EKOLOGIJA. 2006. Nr. 4. P. 70–75

Control of bloodsucking black fly (Simuliidae) populations in Lithuania

Ilona Bartninkaitė[1],

Rasa Bernotienė[1],

Saulius Pakalniškis[1],

Milda Žygutienė[2]

[1] *Institute of Ecology of Vilnius University, Akademijos 2, LT-08421 Vilnius, Lithuania, e-mail: rasab@ekoi.lt*

[2] *Centre for Communicable Diseases Prevention and Control, Kalvarijų 153, LT-08221 Vilnius, Lithuania E-mail: m.zygutiene@ulpkc.lt*

The outbreak of bloodsucking black flies began in the 70s of the 20th century in the south-eastern part of Lithuania. By 1990, the biting activity of bloodsucking black flies increased and had become a serious problem. The bloodsucking insects caused losses of cattle and domestic birds and tormented holiday-makers in the Druskininkai health-resort.

Biological larvicide based on *Bacillus thuringiensis* var. *israelensis* was used for bloodsucking black fly control in 1999–2005. The larvicide was introduced into the Nemunas River stream in one point directly from the river bank. A sufficient efficacy was achieved in a 164 km long segment of the river every year.

Key words: black fly control, *Simulium maculatum*, *Bacillus thuringiensis* var. *israelensis*, Simuliidae, Lithuania

INTRODUCTION

No other biting flies inspire such apprehension as do black flies. Outbreaks of bloodsucking black flies (Diptera: Simuliidae) were observed in Denmark at the beginning of the 20th century (Jensen, 1984), in Russia, in Ukraine (Прудкина et al., 1992), Serbia and Montenegro (Zgomba et. al., 2004) during the two last decades. They could be caused by natural fluctuations of black fly population abundance, by organic or thermal pollution (Рубцов, 1978) and vegetation overgrowths of streams. The real causes of bloodsucking black fly outbreaks have not yet been established.

Black flies are serious pests because of their painful bites and the enormous numbers involved in attacks. Bites of black flies may cause localised swelling, inflammation and intense skin irritation for some days or even weeks. (Rozendaal, 1997). A combination of anaphylactic shock caused by bites, blood loss and respiratory problems due to the inhalation of flies can be the reason of cattle death. Bloodsucking black flies are vectors of onchocerciasis, the so-called river blindness in Africa and in Central and South America. In some regions, up to 15% of people have been blinded by the disease (http://news.bbc.co.uk/1/hi/in_depth/sci_tech/2000/festival_of_science/914616.stm). Myxomatosis, Leucocytozoon and avian trypanosomes can infect both domestic and wild birds. The mentioned parasites can be carried by mechanical transmission (Hutchinson, 1999).

Black flies lay eggs in running water. Larvae and pupae are usually attached to submerged vegetation, stones and other substrates. Larvae are filter feeders. The larval stage lasts from one week to several months, depending on climate conditions. Adults emerge from pupae in 2–6 days. Bloodsucking is not a universal habit, but females of most species feed on warm-blooded vertebrates, often preferring either birds or mammals (Evenhuis, 2004). Most species feed predominantly on birds or mammals (Rozendaal, 1997). Adult black flies do not bite indoors usually.

Outbreak of bloodsucking black flies began in the 70s of the 20th century in the south-eastern part of Lithuania. By 1990, the biting activity of bloodsucking black flies increased and had become a serious problem. The bloodsucking insects caused losses of cattle and domestic birds and annoyed holiday-makers of the Druskininkai health-resort. This prompted investigations of Simuliidae in Lithuania. The main bloodsucking species was determined to be *Simulium (Byssodon) maculatum* Mg. (Žygutienė, Pakalniškis, 1997). The *Simulium (Byssodon) maculatum* Mg. blackfly species was described by Meigen from Germany in 1804. This species vanished in Germany and is very rare in Europe now (Zwick, 1995). We have data that *S. maculatum* occurs in Belarus, Ukraine, Russia, Kazakhstan, China (Рубцов, 1956; Янковский, 2002). Larvae of *S. maculatum* develop in large warm rivers where water is rich in organic matter (Янковский, 2002).

