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Gypsy Moth in Central Serbia Over the Previous Fifty Years

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Abstract: The gypsy moth, *Lymantria dispar* L., after an unusually long latency period (30 years), has demonstrated all the characteristics of an outbreak in the forests of central Serbia three times since 1994. During the first outbreak (1994-1998), 24.8% of the broadleaf forests were endangered by the gypsy moth, during the second one (2002-2005), 18.8% of the broadleaf forests were endangered, and during the third one, which still ongoing, 2% of the broadleaf forests were endangered. The average number of eggs in an egg mass ranged from 191.7 to 654.1. The percentage of vital and parasitized eggs in the total number of eggs ranged on average from 79.2 to 98.7 and from 0.5 to 20.0 respectively. During the observed period, in the gypsy moth populations, the activity of 56 natural enemies of this insect – 19 predators, 28 parasites, 7 parasites or saprophages and 2 pathogens – was reported. A higher mortality rate of the older gypsy moth larval instars was reported in the forest complexes of Belgrade and Valjevo region, in the culmination phase of the new outbreak. By field and laboratory studies of the causes of their death, the presence of conidia and resting spores of the entomopathogenic fungus *Entomophaga maimaiga* was reported in dead caterpillars. During every year of an outbreak, the gypsy moth was suppressed in the egg stage, and in the larval stage during the culmination phases.

Key words: Central Serbia, *Lymantria dispar*, outbreaks, natural enemies, control

Introduction

The economically harmful insect species *Lymantria dispar* L. (Lepidoptera: Lymantriidae) is one of the major pests of broadleaf forests in Central Serbia. Its population size is occasionally great. *O*utbreaks of gypsy moths occurred 17 times over a 150-year-period (Tabakovic-Tosic and Jovanovic 2007), and the 18th outbreak started in 2009. It used to be believed that the outbreaks of the gypsy moth occured every eight to ten years (Mihajlović *et al.* 1998), but the events which have occurred over the previous 50 years show that this view might not be correct. Over the past eighteen years, after a thirty year long latency period, the gypsy moth has had 3 outbreaks of the acute type, i.e. 1995–1999, 2003-2006 and 2009-?. During the outbreak in Central Serbia, the gypsy moth frequently spread in the broadleaf forests which cover an area of several hundred thousand hectares (Nevenic *et al.* 2009, Tabakovic-Tosic and Jovanovic 2007).

The damage caused by the gypsy moth is twofold: direct – defoliation or the loss of leaf mass, and indirect, expressed in the consequences. Defoliation caused by caterpillar feeding leads to the loss of increment, absence of fructification, physiological wakening and tree dying, as well as the creation of favourable conditions for the infestation of phytopathogenic microorganisms, fungi and xylophagous insects, disturbance of the aesthetical appearance, etc. Numerous literature sources show that during defoliation, depending on the tree species and forest type, volume increment decreases by 30-70% (Mirkovic and Miscevic 1960), and during a partial defoliation, by 20-25%.

The integral protection of the forest implies the continuous application of protective measures in the aim of the undisturbed growth and increment of trees, as well as the creation of wood volume of the best possible quality, which in turn, implies the inclusive and maximum protection from the harmful effects of various abiotic and biotic factors (Tabakovic-Tosic 2006).

For control of the gypsy moth in Serbian forests, expensive bacterial and chemical insecticides, which not only affect the target species, but other representatives of entomofauna as well, were frequently used. The necessity for the reduction of the adverse effects of insectides and the preservation of biological diversity in the natural ecosystems, has imposed the need for the study and use of new types of peculiar biological agents and methods for the control of this and other species of pests (Tabakovic-Tosic 2006, 2008, Tabakovic-Tosic *et al.* 2011).

Recent emphasis on the development of an integrated control program for the gypsy moth has necessitated an understanding of its mortality-causing biological agents. Throughout the Central Serbian region there is a wide range of natural enemies of this insect. Parasitic and predatory insects, many species of spiders, several species of birds and common woodland mammals play an important role during periods when gypsy moth populations are sparse. Diseases caused by viruses, bacteria or fungi also contribute to the decline of gypsy moth populations. This paper presents the results of the author's survey of the last three outbreaks (twenty year period) of the gypsy moth and its natural enemies in the forests of Central Serbia.

