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Black Fly Immature Stages Control Efficacy (Diptera: Simuliidae) by Means of Bti Preparations

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Abstract

In the Valencian Autonomous Region, eastern Spain, for approximately 20 years, there has been a constant increase in populations of Black flies (Diptera: Simuliidae). This observation has been evidenced mainly by the medical reports from health centers and hospitals. Due to the inconvenience and damage caused by this insect to the human being, one of the municipalities most affected by the simuliids was chosen, to verify the effectiveness of Bti preparations on immature black fly states. In this article, the results of this scientific experience are presented and its high efficiency in the control of the immature populations of this harmful arthropod is corroborated.

Keywords: *Simuliidae, Control Efficacy, Bacillus thuringiensis variety israelensis, East of Spain.*

1. Introduction

The population control of some species of simuliids, the ones with hematophagy, is directed to the reduction or elimination of the contact between females and their hosts [1]. The adult state of the simuliids represents a great challenge when it comes to keeping their populations below a certain size, which do not reach the threshold of damage or discomfort. This casuistry is mainly due to their behavioral habits that include their possible dispersion over long distances, which makes a focused, controlled and effective treatment difficult, and which, if carried out, only turns out to be effective in a limited temporally and geographically [1].

On the contrary, the immature states of this nematocerous diptera, show aggregation patterns, as a result of the females of some species laying their eggs together. In fact, even the larvae and pupae stages can be found gathered and held on a concrete substrate with a notable increase in their population density. These situations facilitate the treatment of those larval populations whose size make them be classified as a plague. Water is an environment that allows them to be bound in a physical place, a circumstance that facilitates the fight against this type of arthropod that cause discomfort directly and indirectly to both wild and domestic animals, as well as to humans.

Therefore, black fly control can be more effective when it is mainly based on the elimination of the largest possible number of larvae in their aquatic environment, in order to avoid as far as possible the appearance of adults, the harmful state.

Originally simuliid chemical control was carried out using non-specific synthetic insecticides such as dichlorodiphenyltrichloroethane (DDT) or more specifically 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane, whose formula is (ClC6H4)2CH(CCl3), an organochlorine compound. DDT was a second-generation insecticide synthesized in 1874 and used for the first time against simuliids in 1944 in Guatemala [1]. Subsequently it was confirmed that it not only caused the death of insects harmful to the crops for which it was intended, but also affected predators and parasitoids of those same species, as well as other insects beneficial to humans. After it being found that some insects had managed to develop resistance to this product, together with the confirmation of the harmful effects that this product caused in animal and human reproduction, DDT was replaced in the late 1960s by methoxychlor, another organochlorine but ecologically more acceptable [2]. Finally, and after innumerable negative evidence (slow conversion into non-toxic substances, average period of active latency of eight years, accumulative in adipose tissue due to its high lipid solubility and low water solubility, which did not favor elimination and urine excretion, etc.), the United States Environmental Protection Agency (EPA) banned the use of DDT in 1972 and it was excluded from the list of authorized active substances. In Spain, DDT was widely used as a pesticide from the mid-1950s to the mid-1970s, and to a lesser extent later, although the ban on its use became effective in 1977.

From the early 1980s to the present, the fight against black flies has been carried out through the use of a non-persistent and non-polluting biological control agent, the *Bacillus thuringiensis* Berliner 1915 variety israelensis serotype H-14 [2; 3] widely known by the acronym (Bti). It is a strict aerobic gram positive bacterium, which has been found to be specific for several families of diptera, lepidoptera and nematoda, but it does not pose a danger to other vertebrate or invertebrate animal species [2; 4], but against simuliids it is highly effective, causing high mortality in the larval preimaginal stage. This bacillus reproduces by means of the formation of spores. As a result of sporulation, protein crystals called delta endotoxins (δ-endotoxins) are produced, which are released when the bacteria dies and cell walls break down then they are ingested by the larvae affecting to them. The symptoms they cause are the interruption of nutrient intake, gut paralysis, regurgitation, total paralysis and finally death [5]. The action mechanism of these proteins are a process that consists of the formation of soluble crystals, protoxin processing, receptor binding, membrane insertion, aggregation, pore formation and cytolysis.