The use of products based on *Bacillus thuringiensis* var. israelensis against black fly larvae in rivers has been a long-lasting practice in many countries. Treatments of small streams, bringing a good effect in segments of a few hundred metres (Molloy, 1990), application of the preparation in larger rivers, efficient in segments of some kilometres (Riley, Fusce 1990), as well as expensive aerial and ship-board sprayings of biological larvicide in long segments (black fly accumulation places) of larger rivers have been reported.

MATERIALS AND METHODS

Study object

The main bloodsucking black fly species, *Simulium (Byssodon) maculatum* Mg., is a transpalaearctic species adapted to the continental climate. The abundance of blackfly larvae was estimated on water plants. Leaves of *Glyceria maxima* (Poaceae), being very uniformly band-shaped, were collected. We collected three samples at a time. The effect of the biopreparation on other aquatic invertebrates was estimated in chosen study sites using the kick-sampling method. Larvae of black flies as well as aquatic invertebrates were collected before and after the application of the biological larvicide. Adult bloodsucking black flies were collected from several persons in a 10-min period after 5 min of waiting.

Study area

Two rivers (Fig. 1), the Middle Nemunas and Neris, were colonised by *S. maculatum* (Bernotienė, 2001). The Lower Nemunas, downstream the Kaunas water reservoir, stands out for the unstable ground and very poor aquatic vegetation (a settlement substratum for black fly larvae), thus it is much less suitable for the *S. maculatum* breeding. *S. maculatum* is not abundant in the Lower Neris River.

The biopreparation was used in the Middle Nemunas River. Permanent study sites were chosen to establish the efficacy of the biopreparation.

Microbiological investigations

Samples of aquatic plants and black fly larvae were taken in the study sites in the Nemunas River before and 2, 6 and 37 days after the application of the biopreparation. *B. thuringiensis* bacteria were isolated by usual microbiological methods (Bluzmanas, 1970; Menon, De Mestral, 1985) to determine the elimination dynamics of *Bacillus thuringiensis* bacteria from water. Later, the developed *Bacillus* colonies 2 mm in diameter were counted and bacterial smears from each colony were prepared. The colonies with inclusion bodies were assigned to *B. thuringiensis*. All investigations were carried out by three replications, and the obtained mathematical data were processed by variation statistics methods (Лакин, 1980).

Stream treatment

The biological larvicide VectoBac 12AS (Valent BioScience Corporation, USA) was used in the Nemunas River in 1999–2005. The way of treatment with the preparation was different and depended on the experience and on the objective possibilities to work simultaneously in the territory of the neighbouring Belarus.

The Nemunas segment (107 km long) from Grodno to Krikštonys (Fig. 1) was treated by 8500 kg of VectoBac 12AS biopreparation in 1999. The preparation was poured evenly into the middle reaches of the stream from a boat. The action lasted for three workdays.

The VectoBac 12AS was applied at a single point (Varviškė) from a bank prominence in 2000, 2002 and 2005. The stream was moving away powerfully from the bank in the mentioned site. The concentration of the preparation remained 15–18 ppm for about two

