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IMPACT OF A 26-MONTH *BACILLUS SPHAERICUS* TRIAL ON THE PREIMAGINAL DENSITY OF *CULEX QUINQUEFASCIATUS* IN AN URBAN AREA OF RECIFE, BRAZIL

MARIA HELENA SILVA-FILHA, LÊDA REGIS,[1] CLÁUDIA MARIA F. OLIVEIRA AND ANDRÉ F. FURTADO

Departamento de Entomologia, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz (FIOCRUZ), Caixa Postal 7472, CEP 50670-420 Recife-PE, Brasil

ABSTRACT. A field trial using the entomopathogen *Bacillus sphaericus* against *Culex quinquefasciatus* was conducted in a district of Recife, Brazil, an area with a high prevalence of lymphatic filariasis. In this urban area more than 2,500 *Cx. quinquefasciatus* breeding sites within a 1.2-km² area were found and subsequently submitted to a long-term treatment with *B. sphaericus*. To evaluate the impact of treatment on the densities of *Cx. quinquefasciatus* preimaginal forms, 26 breeding sites, representative of the major site types found in that area, were monitored for 3 years. Parameters such as mean and maximum densities of larval population as well as the frequency of infested sites were recorded before, during, and after the trial. The level of each parameter was greatly reduced during the treatment period, particularly in the 2nd treatment year. A low density of *Cx. quinquefasciatus* was also maintained throughout the last year, in the absence of treatment. Analysis of the data reported here confirms *B. sphaericus* as a very effective larvicide against *Cx. quinquefasciatus* despite the optimal environmental conditions for mosquito proliferation in the urban area of Recife.

KEY WORDS *Bacillus sphaericus, Culex quinquefasciatus,* vector control, larvicide, biological control

INTRODUCTION

In recent years, chemical insecticides have been gradually replaced by biological control agents, because of the problems of environmental damage and the selection of insect populations resistant to the chemicals. *Bacillus sphaericus* Neide is considered one of the most promising entomopathogenic agents for controlling Culicidae (Karch and Charles 1987, Lacey et al. 1988). This bacterium displays activity against the genera *Culex, Psorophora, Mansonia, Anopheles,* and *Aedes.* The major feature of this pathogen is its narrow activity spectrum making it safe for nontarget fauna (Mulla et al. 1984, Siegel and Shadduck 1990). Nearly 150 strains of this bacterium are toxic toward mosquitoes (Thiéry and de Barjac 1989). In particular, strain 2362 has shown great toxicity against *Culex quinquefasciatus* Say, the main vector of lymphatic filariasis in many regions of the world. This culicid proliferates in urban areas characterized by polluted aquatic habitats. Small- and large-scale trials have been conducted in countries where nuisance or disease are caused by *Cx. quinquefasciatus* in order to assess the efficacy of *B. sphaericus* control (Lacey 1990, Hougard et al. 1993, Sinègre et al. 1993, Barbazan et al. 1997).

Recife City, with a 6.5% mean prevalence rate of microfilaraemia (Maciel et al. 1996), is the major endemic area for bancroftian filariasis in Brazil. A pilot project was implemented in 2 urban districts of Recife (Coque and Mustardinha) that had about a 10% microfilaraemia prevalence rate, associating diethylcarbamazine mass therapy with vector control and community participation (Furtado et al. 1994). *Bacillus sphaericus* 2362 was the main vector control measure used in the Coque district, with the aim being to reduce larval and consequently adult densities to a level incompatible with disease transmission (Regis et al. 1996). The main goal of the present study was to evaluate the impact and effectiveness of a long-term (26-month) *B. sphaericus* treatment on *Cx. quinquefasciatus* preimaginal densities in 26 selected breeding sites in the Coque area.

MATERIALS AND METHODS

This investigation was conducted in Recife, at 8°4′S latitude, where the monthly average temperature ranges from 24 to 28°C and the relative humidity ranges from 60 to 80%. The area has 2 well-defined seasons: a heavy rainy season from May to August and a dry season with sparse rainfall from September to April.

