Integrated Control Measures against *Culex quinquefasciatus*, the Vector of Filariasis in Recife

Lêda Regis, Maria Helena NL Silva-Filha, Cláudia MF de Oliveira, Eugênia M Rios*, Sinara B da Silva, André F Furtado

Centro de Pesquisas Aggeu Magalhães - FIOCRUZ, Caixa Postal 7472, Campus UFPE, 50670-420 Recife, PE, Brasil *Deptº de Antibióticos, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brasil

Integrated control measures against Culex quinquefasciastus *have been implemented in a pilot urban area in Recife, Brazil. About 3,000 breeding sites found within the operational area were responsible for very high mosquito densities recorded during the pretrial period. Physical control measures have been applied to cess pits before starting a series of 37 treatments of the other sites with* Bacillus sphaericus *strain 2362, over 27 months. In spite of the difficulties due to environmental conditions, very significant reductions in preimaginal population of* C. quinquefasciatus *were achieved and, as a consequence, low adult mosquito densities were maintained for a relatively long period of time. Entomological and environmental data gathered in this pilot project can contribute to design an integrated mosquito control program in Recife city.*

Key words: *Culex quinquefasciatus* - filariasis vector control - *Bacillus sphaericus*

Recife, Brazil, on 8°4'3" south latitude, is inhabited by about 1,5 million people living within a 209 km^2 area. Up to 60% of the population have very low income and most of them live in swampy areas. About 25% of the households in Recife are connected to an underground piped sewerage system. The means of disposing of the water after use in the other households are inadequate. Furthermore, continuous human immigration results in unplanned urbanization, thus providing increasing numbers of breeding places for *Culex quinquefasciatus*, the major mosquito species in this endemic area for Bancroftian filariasis.

Bancroftian filariasis in Recife was first recorded in 1952 by Azevedo and Dobbin, who found a microfilaraemia rate of 9.7% in the district of Afogados. A second survey (Rachou et al. 1956) showed the occurrence of *Wuchereria bancrofti* infective larvae in mosquitoes, and a mf rate of 7%, corroborating the existence of autochthonous filariasis. A recent survey carried out by collecting measured thick blood smear (60µl) from a sample of 15,000 people living in 35 *favelas*, showed mf rates ranging from 0.64 to 14.95%. In 16 of these *favelas* the mf rates were higher than 7% (Maciel et al. in preparation).

In order to identify adequate strategies for filariasis control under the specific environmental conditions in Recife, a pilot-project was implemented in a representative area (Coque), by integrating DEC mass therapy with vector control. The vector control measures employed included biological control, the use of floating layers of polystyrene beads, as well as environmental education actions for the local community. *Bacillus sphaericus* was selected as the biological control agent because of its high activity against *C. quinquefasciatus* larvae, its safety to human and other non target species, its ability to persist in polluted waters (de Barjac & Southerland 1990) and the availability of facilities for local production. We report here the effects of such control actions on the *C. quinquefasciatus* population, emphasizing the biological control with *B. sphaericus*.

MATERIALS AND METHODS

The study areas - The Operational Area (OA) measuring 1.2 km^2 is located in Coque, within Recife urban area. It is limited at the North and West by two arms of the Capibaribe River and at the East by a 300 m wide uninhabited parking area. A 0.17 km^2 Evaluation Zone (EZ) was established in the center of the OA and the outer area, at least 300m wide, served as a Barrier Zone (BZ). The EZ is inhabited by about 3,000 people which were submitted to DEC mass therapy (Furtado et al. in preparation). The check area (CA), located 3 km far from the OA, presents similar socio-economics and environmental features.

The first search for potential and actual breeding places in the Operational Area was carried

Financial support: UNDP/World Bank/WHO/TDR, CNPq (Brazil) and FACEPE (Brazil)
Received 24 June 1994
Accepted 13 September 1994

out during the dry season, from November 1990 to February 1991. Searches for new breeding sites were carried out periodically. All the data obtained about each breeding site, like location, surface area, depth, vegetation, water pollution level and so on, have been collated using the computer program Epi Info (WHO).

