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ARTICLE

On the Biological Regulators of Bloodsucking Blackflies (Diptear: Simuliidae) of Mixed Forests of Belorussian and Ukrainian Woodlands

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ABSTRACT

This article considers the natural regulators of blood-sucking Simuliidae subzone of mixed forests on the territory of Belarus and Ukraine. In the mixed forests, the major regulators of preimaginal phases of bloodsucking blackflies are microsporidia (*Polidyspirenia simulii, Polidyspirenia sp., Thelohania fibrata, Amblyospora bracteata, A. varians*), fungi and mermithides (*Gastromermis boophthorae*). Caddisfly larvae (*Hydropsyche angustipennis, Neureclipsis bimaculata, Polycentropus flavomaculatus, Cyrnus flavidus, Oligostomis reticulata, Brachycentrus subnubilus and Rhyacophila nubila*) and fishes (*Scardinius erythrophthalmus, Rutilus rutilus, Carassius carassius, Gobio gobio*) significantly reduce the number of blackflies larvae and pupae. Adult blackflies are eaten by spiders (*Araneus diadematus*) dragonflies, robberflies, wasps, frogs (*Rana temporaria and Rana terrestris*), and insectivorous birds (*Delichon urbicum, Hirundo rustica, Apus apus*). Bactolarvicid and BLP-2477 are among the most effective biological products.

1. Introduction

The territory of mixed forests belongs to the densely populated regions of Europe, and therefore the protection of the environment is one of the important problems. The integrated control of bloodsucking blackflies includes environmental, biological, and chemical methods, and the chemical ones are predominant. The biological method of the bloodsucking blackflies' number regulation involves the use of their natural enemies — parasites, predators, and biologicals developed on the base of *Bacillus thuringiensis var. israelensis* bacteria (H_{14}). Attempts to control the number of simuliidae should focus on the biological method. Its rational combination with ecological and chemical methods will stabilize the entomological situation in areas of high human and animal concentration, and also improve the environmental situation.

2. Material and Methods

Biological regulators of the number of the bloodsucking blackflies were studied (2000-2020) in eight stations in the western, central and eastern parts of the Belarussian and Ukrainian Woodlands and by routing method on the territory of the region under study. The parasites and predators of the aquatic stage of development of simuliid species were collected, and the effectiveness of biological products was analyzed by conventional meth-

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ods by Rubzov [1, 2], supplemented by Kaplich et al. [3, 4], Voronin, Issy[5], Dubitskiy [6], Baba, Takaoka [7] Adler et al. [8]. Altogether 518 parasites (10915 parasitized larvae and pupae were investigated) and 540 predator samples were selected. Autopsy of aquatic organism intestines was performed: 1067 caddis fly larvae, 180 dragonflies larvae, 137 mayflies, 127 leeches, and 520 fish. Altogether 480 micropreparations of blackflies were prepared for microsporidiosis investigation, 150 — for fungal disease, 33 — for water mites. All quantitative parameters (indices of dominance — ID, frequency index — FI) were determined according to Beklemishev [9]. The indicators of relative abundance (mass, numerous, small, and solitary species) were adopted from those proposed by Skufjin [10] for horseflies. The separation of genera in this article accepted by R. W. Crosskey[11] and P. H. Adler [12].

3. Results

Currently, a lot of attention is paid to the regional study of parasites and predators, since each landscape-climatic zone is characterized by its own Diptera fauna and natural regulators of their population.

3.1 Parasites

The perspective of parasites used in the development of the biological control method is defined by the high specificity for the host, the ability to self-propagate in the host population, to preserve steadily in reservoirs and to limit the number of hosts to a low level without causing harm to helpful aquatic organisms.

In the study area the common mycosis was identified by family Microsporidea, which are found in 271 streams of different types (46.9%). More often microsporidia are found in irrigation ditches (21.9%). Infection by fungi of blackflies was registered in 154 basins (26.7%). Different bacteria infected the collected larvae of blackflies from 86 small rivers and drainage canals. The nematodes from family Mermithidae were found only in 38 basins (6.6%), because they are quite sensitive to water pollution. Water mites are found only in 16 streams and 12 small rivers (4.9%).

