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IMPACT OF BIOLARVICIDING ON *SIMULIUM ORNATUM* MEIGEN 1818 (COMPLEX) POPULATIONS AND RELATED BITING RISK

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ABSTRACT – Recent studies of blackflies in the region of Novi Sad confirmed the dominance of *Simulium ornatum* Meigen, 1818 (complex) in small watercourses. Highly productive breeding sites of this polyvoltine mammophilic species were detected in the majority of the streams flowing down from the slopes of the Fruška Gora Hills, especially in sections running through or close to populated zones.

Routine treatments of the simuliids breeding sites with biologicals (B.t.i.) were conducted for the first time in the region in 2005. Since then, the treatments have been performed on a regular basis following monitoring of larval abundance. The timing of the treatments was chosen carefully for each watercourse in accordance with density and age composition of the populations. It has been demonstrated that the optimal moment for larvicide application is the onset of the pupal stage. Application rates and duration of the treatments were calculated and defined depending on the flow velocity and water temperature established for each stream. The product dosage and duration of application ranged within the intervals of 5 to 25 ppm and 10-20 min, respectively. The registered carry of the B.t.i. was 500 m downstream. Systematically performed larvicide treatments affected the number of adult specimens subsequently captured in traps. In the given circumstances, four larvicide treatments during the spring and summer were sufficient to keep the adult population level low. When the overwintering generation was suppressed by an autumn treatment, two or three spring/ summer larvicide treatments were sufficient to maintain the adult population density constantly under the nuisance threshold.

KEY WORDS: Simuliidae, black flies, *Simulium ornatum*, B.t.i., control

INTRODUCTION

Blackflies can cause a serious nuisance to both people and domestic animals. Their importance is more pronounced due to their serving as vectors of several very serious disease agents.

Their bites are painful and can cause serious dermal problems, especially to sensitive people. Domestic animals also suffer from blackfly bites, and death can be caused by the toxin in the saliva.

The Novi Sad region offers convenient breeding sites to many different blackfly species. The slopes of the Fruška Gora Hills, which are rich in streams running down to the Danube River, seem to be the most prolific in producing masses of all black flies. The Danube itself is also registered as a fruitful blackfly breeding site.

Among the 12 species of the family Simuliidae registered in the region of Novi Sad during the period of 2001-2006, the three most aboundant species were found to be strongly anthropophilic. They were as follows: *Simulium (Simulium) ornatum* Meigen, 1818 (complex); *Simulium (Boophthora) erythrocephalum* (De Geer, 1776); and *Simulium (Wilhelmia) balcanicum* (Enderlein, 1924) (IGNJATOVIĆ-ĆUPINA ET AL., 2003, 2006). *S. ornatum* (complex) is the species with the highest preference for humans and the most aggressive species in the region (IGNJATOVIĆ-ĆUPINA ET AL., 2006). Eggs, larvae and pupae of the targeted species were recorded in abundance in streams along the right bank of the Danube while adult specimens were registered in every dry ice baited trap on the same side of the river. Some adults were recorded on the other side of the river as well, but just in several traps, indicating that the species doesn't spread far away from its breeding place (unpublished data).

MATERIAL AND METHOD

During the period from 2004 to 2006, five streams, the Bukovački, Novoselski, Kamenarski, Šandrovac, and Rakovački Brooks (45° 09' 36,5" - 45° 15' 1,9" N and 19° 46' 22,7" - 19° 54' 29,9" E) were monitored throughout the whole year. The streams were 5400 – 8640 m long, 0,7 – 3,0 m wide, and 7 – 42 cm deep, with discharges between 0,03 and 0,38 m^3/s. In this study two streams were chosen to show the effects of treatments conducted over a period of two and a half years. One of them, the Kamenarski Brook, was treated for the first time in December of 2004, and treatments were continually repeated during the seasons of 2005 and 2006. The second one, the Bukovački Brook, was treated for the first time in early spring of 2005, continually until the end of that season and in the following year too. Characteristics of the targeted streams important for the treatments were similar, with a discharge of running water of 0,03-0,35 m^3/s in the Kamenarski Brook and 0,05-0,30 m^3/s in the Bukovački Brook. Both streams were examined at several checkpoints before and after each treatment.