The trial was conducted from September 1991 to November 1993 in a 1.2-km² area located in the Coque district, where more than 2,500 *Cx. quinquefasciatus* breeding sites of different types were mapped and treated with *B. sphaericus* 2362 (Regis et al. 1995). Entomological data such as mosquito preimaginal and imaginal densities were assessed throughout the trial. To evaluate the impact of treatment on preimaginal densities, 26 small-sized breeding sites, all initially colonized by *Cx. quinquefasciatus* larvae and representative of the major types of sites found in the study area, were chosen and monitored. The sites selected consisted of 11 cesspits, 7 inspection boxes, 3 drains, 3 wastewater pools, and 2 tanks. All breeding sites, except the tanks, contained organically enriched water.

The pretreatment (PRE) densities from all sites

[1] To whom correspondence should be addressed.

Table 1. Preimaginal population densities of *Culex quinquefasciatus* recorded in 26 breeding sites located in Coque district (Recife, Brazil), before a 26-month *Bacillus sphaericus* trial.

Site type	No. sites	Mean ± SD (m^2)	Mean LP/D[1] ± SD (m^2)	Max.[2]
Cesspit	11	1.9 ± 0.6	171.4 ± 196.3	689
Inspection box	07	0.5 ± 0.3	292.2 ± 419.7	963
Drain	03	13.2 ± 5.5	306.3 ± 145.5	425
Wastewater pool	03	30.3 ± 29.2	942.1 ± 832.3	1903
Tank	02	2.1 ± 0.4	117.2 ± 131.5	210

[1] Mean density (3rd- and 4th-stage larvae and pupae/dip) ± standard deviation.
[2] Maximum density (3rd- and 4th-stage larvae and pupae/dip) recorded in each site type.

were assessed just before the 1st treatment with *B. sphaericus*. *Culex quinquefasciatus* densities were then monitored weekly during the 1st treatment year (YI); fortnightly during the 2nd treatment year (YII), and monthly during the 3rd (posttreatment) year (POS). Evaluation was based on 26 breeding sites but, eventually, sampling of some sites in given moment could not be carried out because of periods of site dryness or to other operational limitations. Sampling variation (number of observations) during the trial is reported in Table 2.

Preimaginal density records were based on the dipping method. The density evaluation of each breeding site consisted of collecting a certain number of dips (150 ml) and obtaining the mean number of larvae and pupae per dip (LP/D). The number of dips collected per site was 5 for cesspits, inspection boxes, and tanks; 1 dip/m of the length of drains; and 1 dip/m along the margins of wastewater pools. Only 3rd- and 4th-stage larvae and pupae were recorded in order to determine the LP/D.

Throughout the trial all the existing breeding sites within the operational area, including the 26 monitored sites, were treated with a locally produced *B. sphaericus* 2362 final whole culture (Rios and Silva-Filha 1992). A *B. sphaericus* 2362 flowable concentrate (Spherimos®/Novo Nordisk, Bagsvaerd, Denmark) was occasionally used instead of the final whole culture. The rates applied were 20–40 ml/m^2 and 5–10 ml/m^2 of final whole culture and Spherimos, respectively. Those amounts were diluted in tap water and applied with a manual sprayer. The 1st treatment was conducted just after the pretreatment sampling and the subsequent treatments were carried out only when increasing preimaginal density was observed in the breeding sites. All data obtained during the trial were collated using the Epi Info (World Health Organization, Geneva, Switzerland) computer program. The impact of *B. sphaericus* treatment throughout the 2 treatment years (YI, YII) and the posttreatment year (POS) was evaluated using the following parameters: the general mean density, the mean density per site type, the maximum mean density, the maximum density observed per site type, the percentage of infested samples per site type (percentage of positives samples for *Cx. quinquefasciatus*), and the persistence period during which at least an 80% density reduction was maintained. All reductions of percentages were based on the values recorded at the pretreatment sampling.

