

GYPSY MOTH OUTBREAKS IN FOREST COMPLEXES OF JABLANICA REGION (SOUTHERN SERBIA) IN THE PERIOD 1996–2014

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Abstract

In the area of Jablanica Region (Public Enterprise Srbijasume, Forest Estate Leskovac), after a thirty-year period of latency, significant increase of gypsy moth population level occurred three times (1996–1997, 2002–2005 and 2011–2014). In the culmination phases, attacked areas were: 22,425 ha (1996), 38,856 ha (2004) and 18,272 ha (2013). The retrogradation phases occurred in the autumn of 1998, 2005 and 2014, so it was the result of effective aerial control in the larval instar with microbiological (Foray 48B) and biotechnical (Dimilin SC-48) insecticides as well as of the increased activity of natural enemies. The analysis of gypsy moth egg masses was made on a yearly basis. The average number of eggs ranged from 297.7 (1997; 320 investigated egg masses) to 507.2 (2013; 580 investigated egg masses). The percentage of vital eggs ranged from 89.5 in 2013 to 97.2 in 2012. Average parasitism of eggs ranged from 2.5 % in 2012 to 25.4 % in 2004. Every year only two species of egg parasites *Anastatus japonicus* Ashmead and *Oencyrtus kuwanae* (Howard) were present. Every year the ratio of them was relatively equal with the clear dominance of *O. kuwanae*. In the spring of 2012, 2013 and 2014, the increased mortality rate of larvae was reported and analysed. During the field research clear symptoms of disease caused by *Lymantria dispar* multicapsid nuclear polyhedrosis virus (LdMNPV) and typical symptoms of the fungal diseases caused by *Entomophaga maimaiga* Humber, Shimazu and R. S. Soper were found on dead gypsy moth caterpillars. By microscopic analysis of dead caterpillars, the presence of LdMNPV occlusion bodies, conidiospores and azigospores of *E. maimaiga* was confirmed.

Key words: control, egg masses analysis, *Lymantria dispar*, natural enemies.

Introduction

Gypsy moth (*Lymantria dispar* Linnaeus, 1758), insect from Lepidoptera order (Fam. Erebidae), is one of the major pests of broadleaf forests and orchards. It is characterised by high reproductive capacity, a considerable ecological plasticity and polyphagy. Although present in four continents (North Africa, Asia, Europe, North America), the most damage has been inflicted in

the forests of Balkan Peninsula (Bulgaria, FYR Macedonia, Serbia, Bosnia and Herzegovina, Croatia), Eastern (Romania) and Central Europe (Hungary, Slovakia, Czech Republic, etc), where all environmental conditions for its development are favourable (Tabakovic-Tosic 2004).

Damage caused by gypsy moth is two-fold: both direct – defoliation or a loss of leaf mass caused by feeding of caterpillars, and indirect, manifested through the ef-

fects of defoliation. Defoliations caused by feeding of caterpillars lead to reduction of increment, lack of acorn crop, physiological weakening and drying of trees, as well as the creation of favourable conditions for attacks of phytopathogenic microorganisms, fungi and xylophagous insects, disruption of spatial aesthetics and other.

The gypsy moth is characterized by cyclical abundance of fluctuations resulting in defoliation of large forest areas (Villemant and Fraval 1998, McManus and Csóka 2007, Tabakovic-Tosic 2013). The occurrence of an outbreak, on one hand, depends on a gypsy moth physiological constitution, and on the other, on impact of external factors – type and quality of food, geographical gradients, meteorological conditions and biotic factors (diseases, natural enemies – mainly *Entomophaga maimaiga* Humber, Shimazu, and R. S. Soper, and competitor species) (Csóka 1997; Klapwijk et al 2013; Marovic et al. 1998; McNamara 1996; Mihajlovic et al. 1998; Tabakovic-Tosic 2012, 2013, 2014a, 2014b; Vanhanen et al 2007; Weiser 1987). Studies on gypsy moth outbreak periodicity conducted in Europe, North America, and Asia suggested oscillation cycles between 4 and 12 years (Hlásny et al. 2015; Johnson et al. 2005, 2006; Marovic et al. 1998; Tabakovic-Tosic and Jovanovic 2007; Weiser 1987).

Material and Method

Jablanica region (administrative districts: Leskovac, Predejane, Vlasotince, Vucje, Lebane, Bojnik, Medvedja) is located in the southeastern part of Serbia and covers an area of 2,769 km², with forest complexes spreading over 113,831 hectares, out of which 40,566 ha or 35.6 % is in state, and 73,265 or 64.4 % in private ownership (timber volume amounts to 6,898,554 m³). This

area is a natural habitat for deciduous tree species (*Quercus cerris* L., *Quercus petraea* Liebl., *Quercus frainetto* Ten., *Fagus sylvatica* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Fraxinus ornus* L. and other).