Infection by parasites was found in 18 species of 6 subgenera *Nevermannia, Eusimulium, Schoenbaueria, Wilhelmia, Boophthora,* and *Simulium* of genera *Simulium*, although 65 species inhabit the subzone of mixed forests.

We reported bacterial infection in the larvae of 10 species: *S. pusillum, S. nigrum, S. equinum, S. erythrocephalum, S. ornatum, S. dolini, S. noelleri, S. paramorsitans, S. promorsitans, and S. morsitans*. Bacterial infection was found in 14.9% of the streams surveyed. Bacteria are more rapidly developing in the blackflies that live in β-mesosaprobic streams (Table 1).

In the streams of the studied area, the death of blackfly larvae and pupae coated with thin hyphae of Saprolegnia fungi is registered (*S. pusillum, S. nigrum, S. erythrocephalum, S. dolini, S. noelleri, S. morsitans*). Most often pupae are affected, because the affected larvae die and are drifted downstream, while pupae remain attached to the substrate. The prevalence of the infection of blood-sucking blackflies by Saprolegnia fungi depends primarily on the weather conditions of the year. For example, early and warm springs, when the aquatic stage of development was completed quickly, caused the incidence of infection at the level 0.5-1.5% in the collected larvae and pupae. During cold and long springs the preimaginal development phase was longer, and the level of mycosis reached 3-4%. Larvae and pupae of blackflies can also be damaged by *Entomophthora muscae* and *Batrachochytrium dendrobatidis.*

Table 1. Relative abundance (%) of parasites in different types of streams

Pathogen	Stream	
	oligosaprobic	β-mesosaprobic
Bacteria	26.5	71.5
Fungi	30.8	69.2
mycosis of Microsporidea	31.6	68.4
Mermithidae	100	–
Water mites	73.8	26.2

Our collections of fungi contain division Zygomycota, class Zygomycetes, order Mucorales; division Oomycota, class Chytridiomycetes, order Blastocladiales, genus *Coelomomyces* (Figure 1). Fungal diseases have been found in 11 species of blackflies. Most often fungal infection is found in *S. erythrocephalum* (Index of dominance (ID) = 36.3), and *S. ornatum* (ID = 9.6). The lowest infection rate was recorded in *S. volhynicum* (ID = 1.3) and *S. aureum* (ID = 0.7). Larvae and pupae, infected with fungi, were found in medium and small rivers, streams and irrigation ditches, throughout the warm season, with peaks in June (34.3%) and September (38.7%). Fungi developed intensively in blackflies that live in slightly polluted (mesosaprobic) streams (FI = 69.2%), overgrown with phreatophyte (the deep routed plants), in areas with a small flow rate (0.2-0.4 m/s). The incidence of infection was on average 14%.

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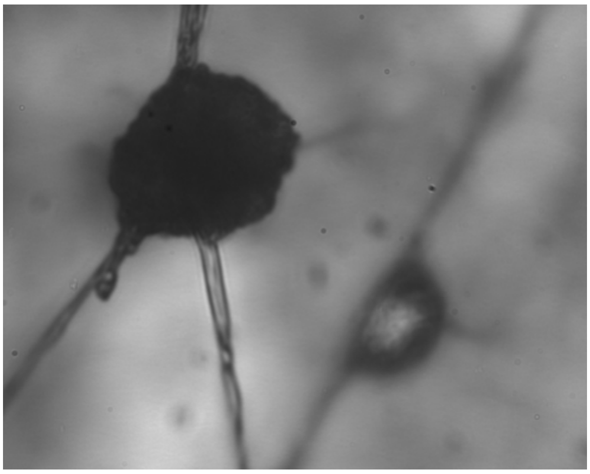

Figure 1a. Mycosis of blackflies (x1630): fungi from the division Zygomycota, order Mucorales