Materials and Methods

Central Serbia covers an area of about 55,000 square kilometers. It is located between 42 and 45 degrees northern latitude and between 19 and 23 degrees eastern longitude. It is a very complex and heterogenous area. In regards to orography, it is heterogenous and made up of plains (100-200 m a.s.l.), gentle hillsides (200-500 m a.s.l.), low mountains (500-1,200 m a.s.l.), high mountains (1,200-2,500 m a.s.l.), and numerous internal basins and river valleys. It is divided into low and high parts (located in the north and south, respectively). There are several types of temperate continental climate. The mean annual air temperatures range from 8 to 12 °C. The mean annual precipitation ranges from 600 millimeters (in the low parts) to more than 1,500 millimeters (in the mountains) (Ducic and Radovanovic 2005).

In Central Serbia, the forests cover an area of 2,098,400 hectares (37.6% of the total area), 87.7% (1,842,400 hectares) of which are pure and mixed broadleaf stands. Timber volume amounts to 333 million m^3 (59.2% in state-owned forests and 40.8% in privately-owned forests), and the volume increment is 3.9 m^3/ ha (56.6% in state-owned and 43.4% in privately-owned forests); broadleaves account for 86.9% of the total timber volume (beech 43.9%, oaks 26.3%, other broadleaves 29.8%) (Bankovic *et al.* 2009).

For many decades, in all broadleaf forests, the population size of the gypsy moth has been controlled by using the permanent and temporary sample plots, march-route method and pheromone traps (Tabakovic-Tosic 2004, 2005). The following scale was applied for the determination of the intensity of attack: 10 egg masses/hectare – weak, 11-100 – average, 101-500 – strong and over 500 – very strong.

The detailed quantitative and qualitative surveys of the sampled egg masses were conducted in the laboratory of the Institute of Forestry, and depending on the analyzed parameter, either the ocular method or the method implying the use of the binocular magnifier was applied.

In addition, under laboratory conditions, the dynamics of the hatching of the imago parasitoids of the gypsy moth eggs from the previously studied egg masses was observed over the winter. A total of 100 randomly sampled, previously cleaned eggs, from each egg mass, were placed in the specially prepared test tubes. During the experiment, temperature and light conditions were constant (temperature 19°C, light regime – 10 hours a night, 14 hours a day). Hatching was reported every day until the end of the process.

A survey of the main predators, parasitoids and pathogens was conducted from May to late November in Central Serbia during studies of the population dynamics and outbreaks of the gypsy moth. The studies for the presence and density of the main predator species of the gypsy moth were conducted by using the method of hunting, typical for some families to which the insects belong (different kinds of traps, manual method, method of mowing by using an entomological net).

The general technique applied for the study of the parasitisation rate of the gypsy moth in larval and pupae instars consisted of weekly collections of up to 100 larvae or pupae per site from all sites in each area. The larvae were collected once a week from April to June. The field-collected larvae were grown under laboratory conditions in the climate chamber. During the experiment, temperature and light conditions were constant (temperature 21 °C, light regime – 8 hours night, 16 hours a day). The larvae were fed on a daily basis with fresh leaves of the main type of the host plant, brought from the sample plots (oak or beech). The field-collected pupae were transferred to 500 g plastic containers (maximum of 10 per container) and held at room temperature.

Larvae and pupae were examined twice a week, and upon indication of being parasitized were removed from the containers and placed in Petri dishes. The immature and adult stages of the parasites were identified at a later stage.

The studies for the presence of entomopathogenic viruses, bacteria and fungi in the dead gypsy moth larvae were conducted in the field and in laboratory conditions. In field conditions the characteristic symptoms of some diseases were identified by using the ocular method, while in laboratory conditions, they were identified by dissection of the dead larvae and using the microscope survey.

Results and Discussion

The outbreak of the gypsy moth is the result of its physiological constitution, the external factors – type and quality of food, weather conditions and biotic factors (diseases, natural enemies and rival species), and the preventive and control activities of humans. During the latency period in Central Serbia, the gypsy moth foci are frequently found in the forest biocoenosis. However, the pest occassionally (when one or several environmental factors change) reacts by outbreaking.

