Acta entomologyca serbica 1998, 3 (1/2): 127-137 UDC 632.79:632.78 (497.1)

A CONTRIBUTION TO THE STUDY OF GYPSY MOTH EGG PARASITOIDS *(LYMANTRIA DISPAR* L.) IN YUGOSLAVIA

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This paper presents the results of four-year investigation on gypsy moth egg parasitism with two species of *Hymenoptera* in Yugoslavia. *O. kuwanae* species is more significant than A. *disparis* in the regulation of gypsy moth population density. Vertical distribution percentage of parasite *O. kuwanae* is higher from 200 to 400 m than other vertical level. Also, the percentage of *O. kuwanae* is higher on the south side than on other exposures.

KEY WORDS: gypsy moth, egg parasitoid, *O. kuwanae*, *A. disparis*, distribution, exposure

INTRODUCTION

Gypsy moth (*Lymantria dispar* L.) is a very significant forest pest with a wide range of distribution. In Yugoslavia, it occurs in the continental and Mediterranean regions. Gypsy moth populations overpopulate acyclically when, in addition to forest trees, they also attack fruit trees, causing great damage. The main reasons and mechanisms of gypsy moth periodic overpopulation are still unknown despite detailed investigations. The results of numerous investigations indicate that many ecological factors (biotic and abiotic) affect the changes in gypsy moth population density.

The latest gypsy moth outbreak in Yugoslavia started in 1995, when about 10,000 ha of forests were attacked. In the following year (1996), gypsy moth occurred on about 80,000 ha, and during 1997, the year of outbreak culmination, it overpopulated on about 500,000 ha of forests, orchards and parks. During 1998, gypsy moth was recorded on about 400,000 ha and during 1999 its presence is expected on about 10,000 ha (MIHAJLOVIĆ, 1998). The measures of control applied against this

dangerous pest depended on population density and the stage of development at the time of suppression. In cases of weak (up to 10 clusters per ha) and medium attacks (11-100 clusters per ha) in egg stage, mechanical and chemical treatments were applied. Aerial spraying was applied in high (101-500 clusters per ha) and very high population densities (more than 500 clusters per ha), by treating the second and third larval instars by the biological preparation based on *Bacillus thurigienzis* var. *kurstaki*.

A significant role in the regulation of gypsy moth population density is played by entomophagous insects (predators and parasitoids), trophically linked with gypsy moth. The dominant ones are parasitoids - 105 species, i.e. 77 in the order Hymenoptera and 29 in the order Diptera (ZEROVA M. D. et al 1989). In the complex of parasitoids, the species that parasitize gypsy moth eggs are the fewest. In the former Yugoslavia until 1957, there was only one identified species *Anastatus disparis* Ruschka (Chalcidoidea, Eupelmidae), (VASIĆ, 1957), but already in 1958 another egg parasitoid species *Ooencyrtus kuwanae* How. (Chalcidoidea, Encyrtidae) (TADIĆ & BINČEV 1959; VASIĆ & SALATIĆ, 1959) was found. The literature also describes the species *Eremioscelio lymantriae* Masnil (Proctotrupoidea, Scelionidae) (MAKSIMOVIĆ,1997; RISTIĆ et al 1998) for the region of former Yugoslavia.