The larvicide treatment with Bti is usually accompanied by physical and/or mechanical preimaginal control procedures [1] such as the removal of riparian vegetation in contact with water or algae in the center of the riverbed, which are substrates that support them [6]. Another method consists of altering the water flow of the basin in question by opening and closing the doors of dams, reservoirs and swamps. Since the temporary reduction of both level and water flow induces the larvae to release themselves from the substrate in search of more optimal areas for feeding, the restoration of the normal channel entails the elimination of larvae by current dragging. Therefore, biological and physical or mechanical treatment are complementary strategies [1].

Likewise, the lotic environment, in which the larvae live, is of great help since the linear confinement of these in the streams, torrents and rivers restrict the target area where the insecticide has to act, since it is necessary to be applied at a few strategic points [1]. In addition, the flow ensures that the insecticide product is transported over long distances by the current and reaches in this way the areas where fixed larvae are.

The different larval phases are susceptible to very low doses of larvicide. However, frequent applications of the product are necessary since the period of the juvenile stage is short, usually between seven and ten days, and because the insecticide has no effect either on egg or on pupa stages [1].

The main active matter of larvicides is based on the use of the Bti bacteria, whose success is due to its ability to produce spores and protein crystals. These, when ingested by the larvae after filtering the water, damage the diptera intestinal wall inducing death in a short space of time [1].

In Valencian Autonomous Region, for approximately 20 years, there has been a constant increase of Simuliidae populations. This fact has been evidenced mainly by the medical reports from health centers and hospitals [7]. These make mention of allergic reactions and inflammatory processes caused by the female bites of certain species with hematophagous habits. The cited reports were from citizens living in human populations located near the watercourses which simuliids inhabit.

A representative example of this problem that affects many municipalities of Valencian Autonomous Region is the one from the town of Villalonga, a Valencian town of 4,350 inhabitants, located at 120 m altitude and on the banks of the Serpis River. This river runs along 50 km between the towns of Alcoy (Alicante) and Gandía (Valencia). Moreover, a great abundance of simuliid species have been registered along its route, whose populations, especially between May and September, can become very significant. Therefore, lesions demanding hospital assistance in the population make it possible to consider black flies with human tropism as a Public Health interest species. Control is carried out by means of biological treatments based on the use of (Bti), with the objective of minimizing the sanitary risks that they can cause.

The main objective has been to reduce simuliid populations to tolerable levels to minimize health risks and minimize citizen complaints, mainly during the summer months, through a Bti treatment program in the municipality of Villalonga.

In order to carry out effective control of simuliid populations, it is essential to catalog both active and potential breeding points. That is, those where the presence of this diptera is verified. When planning a vector control campaign, the duration of its life cycle is taken into account. It depends on the local area and on the season, which will be determined by weather factors, especially temperature, among others. Therefore, the simuliid species must be identified, as well as its spatial distribution and density studied, what is more, the biological cycle must be known and a specific and selective insecticide must be selected so as to limit the impact on environment and public health, always choosing the most suitable application system.

What this scientific work has achieved demonstrates the great effectiveness of this type of biological treatment over pre-imaginal simuliid populations.

2. Material and methods

To carry out the simuliid control program, the sampling stations were analyzed both before the treatments to identify species and their population size, as well as afterwards, in order to assess effectiveness.

After locating several breeding points, four stations close to the municipality were chosen because of two reasons. Firstly because their high human population density and secondly due to their potential leisure characteristics such as bathing, hiking, cycling or because of agricultural activities such as the citrus terrace growing, as well as horticultural crops. The nomenclature of the places under treatment, following the water direction to the mouth is as follows: station 1, station 2, station 3 and station 4 (Fig. 1 a) and b)).