In central Serbia, after three subsequent outbreaks, which occurred over the period 1947-1966, the latency stage of the gypsy moth lasted for three decades. Over the periods 1970-1973 and 1984-1987 (Marovic *et al.* 1998), the population size increased, but not considerably. Also, it occurred in a relatively small area, and no harmful results, such as the defoliation of the host plants, were reported, i.e. it did not have the characteristics of an outbreak. Although there are no concrete literature data on the measures taken in order to suppress this outbreak, it is assumed that they were very effective.

After the period mentioned above, characterized by the relatively low population size, the gypsy moth demonstrated all the features of an outbreaking

Table 1. Outspread of gypsy moth in the forests of Central Serbia in the period 1994-2011 (egg masses oviposited in late summer**).**

Year	**Attack intensity and attacked area**								
	Low		**Medium**		**High**		**Severe**		**Total**
	ha	%	ha	%	ha	%	ha	%	
1994	8.997	90.0	823	8.2	180	1.8	0	0	10.000
1995	12.081	15.1	30.655	38.3	13.105	16.4	24.159	30.2	80.000
1996	111.292	24.4	81.900	18.1	98.322	21.6	163.573	35.9	455.087
1997	134.605	29.4	105.604	23.1	68.124	14.9	149.386	32.6	457.719
1998	10.000	100	0	0	0	0	0	0	10.000
1999-2001 gypsy moth latency period									
2002	2.700	88.7	344	11.3	0	0	0	0	3.044
2003	69.655	48.9	39.538	27.7	19.896	14.0	13.454	9.4	142.543
2004	72.519	20.9	58.819	17.0	64.668	18.6	150.973	43.5	346.980
2005	18.120	29.0	7.937	12.7	10.143	16.3	26.195	42.0	62.395
2006-2008 gypsy moth latency period									
2009	1.287	28.2	2.024	44.4	865	19.0	382	8.4	4.558
2010	5.548	59.9	2.793	30.2	895	9.7	21	0.2	9.257
2011	25.033	67.5	10.497	28.3	1.436	3.9	99	0.3	37.065

and economically harmful species in the forests of Central Serbia three times during the subsequent 18 years (Table 1).

The forests of Central Serbia, where the gypsy moth egg masses were reported, covered an area of 457,719 hectares over the period 1994-1998, and an area of 346,980 hectares over the period 2002-2005. An area of 37,065 hectares is subject to the infection during the latest outbreak, which is still ongoing (Table 1).

When these data are compared with the total area of the broadleaf forests of Central Serbia (1,842,400 hectares), the following results are obtained: during the first outbreak, 24.8% of the broadleaf forests were endangered, during the second one 18.8%, and during the third one, i.e. the infection that is yet to spread, 2%. The outbreaks also varied in the spatial distribution. Some regions, such as Pirotski, Knjazevacki, Negotinski, Zajecarski, Borski were subject to the harmful influence of the gypsy moth only during the first outbreak, while others, such as the broadleaf forests in Belgrade, Valjevski, Loznicki and Kladovski regions, sustained damage during all three outbreaks (Fig. 1).

During the culmination phases of the outbreak, the greatest areas were subject to very high infection rates (1996 – 35.9%, 2004 – 43.5%, 2012 – ?), when tens of thousands of gypsy moth egg masses were laid on the unit of area (Table 1).

The analysis of gypsy moth egg masses, collected in the area of the Central Serbia, had been conducted at the laboratory of the Institute of Forestry every year during the outbreaks. The average number of eggs in an egg mass ranged from 191.7 (2005) to 654.1 (2003). The egg mass with the largest number of eggs (1449) was submitted to the Institute in 2003 from the Belgrade region. The percentage share of vital eggs in the total number of eggs ranged, on average, from 79.2% in 2004 to 98.7% in 1995 (Table 2).