The population density of blackflies in each stream was recorded at five to seven checkpoints. The samples at each checkpoint were part of water debris. The most prominent and representative types of debris were twigs. The number of larvae and pupae on three twigs (10 cm long, 5 mm in diameter) was registered at each point on a regular basis. Their density was estimated in a range of five categories: absent (0 immature stages specimens/twig), low (1-5 specimens/twig), moderate (6-10 specimens/twig), high (11-25 specimens/twig), very high (26-50 specimens/twig), and extremely high (> 50 specimens/twig). Recommendations for treatments were given after recording at least a moderate number of specimens per twig. The same procedure was performed before and after each larvicide treatment in each studied stream.

The larvicide used in all treatments was VectoBac 12 AS, a biological agent based on *Bacillus*

thuringiensis var. *israelensis* (B.t.i.). The carry of the product was estimated to be up to 500 meters for each stream (ZGOMBA ET AL., 2004). The concentration and duration of application were chosen according to the temperature (which ranged from 8° to 21°C) and pollution of the water in each stream. Recommended concentrations of the larvicide were from 5 to 25 ppm, and the duration of application (depending on conditions in the stream) ranged from 10 to 20 minutes at each treatment point. Efficacies of each treatment were calculated and treatments sorted into the following three groups: no impact - efficacy between 0 and 55 %; low impact - efficacy between 56 and 85%; and high impact - efficacy between 85 and 100%.

Adult blackfly species were captured in 28 dry ice-baited traps, type NS-2, containing CO_2 as the attractant (PETRIĆ ET AL., 2000; ĆUPINA ET AL., 2002). Traps were set each season at weekly intervals from March or April to September. The operating time for each trap was from early afternoon until late morning of the following day. Thirteen traps were set on the flat left bank of the Danube and 15 on flood plains and slopes of the Fruška Gora hills on the right bank of the river. Sampled specimens were identified on the basis of descriptions given in available identification keys (KNOZ, 1965; DAVIES, 1968; RIVOSECCHI, 1978). The biting risk to humans was estimated according to the number of specimens of *S. ornatum* (complex) in trapped samples and the ratios given by IGNJATOVIĆ-ĆUPINA ET AL. (2006). By using this scale, it is possible to predict the number of bites during the five hours before sunset.

RESULTS AND DISCUSSION

Found in the majority of studied watercourses, *S. ornatum* was the most frequently occuring blackfly species in former Yugoslavia (ŽIVKOVIĆ, 1961). It was equally present in slowly flowing lowland streams and in fast streams at altitudes higher than 1000 m; in cold waters where the temperature during the summer is about 9°C and under conditions where the temperature exceeds 25°C; and in clean and polluted watercourses with different structure of the stream bed (bedrock, gravel, sand, or mud) (ŽIVKOVIĆ, 1961).

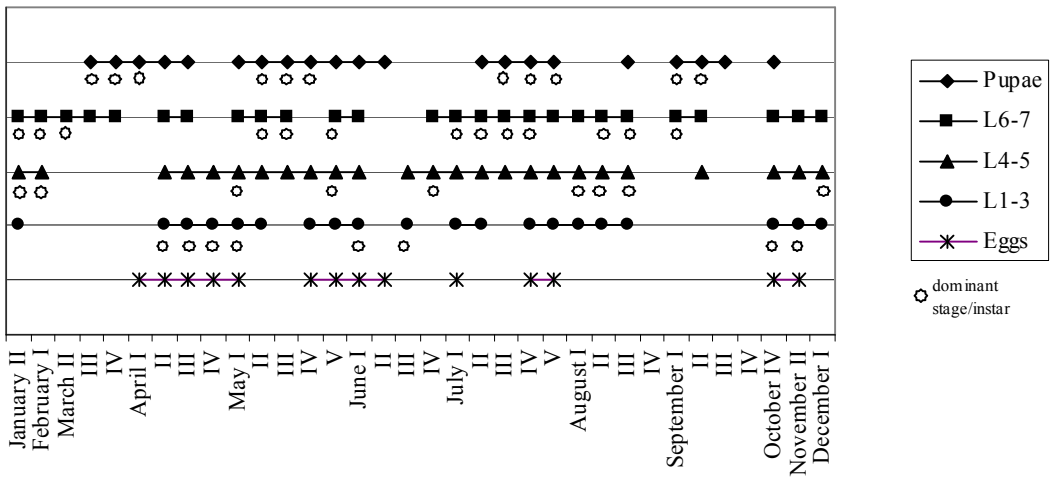

Fig. 1. Life cycle of *Simulium ornatum* Meigen, 1818 (complex) in Fruška Gora streams.

