normally functioning population of the pest in this habitat.

In the egg stage, the most significant factor in reducing the number of *T. pityocampa* is the impact of parasitoids, which is typical of it, and not any specific characteristic of the generations now being compared. Its effect is not a constant value, but varies widely, reaching a difference of nearly 2 times. The proportion of eggs destroyed by predators is insignificant.

A distinctive feature of the Marikostinovo site is the high relative proportion of dead caterpillars, sterile and empty eggs, with values significantly higher than those of other regions of the species' world range. The reasons for this can be found in the specific climatic conditions. The high values of this indicator are also formed by the large number of unfertilised, sterile eggs, reaching in 1994 up to 1/5 of the eggs in the sample. The reasons for which are not clear.

In this region with extremely high temperatures during the summer months, the dominant species, with constant presence in all samples, is *O. pityocampae*. It is suggested that the presence and abundance of the two polyphages *O. pityocampae* and *A. bifasciatus* depends not so much on the temperature conditions, but on the floristic richness of the given biotope providing an environment for the abundance of their alternative hosts. There is no reasonable explanation for the low number of the specific parasitoid *B. servadeii*.

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