Average parasitism of eggs in egg masses at the study localities ranged from 0.5% in 1995 to 20.0% in 2004 (Table 2). The average rates of parasitism should not be taken as final ones, because under

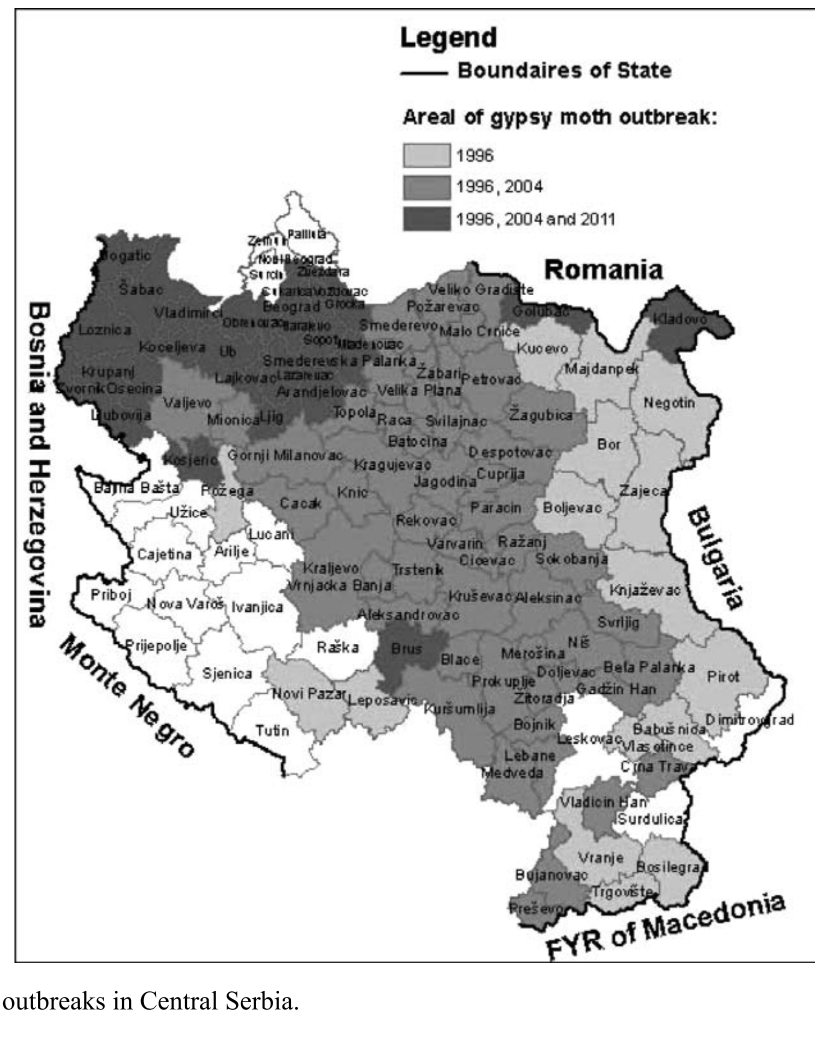

Fig. 1. Gypsy moth outbreaks in Central Serbia.

Table 2. Laboratory analysis of gypsy moth egg masses sampled from representative sample plots in the area of Central Serbia.

Year	Number of		Average number of eggs in an egg mass						
			Fertilised				Unfertilised		Total
	Trial plots	Egg masses	Vital		Parasitised				
			N	%	N	%	N	%	N
1993	27	270	298.9	86.4	44.6	12.9	2.4	0.7	346.1
1994	35	350	555.9	95.0	20.5	3.5	8.8	1.5	585.2
1995	69	690	543.8	98.7	2.8	0.5	4.4	0.8	551.0
1996	427	4,270	348.4	85.9	43.8	10.8	13.4	3.3	405.6
1997	725	7,250	277.7	80.7	41.6	12.1	24.8	7.2	344.1
1999-2001 gypsy moth latency period									
2002	70	700	477.6	95.5	17.3	3.5	5.1	1.0	500.0
2003	431	4,310	570.7	88.3	79.9	11.2	3.5	0.5	654.1
2004	1023	10,230	386.1	79.2	97.4	20.0	4.0	0.8	487.5
2005	266	2,660	162.5	82.4	32.0	16.2	2.6	1.3	191.7
2006-2008 gypsy moth latency period									
2009	58	580	502.5	91.6	42.3	7.7	3.6	0.7	548.4
2010	80	800	526.9	90.9	48.3	8.3	4.5	0.8	579.7
2011	120	1200	465.8	92.2	34.5	6.9	4.5	0.9	504.8

these laboratory conditions it is not possible to determine the effect of a series of parasites and predators, to which egg masses are exposed to in the field. Regarding the species of egg parasites, in the period 1994-2011 *Anastatus japonicus* Ashmead, 1904 (syn. *A. disparis* Ruschka) accounted for the range from 0 to 31%, and *Ooencyrtus kuwanae* (Howard, 1910) for 69-100%.