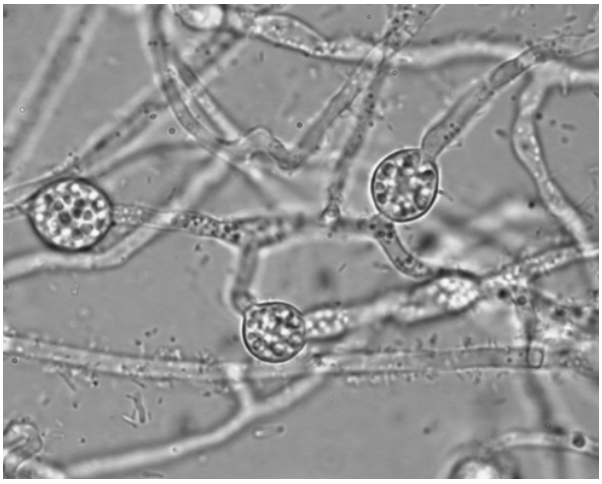

Figure 1b. Mycosis of blackflies (x1630): fungi from the division Oomycota, order Blastocladiales, genus *Coelomomyces*

One of the important regulators of the numbers of larvae is microsporidia. The incidence of infection was about 3-50%. In the studied area the incidence of infection of larvae ranged from 3-8% in the early spring to 3-40% at the end of the summer.

Microsporidia have been found in 18 species of blackflies. Most often they infected larvae of *S. erythrocephalum* (FI = 19.3, ID = 28.3), *S. ornatum* (FI = 9.4, ID = 14.2), *S. pusillum* (FI = 8.4, ID = 12.4). The larvae resistant to microsporidiosis were *S. angustipes* (FI = 0.4, ID = 0.5) and *S. noelleri* (FI = 0.5, ID = 0.8).

Larvae of blackflies were infected with 5 species of microsporidia from 3 genera: *Polidyspirenia simulii* (Lutz, Splendor, 1904), *Polidyspirenia sp., Thelohania fibrata* Strickland, 1913, *Amblyospora bracteata* Strickland, 1913*, A. varians* Leger, 1897. They are located in the fat body of the larvae (Figure 2).

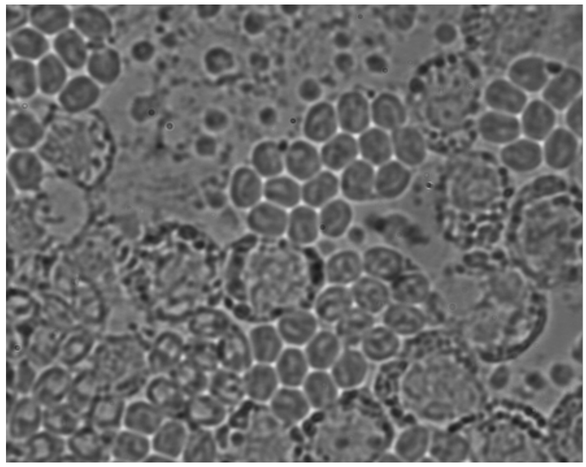

Figure 2a. Microsporidia mycosis in the blackflies (x1630): *Amblyospora varians* from the *S. volhynicum* larvae

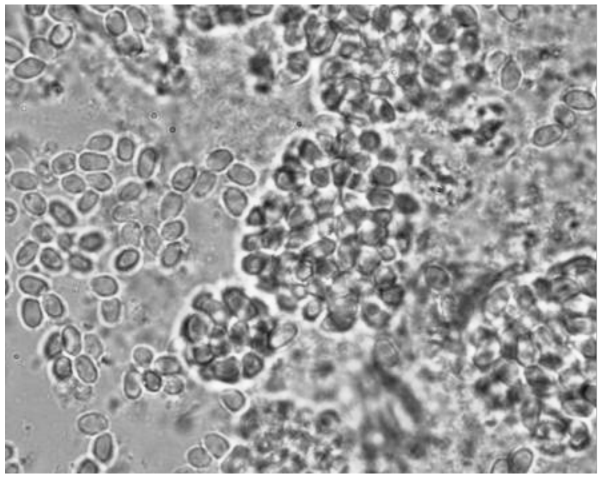

Figure 2b. Microsporidia mycosis in the blackflies (x1630): *Polidyspirenia simulii* form the *S. erythrocephalum* larvae