Gypsy moth population control measures were ordered in all deciduous forests every year, regardless of their ownership category (state or private), and in accordance with the Instructions supplied by the officials responsible for Report-diagnostic forecast in the domain of plant protection – forest protection. Gypsy moth population survey in forests was carried out by means of a permanent (25×25 m) and a temporary (10×10 m) sample plot method, as well as a transect method. Permanent sample plots were surveyed every year, whereas temporary sample plots were surveyed only when population increase seemed apparent. The transect method and pheromone trap method were applied as additional measures during gypsy moth latency (low population density) period, whereas they were invariably used during the outbreak period.

A detailed quantitative and qualitative analysis of sampled egg masses was conducted at the laboratory of the Institute of Forestry and, depending on the parameter analysed, the visual method or the method of binocular magnifying glass examination was applied. Additionally, the dynamics of emerging gypsy moth egg parasitoid imagoes from previously analysed masses was monitored in the laboratory conditions in winter period.

100 randomly sampled, previously cleaned eggs from each egg mass were placed in specially prepared test tubes – with distilled water at the bottom and a cotton wool layer in the middle, intended to prevent a total immersion of eggs. The test tubes with sampled gypsy moth eggs were held in a climate chamber. During the experiment, the air temperature

and the light regime were constant (the temperature 19 °C, the light regime – 10 hours night, 14 hours day). The recording of emerging parasitoids was conducted daily until it ceased.

A survey of main predators, parasitoids and pathogens was conducted from May to late November by using the method of hunting, typical for some families to which the insects belong (different kinds of traps, manual method, and method of mowing by using the entomological net).

For different laboratory analyses, from all sites, in the summer and autumn of the period 2010–2014, dead gypsy moth larvae were collected manually from foliage in the lower parts of tree crowns and tree branches and trunks, two to three times per year.

Microscopic (magnification 1200 times) analyses of some dead gypsy moth larvae with typical symptoms caused by *L. dispar* multicapsid nuclear polyhedrosis virus (*LdMNPV*) was conducted immediately using the standard method of Giemsa's differential staining.

The dead larvae with typical symptoms caused by the entomopathogenic fungus *E. maimaiga* were also placed in Petri dishes and detailed microscope survey of the cadavers was done later. The evaluation of *E. maimaiga* infections was recorded as positive when azygospores and conidiospores were detected in the cadavers of dead gypsy moth larvae. The species identification was based on the size, shape and structural characteristics of different life forms of the fungus – azygospores, conidiospores and mycelia.

Results and Discussion

In the course of those investigations, during the process of establishing the num-

ber of gypsy moth egg masses per surface unit (1 hectare), particular attention was paid on inclusion of all forests complexes. This aspect was of major importance for the areas in which occurrence of a particularly intense attack was observed (over 500 egg masses per hectare), as the amount of damage manifested through leaf mass injury, to be caused by hatched larvae, is not the same under the attack of 501 and, for instance, 50,000 egg masses per hectare.

As can be seen from the data in Table 1, in the area of Jablanica Region (Public Enterprise Srbijasume, Forest Estate Leskovac), after the thirty-year period of latency, the significant growth of gypsy moth population level occurred three times (1996–1997, 2002–2005 and 2011–2014). In the culmination phases, attacked areas were: 22,425 ha (1996), 38,856 ha (2004) and 18,272 ha (2013). The retrogradation phases occurred in the autumn of 1998, 2005 and 2014, so it was a result of effective aerial control in the larval instar by microbiological (Foray 48B in 1998 and 2014) and biotechnical (Dimilin SC-48 in 2005) insecticides as well as of the increased activity of *E. maimaiga* (primarily), *LdMNPV* and other gypsy moth natural enemies (parasites and predators).

Mechanical and chemical suppression measures undertaken in the egg stadium, did not produce satisfactory results. In the summer period of 2004, air-suppression in the larval instar was conducted by means of a biological preparation D-stop (active ingredient: spores and crystals *Bacillus thuringiensis* ssp. *kurstaki* Berliner, 1915), which also proved ineffective. A partial or total defoliation occurred in the entire area. On the account of an enormous number of voracious larvae and lack of food, a large number of caterpillars died,

Table 1. Outspread of gypsy moth in the forests of Jablanica region in the period 1996–2014.