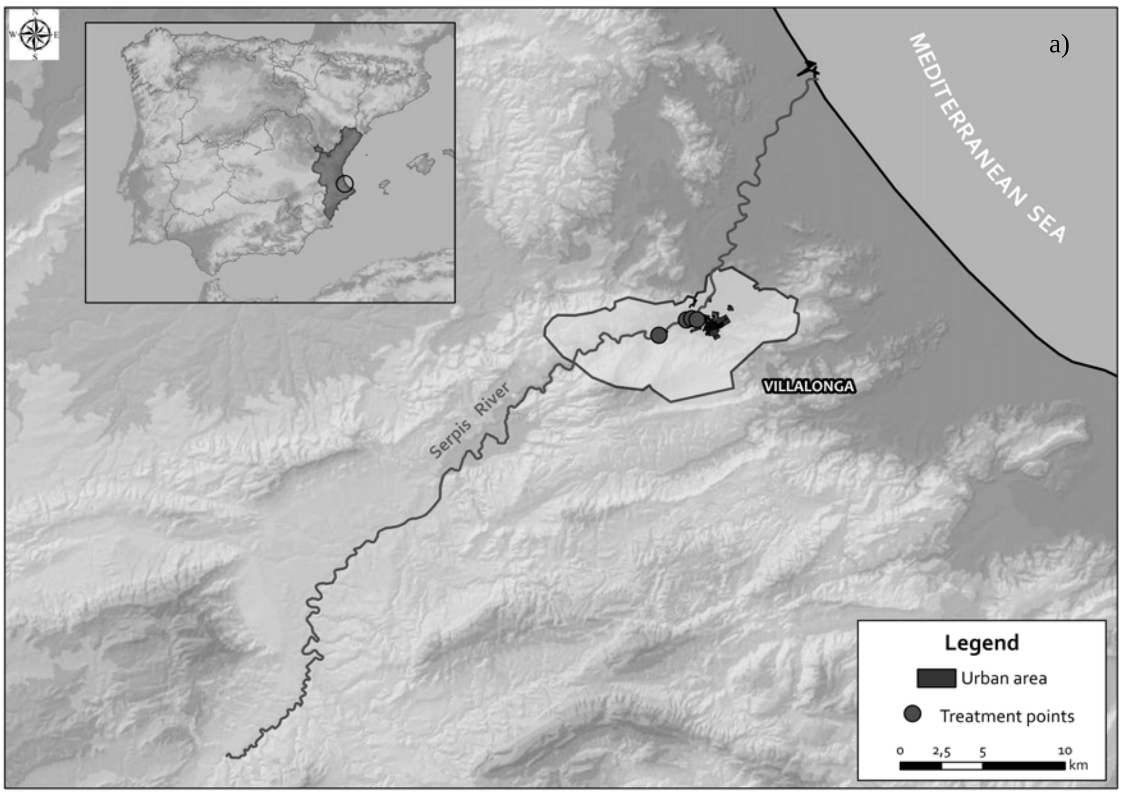

Fig.1 a) Area were the study has been done.

Previously, in each of them, the species present were determined and the population size was estimated. Then, the biological treatment of larvicidal action was applied, using preparations of *Bacillus thuringiensis*. This was always carried out uniformly by specialized and qualified staff during successive treatments and at the appropriate time, that is to say, during the early larvae development stages where the product is most effective for its target. Specifically, the product known under the trade name of Gnatrol SC was chosen. This product is considered as environmentally friendly and, therefore, its effect on pollinators, humans or wildlife in general is minimal or non-existent.

Every application was made by using pre-pressure spraying equipment, with the authorized dose. After the inspection of 4 treatment points, a total number of nine treatments were applied separated from each other for a period of time recommended by the product manufacturer (Fig. 1b).

