*Boll. Zool. agr. Bachic.*
Ser. II, 30 (2): 131-140

30 December 1998

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# The egg parasitoids of the pine processionary moth *Thaumetopoea* (*) *pityocampa* (Den. & Schiff.) in the Eastern Rhodopes, Bulgaria (**)

**Abstract** - In a forest of *Pinus nigra* Arnold, a total of 67 egg batches of two generations of *Thaumetopoea pityocampa* (Den. & Schiff.) were collected at Janino, Kardjali County, in 1995 and 1996. Directly after sampling the batches were put singly in test tubes with cotton stoppers and held under laboratory conditions at 20-22°C. Oviposition always started from the base towards the tip of the needles. After removal of the scales, the numbers of caterpillars hatched were counted. The emergence of egg parasitoids were controlled daily, they were removed and put into small capsules for identification. The final analysis was made after termination of parasitoid emergence. For that all eggs were opened carefully. The impact of the parasitoid species was evaluated by their meconia and other remains.
The average number of eggs per batch was 200 from which 66-72% developed to hatching caterillars. The number of parasitized eggs amounted to 24-27%. Six species of parasitoids were identified: *Baryscapus servadeii* (Dom.), *Ooencyrtus pityòcampae* (Mercet), *Anastatus bifasciatus* (Fonsc.), *Baryscapus transversalis* Graham, *Trichogramma embryophagum* (Htg.), and *Pediobius* sp. The most frequent parasitoid was *B. servadeii* (82-92.5% of the total parasitoids counted) emerging mainly after hibernation. The high percentage (17.6%) of female *A. bifasciatus* was surprising reaching maturity.

**Riassunto** - *Parassitoidi oofagi della Processionaria del pino (*Thaumetopoea pityocampa *(Den. & Schiff.)) nel Rhodopes orientale, Bulgaria.*

In foreste di *Pinus nigra* Arnold, 67 ovature di due generazioni *di Thaumetopoea pityocampa* (Den. & Schiff.) sono state raccolte a Janino, Provincia di Kardjali, nel 1995 e 1996. Immediatamente dopo la raccolta le ovature sono state poste singolarmente in provette con tappo di cotone e mantenute in laboratorio a 20-22°C. Si è verificato che l'ovideposizione della processionaria inizia verso l'apice degli aghi. Dopo la rimozione delle squame è stato contato il numero di larve

(*) Syn. *Traumatocampa* Wallengren, 1871 (Freina J. & Witt T., 1987: Die Bombyces und Sphinges der Westpalaearktis, Lepidoptera. Forschung & Wissenschaft, München).

(**) The investigations were supported by the Government of Lower Saxony, Germany.

nate. La schiusura delle uova dei parassitoidi è stata controllata quotidianamente, e i parassitoidi messi in piccole piastre per l'identificazione. L'analisi finale è stata fatta al termine dello sfarfallamento dei parassitoidi. L'identificazione dei parassitoidi è stato valutata dalla presenza del meconio e di altri residui.
Il numero medio di uova per ovatura è di 200 a, di cui il 66-77% ha dato origine a larve. Il numero di uova parassitizzate ammonta al 24-27%. Sono state identificate sei specie di parassitoidi: *Baryscapus servadeii* (Dom.), *Ooecyrtus pityocampae* (Mercet), *Anastatus bifasciatus* (Fonsc.), *Baryscafus transversalis* Graham, *Trichogramma embryophagum* (Htg.) and *Pediobius* sp. Il parassitoide più frequente è *Baryscapus servadeii* (82-92.5% del totale dei parassitoidi raccolti) sfarfallato soprattutto dopo l'ibernazione. Un'alta percentuale (17.6%) di femmine di *Anastatus bifasciatus* ha raggiunto la maturità.

**Key words**: *Thaumetopoea pityocampa*, egg parasitoids, *Baryscapus servadeii, Ooencyrtus pityocampae, Anastatus bifasciatus, Baryscapus transversalis, Trichogramma embryophagum, Pediobius sp.,* parasitoid impact, eastern Rhodopes, Bulgaria.

## INTRODUCTION

Plantations of Austrian pine (*Pinus nigra* Arn.) and Scots pine (*Pinus sylvestris* L.) were established on large areas of different levels of erosion in the Eastern Rhodopes, throughout the recent 50 years. The Eastern Rhodopes provide conditions favourable for the development of pine processionary moth. Their low-mountain aspect,-64.4% of the territory belong to the low-mountain belt of transitional mediterranean climate -, favoured the growth of Austrian and Scots pines.

