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CONTROL OF *CULEX PIPIENS* BY *BACILLUS SPHAERICUS* AND ROLE OF NONTARGET ARTHROPODS IN ITS RECYCLING

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ABSTRACT. *Bacillus sphaericus* was used to control *Culex pipiens* breeding in a water treatment settling basin near Montpellier, France. Four treatments with 4 liters/ha (3.6 lb/acre) of commercially available *B. sphaericus* formulation (Vectolex®) reduced the larval population of *Cx. pipiens* with 50–600 spores of *B. sphaericus*/ml recorded in the treated portions of the water plant 14 days following the last treatment. Natural recycling of the bacteria was shown to take place at the water surface where germination of spores was evidenced. The appearance of vegetative forms of *B. sphaericus* may be linked to the passage through larval guts of several filter-feeding arthropods.

INTRODUCTION

Two bacteria, *Bacillus sphaericus* Neide and *B. thuringiensis* H-14, have been introduced commercially and used as microbiological control agents against mosquito larvae. *Bacillus sphaericus* has a narrower spectrum of activity than *B. thuringiensis* H-14, but it seems to be more active against larvae of certain genera of culicine species (Sinègre et al. 1980, Davidson et al. 1981, Hertlein et al. 1981, Lacey and Singer 1982). Its spores may persist for a long time in the environment (Hertlein et al. 1979, Mulligan et al. 1980, Des Rochers and Garcia 1984). An attractive feature of this organism is its potential to recycle in the larval gut of *Culex* species (Karch and Coz 1986, Charles and Nicolas 1986) by spore germination, vegetative multiplication and resporulation after the death of the larvae. This recycling in the larval habitat may lead to the control of more than one generation of mosquitoes. Hertlein et al. (1979) recovered spores of *B. sphaericus* 9 months after treatment in a roadside ditch in southwest Florida and Hornby et al. (1984) reported that it persisted several months in sewage plants. More recently, Karch et al. (1988) reported a 4-year persistance in a natural pond and discussed the possible role nontarget fauna could play in this process. In a laboratory study, Karch et al. (1989) observed germination of *B. sphaericus* spores in the larval guts of *Chironomus* sp. and *Culiseta annulata* (Schrank), thus suggesting the possibility that these species may have some influence in the maintenance and seasonal occurrence of *B. sphaericus*.

This study investigated the dynamics of a *Culex pipiens* Linn. population after treatment with *Bacillus sphaericus* and the ability of the bacteria to recycle in nontarget arthropod species found in association with *Culex* spp. in natural breeding sites.

MATERIALS AND METHODS

Mosquito breeding sites: A regional program for mosquito control is carried out every year in the south of France. Between June and August 1988 we were able to join the staff and to study the dynamics of a *Culex pipiens* population in the settling basin of a wastewater treatment plant situated at Fabrègues, in the area of Montpellier. The basin is approximately 1,600 m^2 and 120–150 cm deep. A large variety of aquatic organisms occur in association with *Cx. pipiens* larvae (Table 1). The larval population of *Cx. pipiens* is found under the vegetation which grows 1–2 m in the width along the periphery of the basin where the depth never exceeds 40 cm. This vegetation contains duckweed, algae, arthropods and zooplankton. Three sampling sites were chosen in the plant: site 1 corresponds to the water supply of the lagoon with permanent replenishment, site 2 is lateral to the water flow and site 3 is the point (directly opposite to 1) where water discharges by overflow into a second basin. Water level remains constant; it corresponds to the overflow line. The second basin (2,200 m^2) remained untreated and was used as control for nontarget organisms until mid-July when local authorities were urged to put an end to mosquito annoyance in that area. Observations unfortunately were stopped at the end of July with the emptying of the first basin for cleaning.

The main physical-chemical characteristics of water in the treatment plant (and in the control plant) in June are summarized as follows: acidic organic matter 64.5 (43.5) mg/O_2/liter; alkaline organic matter 30.5 (15.9) mg/O_2/liter; ammoniacal nitrogen 36.0 (36.0) mg/liter; NO_2^- 0.25 (0.20) mg/liter; Cl^- 106.5 (88.7) mg/liter; CO_3H^- 475.5 (512.4) mg/liter; pH 7.5 (7.8); pollution index 212.2 (201.8). Water temperature was taken at 0900 h with a thermometric sonde 20 cm deep.