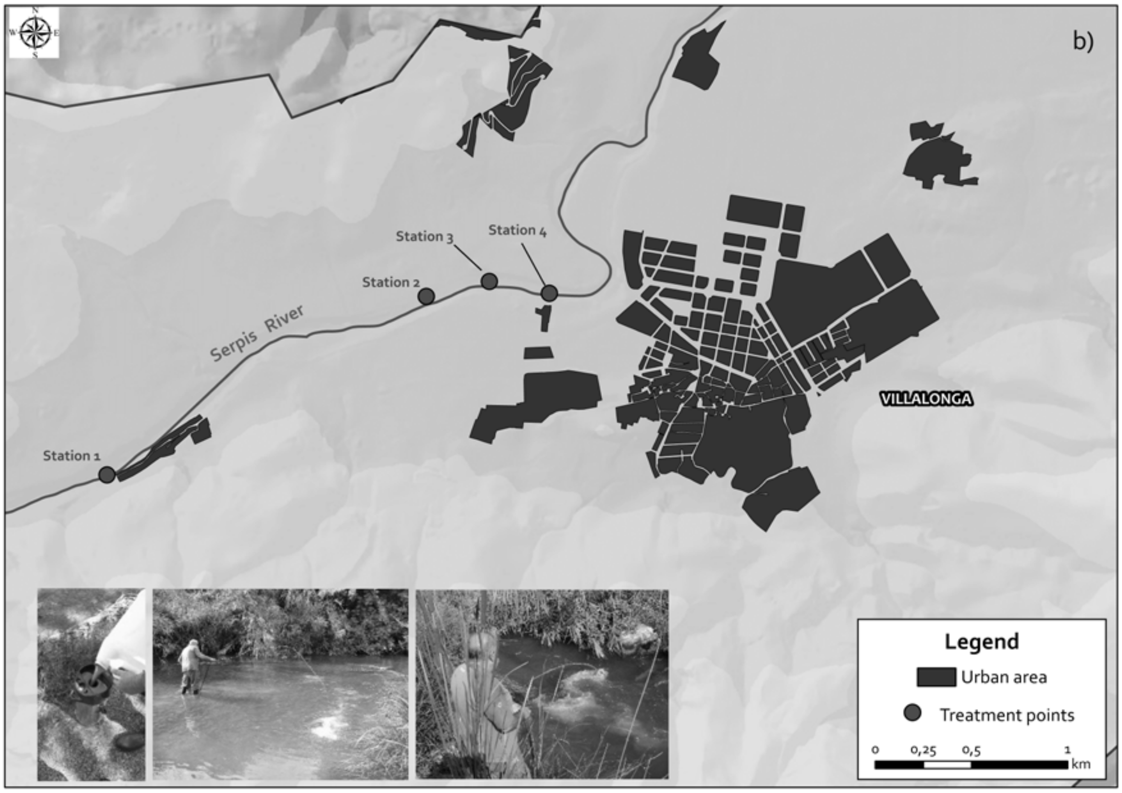

Fig. 1 b) Treatment and control points, used instruments set and applying methodology.

3. Results

The first treatment was carried out on April 24, 2014, evaluation was carried out one month after the application of the product. This verification revealed a practically zero effectiveness in all the points chosen for this purpose, since the populations of the different species present, prior to the treatment, had increased instead of decreasing as expected. Analyzed by station: in station 1 the three initial species continued, in station 3 the same casuistry occurred, but with the appearance of two new species: *S. angustitarse* (Lundström, 1911) and *S. pseudequinum* Séguy, 1921, even though in extraordinarily low population sizes. In station 2 the same situation took place as in the previous one, and in station 4 the same species remained, although the species *S. pseudequinum* had appeared.

Therefore, the time between treatments was adjusted, reducing from one month to fifteen days. After the review of the second treatment of May 23, 2014, the results gave positive and very satisfactory data. The populations had disappeared at all application points except at station 1 where a specimen was detected in a pupa stage.

After the apparent effectiveness, treatment application continued biweekly and not monthly. However, after the review carried out after the third treatment on June 4, 2014, low populations were found in three of the four stations, it was monospecific in stations 2 and 3, and two species in station 1.

After the review of the fourth treatment on June 18, 2014, the same result was obtained, although with a greater reduction in blackfly populations. In fact, in station 3 the simuliids disappeared completely and, in station 1, it went from two species to just one.