RESULTS

Culex quinquefasciatus pretreatment density

The pretreatment (PRE) mean densities in all site categories were found to be very high (Table 1). The general PRE mean density, calculated from samples collected in 26 selected breeding sites just before the 1st *B. sphaericus* spraying, was 299.7 ± 417.1 LP/D (Fig. 1). The high standard deviation observed indicates considerable variation among the densities recorded within each category. The maximum density recorded in each category showed the high potential for mosquito production in those sites (Table 1).

Impact of *B. sphaericus* treatment on the preimaginal population

On average 7.8 ± 2.8 treatments were carried out per site during the 1st treatment year (YI). The mean density calculated from 1,009 samples collected throughout this period was 18.5 ± 18.8 LP/D (Fig. 1), which represents a 93.8% reduction compared to the PRE densities. Nevertheless, the maximum density mean, calculated from the LP/D peaks recorded in the 26 sites during YI, was still high (177.5 ± 164.7 LP/D). In terms of breeding site recolonization, 53% of the samples analyzed were positive for preimaginal forms of *Cx. quinquefasciatus* (Fig. 1) against 100% found at the pretrial sampling. When analyzed per site type, the results recorded in YI showed a sharp decrease of larval density in each category. Indeed, remarkable reductions in the mean LP/D per type, 94.1%, 88%, 92.1%, 99%, and 92.1%, were recorded respectively in inspection boxes, cesspits, drains, wastewater pools, and tanks. However, the maximal densities detected per site type were still high, except for wastewater pools (Table 2). The percentage of infested sites did not show significant variation among the different breeding site types. A minimum of 42% was found in wastewater pools and a maximum of 62% in tanks (Table 2). Persistence of *B. sphaericus* for as long as 92 days was recorded in a cesspit and as long as 45 days in a wastewater

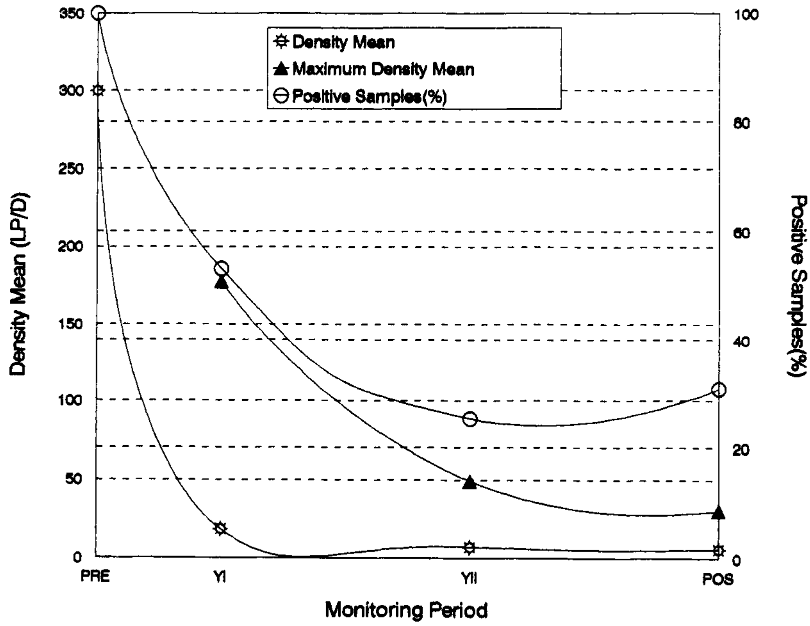

Fig. 1. Impact of *Bacillus sphaericus* treatment on *Culex quinquefasciatus* preimaginal population densities in 26 sites selected in Coque, Recife, Brazil. PRE, pretreatment; YI, 1st treatment year; YII, 2nd treatment year; POS, posttreatment year.

pool. Persistence periods were shorter in inspection boxes and drains, where control persistence did not exceed 14 or 17 days, respectively.