Physical control measures - As a large number of productive cesspits was found, some physical measures such as replacement of covers, protection of vent-pipes with nylon net and/or isolation of the water surface using a 3 cm layer of polystyrene beads (BASF), according to the technique described by Reiter (1978, 1985), were applied to those sites in order to reduce the number of places to be treated with *B. sphaericus*.

Biological control agent - A sample of *B. sphaericus* 2362 from Institut Pasteur was employed to initiate the production of about 90 liters per month of a final whole culture (FWC). The fermentation process, lasting 48 hr and using MBS medium (Kalfon et al. 1983), was conducted at the Department of Antibiotics, Federal University of Pernambuco, as described by Rios and Silva-Filha (1992). Each batch of FWC was submitted to spore count as well as tested for the presence of contaminants and then bioassayed against fourth instar larvae of *C. quinquefasciatus* laboratory strain. The materials were bioassayed again when they were stored (at 4^oC) for more than one month before use. This FWC was employed at most treatments. A flowable concentrate of *B. sphaericus* 2362 (Spherimos®, Novo Nordisk) was eventually used instead of the FWC.

Control strategy - The adequate control measure for each breeding site type was chosen according to a detailed description of the OA. The area mapping was done during the first four months. Physical measures were then applied over the following five months. Since September 1991 all the other places were periodically treated with *B. sphaericus*, over a 27 month period.

The frequency of *Bacillus* applications as well as its rate was initially established based on previous field trials carried out in ten representative breeding places, preceded by the assessment of *C. quinquefasciatus* local population susceptibility to that bacterium (Silva-Filha et al. in preparation). The following control strategy was designed according to the environmental and entomological baseline data gathered during the pretrial phase: (a) large scale treatment with *B. sphaericus* started in September, the beginning of the dry season; (b) at the beginning all potential and real breeding sites were treated every month. As the project progressed, intervals between treatments changed according to the percentage of places reinfested with *Culex* larvae. More polluted sites like cess pits and "inspection boxes" were treated with 40 g/m^2 of FWC or 5 g/m^2 of Spherimos. The other ones received 10 g/m^2 of the former material or 2 g/m^2 of Spherimos. The required amount for each rate was mixed with tap water and applied with a 5 liters pressurized hand sprayer; (c) to make easier the treatments with *B. sphaericus*, the OA was divided in 11 sections. A team of two workers was responsible for two or three sections. Breeding sites located in the EZ were monitored for the presence of mosquito larvae just before each treatment. Other information like "dried" or "fast water flow" or "sealed place" were also recorded on the breeding site lists by field workers. Data were analyzed using the EPI INFO program; (d) entomological indices were gathered over 41 months (from December 1990 to April 1994). This included a six month follow up phase, after the interruption of the treatments.

Entomological indices - The intensity of colonization of the breeding sites by *C. quinquefasciatus* was assessed before, during and after control measure applications in 20 sites representatives of the major types existing in the OA, by monitoring larvae/pupae density. Samples were collected weekly/fortnightly using a 150 ml dip. They were examined the same day in the laboratory. The larvae-pupae relative density (LPD) was estimated by counting only third/fourth instar larvae and pupae from 5 to 10 dips collected per site, depending on its surface area.

Adult mosquito relative density was assessed by using CDC-miniature light traps set at both operational and check areas. Twelve catch stations (households) were distributed within the EZ and eight in the BZ of the former and nine stations in the CA. Counting of mosquitoes was done in the laboratory and the females were dissected to estimate infection and infective rates.

Climate - Both temperature and atmospheric humidity in Recife are very favorable to mosquito breeding throughout the year. Temperatures averages range between 24 and 28^oC monthly and humidity between 67 and 85%. These climatic conditions are propitious to a short life cycle and a high fecundity throughout the year. The post embryonic development from L1 hatching to adult emergence can last only 6-8 days in laboratory, at room temperature. Rainfall regime is the most important data related to the design of mosquito control strategies. During the rain season in Recife (March-August), it rains almost continuously and heavily. Sparse rainfall occurs in the dry season. In fact, it rains almost every month throughout the year (Fig. 1).