Table 1. Population density and age composition of immature stages of *S. ornatum* (complex) before and after larvicide treatments in the Kamenarski Brook in late autumn of 2004 and during the 2005 season.

Date of tretament		December 6th 2004						March 22nd 2005						June 20th 2005						August 3rd 2005						November 2nd 2005					
Targeted generation		Over-wintering generation						First generation						Second and Third generation						Third and Fourth generation						Over-wintering generation					
	Control point	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Pre tretment	Population density	H	VH	EH	VH	EH	VH	A	A	A	M	A	A	L	M	M	H	H	M	M	M	L	H	H	L	M	H	H	H	H	M
	Eggs																+				+		+	+				+	+		
	L 1-3	+	+	+	+	+	+										+							+		+	•	•	•	•	•
	L 4-5	•	•	•	•	•	•				+				+	•	•	+	•		+	+	+	+	+	•	•	•	•	•	+
	L 6-7		+	+		+					•			+	•	•	•	•	•	•	•	•	•	•	+	+	+	+	+	+	+
	Pupae		+								+			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		
Post tretment	Population density	A	A	A	H	VH	A	A	A	A	A	A	A	L	L	L	M	M	L	L	A	L	L	L	A	L	L	L	L	L	A
	Eggs																+											+			
	L 1-3				+	+																						+	+	+	
	L 4-5				•	•																									
	L 6-7					+																									
	Pupae													+	+	+	+	+	+	+		+	+	+		+	+	+	+		

+ present stage/instar
• dominant stage/instar

*categories that represent different numbers of specimens per 10 cm of twig (A-absent = 0; L-low = 1-5; M-moderate = 6-10; H-high = 11-25; VH-very high = 26-50 L; EH-extremely high ≥ 51)

Simulium ornatum (complex) has four overlapping generations per year in the region of Novi Sad (ĆUPINA ET AL., 2003). Pupation of the first, over wintering generation takes place from March to April; that of the second generation starts in May and ends in June; the third generation pupates in July and partly at the beginning of August; and the last (fourth) generation pupates at the end of August and in September (Fig. 1). The species overwinters the different larval instars. Masses of eggs can be found from the beginning of spring until the end of autumn. Larvae are present in the streams thoroughout the whole year, and pupae can be found from March almost until the end of October. Adults are on the wing from very early spring until early winter.

Treatments of blackflies with B.t.i. were not carried out until late autumn in 2004, when four out of five infested streams were treated. The **Kamenarski Brook** was treated for the first time in December of 2004, with VectoBac 12 AS in a concentration of 25 ppm for 20 minutes. The targeted generation was the overwintering one: larvae were from second to fifth instars. No egg masses were registered in the stream before or after the treatment (Table 1). Population density was high at one out of seven checkpoints, very high at three checkpoints and extremely at two checkpoints. After the treatment, at four checkpoints there was excellent efficacy, with no larvae found, while at two checkpoints population density was still high and very high. The following spring, the first treatment was performed in March; the applied concentration of larvicide was the same as before, 25 ppm over 20 minutes. The targeted generation was the first ones of *S. ornatum* (complex) species. Population density was moderate at one checkpoint. Spring treatment successfully reduced larvae at each checkpoint. Occasionally pupae survived and emerged in the meantime. Recommendation for the next treatment was given when population density of second- and third-generation larvae was moderate and high at almost all checkpoints. At the same time, egg masses and newly hatched larvae were still present in the stream. The next treatment was conducted in June of 2005, with 10 ppm of VectoBac 12 AS over 10 minutes. Before the treatment, low density of larvae was registered at one out of six checkpoints, moderate density was registered at three checkpoints, and high density in two. After the treatment, moderate density was estimated at two checkpoints and low density at all remaining ones, where pupae were the only surviving stage. New egg masses were registered at one checkpoint. Population density of the third and fourth generation at the beginning of August of 2005 was estimated as at high two out of six checkpoints, moderate at two, and low at two others. After ten minute treatment with a 5 ppm concentration of VectoBac 12 AS in August, two checkpoints showed absence of larvae and all the rest had only low density of pupae. The last treatment in 2005 was conducted in order to reduce moderate and high population density of the overwintering generation at all checkpoints on the stream. While the dominant larvae were still young (small and medium-sized), water temperature was still high enough to keep them active in feeding. Treatment was carried out at the beginning of November, when the applied concentration of VectoBac 12 AS was 25 ppm and duration of the application was 15 minutes. Effects of the treatment were good, but pupae and small larvae with low density survived at five out of six checkpoints. Egg masses were registered at one checkpoint after that autumn treatment. This was the reason why higher numbers of blackflies were captured in the spring of 2006 then in the spring of 2005.