A mean of 8.7 ± 4.4 treatments per site were carried out during the 2nd treatment year (YII). Analysis of data from 162 samples collected during this period showed that the general mean density of *Cx. quinquefasciatus* larvae was even lower than that observed in YI, corresponding to only 2.4 % of the PRE mean density (Fig. 1). A significant decrease of the maximum density mean was recorded, dropping from 177.5 ± 164.7 LP/D at YI to 49.23 ± 53.6 LP/D (Fig. 1). Furthermore, a lower rate of site recolonization by *Culex* was observed: only 25% of samples were positive, indicating a strong reduction of the *Cx. quinquefasciatus* adult population throughout YII (Fig. 1). The mean densities per site type obtained in YII were lower than those observed in the preceding year, except for the inspection box type, which maintained the same density level as in YI (Table 2). The maximal densities per site type showed a major decline in each site type, when compared to the peaks observed before treatment (Table 2). Analysis of the recolonization level showed that the percentages of infested samples from cesspits and tanks were even below the general average (25%) for this period (Table 2). In wastewater pools, no positive samples were found during that period.

General analysis of the posttreatment year (POS) shows that the densities of *Cx. quinquefasciatus* in the breeding sites were maintained at a low level throughout the period (Fig. 1). The mean density, as well as the maximum density mean, were even lower than the values recorded during YII. The analysis per site showed that despite interruption of

Table 2. Impact of a 26-month *Bacillus sphaericus* trial on *Culex quinquefasciatus* preimaginal densities and recolonization, in different breeding site types found in an urban area of Recife.[1]

Site type	1st year treatment				2nd year treatment				Posttreatment year			
	Obs	LP/D ± SD	Max	+ %	Obs	LP/D ± SD	Max	+ %	Obs	LP/D ± SD	Max	+ %
Cesspit	421	20.6 ± 3.4	603	52	62	4.5 ± 14.4	76	18	83	5.2 ± 16.1	95	18
Inspection box	196	17.2 ± 53.1	480	50	60	15.3 ± 39.6	177	33	71	9.0 ± 20.1	113	46
Drain	171	24.3 ± 43.7	222	61	20	6.7 ± 13.7	57	40	22	10.1 ± 19.5	80	36
Wastewater pool	116	9.1 ± 25.5	175	42	10	0	0	0	13	0.7 ± 1.7	5	15
Tank	105	9.3 ± 20.3	138	62	10	1.8 ± 4.1	12	20	12	2.4 ± 6.6	23	33

[1] Obs, number of observations; LP/D ± SD, mean density (3rd- and 4th-stage larvae and pupae/dip) ± standard deviation; Max, maximum density (3rd- and 4th-stage larvae and pupae/dip) observed; + %, percentage of infested samples (positive for *C. quinquefasciatus*).

Table 3. Effects of *Bacillus sphaericus* on *Culex quinquefasciatus* preimaginal population in 3 selected breeding sites exposed to negative environmental factors (exposure to sunlight, rainfall, and water flow).[1]

	Inspection box			Cesspit			Drain		
	LP/D ± SD	Max	+ %	LP/D ± SD	Max	+ %	LP/D ± SD	Max	+ %
PRE	30	—	—	100	—	—	350	—	—
YI	16 ± 26.7	114	63	62 ± 124.1	603	66	10 ± 28.9	173	30
YII	31 ± 39.7	134	90	11 ± 25.5	76	30	6 ± 9.1	22	56
POS	40 ± 33.9	113	100	34 ± 29.6	95	83	22 ± 24.2	80	80

[1] LP/D ± SD, mean density (3rd- and 4th-stage larvae and pupae/dip) ± standard deviation; Max, maximum density (3rd- and 4th-stage larvae and pupae/dip) observed; + %, percentage of infested samples (positive for *Cx. quinquefasciatus*); PRE, pretreatment; YI, 1st treatment year; YII, 2nd treatment year; POS, posttreatment year.