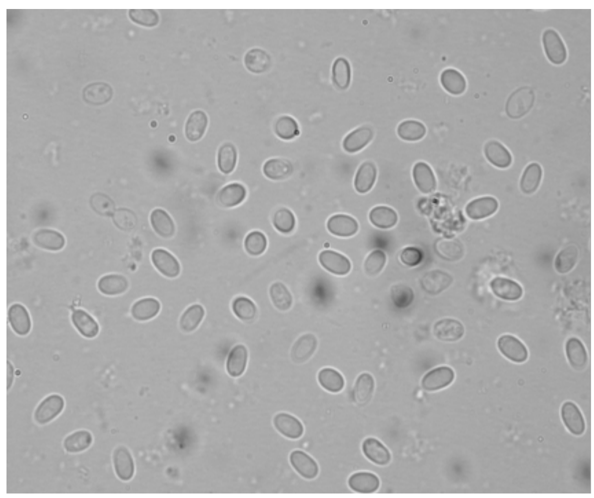

Figure 2c. Microsporidia mycosis in the blackflies (x1630): *The lohania fibrata* from the *S. erythrocephalum* larvae

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The degree of larvae invasion by microsporidia depends primarily on the conditions of the environment. In middle-sized rivers during the summer period two increases of larvae infestation can be traced - in spring (3rd decade of May - 1st decade of June) and in late summer (3rd decade of July – 2nd decade of August). The largest percentage (till 50%) of infected larvae is observed in the second half of the summer, due to the deteriorated environmental conditions. Usually, the flow velocity of rivers and the content of dissolved oxygen decrease at this time, siltation of water occurs, and the channel bed is overgrown by flora. Therefore, the conditions are favourable for larval reinfestation.

Three species of microsporidia — *T. fibrata* (FI = 40.9), *P. simulii* (FI = 25.0), and *A. bracteata* (FI = 22.6) are widespread, sometimes *A. varians* (FI = 1.4) are observed.

Microsporidia mycosis, as well as other species of parasites, prefer to live in slightly polluted streams. The incidence of microsporidia in mesosaprobic reservoirs is 68.4%.

Larvae infected with microsporidia were detected in all types of streams, in different generations of blackflies throughout the year, with a peak in spring (3rd decade of May) and the second half of the summer (1st decade of August). In mid-summer on average 15–25% of larvae were infested by microsporidia. Sometimes blackflies infested with larvae were found in winter. Until the end of April the extensiveness of larvae invasion reached 3–10%. The highest infestation of simuliidae was recorded in late summer due to the reinfection of larvae population. Larvae infected by microsporidia were not able to pupate and died in autumn.

The outbreaks of mermithids-infestation were registered in all oligosaprobic streams, but compared to other parasites they are less common. Infestation of larvae with *Gastromermis boophthorae* (Welch & Rubzov, 1965) was observed in seven species of blackflies. The incidence of blackfly infection was low (1–14%). The intensity of infection was 1–2 parasites on the host organism. Infected larvae were found from July to September, with a peak in August (54%). Populations of mermithids developed only in clean streams, not contaminated by industrial and agricultural waste.

Usually, these streams have rocky and sandy or peat and sandy beds that are overgrown with phreatophyte, where the flow rate varies from 0.3 to 0.6 m/s when the dissolved oxygen content of the water is 75–97% and water temperature is +3 – +21°C. Free mermithids are found in flowing water reservoirs with temperatures of +11 – +13°C. We registered the case of joint parasitism of microsporidia *P. simulii* and a mermithid *G. boophthorae* in larvae of *S. erythrocephalum.*

Water mites are parasitic on pupae of blackflies [13]. In the streams studied in May water mites *Sperchon setiger* were found (Thor, 1898) on pupae of *S. noelleri, S. dolini,* and in the 3rd decade of June — on larvae of *S. morsitans.* The bottom of streambeds, habitated by affected simuliidae, is stony and sandy, along the banks — muddy, with water flow velocity till 0.35 m/s, dissolved oxygen content 73–80% and temperature +14 — +18°C. Substrate for the mites were stones, branches at a depth of 0.25 m. Water mites were found mainly (73.8% of all registered ones) in oligosaprobic streams. Their oviposition was also observed here, from 9 to 28 eggs. Eggs were oval, tightly attached to the substrate, and covered with a transparent top spider cocoon. The colour of the eggs as they matured varied from white to red.