Staff - The project was conducted by two Entomologists, three trainees (biologists) and two technicians; *B. sphaericus* was produced by a microbiologist and two technicians; eight full-time sanitary agents from the FNS (Health Na-

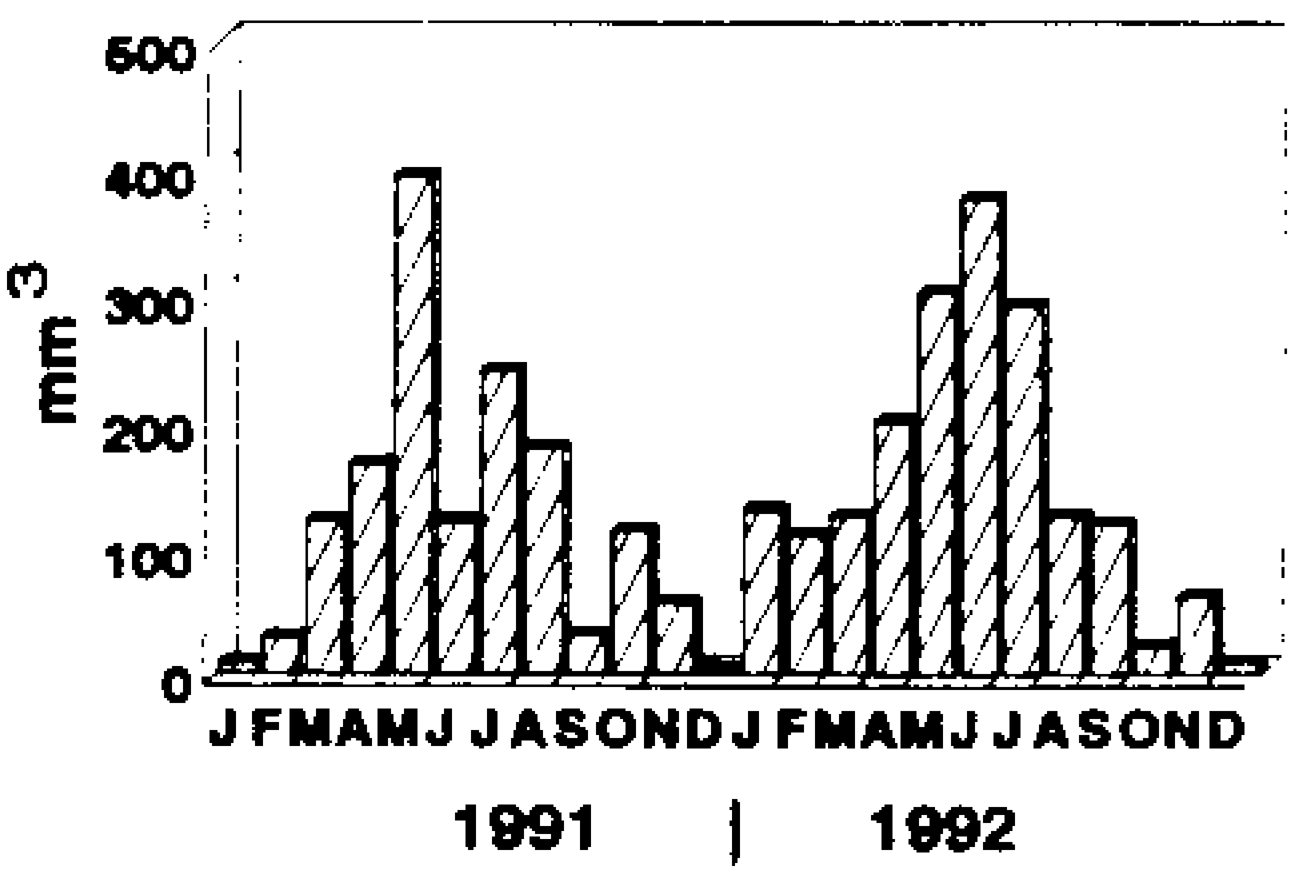

Fig 1: rainfall in Recife, Brazil. 1991= 1.47 m^3; 1993= 1.87 m^3.

tional Foundation) and a driver performed the field works. About 30 "Vector Vigilantes", a volunteer group from the local school, participated in field works.