In 2006, the first treatment on Kamenarski Brook was performed in late March, with an applied concentration of VectoBac of 25 ppm and duration of application of 20 minutes. Population density of the targeted first generation was estimated high at three out of six checkpoints (Table 2) moderate at two, and low at only one out of six checkpoints. Post-treatment examination of the checkpoints showed low density of old larvae and pupae at one checkpoint and absence of blackfly larvae at all other checkpoints on the stream. Recommendation for the next treatment was given in the first decade of May, when population density of the targeted generation of larvae was mod-

Table 2. Population density and age composition of immature stages of *S. ornatum* (complex) before and after larvicide treatments in the Kamenarski Brook during the 2006 season.

Date of treatment		March 27th 2006						May 10th 2006 Pretreatment only							May 29th 2006							June 14th 2006							July 18th 2006						
Targeted generation		First generation						Second generation							Second/third generation							Second/third generation							Third/fourth generation						
Control point		1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Pre treatment	Population density	M	H	H	H	M	L	M	M	M	M	M	M	H	M	H	H	VH	H	H	VH	H	H	H	VH	VH	M	L	M	H	H	VH	VH	M	L
	eggs										+			+					+				+		+	+		+			+	+	+		+
	L_{1-3}								+	+	+	+	+	•					+	+	+											+			
	L_{4-5}	+		+	+	•	•	•	•	•	+	+	•	+	+	+	+		•	•	•									+	+	+	+	+	+	+
	L_{6-7}	•	•	•	•	+	+	•	•	•	•	•			•	•	•	•	•	•	+				+				•	•	•	•	•	•	•
	Pupae	+	+		+						+	+			+	•	•	•	+	+		+	+	+	•	+	+	+	+	+	+	+	+		
Post treatment	Population density	A	L	A	A	A	A								H	H	H	VH	VH	M	L	A	A	H	H	VH	L	M	A	L	A	A	M	A	A
	eggs															+		+	+		+			+	+	+		+							
	L_{1-3}																							•	•	•	•	•							
	L_{4-5}																													+			•		
	L_{6-7}		+															+												+			+		
	Pupae		+												+	+	+	•	+	+	+									+						

+ present stage/instar
• dominant stage/instar

*categories that represent different numbers of specimens per 10 cm of twig (A-absent = 0; L-low = 1-5; M-moderate = 6-10; H-high = 11-25; VH-very high = 26-50 L; EH-extremely high ≥ 51)

Table 3. Population density and age composition of immature stages of *S. ornatum* (complex) before and after larvicide treatments in the Bukovački Brook during the 2005 season.

Date of treatment		March 21st 2005							June 21st 2005							July 18th 2005							August 17th 2005							October 31st 2005						
Targeted generation		First generation							Second and third generation							Third and fourth generation							Fourth generation							Over wintering generation						
	Control point	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Pre tretment	Population density	A	M	M	H	H	H	M	A	M	H	H	H	M	M	H	M	H	H	H	M	M	L	M	H	M	H	M	L	M	M	H	H	H	H	L
	Eggs										+		+			+		+						+	+		+	+			+		+	+		
	L 1-3		+	+			+				+	•	+			+	+	+	+	+	+	•		+	+		+	+		+	•	•	•	•	•	+
	L 4-5		+	+	+	+	+	+			•	•	•	•	•	+	+	+	•	•	•	+	+	•	•	•	•	•	+	•	•	•	•	•	•	+
	L 6-7		•	•	•	•	•	•		•	•	•	+	•	+	•	•	•	•	•	•		+	+	+	+	+	+	+	+	+	+	+	+	+	
	Pupae		+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+		+		
Post treatment	Population density	A	L	L	L	L	L	L	A	L	M	L	M	L	L	A	L	L	L	L	L	A	L	L	L	L	L	L	L	L	L	L	A	L	A	L
	Eggs									+	+	+	+				+			+				+		+										+
	L 1-3										+		+				+											+	+								
	L 4-5																									+	+	+	+							
	L 6-7																												+							
	Pupae		+	+	+	+	+	+		+	+	+	+	+	+			+	+	+	+		+	+	+				+	+	+	+		+		