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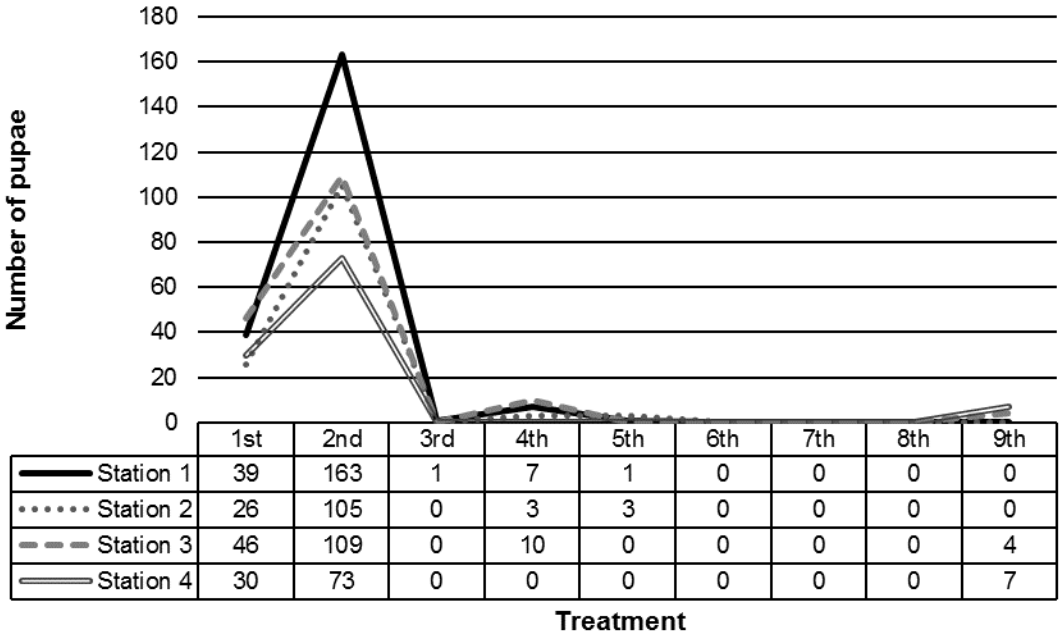
However, from the fifth treatment up to the eighth one (June 30, July 16, July 30 and August 14, 2014 respectively), all revisions were negative for the presence of this diptera. No trace of larvae phases or pupae were found in any of the observed substrates.

Finally, the review carried out after the treatment of September 3, 2014, which concluded the planned program, revealed simuliid absence, except in station 3 where three specimens of *S. sergenti* Edwards, 1923 and one of *S. velutinum* (Santos Abreu, 1922), as well as in station 4 where an individual of *S. pseudequinum*, five of *S. sergenti* and one of *S. velutinum* were counted. In any case, the populations were scarcely present and without great importance since they were within threshold levels that did not cause problems for citizens or animals.

In August the treatments were interrupted, ending in this way the pre-stablished program with the absence of discomfort and annoyance for citizens. A year later, we returned to the treated areas and we found that these places had been colonized again by the same species that were found in the first instance, as well as some new ones such as *S. petricolum* (Rivosecchi, 1963), *S. tuberosum* (Lundström, 1911) and *S. velutinum*.

Analyzing the evolution in each treatment point, the data allow us to affirm the absence of preimaginal states of larvae and pupae in all the control points from the fifth treatment to the ninth. In addition, in some others the absence was registered from before the fifth treatment, as in station 4, where from the third treatment no immature blackfly stages were found, a fact that can be observed globally in Fig. 2.



	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Station 1	39	163	1	7	1	0	0	0	0
Station 2	26	105	0	3	3	0	0	0	0
Station 3	46	109	0	10	0	0	0	0	4
Station 4	30	73	0	0	0	0	0	0	7

Fig. 2 Population size variation calculated by adding the total number of pupae of all simuliid species present in each of the four areas through the nine applied treatments.

In addition, as the treatments and subsequent revisions were carried out to verify whether they were effective or not, the generalized trend could be observed in every one of the places chosen for this purpose; this was a gradual population decrease of the different species at each application point, until they reach their complete disappearance (Fig. 3, 4, 5 and 6).