RESULTS

A detailed search for breeding sites in both Evaluation and Barrier Zones was carried out prior to the implementation of control measures, to understand the mosquitogenic conditions existing within the OA. Over 3,000 potential or actual breeding places were recorded, of which 1,200 are located in the EZ. There is a local piped sewerage system covering part of this zone. It consists of a cess pit for four households from which water excess is drained by an underground piped system presenting a large number of small covered concrete boxes, called "inspection boxes". The pipes from four streets converge toward a big concrete catch basin, the water of which is disposed into a channel. This channel runs directly to the Capibaribe River. In the streets not served by the sewerage system, there is usually a cess pit per household and the usual means of disposing of water after use are open drains running through the streets.

Numerically the major breeding site types existing in EZ are "inspection boxes" and cess pits. The latter type always contains free water because of local soil conditions. Both represent 79% of the sites recorded, followed by open drains (14%), rainpools (5%) and others such as swamps, marshes, tanks, channel, lagoons, etc.

The sprayable water surface in the EZ was approximately 10,000 m^2. The lagoon is the major surface area, however *Culex* population seems to be naturally controlled here by an important indigenous population of the larvivorous *Gambusia* fish living there. In fact *Culex* larvae have never been found in the lagoon. The second largest surface area, the channel (2,100m^2 within the EZ) was almost never infested by *Culex*, for unknown reasons. The surface area sum of the inspection boxes found in the EZ is 440m^2. However they were considered as the main breeding source, for the following reasons: (a) 50% of the "inspection boxes" were real breeding places before *Bacillus* application; (b) they presented the highest larval densities. Furthermore, the intermittent water flow occurring in that site type represents an additional problem for bioinsecticide persistence. Special attention has also been paid to cess pits, because most of them were not mosquito proof and 17% were colonized by *Culex* before the treatment period start.

Prior to initiate the treatment with *B. sphaericus*, some physical measures were applied to cess pits. The vent pipe of every cesspit in EZ was protected with a nylon net. Eleven pits received concrete covers and 116 cracks on cesspit walls were repaired. From up to 200 pits examined only 29, all located in the BZ, presented the required conditions to be treated with a layer of polystyrene beads. That measure resulted in an excellent control of mosquito breeding. Detailed results will be presented elsewhere (Silva et al. in preparation). Besides to encourage the community to survey their cess pits, the applied physical measures promoted a decrease in the number of sites to be treated with *B. sphaericus*.

The first treatment of the sites with *B. sphaericus* was carried out in September 1991, just after the heavy rainfall period. Every potential or actual breeding place previously described in the OA had been treated. Information about the treatment was then recorded on the site's lists. This same schedule was followed for the subsequent treatments. At the first one (T1) 290 places were treated in the EZ (Fig. 2) and 42% of them examined for *Culex* presence. The number of treated sites increased thereafter reaching 821 at T30. This increase was due to the appearance of new sites after rainfall periods, family migration into the area and as a result of further careful surveys carried out periodically.

As many as 45 actual breeding sites were recorded at T1, i.e. 38% of the sites examined. The percent of positive sites has progressively decreased to 29.8% and 15% at T2 and T3 respec-