+ present stage/instar
• dominant stage/instar

*categories that represent different numbers of specimens per 10cm of twig (A-absent = 0; L-low = 1-5; M-moderate = 6-10; H-high = 11-25; VH-very high = 26-50L; EH-extremely high ≥ 51)

erate at six checkpoints and high at one. Treatment with 25 ppm VectoBac 12 AS over 15 minutes was performed two weeks later than recommended. That time window allowed the larval population to increase in age and size and caused low efficacy of the treatment. The main reason for this situation was pupation of almost all old larvae in the stream. At the same time, new egg masses were registered at four checkpoints. The next treatment was conducted on the 14th of June; again, it was not in time, since many pupae and still unhatched new eggs were registered at that time. Post-treatment evaluation of checkpoints on the stream therefore showed still very high, high, and moderate population density of the first and second instars of blackfly larvae, which hatched after the treatment. Third- and fourth-generation larvae were successfully reduced after treatment performed on the 18th of July. The population density of all instars of blackfly larvae declined from very high, high, and moderate number (at two out of seven checkpoints) to moderate and low at one checkpoint and absence of larvae at the other checkpoints on the stream. The concentration of larvicide used in this treatment was 10 ppm, the duration of application 10 minutes.

The Bukovački Brook was the only stream where no winter treatment was performed during 2004. The first treatments on this stream were performed in the spring, on the 21st of March, 2005. The applied concentration of VectoBac 12 AS was 25 ppm, the duration of application 20 minutes. The estimated population density decreased from high and moderate before the treatment to low at checkpoints evaluated in post-treatment inspection of the stream (Table 3). The targeted generations for the next treatment, performed in June of 2005, were the second and third generation of *S. ornatum*, whose immature stages were present in high and moderate population density at three out of seven checkpoints before the treatment. Treatment was performed using VectoBac 12 AS in a concentration of 5 ppm over 10 minutes. Population density was reduced to moderate at two checkpoints, and low at the rest of them. Post-treatment examination showed that only pupae and very small larvae, which hatched in the meantime, survived. Immature stages of the third and fourth generation were treated in July, when their population density was estimated as high at four out of seven checkpoints and moderate at three. The number of specimens per twig was reduced and estimated as low at five out of seven checkpoints, larvae were absent at two. The next treatment was performed in August, when the density of larvae was estimated as high at two out of seven checkpoints, moderate at three, and low at two. The concentration of larvicide used was 10 ppm, the duration of application 10 minutes. Post-treatment inspection of checkpoints showed low density of blackfly larvae at each of them. Autumn treatment was performed at the end of October, when population density of all immature stages was estimated as high at four out of seven checkpoints, moderate at two, and low at only one. Treatment was performed using VectoBac in a concentration of 25 ppm over 15 minutes. Old larvae pupated and survived this treatment in estimated low density at five out of seven checkpoints, and the presence of freshly laid eggs was also recorded.

The first inspection of the stream in March of 2006 showed that the population density of immature stages of *S. ornatum* (complex) was high at two out of seven checkpoints, moderate at two, and low at one. Absence of larvae was registered at two checkpoints (Table 4). The first treatment in 2006 was performed in March using a 25 ppm concentration of larvicide and on application period of 20 minutes. Reduction of larvae was good, and population density was estimated as low at two out of five checkpoints, while no larvae were found at the remaining ones. Larvae of the second generation in the stream were reduced by the treatment performed on May 12th, which was a larvicide with concentration of 10 ppm and an application time of 10 minutes. This treatment, done at the right time and under good conditions, gave excellent results. Population density throughout the entire length of the stream was estimated as absent in post-treatment inspection. In June, new egg masses and larvae were registered in high, moderate, and low density at two check-

Table 4. Population density and age composition of immature stages of *S. ornatum* (complex) before and after larvicide treatments in the Bukovački Brook during the 2006 season.