Bacterial treatments: Bacillus sphaericus

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Table 1. Abundance of arthropod species found breeding in association with *Culex pipiens* in Montpellier, France, June and July 1988.

Treatment date	Species collected in 200 ml of water sample[1]																				
	Co. punctata (Hemiptera)		*N. cinera* (Hemiptera)		*Cl. dipterium* (Ephemeroptera)		*E. melanocephalus* (Coleoptera)				Chironomidae (Diptera)		Anisoptera (Odonata)		*Daphnia* sp. (Crustacea)		*Isopoda* (Crustacea)		*Cypris* sp. (Ostracoda)	
							Larvae		Adults				Larvae							
	C[2]	T[3]	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
June 3 (T1)	+	+	+	–	+	++	–	–	–	–	+	+	–	–	+++	+++	–	–	++	++
June 20 (T2)	++	+	+	–	++	+++	–	+	–	–	+	+	+	–	+++	+++	–	–	++	+++
June 28 (T3)	++	++	+	+	++	+++	+	–	–	–	+	+	+	+	+++	+++	+	–	+++	+++
July 11 (T4)	++	++	+	+	+++	+++	+	+	+	+	+	+	+	+	+++	+++	++	++	+++	+++
July 25[4]	++	++	+	+	+++	+++	+	+	+	+	+	+	+	+	+++	+++	++	++	+++	+++

[1] No. specimens of each species counted in 200 ml sample as follows: +, 1–50; ++, 50–100; +++, >100.
[2] C = control.
[3] T = treated.
[4] Observation only, no treatment.

strain 2362 (Vectolex®, from Abbott Laboratories, U.S.A., containing 1.2×10^9 spores/ml) was used at a final concentration of 4.0 liters/ha (3.6 lb/acre, 4.8 10^{12} spores/ha). The treatment was applied with a pressurized hand sprayer in a 2 m width around the periphery of the water treatment plant.

Sample collection: Samples (200 ml) of water and arthropods were taken from the surface around the basin throughout the observation period at least once a week and before each treatment. For the bacteriological analyses, samples were taken 2 h after each treatment at the 3 sites, both on the surface and at the bottom of the treated zone.

Bacteriological counts: After harvesting, water samples and 10 arthropods ground in 1 ml of sterile water were split in 2 subsamples, one of which was submitted to heat shock (80°C for 12 min) to kill vegetative cells and nonspore-forming bacteria. Then, 0.1 ml of each subsample or a dilution of it was plated on MBS solid medium (Kalfon et al. 1983) in Petri dishes containing 100 mg/liter of streptomycin. The colony counts were recorded after a 24-h growth period at 35°C. Identification of *B. sphaericus* was confirmed by morphological and microscopic observations. In the 3 sampling sites of the lagoon, the concentration of *B. sphaericus* was evaluated by the mean of 3 bacteriological counts.

Laboratory studies: Tests were performed with filter feeders (*Daphnia pulex* and *Cypris* sp.) to determine their ability to ingest and release *B. sphaericus* as already described elsewhere for *Culiseta annulata* and *Chironomus* sp. (Karch et al. 1989). Several samples of 10 organisms were placed in 10 ml of a suspension at 0.1 mg/liter. After a contact period of 2, 5 and 24 h, one sample was removed, rinsed with sterile distilled water, diluted 100-fold and treated as above for bacteriological counts. After 2 h contact, 2 other samples were removed, rinsed and placed in 10 ml sterile water. Two and 24 h later, they were submitted for bacteriological counts as well. Counts were also made in the sterile water itself by plating 0.1 ml.

RESULTS

Efficacy of B. sphaericus *for the control of* Cx. pipiens *under field conditions:* Four treatments using *B. sphaericus* were sufficient to limit the preimaginal population of *Cx. pipiens* during the observation period of June–July 1988. The interval between 2 treatments was chosen in relation to the occurrence of a new larval population of *Cx. pipiens*. Figure 1 shows that the effectiveness of the product varies with the sampling sites. This difference is due to their location with regard to the water flow. The lower