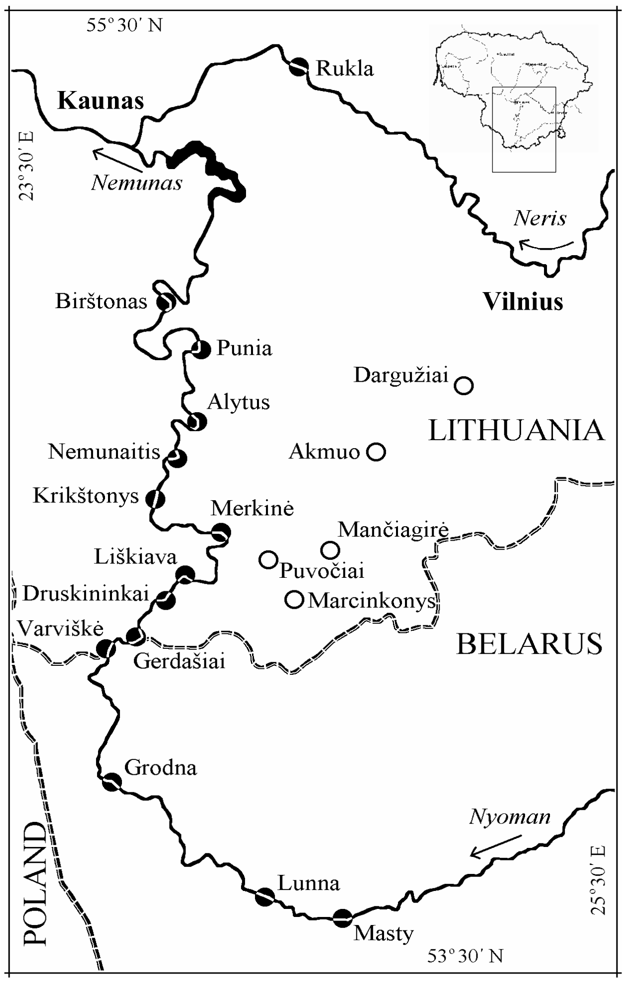

Fig. 1. Study sites in the terrain (100 × 170 km) surrounding the middle Nemunas river. Channel length: from Masty to Varviškė 113 km, from Grodna to Varviškė 22 km, from Grodno to Krikštonys 107 km, from Varviškė to Birštonas 164 km

hours. The treatment was repeated two weeks after the first application to affect black fly larvae which had immigrated downstream the river from Belarus. The VectoBac 12AS was applied at two points in 2001, 2003 and 2004 (Varviškė and Masty or Varviškė and Lunna) (Fig. 1). The preparation was used at a week's interval, i. e. one week later, in point upstream.

RESULTS

Black fly investigations

Larvae of *Simulium maculatum* were found in the middle Nemunas in late April. The thickest density of *S. maculatum* larvae was 3464.0 per 1 dm^2 on aquatic vegetation in April 27, 1999 in the study site near Merkinė (Fig. 1). The density of *S. maculatum* was much lower (up to 83.7 larvae per 1 dm^2) in the Neris River. *S. maculatum* pupae were not found each year in the Neris River. According to the postgenal width of larvae (Ross, Merritt, 1978), seven instars are typical of *S. maculatum* larvae in Lithuania.

The mass flying of adults began in late May and continued up to early July in Lithuania. Adults of *S. maculatum* used to flight a great distance away from the river where they had developed. Biting females dispersed over the territory of West Belarus and South Lithuania each June. Larvae of the other black fly species, *S. reptans* L., developed in the Nemunas River in April also. Usually, the development of *S. reptans* larvae began one or two weeks earlier than the development of *S. maculatum* larvae. Larvae of *S. erythrocephalum* began their development two-three weeks later than *S. maculatum* larvae.

The preparation was used when *S. maculatum* larvae dominated among other black fly species and when 4^{th}–5^{th} instar *S. maculatum* larvae dominated among larvae of other instars each year.

Black fly control efficiency

Four to 8.5 t of VectoBac 12AS was used each year. The effect of the preparation depended on the river discharge. The discharge of the Nemunas River fluctuated between 152 m^3/s in 2002 and 363 m^3/s in 2005. The mortality of black fly larvae was 95.9 ± 9.5% within an up to 25 km long segment and 86.5 ± 30.2% within an up to 50 km long segment downstream from the point of application each year for one week after application. The mortality of black flies larvae reached 49.8% in the study site 164 km downstream the point of application 2–3 weeks after application. The density of new born *S. maculatum* larvae has been reducing each year beginning from 1999 (Fig. 2) in the Nemunas River.