A. disparis is an autochthonous species, largely distributed in lowland and upland regions up to the altitude of 1600 m (Kopaonik). The efficiency of this wasp is up to 18.10% (BJEGOVIĆ, 1974), 33.0 % (KRNJAJIĆ, 1967), 70.0 % (KURIR, 1944). *O. kuwanae* How. (Encyrtidae) is an introduced species. BOUČEK (1977) claims that it occurs in Yugoslavia everywhere along with gypsy moth after BJEGOVIĆ (1972), i.e. that it is known in Croatia, Bosnia and Herzegovina and Macedonia. He also claims that it is an egg parasitoid specialized for gypsy moth. The species was originally described in Japan, wherefrom it was introduced to USA at the beginning of this century (CROSSMAN, 1925). From USA *O. kuwanae* was introduced to Spain, Maroco and Tunis. It was also introduced to Turkey and Yugoslavia, and it spread to Bulgaria most probably from Yugoslavia (BOUČEK, 1977). The range of this species, along with Japan and USA extends to Europe and North Africa, where it is the gypsy moth egg parasitoid, but it can also occur as a hyperparasite (TRJAPITZIN, 1989). According to TRJAPITZIN (1989), in addition to parasitizing gypsy moth eggs, this species was also identified as the parasitoid of *Lymantria fumida* Butl. in Japan, then *Eriogyna pyretorum* Westw. (Lepidoptera, Saturnidae) in Taiwan. During the introduction to USA, aiming at the biological control of gypsy moth, the hosts of this species, along with gypsy moth, were brown tail *Nygmia phaerrhoea* Don. and poplar gypsy moth *Stilpnotia salicis* L. According to the same author (TRJAPITZIN, 1989) during the introduction *O. kuwanae* to USA, together

with gypsy moth clusters the species *Tyndarichus navae* How. was also introduced (hyperparasite of gypsy moth, i.e. parasitoid *O. kuwanae*), although it could not acclimatize there. We did not record this species in our investigations, but it should be paid attention to in future, as it is a potential enemy of the species *O. kuwanae*. The effect of *O. kuwanae* on the population dynamics of gypsy moth is very significant (CROSSMAN, 1925), so it was studied in detail in our country (TADIĆ & BINČEV, 1959; VASIĆ & SALATIĆ, 1959; BJEGOVIĆ, 1962; KRNJAJIĆ, 1967; BJEGOVIĆ, 1972; BJEGOVIĆ, 1974). The spontaneous spreading was slow, so the wasps were released at various sites in Serbia and Montenegro (BJEGOVIĆ, 1974), to hasten the spreading. A later analysis of the gypsy moth clusters in different parts of the former Yugoslavia showed its presence at several localities in Serbia, but not in Montenegro, although *O. kuwanae* was released in the Mediterranean part of Montenegro, (Herceg Novi 1959) (BJEGOVIĆ, 1974). The efficiency of *O. kuwanae* ranges up to 27.68 % (TADIĆ & BINČEV, 1959), 29.60 % (BJEGOVIĆ, 1974), 33.60% (THOMAS et al., 1966).

MATERIAL AND METHODS

The presence of gypsy moth egg parasitoids and the percentage of parasitism were determined on gypsy moth clusters collected in the autumn in 1996 and 1997 at different localities in Serbia and in 1994 and 1995 in Montenegro. They were kept in the refrigerator at the temperature of 0 - 2 ^{o}C. Gypsy moth clusters were prepared for the analysis in the following way. The hairs were separated from eggs by rubbing gypsy moth eggs against the frame with stretched cotton and cotton-wool tampons. Cleaned eggs were examined under the binocular, to check the presence of *O. kuwanae* and *A. disparis*.

Vertical distribution and the effect of exposure on the parasitoid efficiency were analyzed only for the species *O. kuwanae*. The calculation of the percentage of parasitism for different altitudes and exposures was based only on parasitized clusters, in order to assess the effect of these two orographic factors on the distribution and efficiency of *O. kuwanae*. In the calculation of the of parasitism per localities, the clusters which were not parasitized were also taken into account. The frequency was calculated by the formula:

$$F = Ncp / Nct * 100$$

Ncp Number of parasitized clusters
Nca Number of non-parasitized clusters
Nct Total number of clusters in the study category
$Nct = Ncp + Nca$

RESULTS

The study clusters were collected at different localities in Serbia in 1997, except for Vrnjačka Banja and Priština, where the analyzed clusters were collected in 1996. Altogether 364 clusters were analyzed. *O. kuwanae* was present in 289, and *A disparis* was present in 25 clusters. Based on the study material, egg parasitoid *O. kuwanae* was present at all localities (19), and *A. disparis* was present only at 9 sites (Table I).