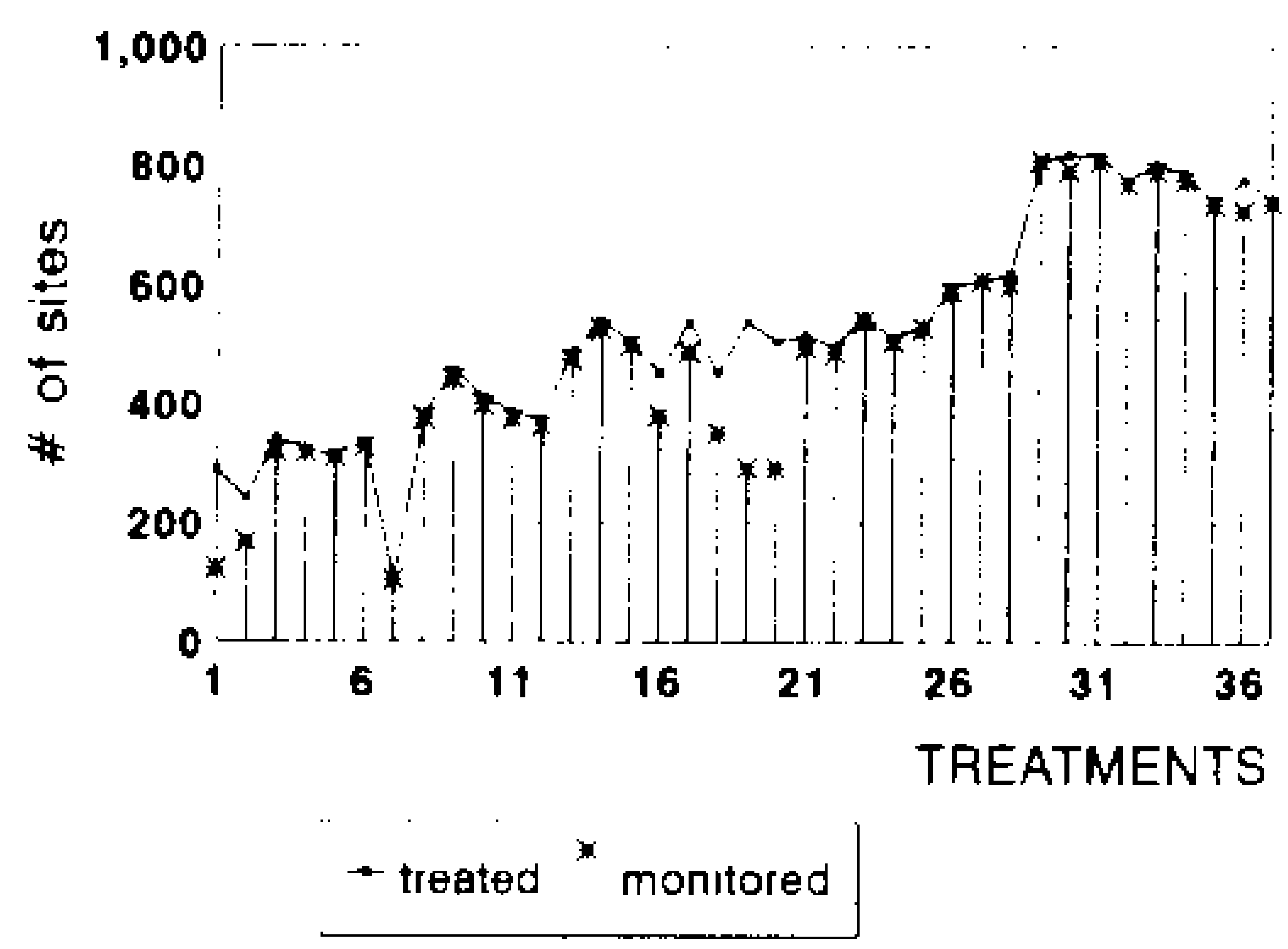

Fig. 2: number of places treated with *Bacillus sphaericus* and places monitored before each treatment, in the Evaluation Zone.

tively and remained thereafter almost always below 10% (Fig. 3). The "inspection boxes" have been more frequently colonized between treatments by *C. quinquefasciatus* than the other breeding site types, indicating lower *B. sphaericus* persistence in that site type.

Both low residual activity noted in the inspection boxes, and the frequent appearance of new habitats seem to be responsible for a relatively high percentage of colonized sites persisting throughout the first year. The strategy was then changed as an attempt to obtain lower frequency of site re-colonization by *Culex*. Thus, from T15 the sites were treated twice a month and the frequency of infested sites was subsequently de-