The relative abundance of black fly species has changed markedly since 1999 in the Nemunas River. In 1997–1999, black fly larvae of the *S. maculatum* and *S. reptans* species, the development phase of which is almost concurrent with that of *S. maculatum* were record-

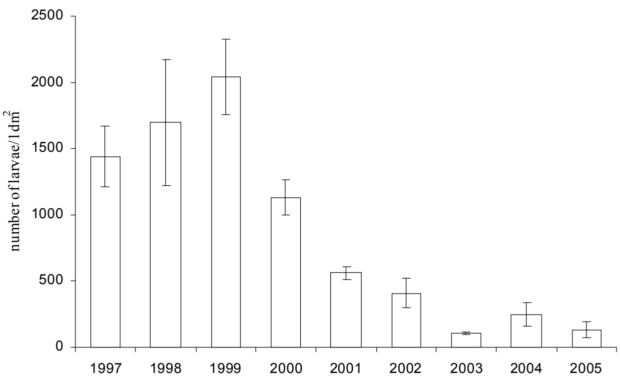

Fig. 2. Density of *Simulium (B.) maculatum* Mg. larvae each year in the Nemunas River (individuals per dm^2 of water vegetation).

ed as dominant, whereas beginning with 2001 the dominant position was occupied by larvae of the subgenus *Wilhelmia* and *S. erythrocephalum* (Bernotienė, 2005). Hence, since 2000 the abundance of *S. maculatum* and *S. reptans* has been decreasing in the Nemunas River with a corresponding increase in the abundance of larvae of other species (Fig. 3). No changes in the total density of black fly larvae on water plants in different years were determined (Bernotienė, 2005).

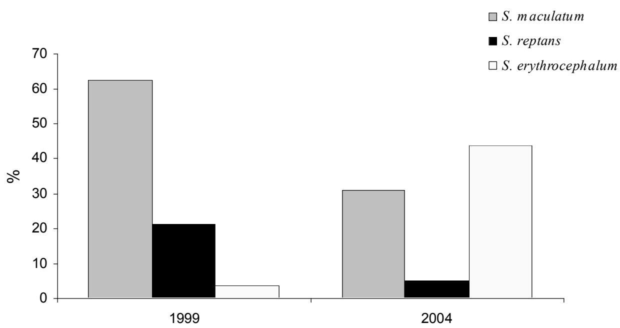

Fig. 3. Relative abundance of different black fly species in the Nemunas river in different years

No mass flying of bloodsucking black flies was observed after 1999 (Fig. 4). The adult *S. maculatum* flying period was shortened from 1–2 weeks (Druskininkai) to 3–4 weeks (Alytus) and lasted 2–3 weeks only. The abundance of bloodsucking *S. maculatum* adults depended only ($R = -0.92$, $p < 0.05$) on the distance to the Nemunas segment where the preparation had not been used.

The decrease of pests (black flies) may have positively influenced the Druskininkai health-resort, because the numbers of guests visiting Druskininkai began to grow from 2001 and have been growing continually (Fig. 5).

The effective application of the VectoBac 12AS biopreparation in the Nemunas River can be explained by the way of stream treatment. A large amount of the preparation (2 kg for 1 m^3/s of yield) was turned to the middle reaches of the stream, so it was borne by the river much farther than in customary cases. Some doses