3.2 Predators

In mixed forests the predators of preimaginal phases of blackflies are the larvae of caddisflies, mayflies, dragonflies, leeches, and fish. The main predators are the following caddisfly larvae: *Hydropsyche angustipennis* (Curtis, 1834), *Neureclipsis bimaculata* (Linnaeus, 1758), *Polycentropus flavomaculatus* (Pictet, 1834), *Cyrnus flavidus* (McLachlan, 1864), *Oligostomis reticulata* (Linnaeus, 1761), *Brachycentrus subnubilus* (Curtis, 1834), and *Rhyacophila nubila* (Zetterstedt, 1840).

Caddisflies live in all types of streams and can partially tolerate their contamination. Eggs and fragments of larvae of 6 subgenera (*Wilhelmia, Nevermannia, Eusimulium, Schoenbaueria, Boophthora, Simulium*) were found in the intestines of 751 blackflies (70.4%) from the 1067 caddisfly larvae studied.

No food preferences of caddis to certain types of blackflies were found. In their intestines, the ratio of of blackflies species typical for a certain period in the stream remained approximately the same. Of the total number of larvae and pupae of blackflies found in the guts of predators, caddisflies accounted for 52%.

The attack of caddis larvae on the larvae and pupae of blackflies is characterized by a certain seasonal pattern. They feed on blackflies more intensely in spring (58%), and sometimes in winter (18%), and less intensely in summer (17%) and autumn (7%). This pattern is explained by the dynamics of the number of larvae simuliidae: in spring their density (2,200 flies/dm^2 on average) is significantly higher than in the autumn (350 flies/dm^2) and in winter (54 flies/dm^2).

Comparing the average number of larvae of blackflies in stream per area unit with their population in the intestines of caddisflies, one can conclude that caddisflies are able to reduce the number of preimaginal phases of simuliidae to 5.1%.

The larvae of mayflies are also regulators of the number of blackflies that inhabit small clean ponds and streams. In the 137 mayflies found in the intestines of 48 (35%) *Isonychia ignota* (Walker, 1853) and *Ecdyonurus venosus* (Fabricius, 1775), the simuliidae eggs were represented in large quantities (from 3 to 20 pcs. in one specimen) as well as fragments of larvae of subgenera *Nevermannia, Eusimulium, Schoenbaueria, Boophthora*, and *Simulium*. Comparing the average number of larvae of blackflies per area unit of the stream with a population in the intestines of mayflies, one can conclude that mayflies are able to reduce the number of preimaginal phases of simuliidae to 0.2%.

Dragonfly larvae live in medium and small rivers, and in drainage canals. They feed on the larvae of blackflies mainly in summer. Following the study of 180 dragonfly larvae, residues of 13.7% blackfly larvae were found in the intestines of 124 individuals (69%). Head capsules and other body parts of larvae of subgenera *Wilhelmia, Nevermannia, Boophthora*, and *Simulium* were found in the intestines of three species of dragonflies (*Calopteryx splendens* Harris, 1780, *Aeshna cyanea* Muller, 1764, *Libellula depressa* Linnaeus, 1758).

It was found that larvae of dragonflies eat up to 1.1% of the larvae of blackflies that live on 1 m^2 of pond area.

Hemolymph of larvae of blackflies is “sucked” by predatory bugs Nepa cinerea (Linnaeus, 1758). They were found mostly in spring (April-May) on underwater objects and plants where the larvae of simuliidae are attached or ovipositions are located. Likewise, these bugs feed on the larvae of mayflies and caddisflies.

Leeches *Erpobdella octoculata* Linnaeus, 1758 and *Haemopis sanguisuda* Linnaeus, 1758, inhabiting streams with varied degrees of contamination and flow velocity, fed on larvae and pupae of blackflies, too. Of the 127 leeches studied, the residues of blackflies were found in 52 individuals (41%), accounting for 5.4% of the larvae found in predators.

In the gut of leeches 311 fragments of larvae of simuliidae (5.4%) were registered, among them subgenera *Boophthora* (4.1%), *Nevermannia* (0.9%), and *Eusimulium* (0.4%). Leeches can reduce the number of blackflies by approximately 0.5% per 1 m^2 of stream.