Table I
Analyses of gypsy moth eggs from different areas in Serbia

Area	Year	Nc.	Ne.	Pf	Ps	Po	Pa
Žagubica	1997	6	329.2	81.5	3.7	14.7	0.1
Štrpce	1997	10	310.8	81.2	8.8	8.4	1.6
Čačak	1997	4	569.0	93.7	3.1	3.2	
Boljevac	1997	16	450.8	85.6	4.9	8.5	1.0
Bor	1997	20	404.7	91.4	3.0	5.2	0.4
D. Milanovac	1997	15	414.5	89.2	2.1	8.7	
Jagodina	1997	5	290.2	70.9	4.0	25.0	
Knjaževac	1997	29	252.9	86.3	6.6	6.6	0.5
Kragujevac	1997	4	261.0	66.5	16.3	17.3	
Kruševac	1997	8	476.9	89.6	3.2	7.2	
Kučevo	1997	129	392.8	91.7	4.2	4.1	
Majdanpek	1997	8	340.1	77.4	6.2	16.4	
Negotin	1997	10	402.6	79.7	3.0	17.0	0.2
Požarevac	1997	19	360.6	88.2	4.7	7.1	
Priština	1996	20	468.3	94.0	5.3	0.6	
Smederevo	1997	10	374.1	86.2	5.1	8.6	0.1
S. Palanka	1997	3	572.3	91.4	2.8	5.8	
Vrnjačka Banja	1996	40	369.9	72.9	2.2	24.9	
Zaječar	1997	8	374.6	83.4	2.6	13.8	0.2
Total		364	383.0	86.7	4.3	8.7	0.2

Nc-number of clusters
Ne-number of eggs per cluster
Pf- % of fertile eggs
Ps- % of sterile eggs
Po- % of parasitized by *O. kuwanae*
Pa- % of parasitized by *A. disparis*

The average number of eggs per cluster ranged from 252.9 at Knjaževac to 572.3 at S. Palanka, and the average for the whole sample of 364 clusters was 383.0 eggs per cluster. The efficiency of egg parasitoid *O. kuwanae* ranged from 0.6 % at Priština to 25.0 % at Jagodina, and the average for the all study regions was 8.7 %.

The efficiency of *A. disparis* was far less and ranged from 0.1% at Žagubica to 1.6 % in the community Štrpce, with the average of 0.2 % for 9 localities, where the presence of this species was recorded. The presence of the species *O. kuwanae* in Serbia was recorded in 289 gypsy moth clusters. Vertical distribution (Tab. II) was calculated for only 204 egg clusters for which the altitude from which the eggs were collected was known.

Table II
Analyses of vertical distribution of gypsy moth egg parasitoid *O. kuwanae*

	Po	Po	Po			
level	average	min	max	Ncp	Nac	F
up to 200	13.7	0.3	58.7	19	3	86.4
from 200 to 400	17.2	0.6	66.5	74	9	89.2
from 400 to 600	9.2	0.5	55.9	75	27	73.5
from 600 to 800	4.5	0.2	20.2	30	16	65.2
above 800 m	10.6	1.1	22.4	6	1	85.7
no data	8.9	0.4	45.3	85	19	81.7
Total	11.0	0.2	66.5	289	75	79.4

Ncp-number of parasitized clusters
Nap-number of clusters without parasite
F- frequency
Po- % of parasitized by *O. kuwanae*
Pa- % of parasitized by A. disparis

The highest percentage of parasitism (17.2) occurred in the altitudinal belt from 200 to 400 m where the cluster with the highest percentage of parasitism (66.5) was also found. The lowest percentage of parasitism occurred in the belt from 600 to 800 m (4.5) where the cluster with the lowest individual maximum (22.4) was also found. The trend of decreasing parasitoid efficiency with higher altitudes is evident, with some anomalies in the belt below 200 m and in the belt above 800 m, which can be explained by a smaller number of analyzed clusters. The same trend also applies for the *O. kuwanae* frequency distribution (F).