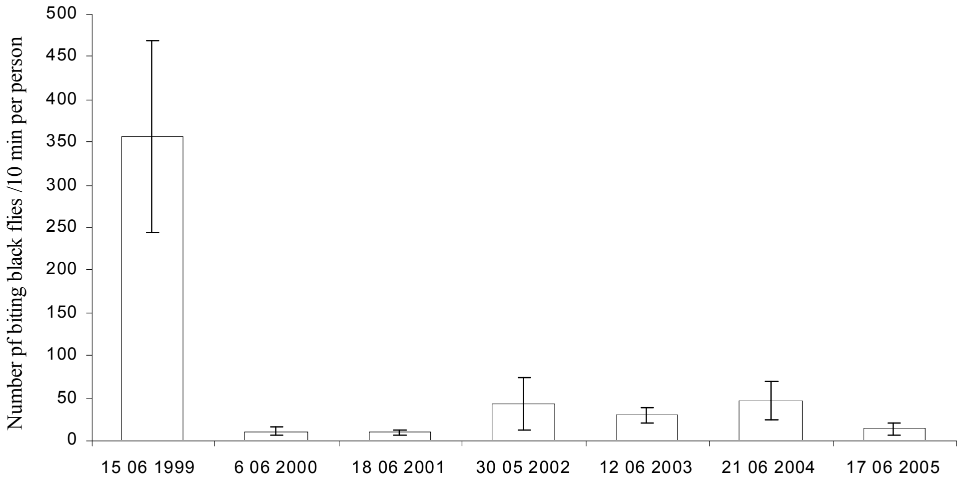

Fig. 4. The number of *Simulium (B.) maculatum* Mg. attacking a person per 10 minutes in days of their maximal activity in south-eastern Lithuania.

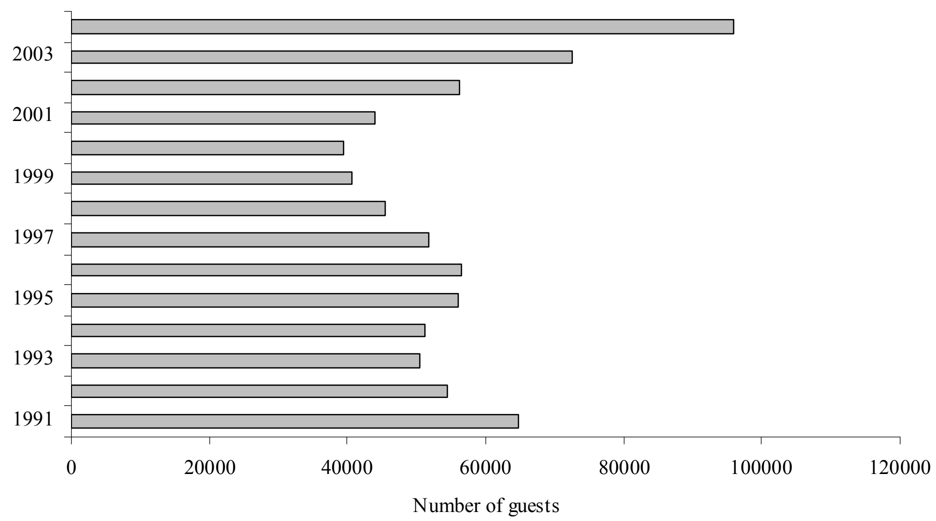

Fig. 5. Yearly number of guests in the Druskininkai health-resort town (data of Druskininkai Tourism & Business information Centre)

of the biological larvicide gradually began reaching the banks and water vegetation with black fly larvae on it, and the bulk of the biological larvicide continued to drift downstream decreasing gradually. However, should we attempt to disperse the biopreparation across the river to get a sudden effect, it would mostly settle in the bank reaches of a few nearest kilometres.

Environmental effects

Bacillus thuringiensis had no effect on earthworms (Oligochaeta), leeches (Hirudinea), molluscs (Gastropoda: Bivalvia), crustaceans (Crustacea: Malacostraca), and larvae of water insects: dragonflies (Odonata), mayflies (Ephemeroptera), caddis flies (Trichoptera) in the Nemunas segment treated by the biopreparation (Bernotienė, 2001). The biopreparation could affect the number of Diptera larvae. Those of the families Tipulidae, Limoniidae, Ceratopogonidae, Dixidae, Ephydridae, Stratiomyidae were found before and after the application in the Nemunas River. Their number was

Table. **Elimination of *Bacillus thuringiensis* bacteria from the environment under the effect of VectoBac 12AS bacterial preparation in 2001**