| Year | Attack intensity and attacked area | | | | | | | | Total, ha |
|---------------------------------------|--------------------------------------|-------|--|-------|---|-------|--|-------|--------------|
| | Low intensity 1–10* egg masses | | Medium intensity 11–100 egg masses | | High intensity 101–500 egg masses | | Severe intensity over 500 egg masses | | |
| | ha | % | ha | % | ha | % | ha | % | |
| 1996 | 1,917.00 | 8.55 | 4,190.00 | 18.68 | 4,886.00 | 21.79 | 11,432.00 | 50.98 | 22,425.00 |
| 1997 | 5,327.00 | 27.27 | 3,903.00 | 19.98 | 10,016.00 | 51.28 | 287.00 | 1.47 | 19,533.00 |
| 1998–2002 – gypsy moth latency period | | | | | | | | | |
| 2002 | 163.00 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 163.00 |
| 2003 | 6,453.00 | 42.35 | 7,782.00 | 51.07 | 25.00 | 0.16 | 979.00 | 6.42 | 15,239.00 |
| 2004 | 2,373.00 | 6.11 | 6,419.00 | 16.52 | 3,920.00 | 10.09 | 26,144.00 | 67.28 | 38,856.00 |
| 2005–2010 – gypsy moth latency period | | | | | | | | | |
| 2011 | 100.14 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100.14 |
| 2012 | 2,063.37 | 59.21 | 1,295.82 | 37.20 | 125.00 | 3.59 | 0 | 0 | 3,484.19 |
| 2013 | 1,434.60 | 7.85 | 5,686.51 | 31.12 | 1,398.16 | 7.65 | 9,752.70 | 53.38 | 18,271.97 |
| 2014 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Note: * Number of oviposited egg masses per one hectare at the end of summer period.

but nevertheless, a sufficient number of them remained, continuing their development. In autumn of 2004, newly-laid gypsy moth egg masses were found in the area of 38,856 ha. Following the partial elimination of egg masses, performed in the autumn-winter period in spring of 2005, a repeated air-suppression was conducted, but this time a third generation chemical insecticide Dimilin SC 48 (active ingredient diflubenzurone) was selected.

The analysis of gypsy moth egg masses, collected in Jablanica region (Table 2), was conducted every year during the investigation period. The average number of eggs in an egg mass ranged from 297.7 (1997; 320 investigated egg masses) to 507.2 (2013; 580 investigated egg masses). The percentage share of vital eggs in

the total number of eggs ranged, on average, was from 89.5 % in 2013 to 97.2 % in 2012. The average egg parasitization rate ranged from 2.5 % in 2012 to 25.4 % in 2004. The above-stated parasitization values should not be considered final, as they concern laboratory conditions, which prevent the activity of a number of parasites and predators, to which egg masses are exposed in nature.

The results of analysed quantitative and qualitative parameters of gypsy moth egg masses confirm the above-stated assertion that the increase of gypsy moth population density above normal values in the forest area of Jablanica region in the period 1996–2014 occurred three times.

The dynamics of emerging parasite imagoes from the previously analysed egg

Table 2. Laboratory analysis of gypsy moth egg masses.

| Year | Number of egg masses | Average number of eggs in an egg mass | | | | | | Total Number |
|-------------------------------------|----------------------|---------------------------------------|------|-------------|------|--------------|-----|--------------|
| | | Fertilised | | | | Unfertilised | | |
| | | Vital | | Parasitised | | | | |
| | | Number | % | Number | % | Number | % | |
| 1996 | 370 | 291.3 | 90.0 | 29.8 | 9.2 | 2.6 | 0.8 | 323.7 |
| 1997 | 320 | 278.5 | 93.5 | 15.7 | 5.3 | 3.5 | 1.2 | 297.7 |
| 1997–2002 gypsy moth latency period | | | | | | | | |
| 2003 | 239 | 417.8 | 93.6 | 25.5 | 5.9 | 2.0 | 0.5 | 445.3 |
| 2004 | 154 | 406.4 | 92.3 | 31. | 25.4 | 2.7 | 0.6 | 440.3 |
| 2005–2010 gypsy moth latency period | | | | | | | | |
| 2011 | 100 | 393.4 | 90.5 | 36.4 | 8.4 | 4.7 | 1.1 | 434.5 |
| 2012 | 53 | 439.3 | 97.2 | 11.4 | 2.5 | 1.1 | 0.3 | 451.8 |
| 2013 | 580 | 456.2 | 89.5 | 47.0 | 9.7 | 4.1 | 0.8 | 507.2 |

masses was monitored in special trials. Only two egg parasites species *Anastatus japonicus* Ashmead (syn. *A. disparis* Ruschka) and *Oencyrtus kuwanae* (Howard) were present every year. Their ratio was relatively consistent in every year – 33 % : 67 % (± 2 %), with clear domination of *O. kuwanae*.