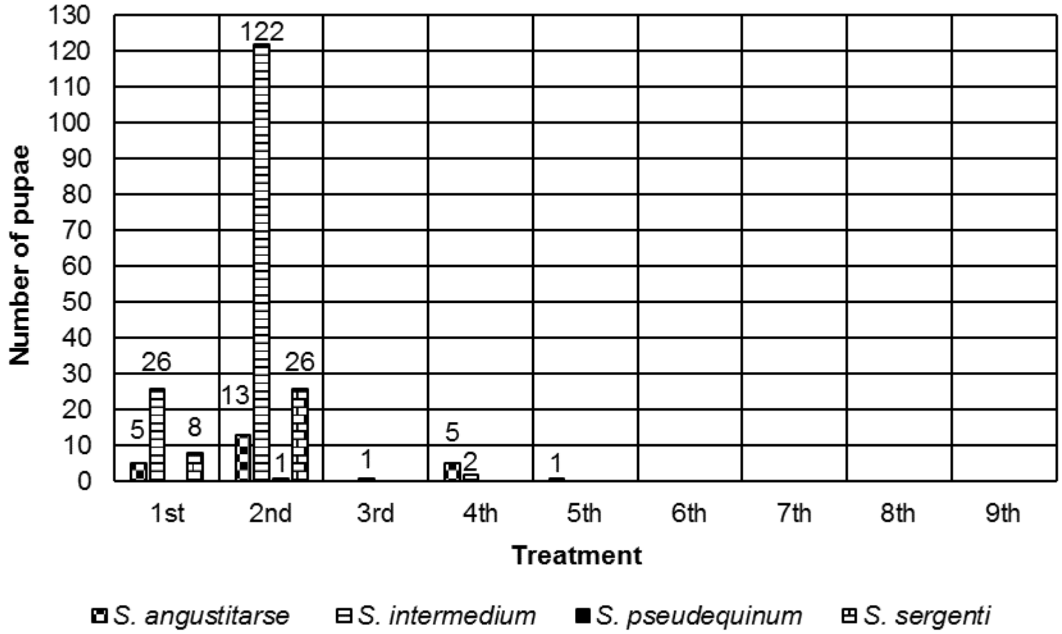


Fig. 3 Population fluctuation of simuliid species in station 1 after the different treatments carried out.

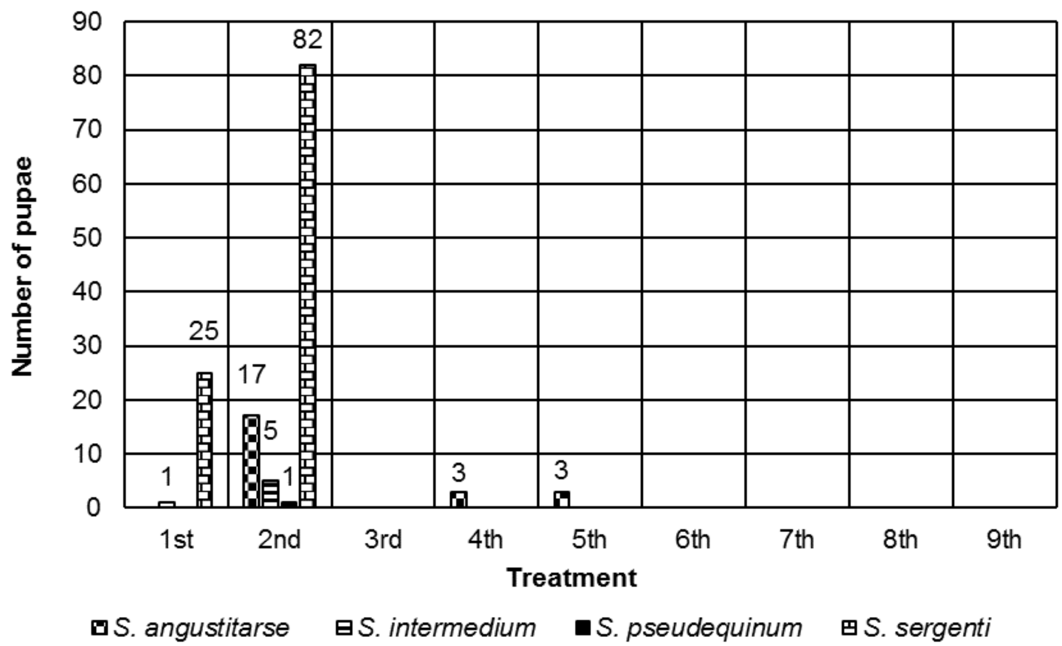


Fig. 4 Population fluctuation of simuliid species in station 2 after the different treatments carried out.