River, locality	Object of study	Average number of *B. thuringiensis* colonies in 1 ml of suspension					t	p
		before treatment	2 days after treatment	12 days after treatment	no treatment	37 days after treatment		
Nemunas, Varviškė	plants			1.7 ± 1.19				
	black fly larvae			6.2 ± 1.04				
Nemunas, Gerdašiai	plants	0	11.0 ± 2.08			0		
	black fly larvae	5.2 ± 0.80	21.8 ± 1.28			5.8 ± 0.64	18.24	<0.001
Nemunas, Merkinė	plants	0	9.3 ± 1.45			0		
	black fly larvae	5.8 ± 0.74	14.4 ± 1.44			4.8 ± 0.64	13.23	<0.001
Nemunas, Birštonas	plants	0	1.0 ± 1.83			0		
	black fly larvae	4.8 ± 0.74	7.0 ± 0.95			5.4 ± 0.93	4.37	<0.01
Neris, Rukla	plants				0			
	black fly larvae				4.0 ± 0.45			

too small to draw any conclusions. The biopreparation had no statistically significant effect ($p < 0.3823$) on benthic larvae of the most abundant family Chironomidae. The abundance of chironomids of the tribe Tanytarsini (cf. *Rheotanytarsus* sp.), abundantly colonising the same plant leaves as black fly larvae, decreased thrice during the first 10 days after treatment. The same process was noticed in the Neris River simultaneously, what may be associated with the pupation and flight of *Rheotanytarsus* in late May.

Microbiological investigations in the Nemunas River after employment of the VectoBac 12AS biological preparation showed that bacteria *Bacillus thuringiensis* var. israelensis present in this preparation were eliminated from all localities on aquatic plants in 37 days after its application. Only atonic amount of bacteria was isolated from insect larvae (Table). Thus, the VectoBac 12AS preparation is quite harmless to a water body because its active stuff (bacteria) is very quickly eliminated from the environment.

ACKNOWLEDGEMENTS

We are grateful to Algirdas Petkevičius and Pierre Bauer for assistance and valuable advice.