The pine pest had not been reported for this region till the beginning of the 1930s (Ruskoff, 1929-1930). An infestation causing appearance of *T. pityocampa* was established in the last 20 years when an permanent application of forest protection operations was needed carried out by aerial treatment with bacterial preparations based on *Bacillus thuringiensis* (BT) and, since 5 years ago, also by application of Dimilin, according to the proportion of the infested sites.

The purpose of the present study was to identify the pattern of the egg parasitoid species in an Austrian pine stand, where the abundance of the pest has been regulated by application of mainly biological preparations throughout the recent two decades.

## MATERIALS AND METHODS

The egg batches were collected in a 35 years old; low-graded (IV level of stand quality) Austrian pine stand of eastern-south-eastern exposition at an altitude of 400 m in the vicinity of the village of Yanino, Kardjali County, the Eastern Rhodopes, in

1995 and 1996. In the Eastern Rhodopes, most of the regions are hilly, up to 200 - 600 m a.s.l. Their climate is transitional mediterranean, with comparatively warm winter and hot summer. The meteorological data pertaining a 40-years period was taken from the Jebel Meteorological Station near the village of Yanino showing 1.4°C for the mean January temperature and 23°C for the mean July temperature. The mean annual precipitation was found at 723 mm.

After collection, the batches were put singly in test tubes with cotton stoppers and transported to the laboratory of the Forest Research Institute at Sofia. All the laboratory studies were carried out at room temperature (20-22°C). The scales of the egg batches were removed, the parasitoids emerging each day were counted, taken away from the test tubes and put into small plastic capsules for identification.

A total of 67 egg batches of two generations of pine processionary moth were analysed. Thirty egg batches deposited in the autumn of 1994 were collected on 13 April, 1995. A very high frequency of pine processionary nests was reported for the time when the egg batches were collected (7.5 nests per tree). On 22 August, 1995, when 22 egg batches of the next generation were collected, all of the caterpillars were hatched. Control by BT was carried out in September, 1995. The second sample of 15 egg batches was taken on 10 April, 1996 for the same generation. Now, the abundance of the pest was found at 0.46 nests per tree.

All the egg batches were controlled at least at the Department of Zoology-Entomology, University of Hannover, in October, 1995 and November, 1996, respectively. Every egg without a hole in its shell was opened carefully, and the meconia and remains of the dead insects were determined by using a stereomicroscope (40 x magnification).

## RESULTS

### *Structure of egg batches*

In all cases, the pine processionary moth started oviposition from the base towards the tip of the needles, at 6 mm from the needle base, on average. All egg batches were formed around two needles, except of two wrapped around four needles. The average needle length was about 85 mm, the shortest being 58 mm and the longest 125 mm. The average egg batch diameter was about 3.5 mm and the average spiral egg row number per batch was 9 (Tab. 1).

The average egg batch length was about 26 mm,. 66.7% of the egg batches collected on 13 April, 1995 were within the range from 25 to 30 mm. The length of egg batches of the second generation, which were collected on 23 August, 1995 and on 10 April, 1996, ranged from 25 to 30 mm for 59.5%.

### *Caterpillars' hatch*

The egg batch analysis revealed that the percentages of the caterpillars hatched from the eggs deposited in 1994 and 1995 were almost equal (Tab. 2). From the eggs collected on 13 April, 1995, 69.3% of the caterpillars hatched, this percentage averaged

*Table 1 - Results from analysis of egg batches* of Thaumetopoea pityocampa *(Den. & Schiff.) collected at Janino (Eastern Rhodopes).*

| Date of collection | 13. 04. 1995 | 23. 08. 1995 | 10. 04. 1996 |
|---|---|---|---|
| Number of egg-batches | 30 | 22 | 15 |
| Length of needles wrapped by an egg batch | 81 (58 - 115) mm) | 86 (59 - 115) mm) | 92 (60 - 125) mm) |
| Length of egg-batches | 26 (7 - 40) mm) | 26 (9 - 34) mm) | 26 (20 - 30) mm) |
| Diameter of egg-batches | 3.4 (3.1 - 4.8) mm) | 3.5 (3.0- 5.0) mm) | 3.5 (3.0 - 4.2) mm) |
| Number of egg rows per batch | 8.6 (6 - 11) | 8.8 (8 - 12) | 9.1 (8 - 10) |
| Distance of egg-batches to base of needles | 6.1 (0 - 18) mm) | 5.9 (2 - 20) mm) | 5.7 (0 - 25) mm) |
| Number of eggs per batch | 34 - 296 | 58 - 256 | 157 - 255 |
| Total number of eggs | 5997 | 4606 | 2957 |
| Mean per batch | 200 | 209 | 197 |

to 68.4%, for the second generation.