Date of treatment		March 24th 2006							May 12th 2006						June 29th 2006						August 17th 2006					
Targeted generation		First							Second						Second and third						Fourth					
Control point		1	2	3	4	5	6	7	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Pre treatment	Population density	A	H	H	M	M	L	A	-	A	H	H	H	L	L	M	M	H	H	L	M	M	M	H	M	L
	eggs										+		+			+	+	+	+	+						
	L_{1-3}										+	+	+	+	+	+	+	+	+	•				+		
	L_{4-5}				+	•	•				•	•	+		•	•	+	+	•	+		+	+	•	+	+
	L_{6-7}		•	•	•	+	+				•	•	•		+	+	•	•	•		•	•	•	•	•	+
	Pupae		+	+			+					+									+	+			+	
Population density	Population density	A	L	L	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	L	L	L	A	A
	eggs																+	+					+	+		
	L_{1-3}																					+	+	+		
	L_{4-5}		+																							
	L_{6-7}		+	+																						
	Pupae		+	+																						

+ present stage/instar
• dominant stage/instar

*categories that represent different numbers of specimens per 10 cm of twig (A-absent = 0; L-low = 1-5; M-moderate = 6-10; H-high = 11-25 VH-very high = 26-50 L; EH-extremely high ≥ 51)

points, and new treatment was performed on the 29th of June. The larvicide concentration was 5 ppm, the duration of application 10 minutes. Post-treatment results were again excellent, as no larvae were found at the checkpoints; however, new fresh egg masses were registered at two checkpoints. The next treatment was recommended after hatching of new larvae in August, when the population density of blackfly larvae was high at one checkpoint, moderate at four, and low at only one out of five checkpoints. After this treatment (performed on the 17th of August with a concentration of 10 ppm over a period of 10 minutes), only fresh egg masses and newly emerged larvae were registered in low population density at three out of six checkpoints.

Assessment of the stream sections infested with immature stages of blackflies before and after each treatment showed that not every treatment was successfull. During 2005, there were five treatments with no impact on immature stages of blackflies, 21 treatments with an efficacy level between 55 and 85%, and 27 treatments with good results, where the efficacy rate was from 85 to 100% (Fig. 2). During 2006, there were still eight treatments with no impact (probably due to lateness of treatments), when the age composition of blackflies did not respond to larvicide treatment. The efficacy of one treatment in 2006 was less than 55%, seven treatments had an efficacy of 55 to 85%, and 48 yielded excellent results. Efficacy in the latter cases was 85-100%, probably due to experience gained by pest control operators in blackfly control.

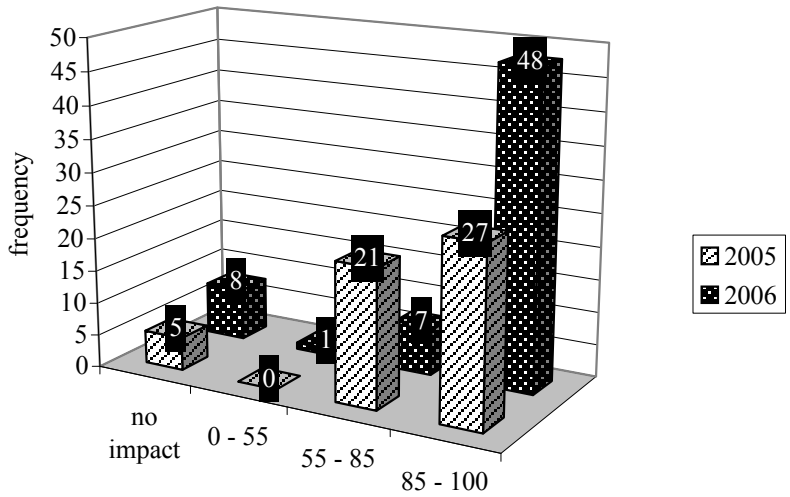

Fig. 2. Frequency of treatments with different efficacy levels.

Our results are comparable with ones obtained in Southern Bohemia (Czech Republic), where streams with similar characteristics (depth 5 cm, width 1,5 m, stream velocity 0,4-0,9 m/s) were treated with B.t.i. in a final concentration of 1x 10^5 spores/cm^3 for a duration of 10 minutes. The mortality of *S. ornatum* larvae in Bohemia ranged between 95 and 100% at sites 0-400 m distant from the treatment point and from 20 to 40% at a distance of 700 m, the population was not affected at all while at the site 1000 m downstream (OLEJNIČEK, 1986). Recolonisation of sites was observed by the same author after 12 days. Routine application of B.t.i. had no effect on supression of the population at temperatures below 4°C.