The effect of exposure on the distribution and efficiency of parasitoids was studied on only 178 gypsy moth clusters (Tab III). The highest percentage of parasitism clusters was recorded at the south exposure (16.8), and the lowest (6.9) at the north exposures.

Table III
Analyses of gypsy moth egg parasitoid *O. kuwnae* distribution at different exposures

	Po	Po	Po	level		Ncp	Nca	F
Exp	average	min	max	min	max			
E	8.2	1.0	19.3	270	800	18	9	66.7
S	13.8	0.3	54.3	180	900	41	9	82.0
SE	16.8	0.5	55.9	50	800	34	5	87.2
SW	14.3	1.4	50.0	190	600	25		100
R	9.0	1.5	16.3	200	540	8		100
N	6.9	0.5	20.2	200	800	31	3	91.2
NE	7.5	1.2	22.4	200	850	10	3	76.9
NW	7.2	0.9	11.6	300	580	6	9	40.0
W	11.6	1.6	33.7	180	720	15	10	60.0
no data	9.6	0.2	66.5	180	600	101	27	78.9
Total	11.0	0.2	66.5	50	900	289	75	79.4

Exp- exposure
Ncp-number of parasitized clusters
Nap-number of clusters without parasite
F- frequency

The highest percentage of parasitism for individual cluster (55.9) was found at southeast, and the lowest at northwest exposure (11.6), where the lowest frequency of this parasitoid in gypsy moth clusters (40%) also occurred.

The study gypsy moth egg clusters in Monte Negro taken at four localities, Čanj, Utjeha, Kruče and Kruta in two years, were parasitized only by *O. kuwanae* How. (Tab. IV). Mean values of parasitism percentage in clusters at four localities in 1994 and 1995 differ. It can be seen that the values are higher in the clusters collected in 1995, which can be related to gypsy moth population dynamics and small clusters characteristic of the retrogradation phase. *O. kuwanae* How. was recorded for the first time at these localities after the wasps were released in the Mediterranean part of Montenegro in 1959 and 1964 by BJEGOVIĆ. These results show that the species spread in the Mediterranean part of Montenegro (Fig 1).