B. sphaericus treatments, a low density level was recorded in all site types (Table 2). However, a 1st sign of recovery in *Cx. quinquefasciatus* proliferation was noticed, because the percentage of infested samples had increased in all breeding site types (Table 2), especially from September to December.

Major environmental factors affecting *B. sphaericus* efficacy

Despite a high efficacy of *B. sphaericus* against *Cx. quinquefasciatus* during this trial, control was less efficient in some sites and this was found to be related to environmental factors. The entomological data from an inspection box, a drain, and a cesspit are presented in Table 3. The lack of cover allowed those breeding sites direct exposure to sunlight and rainfall, whereas the drain and the inspection box were also subject to water flow. In the inspection box, reduction of mosquito density was not recorded and percentages of infested samples as high as 63% and 90% were observed during YI and YII, respectively. Note that both larval density and percentage of infested samples had decreased in the cesspit as well as in the drain, although they did not attain the reduction levels recorded for the other sites. Analysis of data from those sites during the posttreatment year revealed a high *Cx. quinquefasciatus* production as shown by the mean density, maximum density, and percentage of recolonization, which reached the highest level detected in this period among all sites (Table 3).

DISCUSSION

The poor sanitary conditions found in the Coque area provide organically polluted breeding sites able to sustain intense mosquito productivity. In fact, very high pretrial densities were found in all 26 breeding sites that were selected as representative of the major types occurring in that area. The results of the trial carried out over a 26-month period, showed the high efficacy of *B. sphaericus* in reducing *Cx. quinquefasciatus* larval populations. Low densities of mosquito preimaginal forms were maintained throughout the posttreatment year.

The 1st entomological parameter indicating the impact of *B. sphaericus* treatment was the drastic decrease of the LP/D observed in most breeding sites in YI. Lower mean densities were recorded in YII and, surprisingly, during POS, in the absence of *B. sphaericus* treatment. The general mean density recorded at the pretreatment sampling (299.7 larvae and pupae per dip) declined significantly to 18.5, 8.3, and 6.75 LP/D in YI, YII, and POS, respectively. However, despite this drastic reduction of the mean density, high peak densities continued to be observed during YI in different site types. A marked decrease in maximal densities was detected only in the following year. The recolonization frequency of the sites by *Cx. quinquefasciatus,* as indicated by the percentage of positive samples, showed a significant although slow decrease. Before the 1st treatment 100% of the samples collected were infested by *Cx. quinquefasciatus* larvae. The frequency declined to 50% and 25%, respectively, in YI and YII and, during the posttreatment period, 31% of the samples were positive for *Cx. quinquefasciatus* larvae. Although the mean densities suffered a marked and short-term reduction after *B. sphaericus* treatments, note that the reduction of recolonization frequency was gradual.

The impact of *B. sphaericus* treatment of more than 2,500 breeding sites in the area showed a clear relationship with the adult population density recorded in Coque (Regis et al., 1995). The annual average adult densities recorded before treatment decreased in YI and YII, whereas no significant changes in mosquito population densities were found in an untreated area in Recife showing environmental characteristics and size similar to the Coque area (Regis et al. 1995). As for young forms, a major decline of adult population was reached only during the 2nd treatment year. The maintenance of a relatively high density of *Cx. quinquefasciatus* in YI is attributed mainly to the atypical heavy rainfall that occurred from January to April that year, as well as to the lack of control of some breeding sites, allowing the maintenance of *Cx. quinquefasciatus* foci (Regis et al. 1995). The remarkable level of mosquito control reached in YII was caused by the greater effort made to spray every potential breeding site within that area. In addition some other factors seemed to contribute to the maintenance of very low *Cx. quinquefasciatus* density. The absence of lethal effects of *B. sphaericus* on nontarget associated fauna (Mulla et al. 1984), including natural enemies and competitors of *Culex,* is an important factor. Continuity of treatment also contributed to a good protection level at the sites, allowing the availability of *B. sphaericus* toxins in the upper water layer. Slow sedimentation of spores (Nicolas et al. 1987) or even resuspension of recycled spore–toxin complexes might have played an important role in prolonged control. These factors seemed to be essential for promoting a good level of protection at breeding sites even after interrupting treatment in that area. The data gathered here reinforce the idea that the effects of *B. sphaericus* treatment on mosquito preimaginal density in a given area might be cumulative and long lasting, despite the presence of optimal conditions for *Cx. quinquefasciatus* breeding and the high productivity observed there before treatments.