The eggs from which no caterpillars had been hatched, without effect of parasitoids, were about 6%, about the half of these eggs was sterile and the rest of them was developed, but without hatching of caterpillars.

*Egg parasitoid spectrum*

The eggs of the first generation investigated were attacked by parasitoids up to 24.3%. For the second generation, the percentage increased to 27% (27.8% for the first sample and 25.8% for the second one, respectively) (Tab. 2).

Six egg parasitoids were identified (Tab. 3). A female individual of *Pediobius sp.* was found in the egg batches collected on 23 August, 1996.

Twenty eggs of pine processionary moth attacked by *Trichogramma embryophagum* (Htg.) were found in the sample collected on 13 April, 1995, from 14

*Table 2 - Analysis of eggs and hatching rates of caterpillars of* T. pityocampa *from batches sampled at Janino (Eastern Rhodopes).*

| Date of collection | 13. 04. 1995 | 23. 08. 1995 | 10. 04. 1996 |
|---|---|---|---|
| Number of egg-batches | 30 | 22 | 15 |
| caterpillars died without opening | 109 | 114 | 40 |
| caterpillars died with opening | 30 | 14 | 3 |
| caterpillars hatched | 4159 (69.3 %) | 3049 (66.2 %) | 2125 (71.9 %) |
| undeveloped eggs with dried-up yolk | 238 (4 %) | 109 (2.4 %) | 24 (0.8 %) |
| eggs totally empty, without any remains | 5 | 40 | 2 |
| Total number of eggs from which no caterpillar hatched without influence of parasitoids | 382 (6.4 %) | 277 (6.0 %) | 69 (2.3 %) |
| Impact of egg parasitoids | 1456 (24.3 %) | 1280 (27 8 %) | 763 (25.8 %) |

*Table 3 - The egg parasitoids of* T. pityocampa *and their developmental rates in the batches found at Janino (Eastern Rhodopes).*

| Date of collection | 13. 04. 1995 | 23. 08. 1995 | 10. 04. 1996 |
|---|---|---|---|
| Number of egg-batches | 30 | 22 | 15 |
| ***Ooencyrtus pityocampae* (Mercet)** | **2.6%** | **7.0%** | **5.9%** |
| emerged before collection of egg-batches | 20 | 48 | 25 |
| emerged after collection of egg-batches | 8♀♀ | 13 ♀♀ | 20 ♀♀ |
| adults died in eggs without opening | 1 ♀ | 4 ♀♀ | 0 |
| pupae died in eggs | 0 | 12 | 0 |
| larvae of different stages died in eggs | 8 | 11 | 0 |
| ***Anastatus bifasciatus* (Fonsc.)** | **0.3%** | **14.6%** | **0.8%** |
| emerged before collection of egg batches | 0 | 39 | 2 |
| emerged after collection of egg-batches | 4♂♂ | 108 (89 ♂♂, 19 ♀♀) | 3 ♂♂ |
| adults died in eggs without opening | 1 ♂ | 21 ♂♂ | 1 ♂ |
| pupae died in eggs | 0 | 9 | 0 |
| larvae of different stage died in eggs | 0 | 8 | 0 |
| ***Baryscapus servadeii* (Dom.)** | **92.5%** | **77.8%** | **88.6%** |
| emerged before collection of egg batches | 358 | 164 | 41 |
| emerged after collection of egg batches | 893 (884♀♀, 9 ♂♂) | 790 (786♀♀,4♂♂) | 613 ♀♀ |
| adults died in eggs without opening | 36 (22♀♀,14♂♂) | 4 (2♀♀,2♂♂) | 9 (6♀,3♂♂) |
| pupae died in eggs | 24 | 7 | 5 |
| larvae of different stages died in eggs | 36 | 18 | 7 |
| ***Baryscapus transversalis* Graham** | **3.2%** | **0.5%** | **4.7%** |
| emerged before collection of egg-batches | 1 | 0 | 0 |
| emerged after collection of egg-batches | 40 (15♀♀,25♂♂) | 5 (2♀♀,3♂♂) | 36 (♀♀,18♂♂) |
| adults died in eggs without opening | 5 (3♀♀,2♂♂) | 1 | 0 |
| pupae died in eggs | 1 | 0 | 0 |
| ***Pediobius sp.*** | | **0.1** | |
| adults died in eggs without opening | 0 | 1 | 0 |
| undetermined larvae of parasitoids | 0 | 17 | 1 |
| ***Trichogramma embryophagum* (Htg.)** | **1.4% eggs** | | |
| emerged before collection of egg batches | from 14 eggs | 0 | 0 |
| emerged after collection of egg batches | from 6 eggs | 0 | 0 |