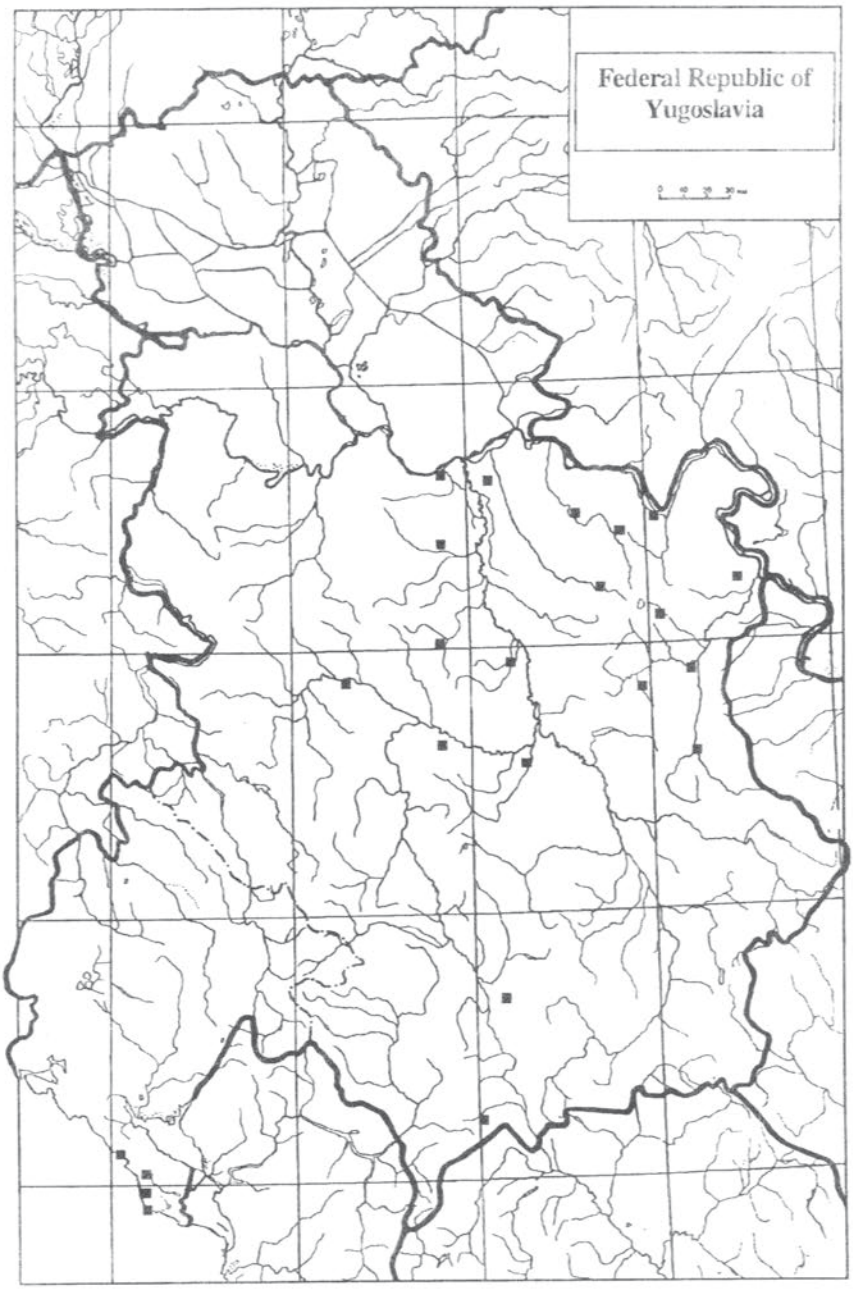

Figure 1 - Map of geographical distribution of egg parasitoid *O. kuwanae* based on our investigation

Table IV
Analyses of gypsy moth eggs from different localities of Montenegro during two years

Locality	1994.				1995			
	Nc	Ne	Po	Pa	Nc	Ne	Po	Pa
Čanj	10	202.0	0.00	0.00	6	325.5	5.97	0.00
Utjeha	3	331.0	4063	0.00	14	201.5	9.90	0.00
Kruče	2	220.0	5.45	0.00	7	332.7	11.18	0.00
Kruta	12	290.8	3.12	0.00	8	295.3	5.79	0.00

Nc-number of clusters
Ne-number of eggs per cluster
Po- % of parasitized by *O. kuwanae*
Pa- % of parasitized by *A. disparis*

DISCUSSION

The dominance of the egg parasitoid *O. kuwanae* over the autochthonous species *A. disparis* in Yugoslavia can be explained partly as the consequence of the applied control treatments which favor the introduced species. During 1996, in Serbia gypsy moth outbreak occurred over more than 80,000 hectares and in 1997 it occurred on more than 500,000 hectares of forests. Different control measures were undertaken. Gypsy moth clusters were destroyed on 43 % of the area in 1996 and in 1997 on about 34 % of the total attacked area (MAROVIĆ, 1997). Gypsy moth clusters were collected and destroyed by burning or soaking with petroleum. In these circumstances egg parasitoids were also destroyed. *A. disparis* suffered a greater loss, since it eclodes during July or August (Parker,1993; KURIR, 1944). *O. kuwanae* was subject to a smaller damage, since it eclodes predominantly in the autumn and its adults overwinter in different shelters in the forest. A significant fact is also that *A. disparis* parasitizes only freshly laid gypsy moth eggs (KOVAČEVIĆ, 1947; SALATIĆ, 1966), while *O. kuwanae* parasitizes gypsy moth eggs, both fresh and several months after oviposition. *O. kuwanae* also develops as a hyperparasite of *A. disparis* pupae and larvae if they are not in diapause, when it often lays two eggs into one gypsy moth egg (BJEGOVIĆ, 1963). *O. kuwanae* has up to seven generations per year (CROSSMAN, 1925), which is also favorable.