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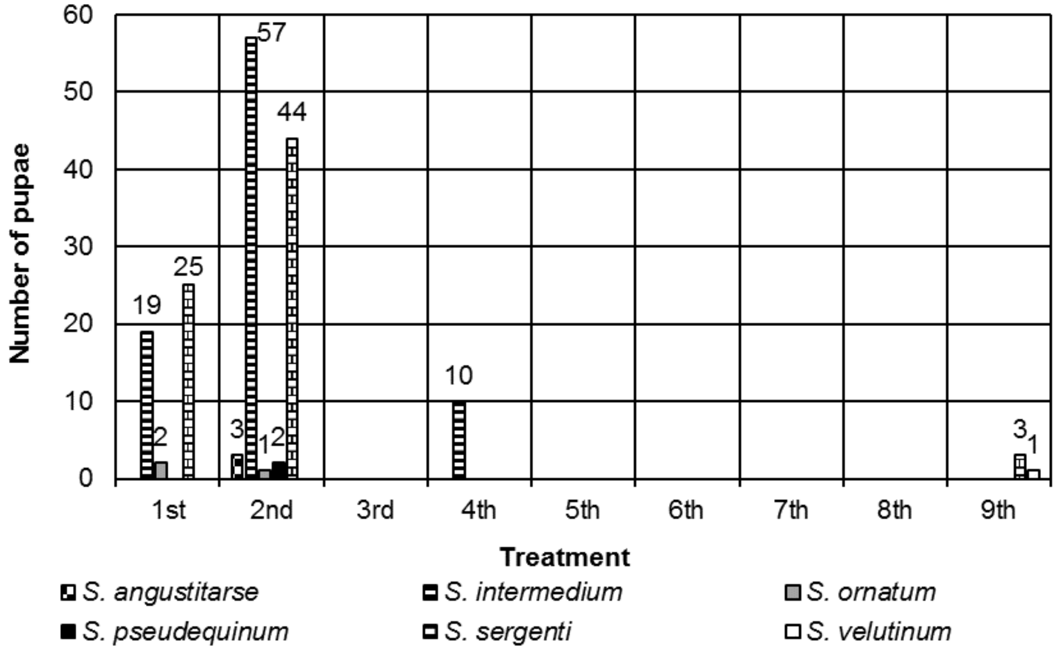

Fig. 5 Population fluctuation of simuliid species in station 3 after the different treatments carried out.

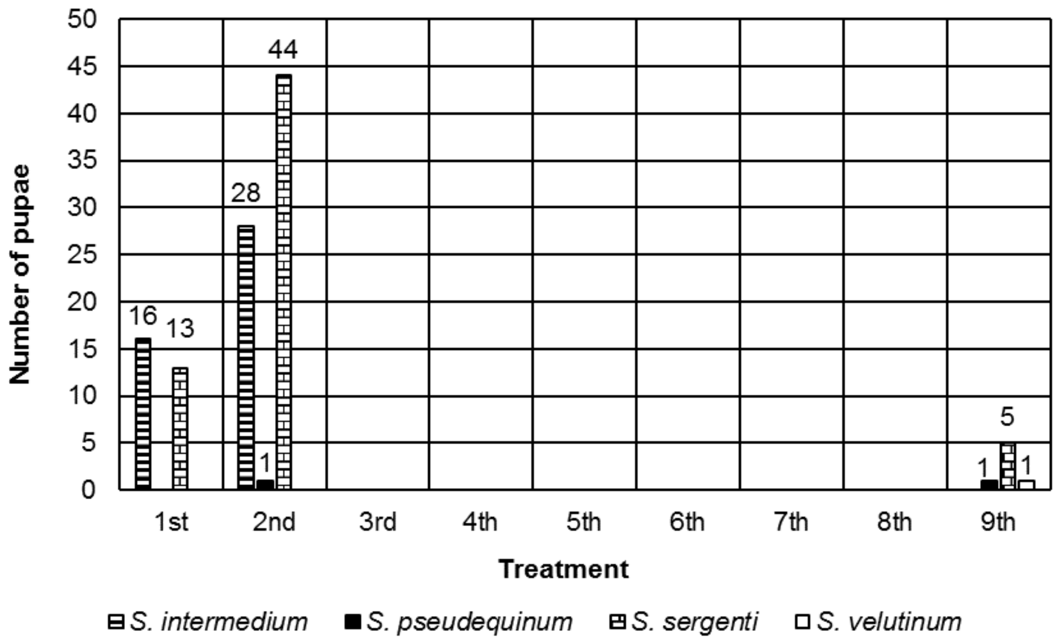

Fig. 6 Population fluctuation of simuliid species in station 4 after the different treatments carried out.

On the other hand, the percentage population size representation of all the simuliid species described, shows how *S. intermedium* and *S. sergenti*, reach the highest relative percentages, and are present in all stations, at least in the initial phases of the treatment program (Table 1).

Table 1: Simuliid species percentage representation at each point throughout the treatments.