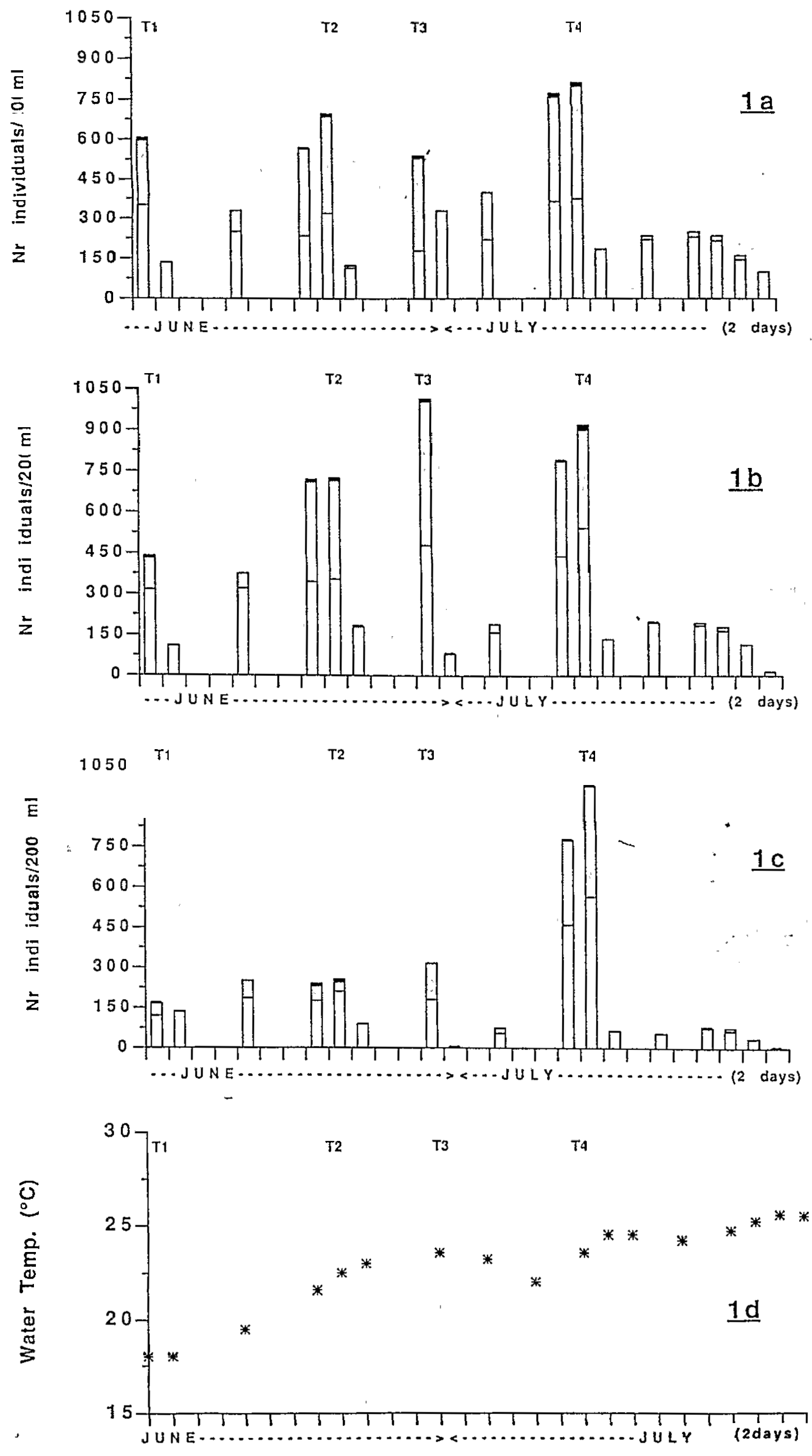

Fig. 1. Density of preimaginal population of *Culex pipiens* in the 3 sites of a lagoon treated with *Bacillus sphaericus* and mean water temperature. 1a = site 1; 1b = site 2; 1c = site 3 and 1d = water temperature at 0900 h. Blank = 1st and 2nd instars; punctate = 3rd and 4th instars; black = pupae; Ti = treatment number i.

efficacy observed at site 1 under treatment was due to leaching by water replenishment but not to the absence of the product or to its ineffectiveness. Oviposition occurred at the same level in both ponds. Egg rafts were observed among the vegetation generally blown to one side by the wind.