The data on egg, larval and pupal parasitism, as well as the data on the state of the gypsy moth predators and pathogens at individual localities should also be included in the study. During the observed period, in the gypsy moth populations, the activity of 56 natural enemies of this insect – nineteen predators, twenty-eight parasites, seven parasites or saprophages and two pathogens was reported. The gypsy moth eggs were attacked by eleven predator species [*Trombidium holosericeum* (Linnaeus, 1758); *Forficula auricularia* Linnaeus, 1758; *Carabus latus* Dejean, 1826; *Dermestes erichsoni* Ganglbauer, 1904; *Megatoma pici* Kalik, 1952; *Megatoma pubescens* (Zetterstedt, 1828); *M. undata* (Linnaeus, 1758); *Globicornis nigripes* (Fabricius, 1792); *Julistus floralis* (Olivier, 1790); *Malachus bipustulatus* (Linnaeus, 1758); *Formica sp.*], larvae were attacked by six species [*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 species [*Calosoma sycophanta* (Linnaeus, 1758); *C. inquisitor* (Linnaeus, 1758)].

There were three parasitic species of the gypsy moth eggs [*Anastatus japonicus* Ashmead, 1904; *Ooencyrtus kuwanae* (Howard, 1910); *Eremioscelio lymantriae* Masnil, 1958], eighteen parasitic species of the gypsy moth larvae [*Casinaria tenuiventris* (Gravenhorst, 1829); *Phobocampe disparis* (Viereck, 1911); *P. pulchella* (Thomson, 1887); *Apanteles glomeratus* (Linnaeus, 1758); *A. lacteicolor* Viereck, 1911; *Cotesia melanoscela* (Ratzeburg, 1844); *C. ocneriae* (Ivanov, 1898); *C. scabricula* (Reinhard, 1880); *Protapanteles liparidis* (Bouček, 1834); *P. porthetrie* Muesebeck, 1954; *P. fulvipes* (Haliday,1834); *Meteorus versicolor* (Wesmael, 1835); *Euplectrus liparidis* Ferrière, 1941; *Exorista larvarum* (Linnaeus, 1758); *Parasetigena silvestris* (Robineau-Desvoidy, 1863); *Compsilura concinnata* (Meigen, 1824); *Blepharipa pratensis* (Meigen, 1824); *B. schineri* (Mesnil, 1939)], and seven parasitic species of the gypsy moth pupae [*Pimpla instigator* Fabricius, 1793; *P. inquisitor* (Scopoli, 1763); *P. turionellae* (Linnaeus, 1758); *Theronia atalantae* (Poda, 1761); *Lymantrichneumon disparis* (Poda, 1761); *Brachimeria intermedia* (Nees, 1834); *B. femorata* (Panzer, 1798)]. Parasites or saprophages of gypsy moth pupae were represented by seven species [*Agria affinis* (Fallén, 1817); *Kramerea schuetzei* (Kramer, 1909); *Parasarcophaga harpax* (Pandelle, 1896); *P. portshinskyi* Rohdendorf, 1937;

P. uliginosa (Kramer, 1908); *Muscina pabulorum* (Fallen, 1817); *M. stabulans* (Fallen, 1817)]. Two pathogenic species (*Ld*NPV and *Entomophaga maimaiga*) were identified.

Regarding the density of some predator species, *Trombidium holosericeum, Forficula auricularia*, *Silpha quadripunctata*, *Calosoma sycophanta* and *Carabus sp.* were the most abundant ones. *Calosoma sycophanta*, which regularly occurs during the outbreak of the gypsy moth, was found more frequently than other predator species, and it reduced the population size of the gypsy moth both in the larval and imago instars.