Our results confirmed that high efficacy of treatments can be achieved throughout the entire stream length if the treatment points are appropriately chosen at distances no greater than 500 m away and if the treatments are adapted to ecological conditions.

With respect to the number of adult specimens of blackflies captured in dry ice-baited traps, 2004 was the most productive year (Fig. 3). Adult specimens of *S. ornatum* (complex) were registered in varying numbers almost each trap set close to the breeding places during the spring and summer months of the season. The year 2003 was very dry, with high temperatures and almost no summer rains. This caused drying of some streams and consequent decrease in abundance of *S. ornatum* (complex). Shrinkage of the breeding areas resulted in low numbers of captured adult specimens and fewer traps with captured adult blackflies. In the following years (2005 and 2006), larvicide treatment was introduced as a control measure, with the result that the number of captured adult specimens of *S. ornatum* (complex) was markedly reduced. The species composition of adults captured in the traps also changed during the last four years (Fig. 4). In 2003 and 2004, when no treatments of streams were performed, the dominant species was *S. ornatum* (complex), which prefers small streams as its breeding site and therefore is never found in big rivers such as the Danube. In the 2005 and 2006, when most of the streams were successfully treated, the relative abundance of *S. ornatum* (complex) adults decreased. Abundance of the species that develops in

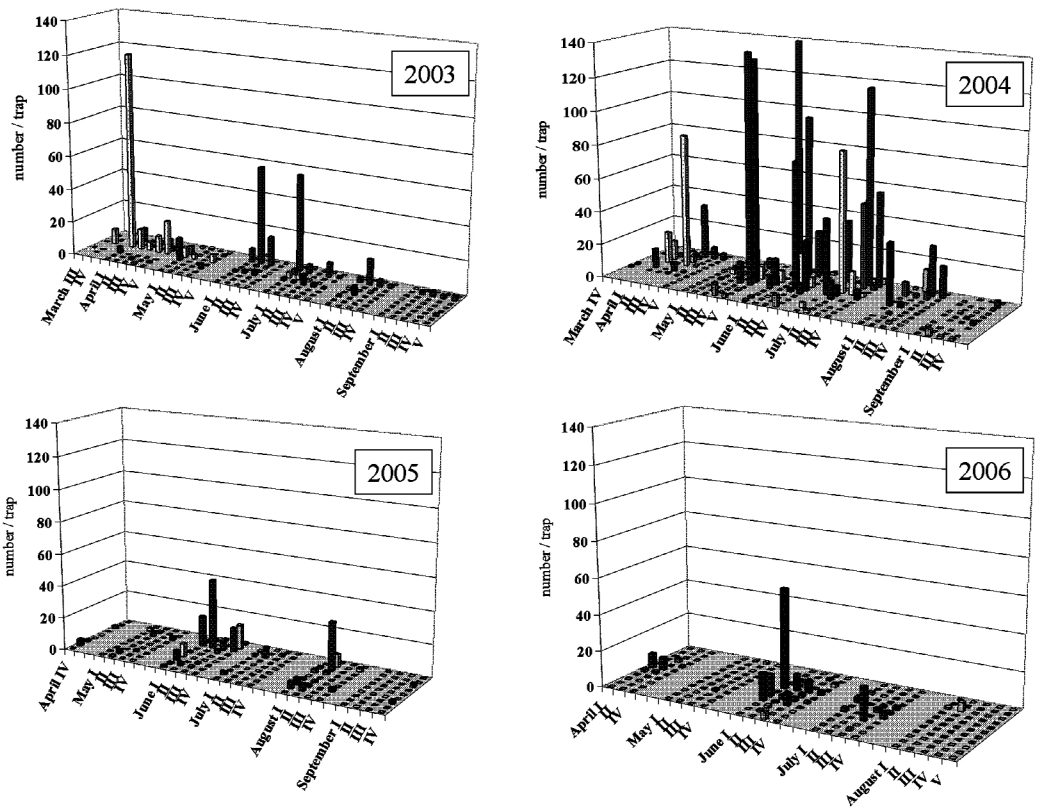

Fig. 3. Overview of *S. ornatum* abundance in dry ice baited traps at different localities during the last four years.