Arthropods found in the treatment and control

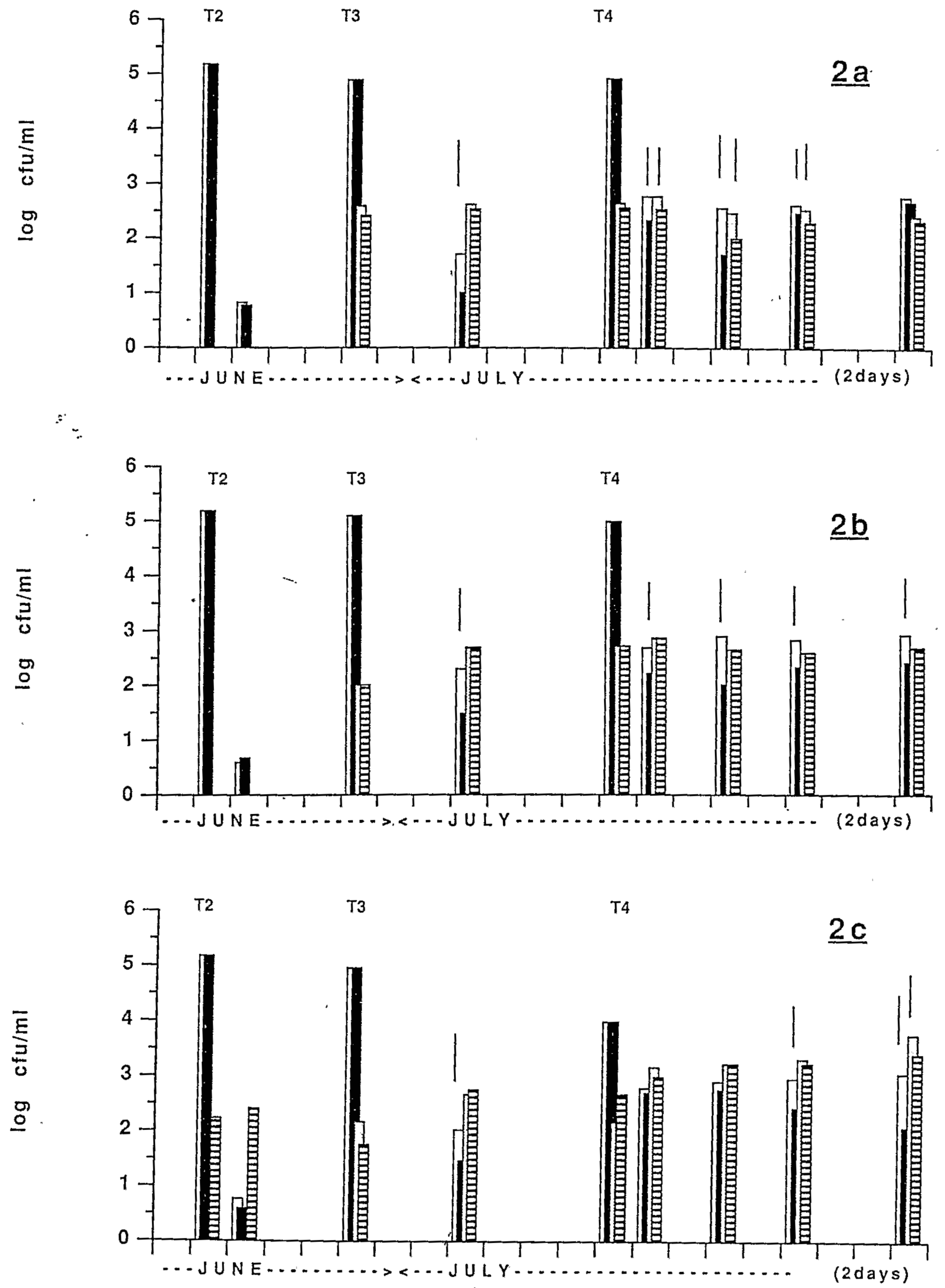

Fig. 2. Total number of bacteria and heat-resistant spores of *B. sphaericus* 2362 present in the 3 sites of a lagoon treated against *Culex pipiens*. Samples were taken at the surface (blank = total bacteria; black = spores) or at the bottom of the pond (punctate = total bacteria; stripes = spores); 2a = site 1; 2b = site 2 and 2c = site 3.