As far as persistence of control is concerned, excellent results were obtained in a cesspit and in a wastewater pool despite the high level of organic matter. In contrast, a lower control persistence was observed in drains and inspection boxes. In fact, these site types have been identified in the present study as the most problematic larval habitats to be controlled. They certainly contributed to the recovery of *Cx. quinquefasciatus* proliferation detected between September and December of the posttrial year.

The major factors affecting the larvicidal efficacy of *B. sphaericus* in this study were found to be direct solar and rainfall exposure and water flow. Low *B. sphaericus* persistence in breeding sites fully exposed to sunlight has been reported (Karch and Charles 1987, Lacey et al. 1988, Jones et al. 1990, Lord 1991). The mechanism underlying solar effects on *B. sphaericus* activity is related to the drastic reduction of spore viability by ultraviolet radiation (Burke et al. 1983), which might affect recycling and consequently prolonged control, in addition to the denaturation of the toxin itself in shallow water (Des Rochers and Garcia 1984). The water flow found in some larval habitats proved to be a limiting factor for *B. sphaericus* effectiveness. Previous trials conducted in breeding site that were flushed by rains or received effluent overflow similarly have shown poor persistence (Karch et al. 1991, Montero-Lago et al. 1991). This is an important point to be considered for trials conducted in areas where rainfall occurs with great intensity, and where intermittent water flow from domestic use occurs. In parallel, direct exposure to rainfall promotes the settlement of spore–toxin complexes resulting in reduced control persistence. In YI, for instance, not only did a marked rainfall season occur but also periods of heavy rainfall occurred during the dry season, which could have reduced the effectiveness of the *B. sphaericus.* In *B. sphaericus* field trials, special attention should be paid to breeding sites that are subject to the negative environmental factors mentioned above, in order to avoid continuous mosquito proliferation in some foci within the target area. The use of improved formulations allowing good dispersal, slow release, and photodegradation protection (Levy et al. 1986, Levy et al. 1990) might ensure better *B. sphaericus* persistence in those sites. Strategies such as environmental management and physical elimination of those sites should also be employed.

On the other hand, analysis of our data on *B. sphaericus* efficacy in cesspits and other polluted breeding sites reinforces the concept that *B. sphaericus* is highly effective in these sites, which are also commonly stressed as being responsible for high *Cx. quinquefasciatus* production in many filariasis-endemic urban areas. Bimonthly applications of *B. sphaericus* in those sites are able to reduce and keep *Cx. quinquefasciatus* larval density at a low level. The diversity of control strategies, including the alternating use of *B. sphaericus* and *B. thuringiensis* var. *israelensis,* should be considered vis a vis the potential development of resistance to *B. sphaericus* in natural mosquito populations. The *Cx. quinquefasciatus* population from Coque has been shown to have developed a low-level (10-fold) resistance after the 26-month treatment period (Silva-Filha et al. 1995, Silva-Filha and Regis 1997).

A detailed characterization of the environmental profiles of breeding sites and the adequate use of *B. sphaericus* products can provide a substantial impact on *Cx. quinquefasciatus* larval density. The dynamics of decreasing field mosquito populations observed in this study point out the importance of continuous and long-term control strategies in order to achieve low levels of *Cx. quinquefasciatus* density and to contribute to the breakdown of filariasis transmission. In conclusion, treatment with *B. sphaericus* 2362 showed great effectiveness as a *Cx. quinquefasciatus* larvicide in an urban area that presented a critical mosquitogenic profile. Further work must now be carried out to extend the use of this biological insecticide.

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