		Simuliid species					
Treatment	**Area**	*S. angustitarse*	*S. intermedium*	*S. ornatum*	*S. pseudequinum*	*S. sergenti*	*S. velutinum*
1st	Station 1	13%	67%	–	–	20%	–
	Station 2	–	4%	–	–	96%	–
	Station 3	–	41%	4%	–	55%	–
	Station 4	–	57%		–	43%	–
2nd	Station 1	8%	75%	–	1%	16%	–
	Station 2	16%	5%	–	1%	78%	–
	Station 3	3%	53%	1%	2%	41%	–
	Station 4	–	38%	–	2%	60%	–
3rd	Station 1	–	100%	–	–	–	–
	Station 2	–	–	–	–	–	–
	Station 3	–	–	–	–	–	–
	Station 4	–	–	–	–	–	–
4th	Station 1	71%	29%	–	–	–	–
	Station 2	100%	–	–	–	–	–
	Station 3	–	100%	–	–	–	–
	Station 4	–	–	–	–	–	–
5th	Station 1	100%	–	–	–	–	–
	Station 2	100%	–	–	–	–	–
	Station 3	–	–	–	–	–	–
	Station 4	–	–	–	–	–	–
6th	Station 1	–	–	–	–	–	–
	Station 2	–	–	–	–	–	–
	Station 3	–	–	–	–	–	–
	Station 4	–	–	–	–	–	–
7th	Station 1	–	–	–	–	–	–
	Station 2	–	–	–	–	–	–
	Station	–	–	–	–	–	–

	Station 3						
	Station 4	–	–	–	–	–	–
8th	Station 1	–	–	–	–	–	–
	Station 2	–	–	–	–	–	–
	Station 3	–	–	–	–	–	–
	Station 4	–	–	–	–	–	–
9th	Station 1	–	–	–	–	–	–
	Station 2	–	–	–	–	–	–
	Station 3	–	–	–	–	75%	25%
	Station 4	–	–	–	14%	72%	14%

The percentages of the detected species were analyzed, before making the decision to carry out the treatment program, it was observed in the previous analysis of the captured samples that *S. intermedium* and *S. sergenti* presented the most numerous populations in the four zones and that predominated over the other simuliid species. Of these two, the one of greatest interest is *S. intermedium* since [8] it is a species whose females mainly cause bites on horse and cattle. However, the rest of the species: *S. angustitarse*, *S. sergenti* and *S. velutinum* have been described as non-hematophagous, with the exception of *S. pseudequinum* whose females attack cattle, horses and pigs [8; 9; 10; 11; 12). This implies that together with *S. intermedium*, there are two species to be taken into account both for animal welfare and for economic efficiency, in addition to *S. ornatum* Meigen, 1818, whose females cause significant annoyance and discomfort to human populations [1; 8; 10; 11; 12; 13]. In any case, after the treatments, the populations of all the species and particularly of the three of interest: *S. intermedium*, *S. pseudequinum* and *S. ornatum*, are gradually reduced considerably until disappearance, thus dissipating the threat and the possible inconvenience and consequences.

After several months of the last treatment, we returned to the four areas and it was possible to observe again the presence of black flies, so simuliids had colonized the treated areas in the absence of the product. In fact, even new species of blackfly appeared attracted by the availability of ecological niches, which were not found in the preliminary study, such as the species *S. velutinum*.

4. Discussion

In conclusion, this experience demonstrates the clear effectiveness of this type of biological treatment on simuliid populations. It can be affirmed that at all the control points, the absence of preimaginal stages of larvae and pupae was observed from the fifth treatment to the ninth, and in some even before as in station 4. In addition, it is important to highlight that in the study a total number of six species were detected: *S. angustitarse*, *S. intermedium*, *S. ornatum*, *S. pseudequinum*, *S. sergenti* and *S. velutinum*. Out of all of them, *S. intermedium* and *S. sergenti* show the highest populations and exert dominance over the rest of the species with which they cohabit. It is important to remark two things: firstly that the population sizes decrease to reach a total absence, and secondly that there is larvicide treatment effectiveness from the third application on. Furthermore, it is important to note that *S. angustitarse* populations are predominant in the fourth and fifth treatment.

Finally, we need to emphasize the importance of maintaining treatment periodicity to avoid possible recolonization, as was verified months after the end of this experience.

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