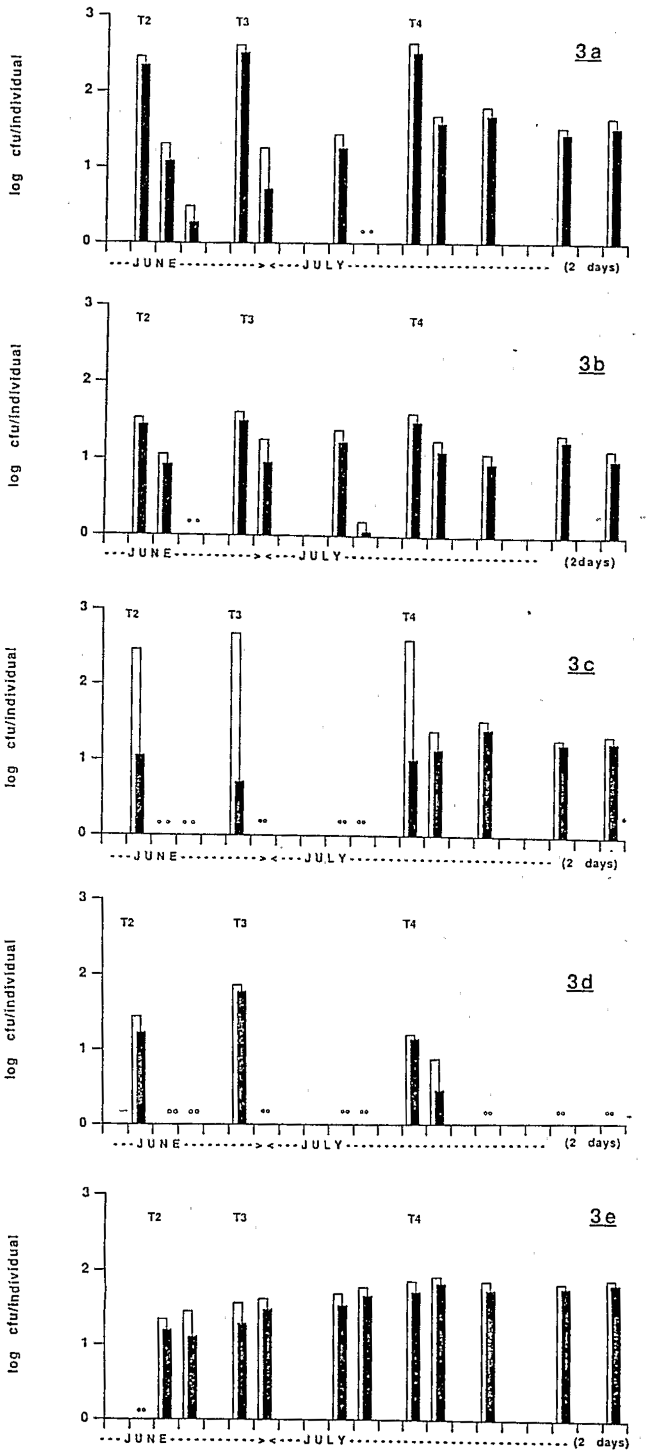

Fig. 3. Total bacteria (blank) and heat resistant spores (black) of *Bacillus sphaericus* present in some arthropods found in breeding site of *Culex pipiens;* °° = no bacterium found in the sample; filter feeders: 3a = *Daphnia pulex* and 3b = *Cypris* sp.; predators: 3c = *Cleon dipterium* and 3d = *Corixa punctata*; bottom feeders: 3e = *Chironomus* sp.

ponds: Table 1 gives an evaluation of the populations of some arthropods found in the breeding sites under study. The abundance levels of these aquatic arthropods is nearly similar whether or not *B. sphaericus* is present. Moreover, the numbers of all species increased gradually in the treated portions as well as in the controls throughout the observation period. In this region of France, other *Cx. pipiens* breeding sites have been treated with chemical insecticides. It has been our observation that insecticides, particularly chlorpyrifos, kill nonselectively and in so doing eliminate predators of mosquito larvae which might otherwise contribute to their natural control.

Fate of B. sphaericus *in the breeding site:* Figure 2 shows that the concentration of *B. sphaericus* was nearly similar in the 3 sampling sites throughout the course of treatments. The sedimentation of this bacteria leads to its disappearance from the feeding zone of the mosquito larvae as early as the first 48 h following each application.

The concentration of the bacteria was as follows:

1) On the surface: At the beginning of the experiment, the number of spores 2 days after spreading ranged from about 0 to 50 per ml, while the bacterial population was 50–600 sp./ml 2 weeks after the 4th treatment. The disappearance of the larval population observed in Fig. 1 is consistent with the results obtained by Hornby et al. (1984) and those of Nicolas et al. (1987) which show that a spore count ranging from 100 to 500 per ml is sufficient to control mosquito larvae.