During the observed period, at the sample plots, gypsy moth eggs were attacked by seven predator species [*Trombidium holosericeum* (Linnaeus, 1758); *Forficula auricularia* Linnaeus, 1758; *Carabus latus* Dejean, 1826; *Dermestes erichsoni* Ganglbauer, 1904; *Julistus floralis* (Olivier, 1790); *Malachus bipustulatus* (Linnaeus, 1758); *Formica* sp.], larvae by six [*Silpha quadripunctata* Schreber, 1759; *Carabus coriaceus* (Linnaeus, 1758); *C. cancellatus* (Linnaeus, 1758); *C. cavernosus* Frivaldsky, 1837; *C. intricatus* (Linnaeus, 1758); *C. scabriusculus bulgarus* Lapouge, 1908] and larvae and pupae by two [*Calosoma sycophanta* (Linnaeus, 1758); *C. inquisitor* (Linnaeus, 1758)]. Regarding the density of some predator species, *Trombidium holosericeum*, *Forficula auricularia*, *Silpha quadripunctata*, *Calosoma sycophanta* and *Carabus*

sp. were most abundant ones. *Calosoma sycophanta*, which regularly occurs during gypsy moth outbreak, was found more frequently than other predator species, and it reduced its population size both in the larval and imago instars.

There were two parasitic species of gypsy moth eggs [*Anastatus japonicus* Ashmead, 1904; *Oencyrtus kuwanae* (Howard, 1910)]; fourteen parasitic species of gypsy moth larvae [*Phobocampe disparis* (Viereck, 1911); *P. pulchella* (Thomson, 1887); *Apanteles glomeratus* (Linnaeus, 1758); *Cotesia melanoscela* (Ratzeburg, 1844); *C. ocnariae* (Ivanov, 1898); *C. scabricula* (Reinhard, 1880); *Protapanteles liparidis* (Bouček, 1834); *P. porthetrie* Muesebeck, 1954; *P. fulvipes* (Haliday, 1834); *Meteorus versicolor* (Wesmael, 1835); *Exorista larvarum* (Linnaeus, 1758); *Parasetigena silvestris* (Robineau-Desvoidy, 1863); *Compsilura concinnata* (Meigen, 1824); *Blepharipa pratensis* (Meigen, 1824)], and four parasitic species of the gypsy moth pupae [*P. inquisitor* (Scopoli, 1763); *P. turionellae* (Linnaeus, 1758); *Lymantrichneumon disparis* (Poda, 1761); *Brachimeria intermedia* (Nees, 1834)]. The cocoons of para-

sitic species from the families Braconidae and Tachinidae were regularly found in spring. Other species were considerably less frequent and were found individually.

During the field research in the spring of 2012, 2013 and 2014, at sample plots in the oak and beech forests, the increased mortality rate of younger and older larval instars in comparison with the expected one was reported and clear symptoms of disease caused by *LdMNPV* and characteristic symptoms of fungal diseases caused by *E. maimaiga* were found on dead gypsy moth caterpillars.

By detailed microscopic analysis, the presence of *LdMNPV* occlusion bodies, conidiospores and azigosporos of entomopathogenic fungus *E. maimaiga* was confirmed. *LdMNPV* occlusion bodies were observed in 7.1 (2012) and 16.3 (2013) percent of the dead older caterpillars (L_4 – L_6). Conidiospores and azigosporos of *E. maimaiga* were at 90.5 (2012), 80.1 (2013) and 90.9 (2014) percent of the reported dead gypsy moth larvae. In the early spring of 2014, the largest number of gypsy moth larvae died in the second instar.

Bulgaria has been the second in the world and the first in Europe country in which *E. maimaiga* was introduced successfully (Pilarska et al. 2006). For a period of 10–12 years it expanded its range (naturally and by introductions) and is now found throughout Bulgaria (Mirchev et al. 2013). This entomopathogenic species has slowly spread along the Balkan Peninsula and Southeast Europe, and so far its presence has been reported also in European part of Turkey (Georgiev et al. 2012), Serbia (Tabakovic-Tosic et al. 2012, 2013), Greece, FYR Macedonia (Georgieva et al. 2013), Croatia (Hrašovec et al. 2013), Hungary (Csóka et al. 2014), Slovakia (Zúbrik et al. 2014), Bosnia and Herzegovina (Milotić et al. 2015). Regard-

ing the situation in Serbia, *E. maimaiga* was introduced or found in many places of the country (Tabaković-Tošić 2014a, 2014b). In the forests area of Jablanica region this entomopathogen was spread naturally from Bulgaria.

Conclusions

In the forest area of Jablanica region (Southern Serbia) in the period 1996–2014, the increase of gypsy moth population density above the normal level occurred three times. Natural enemies of gypsy moth, as well as the control measures undertaken in egg and larval stadium, although adequately prepared and timely applied, did not always produce satisfactory results (first and second outbreak).

In 1998, 2005 and 2014, regressive phases occurred as a result of a successful gypsy moth suppressions in the larval stadium, as well as the increased activities of gypsy moth natural enemies – predators, parasites and pathogens. Disease caused by pathogens – *LdMNPV* and fungus *E. maimaiga* especially, the most contributed to the decline of gypsy moth in broadleaf forests of Jablanica region.

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