eggs of these parasitoids had emerged in the autumn 1994 before hybernation. Eight to ten adult *Trichogramma* individuals per egg emerged after the date of egg batch collection.

*Anastatus bifasciatus* (Fonsc.) attacked the eggs laid in the autumn 1994 to only

0.3% of all the egg parasitoids. Its percentage reached up to 9.4% in the next year, when a comparatively high number (19) of emerged females was found after the date of egg collection, amounting to 17.6% of the emerged adults.

The percentage of *Ooencyrtus pityocampae* (Mercet) was found close to that of *Anastatus bifasciatus*, whilst an increase from 2.6% to 6.5% was observed in the second host generation. All the individuals, which emerged after the date of egg collection, were female.

The percentage of the hyper-parasitoid *Baryscapus transversalis* Graham hold the values of 3.2% and 2.1% for the two years, respectively. The sex ratio of the adults, found after the date of egg collection, was almost 50♀♀:5♂♂.

The highest percentage of parasitized host eggs was attacked by *Baryscapus servadeii* (Dom.). It was 92.5% in the sample collected on 13 April, 1995, and 81.9% in that taken on 10.04.1996. The percentage of males becoming adult after the date of egg collection was 2.5%, in the first year, and only 0.6% in the second one. The host eggs attacked by this parasitoid amounted to 22.5% in the first year and to 21.9% in the second one.

The results presented in Table 3 reveal that most of the individuals of *B. servadeii* became adult after the date of egg collection in both host generations. The individuals that reached maturity in the autumn of the first year were 28.6%, and those which matured in autumn of the second year amounted to 12.7%. The individuals of *B. servadeii* that failed to complete their development were 7.7% and 3.1%, respectively, for the two years.

*Distribution and effectiveness of egg parasitoids*

The parasititoids were irregularly distributed on and in the egg batches (Fig. 1 and 2). In the sample collected on 13 April, 1995, two batches were not parasitized by any species of parasitoids. The maximum percentage of parasitism was found at 59.4% for one egg batch. The results for the second year were analogous to these: parasitism was not found in one batch, and the maximum of parasitism was 54.4% for one batch sampled on 10 April, 1996.

Regarding the distribution of *B. servadeii* parasitoids in different parts of the egg batch (Fig. 2), the eggs at the base and top of the batches were somewhat preferred for parasitism, with some variation and without statistical significance.

## DISCUSSION

The length of the needles of *Pinus nigra* f. *nigricans* varied normally from 80 to 140 mm (Debazac, 1964). Growing under unfavourable conditions, the length of the needles are shorter (Mihaylov, 1983). The average length of the pine needles in the investigated area was within the lower range of values, indicating the not good physiological conditions of the trees and favouring the development of the pine processio-