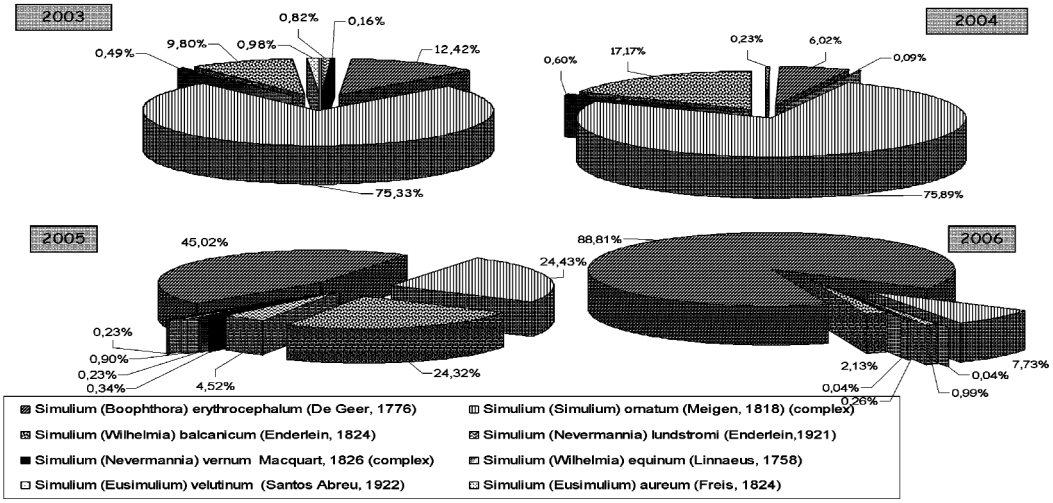

Fig. 4. Species composition of adult black flies sampled by dry ice baited traps (2003-2006).

the Danube, e.g., *S. erythrocephalum*, increased over the last two years and it became the dominant species in traps because larvicide treatments of the Danube are still not conducted.

The highest number of trap days with elevated risk of being bitten by *S. ornatum* (complex) was recorded during 2004 (Table 5). In the following years, 2005 and 2006, several treatments were performed, and the number of trap days with elevated risk to humans of being bitten declined from 42 days in 2004 to nine and 10 in 2005 and 2006, respectively. The number of trap days with estimated high risk to humans of being bitten by *S. ornatum* declined from 14 and 20 during years with no treatments (2003 and 2004) to only four and seven during 2005 and 2006, respectively. The number of trap days with estimated very high biting risk was smaller and decreased from six in 2004, to three in 2005 and two in 2006. Trap days with estimated extremely high risk to humans decreased from 16 during 2004 to two in 2005 and only one in 2006. This shows that adult *S. ornatum* (complex) populations in the region of Novi Sad were significantly suppressed after performance of biolarvicide treatments of streams in the Fruška Gora Hills. Similarly, the effects of larvicide treatments can also be documented by the yearly average number of captured adult speci-

Table 5. *S. ornatum* (complex) biting risks in the years without and with control of larval population.

Year	Number of trap days with elevated biting risk			
	High	Very high	Extremely high	Total
2003	14	3	3	20
2004	20	6	16	42
2005*	4	3	2	9
2006*	7	2	1	10

* larvicide treatments with B.t.i.

Table 6. Comparison of yearly averages of *S. ornatum* (complex) females captured in dry ice baited traps.

Locality	Average No. of females per trap/night				
	2002	2003	2004	2005	2006
1. Bukovac	0 [a]	0.038 [a]	1.136 [b]	0.095 [a]	0.105[a]
5. Ledinci village 1	1.52 [ab]	0.310 [a]	3.263 [b]	0.952 [a]	1.000[a]
6. Ledinci village 2	1.870 [a]	1.227 [a]	16.762 [b]	0.857 [a]	4.000[a]
7. Šandrovac	4.654 [ab]	7.036 [ab]	9.769 [b]	0.476 [a]	0[a]
9. Rakovac Danube	0.375 [ab]	0.536 [ab]	1.706 [b]	0.286 [a]	0.250[a]
10. Popovica	2.091 [a]	7.778 [ab]	32.619 [b]	6.048 [ab]	1.250[a]

[a,b] - numbers in the same row marked with the same letter are not significantly diferrent at the level of p = 0,01

mens of the targeted species per trapping day during the 22 weeks of sampling (Table 6). The average number of trapped adults in 2004 was significantly higher than in 2005 and 2006, with the exception of one trap point (Popovica) in 2005 with a high number of adult black flies, even though no immature stages were ever found in the small stream close to the trapping site.

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