2) At the bottom (0.40 m in depth): From the second week following the first treatment, the number of spores ranged from about 100 to 200 per ml. It was considerably higher at the end of the experiment where it ranged from about 500 to 2,000 spores per ml. This increase is due not only to sedimentation, as the concentration also increased at the surface.

Countings of colony forming units (CFU) after thermic shock of the subsamples reveal that a nonnegligible amount of bacteria may be vegetative forms. This is observable most of the time near the surface but to a lower extent also on the bottom.

Fate of B. sphaericus *in nontarget arthropod species:* The different arthropod species present in the breeding site could contribute to the dispersion of *B. sphaericus* in the treated area, as several of them ingest bacteria. We followed the presence of *B. sphaericus* in some of them collected in the settling tank. Results (Fig. 3) show that filter feeders (*Daphnia* sp. and *Cypris* sp.) ingest *B. sphaericus* as long as the bacteria remains present in their feeding zone which is closely related to vegetation decaying in the water column. A small percentage of *B. sphaericus* in their guts are vegetative forms. The same process is observed for the bottom feeder *Chironomus* sp. which feeds essentially on bacteria. As they filter continuously, it is not possible to determine where germination took place. A laboratory test substantiates the idea of germination inside their guts (Table 2) and spreading of vegetative cells in the environment. The 24-h contact result is not really significant because the suspension had been exhausted by continuous feeding. For predators (*Cleon dipterium* and *Corixa punctata*); *B. sphaericus* is found when present in their prey. The number of CFU is probably related to the number of larvae they ingest. However this would require an additional quantitative study of their feeding habits.

Table 2. Results of a laboratory test concerning the number of *Bacillus sphaericus* ingested or released by 2 filter feeders in 24 h.

Species		In *B. sphaericus* suspension			In sterile water[1]		cfu released in water/individual[2]	
		2 h	5 h	24 h	2 h	24 h	2 h	24 h
	Total bacteria	8,100	15,700	100	7,000	100	1,630	1,000
Daphnia pulex	Heat resistant spores	7,900	10,300	0	6,500	0	1,570	520
	% spores in total bacteria	97.5	65.6	—	92.8	—	96.0	52.0
	Total bacteria	8,900	8,200	1,500	6,300	100	530	1,540
Cypris sp.	Heat resistant spores	8,100	7,100	0	5,700	200	360	1,290
	% spores in total bacteria	91.0	86.6	—	90.5	—	67.9	83.8

[1] All results were obtained with 100-fold dilutions of samples.
[2] Results were obtained from 0.1 ml of sterile water containing 10 individuals/10 ml.

Nevertheless, the disappearance of mosquito larvae will diminish their participation in *B. sphaericus* recycling.

DISCUSSION

Our observations are consistent with those of other workers (Davidson et al. 1984, Mulla et al. 1984) and show that this bacterium used as a larvicide against mosquitoes in aquatic environments does not disturb the natural equilibrium among the various populations of the natural fauna. The most important point is the role likely to be played by this fauna in relation to the larval population of *Cx. pipiens*. On one hand, some species (*Cleon dipterium*, *Corixa punctata* and *Nepa cinerea*) are predators or killers of mosquito larvae and their activity is additive to that of the bacterium; a phenomena which could not have been observed while using chemical insecticides. On the other hand, several species (*Daphnia* sp., *Cypris* sp.) contribute to redistribute the bacterium in the water column.

Therefore, this study suggests that periodicity of treatments with *B. sphaericus* will depend on the density of the nontarget arthropod species. Considerable natural fluctuations will be observed in a breeding site, particularly if this density is high. The 4 treatments applied at 4 liters/ha each ($4 \times 5 \times 10^{12}$ spores/ha) were sufficient to control emergence of adult *Cx. pipiens* during at least 14 days under the present conditions. Unfortunately, observations were stopped before knowing how long the recycling could be effective. Mulla et al. (1988) reported 99% control during 49 days with a single treatment of a wastewater facility at 4.48 kg/ha (3.4×10^{14} spores/ha) which in fact represents 10 times the quantity (2×10^{13} spores/ha) which yielded 100–94% reduction for only 4–7 days in a study reported earlier (Mulla et al. 1984) or the quantity applied in the present study. The cumulative effect of treatments resulting in long lasting mosquito control will depend on several factors such as the type and density of species present (carnivorous versus filter feeders), the physical-chemical characteristics of the water that might allow bacterial reproduction, the presence of vegetation and the possible attraction of the habitat for mosquito oviposition.

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