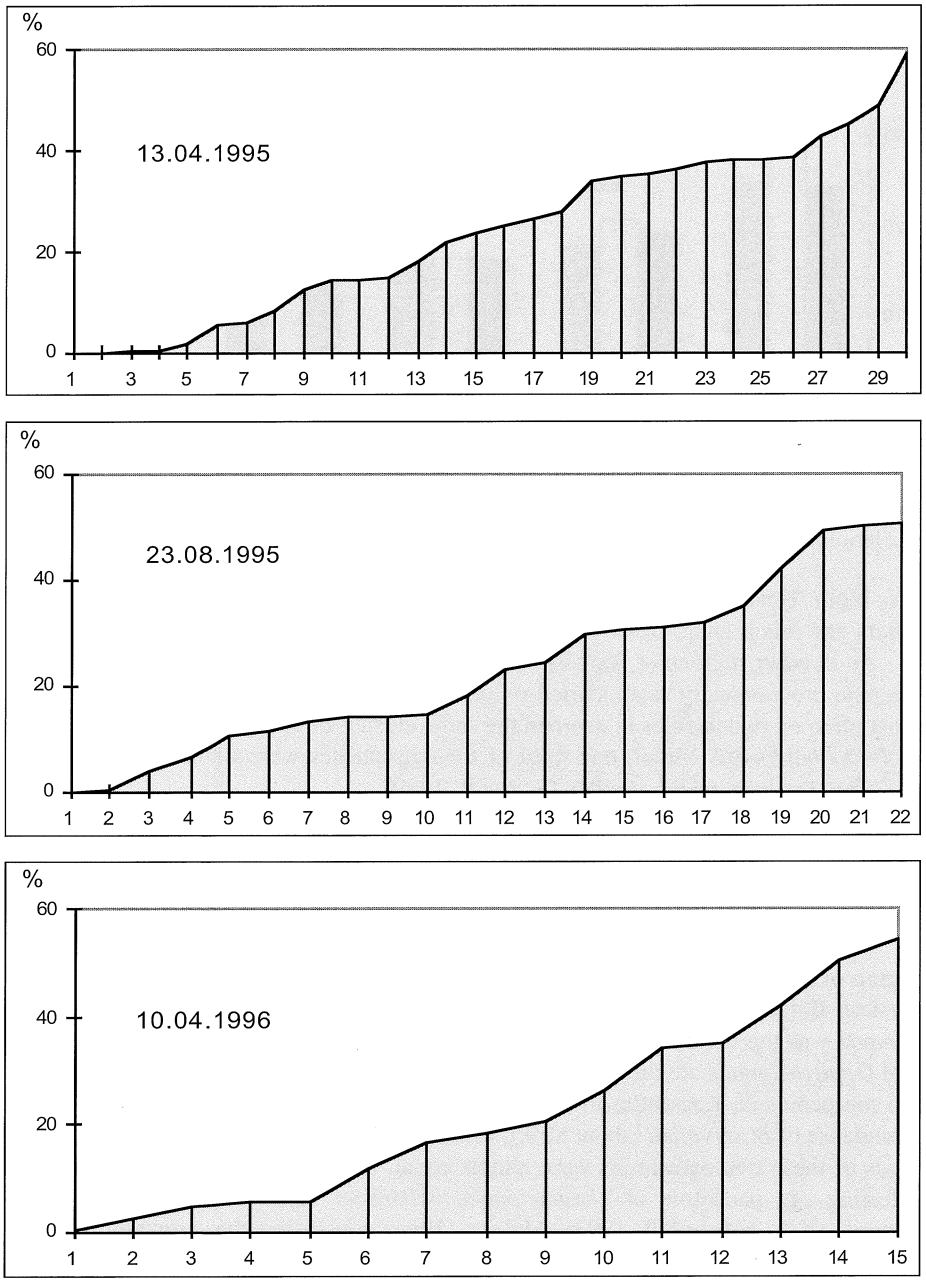


Fig. 1 - Percentage of parasitism of *Baryscapus servadeii* in single egg-batches at Janino (Eastern Rhodopes); abscisse: number of egg-batches studied.