At the selected sites in many regions of Central Serbia the cocoons of the parasitic species from the families Braconidae and Tachinidae were regularly found in spring. Their identification was conducted after they were grown under laboratory conditions and after the emergence of the imagos. (The species from the families Braconidae i Ichneumonidae were determined by Professor Doctor Miloje Brajković [†], from the University of Belgrade – Faculty of Biology.) Other species were considerably less frequent and were found individually.

The activity of *Lymantria dispar* NPV was reported at a few sites, with the extremely high population sizes of the gypsy moth. For example, *Lymantrya dispar* NPV caused the death of about 20% of L_4 gypsy moth instar at one site in the Forest Administration Lipovica (Belgrade region). In addition, a large number of the larvae brought from the field and subsequently grown under the laboratory conditions were infested by this pathogenic species (Tabakovic-Tosic *et al.* 2012).

Higher mortality rates of older gypsy moth larval instars were reported in the forest complexes of Belgrade and Valjevo region, in the culmination phase of the new outbreak of the gypsy moth in Serbia. Field and laboratory studies showed the presence of conidia and resting spores of the entomopathogenic fungus *Entomophaga maimaiga* which was the cause of the mortality. This is the first report of occurrence of this species in Serbia, i.e. Serbia is the third European country in which this fungus is reported. It showed to be a powerful reducer of the population size of the gypsy moth, and in both regions it caused the collapse of the outbreak in 2011 (Tabakovic-Tosic *et al.* 2012).

Since the gypsy moth is one of the economically most harmful insect species in the broadleaf forests of Central Serbia, the great attention is regularly paid to the suppression of it. The repressive methods of the suppression of this pest during the observed years of the outbreak are analyzed in the Table 3.

Since the gypsy moth is one of the most economically harmful insect species in the broadleaf forests of Central Serbia, great efforts are regularly spent for its suppression. The control methods for the suppression of this pest during the observed years of the outbreak are presented in Table 3.

Every year of outbreak, during the period autumn-winter, the gypsy moth was suppressed in the egg stage by mechanical methods (removal and burning) and chemical methods (soaking in oil and petroleum) (Table 3). The suppression in the egg stage alone was not sufficient because most of the egg masses were inaccessible, high in the crowns,

Table 3. The supression of the gypsy moth in the forests of Central Serbia over the period 1994-2011

Year	Control			
	In stage of egg		In stage of larvae	
	Type of control	Area (ha)	Type of insecticide – active ingredient	Area (ha)
1994	-	-	-	-
1995	mechanical	400	*Bacillus thuringiensis ssp. kurstaki*	400
1996	mechanical	17,427	*Bacillus thuringiensis ssp. kurstaki*	9,500
1997	mechanical and chemical	142,000	*Bacillus thuringiensis ssp. kurstaki*	253,525
1998	-	-	*Bacillus thuringiensis ssp. kurstaki*	5,055
2003	mechanical and chemical	77,576	-	-
2004	mechanical and chemical	115,998	*Bacillus thuringiensis ssp. kurstaki*	12,155
2005	mechanical and chemical	20,767	diflubenzuron	71,593
2006			diflubenzuron	21,200
2009	-	-	-	-
2010	mechanical	9,257	-	-
2011	mechanical	4,533	-	-

so control in the larval stage had to be performed by aerial spraying in the spring. The aerial application was mainly by biological and biotechnical preparations which are highly selective and ecologically friendly, and they are applied in ultra low volumes (ULV), which is very important bearing in mind the immense areas of forest complexes and the financial construction of the action (Table 3).

Conclusions

After a thirty-year long interval of latency, the increase of the gypsy moth population density above the normal level occurred three times in the forest areas of Central Serbia in the period 1994-2011. Natural enemies of gypsy moth, as well as the control measures undertaken in egg and larva stadia, although adequately prepared and timely applied, did not always produce satisfactory results (first and second outbreak).

In the period 2009-2011, a regressive phase occurred as a result of the successful gypsy moth suppression in the egg stadium, as well as the increased activities of the gypsy moth natural enemies – predators, parasites and pathogens. Diseases caused by the pathogens – *Lymantria dispar* NPV and fungus *Entomophaga maimaiga*, were the greatest contributors to the decline of the gypsy moths in some parts of Central Serbia.

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