The dominance of the species *O. kuwanae* is also reflected in the frequency of occurrence in the study area. This species was found in egg clusters at all 19 sites in Serbia, whereas the species *A. disparis* was found in egg clusters in 9 regions. The results of the research in the Republic Slovakia are different The species *A. disparis* was present at 7 study sites and the species *O. kuwanae* occurred at 4 out of 10 localities (ZUBRIK & NOVOTNY, 1996). According to the same authors, the share

of the species *A. disparis* in the total percentage of parasitism is 79.5 % and *O. kuwanae* - 20.5%, which is the consequence of the effect of abiotic factors. Based on our investigations, the share of *A. disparis* in the total percentage of parasitism is only 2.3 %, and that of the species *O. kuwanae* is 97.7 %.

Previous study of *O. kuwanae* distribution after its introduction to the regions of Zaječar and Negotin shows that the share of the species *A. disparis* in 1973 in the total percentage of parasitism in the region of Zaječar was 18.1 %, and *O. kuwanae* 8.5 % respectively of the total percentage of parasitism amounting to 26.7 %. In the region of Negotin, the share of the species *A. disparis* was 1.73 %, and *O. kuwanae* 13.41 % respectively. The total percentage of parasitism was 15.14 % (BJEGOVIĆ, 1974).

Our analyses of egg clusters in 1997 show that the share of the species *A. disparis* in the region of Zaječar was 0.2 %, and *O. kuwanae* 13.8 % of the total percentage of parasitism which was 14.0 %. The situation was similar in the region of Negotin. *A. disparis* parasitizes 0.2 %, and *O. kuwanae* 17.0 % of gypsy moth eggs.

The analysis of clusters from anj, Utjeha, Kruče and Kruta (Montenegro) shows only the presence of egg parasitoid *O. kuwanae*. These are the first data that show that this species extended its distribution in Yugoslavia, so that it is present both in continental and Mediterranean climatic conditions (Fig. 1).

Gypsy moth egg-mass survey at four localities in the Mediterranean part of Montenegro in two successive years 1994 and 1995 showed a significant percentage of parasitized clusters by the species *O. kuwanae*.

CONCLUSION

Based on the analysis of gypsy moth clusters collected in 1994, 1995, 1996 and 1997 at several localities in Yugoslavia, as well as based on Yugoslav literature, we can conclude that:

In Yugoslavia, there are two species of gypsy moth egg parasitoids: *A. disparis* and *O. kuwanae*. The species *O. kuwanae* parasitized gypsy moth clusters to a higher percentage (97.7%) than the species A. disparis (2.3%), so its role in the regulation of gypsy moth population density in Yugoslavia is greater and, according to literature data, it is still greater.

O. kuwanae is a species that extended its range of distribution from continental also to Mediterranean region in Yugoslavia.

Vertical distribution of *O. kuwanae* shows that gypsy moth clusters are parasitized to the highest percentage in the altitudinal belt between 200 and 400 m (66.5).

The percentage of parasitism is also affected by the exposure. The highest percentage of parasitism was recorded at south exposures (55.9).

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ПРИЛОГ ПОЗНАВАЊУ ЈАЈНИХ ПАРАЗИТОИДА ГУБАРА (*LYMANTRIA DISPAR* L.) У ЈУГОСЛАВИЈИ

С.МИЛАНОВИЋ, С. КРЊАЈИЋ И Љ.МИХАЈЛОВИЋ

Извод

У раду су приказани резултати вишегодишњих истраживања јаних паразитоида губара на простору Југославије.

Утврђене су две врсте јајних паразитоида губара и то и *Anastatus disparis* Ruschka и *Ooencyrtus kuwanae* How. где је друга врста имала значајнију улогу у редукцији бројности популације губара.

У раду се износе расположиви подаци о распрострањености и релативној бројности за обе врсте, као и подаци о вертикалној дистрибуцији и дистрибуцији по различитим експозицијама за врсту *O. kuwanae* How.

Received January 20, 1999
Accepted March 10, 1999