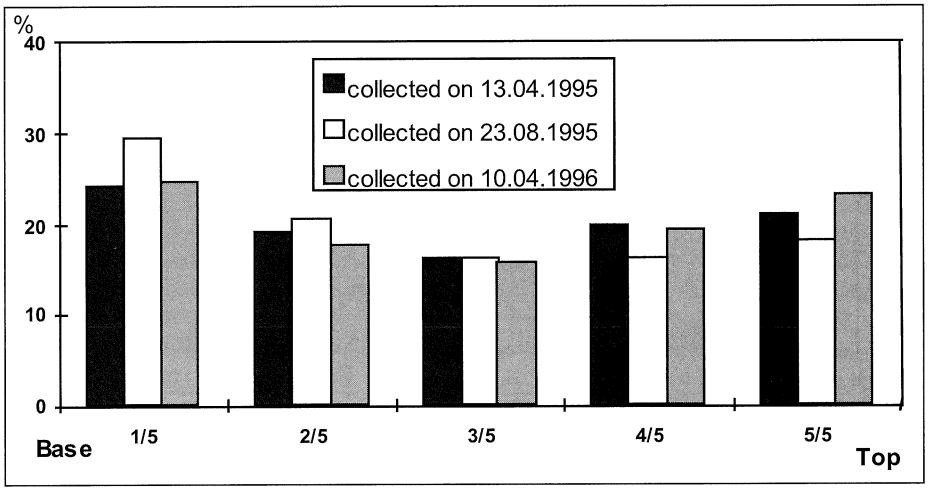


Fig. 2 - Oviposition pattern of *Baryscapus servadeii* in egg-batches of *T. pityocampa* from Janino (Eastern Rhodopes).

nary moth. Thus, BT application was needed to reduce the abundance of the pest caterpillars and much lesser nests were formed for the winter.

As in other regions of Bulgaria (Tsankov *et al.*, 1996), our studies revealed that the pine processionary moth started oviposition at the base of Austrian pine needles, most often on two needles in one and the same cluster. In southern Greece, on needles of *Pinus halepensis* Miller, one third of the egg-batches were found deposited from the tip to the base of the needles (Schmidt, 1990).

The average number of the eggs per batch was 200, this is somewhat less than found near the village of Marikostinovo at Struma river valley, in south-western Bulgaria (Tsankov *et al.*, 1996, 1998). Schmidt (1990) reported 208 eggs per batch on *Pinus halepensis* in the regions of Kalogria and Delphi (Peloponnese, Greece).

The results of the investigations reveal a high domination of *B. servadeii* in the region of the Eastern Rhodopes, reaching about 90% of all egg parasitoids. In south-western Bulgaria (Tsankov & Mirchev, 1983; Tsankov *et al.*, 1996, 1998) *O. pityocampae* was the dominating parasitoid. In Italy, Tiberi (1990) found that *B. servadeii* and *O. pityocampae* are the most abundant parasitoids dominating in different regions. On the peninsula Kassandra in Greece, Bellin *et al.* (1990) reported a high relative abundance of *B. servadeii* (about 80%), whereas at Kalogria (Peloponnese), the percentages of these two parasitoids were almost equal. In Israel *B. servadeii* was the most infesting egg parasitoid of *Thaumetopoea wilkinsoni* Tams, followed by *O. pityocampae* (Kitt & Schmidt, 1993). Masutti (1964) considered the temperature as the environmental factor which limits the abundance of one of these parasitoids in a certain region. *B. servadeii* is a species of higher ecological plasticity, whilst temperatures over 30°C are not favourable for the development of *O. pityocampae*.

Regarding the parasitism of *B. servadeii*, 1251 individuals developed in April 1995 from 30 egg-batches, in April 1996 the number would be 1308 individuals for the same number of batches, this means that in spite of the reduction of the winter nests the relative effect of parasitoids remained more or less equal. BT seems to have no effect on the egg parasitoids of the processionary moth.

The high percentage of female *A. bifasciatus* is of most interest, because Haleperin (1990a) reported that eggs of the pine processionary moth produced females only in Cyprus.

In the Eastern Rhodopes, about 22% of the eggs of the pine processionary moth were parasitized by *B. servadeii*, and 59.4% being the maximum percentage of attacked eggs in one batch, but with high variation. For Israel, Halperin (1990b) found 13% parasitism by this species, the maximum being 37% in one batch.

A certain difference in the abundance of the hyper-parasitoid *B. transversalis* has been noticed. The egg batches collected at the end of the summer (23 August, 1995) were much less attacked than those collected in the spring (10 April, 1996), reaching a higher rate than in the year before. The difference between the autumn and spring collections due to the fact that *B. transversalis* has to wait for the development of the primary parasitoids reaching a suitable stage for hyper-parasitism, which may be present first in the late autumn. But the offspring of *B. transversalis* can emerge after some weeks to hyper-parasitize again in the same year decreasing the abundance of the primary parasitoid (Bellin, 1995). In the Eastern Rhodopes the influence of the hyper-parasitoid can be considered as low, less than 3% of the primary parasitoids were killed.

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Accepted 5 November 1998