Our observations indicate that fish are one of the most important regulators of the numbers of simuliidae. 520 individual fish from 4 species (*Scardinius erythrophthalmus* Linnaeus, 1758, *Rutilus rutilus* Linnaeus, 1758, *Carassius carassius* Linnaeus, 1758, *Gobio gobio* Linnaeus, 1758) were investigated. Larvae and pupae of blackflies were found in the stomachs of 250 individuals (48%), which is 23.4% of all simuliidae found in predators. In medium rivers rudd (*S. erythrophthalmus*) and gudgeon (*G. gobio*) are found. The predominant fragments of larvae are from subgenera *Boophthora*, *Wilhelmia*, and *Simulium*. In general, in the intestines of these fish 9.5% of all blackflies, which died from predators, were found.

Adult blackflies are eaten by dragonflies, robberflies, wasps, swallows (*Delichon urbicum* (Linnaeus, 1758), *Hirundo rustica* Linnaeus, 1758), and swifts (*Apus apus* Linnaeus, 1758). Cases of assault by predatory flies of genes *Xenomyia* and *Ochthera* on adult blackflies were described [14]. Deaths of blackflies from spiders are registered. Their trapping grids contained between 1 and 12 of simuliidae females. For example, in the trapping grid of diadem spider (*Araneus diadematus* Clerck, 1757) in a pine forest 7 females of *S. erythrocephalum* were registered.

Amphibians also hunt simuliidae. Throughout the day, more often at the beginning (7 hr.) and at the end (19-21 hrs.) of blackfly flight, when humidity is increased, they become the bag of herbal (*Rana temporaria* Linnaeus, 1758) and moor (*Rana terrestris* Andrzejewski, 1832) frogs.

4. Biologicals

The usage of biologicals today is virtually the only method that allows for the effective control and regulation of the number of bloodsucking insects larvae in streams of economic importance, although the specificity of biologicals to a particular group of insects limits the scope of their use [15,16,17,18] .

The comparative analysis of the efficiency of stream delarvation by the three biological agents (bactoculicid, bactolarvicid, and BLP-2477) showed that the control of the number of preimaginal blackflies is achieved most effectively by BLP-2477 and bactolarvicid. In drainage canals of up to 1.5 km length, up to 2 m width, 0.5 m depth, and a flow rate of 0.7 m/s, the optimal dose for the death of simuliidae larvae is 400 grams of the drug. The processing of streams by biologicals is best to be performed in late April — early May in the southern zone, and in May — early June in northern areas by the progressive introduction of the drug in the stream. The use of chemicals in rivers and major drainage canals to destroy the aquatic stage of development of simuliidae is believed to be impractical because there is pollution of drinking

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water, which kills useful aquatic species, whose number is recovering slowly.

5. Conclusions

The principal biological regulators of preimaginal phases of blood-sucking blackflies in the investigated region are microsporidia, caddisfly larvae and fish, while those of adult blackflies are insectivorous birds.

All species of parasites are more rapidly develop in the blackflies living in β-mesosaprobic streams. The incidence of fungal infection (Mucorales and *Coelomomyces*) was on average 14%. Infection with microsporidia (*Polidyspirenia simulii, Polidyspirenia sp., Thelohania fibrata, Amblyospora bracteata, A. varians*) reaches 50% in the second half of the summer. The mermithids-infestation *Gastromermis boophthorae* of blackfly was low (1-14%). The intensity of infestation was 1-2 parasites on the host organism.

Caddisflies (*Hydropsyche angustipennis, Neureclipsis bimaculata, Polycentropus flavomaculatus, Cyrnus flavidus, Oligostomis reticulata, Brachycentrus subnubilus u Rhyacophila nubila*) are able to reduce the number of preimaginal phases of simuliidae to 5.1%. Dragonfly larvae (*Calopteryx splendens, Aeshna cyanea, Libellula depressa*) eat up to 1.1% of blackflies larvae. Mayflies (*Isonychia ignota, Ecdyonurus venosus*) are able to reduce the number of preimaginal phases of simuliidae to 0.2%. Leeches *Erpobdella octoculata* and *Haemopis sanguisuda* can reduce the number of blackflies by approximately 0.5%. Fishes (*Scardinius erythrophthalmus, Rutilus rutilus, Carassius carassius, Gobio gobio*) are able to reduce the number of preimaginal phases of simuliidae to 9.5%.

Among biologicals, BLP 2477 and bactolarvicid have shown the highest larvicidal efficacy.

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