Received 15 June 2006
Accepted 20 October 2006

References

1. Bernotienė R. 2001. The effect of application of *Bacillus thuringiensis* var. *israelensis* (H 14) on bloodsucking blackflies (Diptera, Simuliidae) in Lithuania. *Norwegian Journal of Entomology*. Vol. 48. P. 155–120.
2. Bernotienė R. 2005. Long-term investigations of blackflies (Simuliidae) in the environs of the Čepkeliai state strict nature reserve. *Acta Zoologica Lituanica*. Vol. 15(2). P. 96–99.
3. Bluzmanas P. 1970. *Mikrobiologinė technika*. Vilnius: Mokslas.
4. Evenhuis 2004. http://hbs.bishopmuseum.org/aocat/aocathome.html
5. http://news.bbc.co.uk/1/hi/in_depth/sci_tech/2000/festival_of_science/914616.stm
6. Hutchinson R. 1999. www.roberth.u-net.com/blackfies.htm
7. Jensen F. 1984. A revision of the taxonomy and distribution of the Danish black-flies (Diptera: Simuliidae), with keys to the larval and pupae stages. *Natura Jutlandica*. Vol. 21 (6). P. 69–116.
8. Menon A. S, De Mestral J. 1985. Survival of *Bacillus thuringiensis* var. *kurstaki* in waters. *Water, Air and Soil Pollution*. Vol. 25. P. 265–274.
9. Molloy D. P. 1990. Progress in the biological control of black flies with *Bacillus thuringiensis israelensis*, with emphasis on temperate climates. In: Barjac H. de, Sutherland, D.J. (eds.). *Bacterial Control of Mosquitoes and Black Flies*. Rutgers University Press, New Brunswick, New Jersey. P. 161–186.
10. Riley C. M., Fusce R. 1990. Field efficacy of VectoBac-12AS and Vectobac-24AS against black fly larvae in New Brunswick streams (Diptera: Simuliidae). *Journal of the American Mosquito Control Association*. Vol. 6(1). P. 43–46.
11. Ross H. D., Merritt R. W. 1978. The larval instars and population dynamics of five species of black flies (Diptera: Simuliidae) and their responses to selected environmental factors. *Canadian Journal of Zoology*. Vol. 56(8). P. 1633–1642.
12. Rozendaal J. A. 1997. *Vector Control*. WHO. Geneva. P. 17–18.
13. Wilson M. D., Akpabey F. J., Osei-Atweneboana M. Y., Boakye D. A., Ocran M., Kurtak D.C., Cheke R. A., Mensah G. E., Birkhold D., Cibulsky R. 2005. Field and laboratory studies on water conditions affecting the potency of VectoBac® (*Bacillus thuringiensis* serotype H-14) against larvae of the blackfly, *Simulium damnosum*. *Medical and Veterinary Entomology*. Vol. 19(4). P. 404–412.
14. Zgomba M., Petrić D., Ćupina A., Konjević A., Marinković D. 2004. Application of *Bacillus thuringiensis* var. israelensis in control of *Simulium ornatum* Meigen, 1818 (complex) (Diptera: Simuliidae), the most abundant mammophilic blackfly species in the region of Novi Sad. T*he 3rd European Mosquito Control Association Workshop*. Osijek, Croatia. P. 23–24.
15. Zwick H. 1995. Contribution to the European blackfly taxa (Diptera: Simuliidae). *Aquatic Insects*. Vol. 17(3). P. 129–173.
16. Žygutienė M., Pakalniškis S. 1997. Upiniai mašalai (Diptera, Simuliidae) Lietuvoje. *Higiena ir epidemiologija*. T. 5(61). P. 21–24.
17. Янковский А. В. 2002. *Определитель мошек (Diptera, Simuliidae) России и сопредельных стран*. Санкт-Петербург. С. 570.
18. Лакин Г. Ф. 1980. *Биометрия*. Москва: Наука. 293 с.
19. Прудкина Н. С., Солодовникова В. С., Гусакова В. А. 1992. Массовое размножение мошек в восточной Украине. В кн.: Нарчук Э.П. (ред.). *Кровососущие и зоофильные двукрылые (Insecta: Diptera)*. Зооинститут АНР, С.-Петербург. С. 69–83.
20. Рубцов И. А. 1956. *Фауна СССР: двукрылые насекомые*. Москва–Ленинград. С. 853.
21. Рубцов И. А. 1978. Мошки как индикаторы загрязнения текущих вод. В кн.: Смирнов Н. Н. *Биологические методы оценки природной среды*. Москва: Наука. С. 141–151.

Ilona Bartninkaitė, Rasa Bernotienė, Saulius Pakalniškis, Milda Žygutienė

KRAUJASIURBIŲ UPINIŲ MAŠALŲ (SIMULIIDAE) POPULIACIJŲ GAUSUMO REGULIAVIMAS LIETUVOJE

Santrauka

Kraujasiurbių upinių mašalų gausumo protrūkis Pietryčių Lietuvoje prasidėjo XX a. aštuntajame dešimtmetyje. Kraujasiurbių mašalų puolimo intensyvumas kasmet augo ir iki 1990 m. tapo svarbia problema regiono, ypač Druskininkų kurorto gyventojams.

Biologinis preparatas, kurio aktyvųjį pagrindą sudaro bakterijos *Bacillus thuringiensis* var. *israelensis*, 1999–2005 m. panaudotas kraujasiurbių upinių mašalų populiacijų gausumui reguliuoti. Biologinis preparatas buvo paskleistas kasmet nuo kranto viename upės taške ir efektyviai veikė 164 km ilgio upės atkarpoje.

Raktažodžiai: mašalų gausumo reguliavimas, *Simulium maculatum*, *Bacillus thuringiensis* var. *israelensis*, Simuliidae, Lietuva