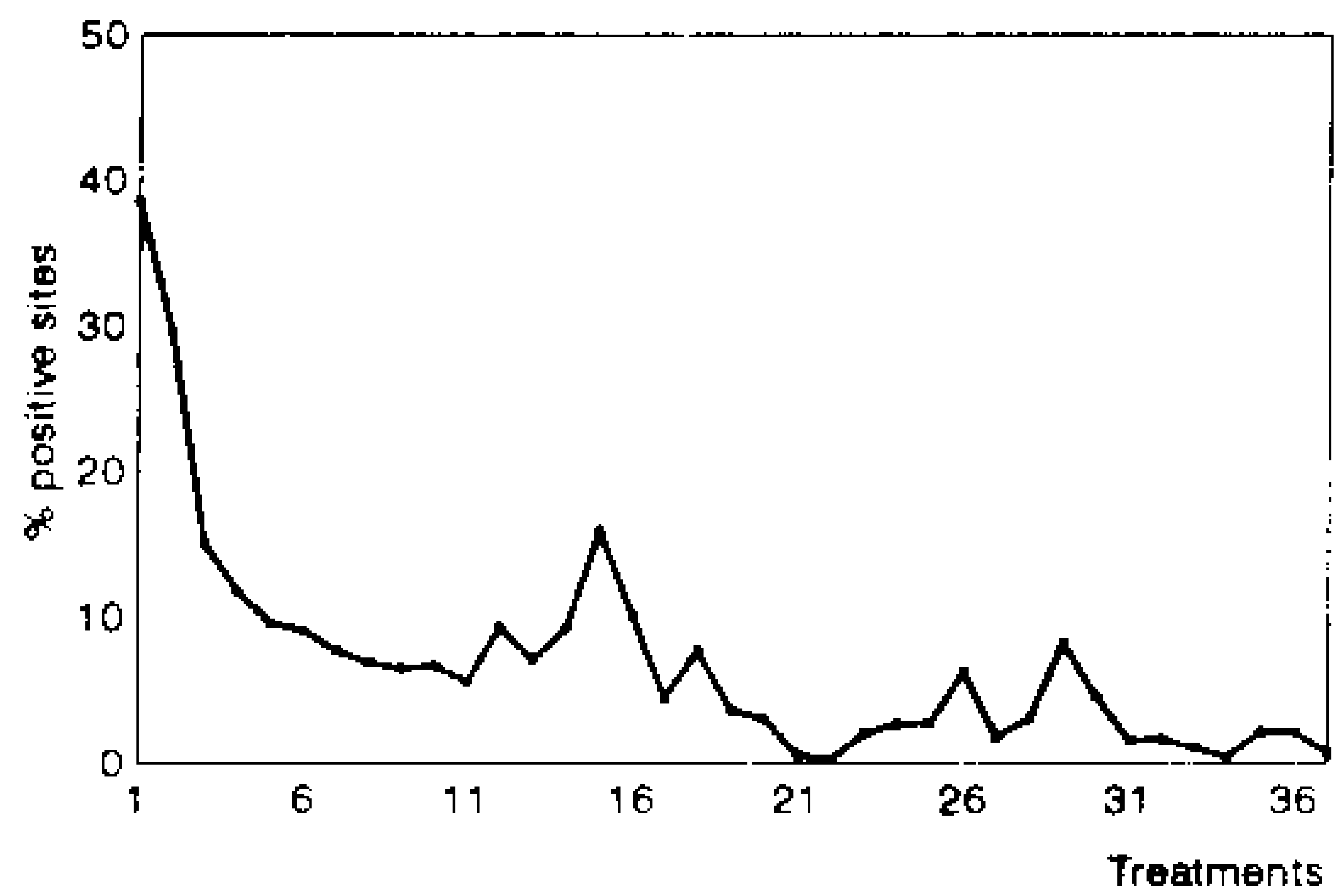

Fig 3: percentage of places found colonized by *Culex quinquefasciatus* larvae before each treatment with *Bacillus sphaericus*, in the Evaluation Zone.

creased. *Culex* larval densities, estimated from 17 sampled places just before T1 were extremely high: an average of 437.8 larvae + pupae per dip. At the subsequent inspections a dramatic decline was observed, the values oscillating between 0.02 and 30 (Fig. 4).

The profile of adult mosquito density in the CA showed monthly oscillations ranging from 20 to 60 mosquitoes per room per night (m/r/n) with one peak of 95-100 m/r/n occurring in the middle of the heavy rainy season (Fig. 5A). In the OA, averages of 60 to 120 m/r/n were recorded before starting the implementation of physical control measures (December 1990-April 1991) (Fig. 5B). Such a high mosquito density reflects the severe environmental conditions existing there. The abundance of inspection boxes in this area, on one hand, and the presence of *Gambusia* sp. in most drains, the main *Culex* breeding place type in the CA, on the other hand, could explain the marked difference observed in mosquito densities

From the beginning of control interventions in the OA, adult mosquito density decreased progressively to 5.7 m/r/n but population size was recovered quickly the following month, persist-

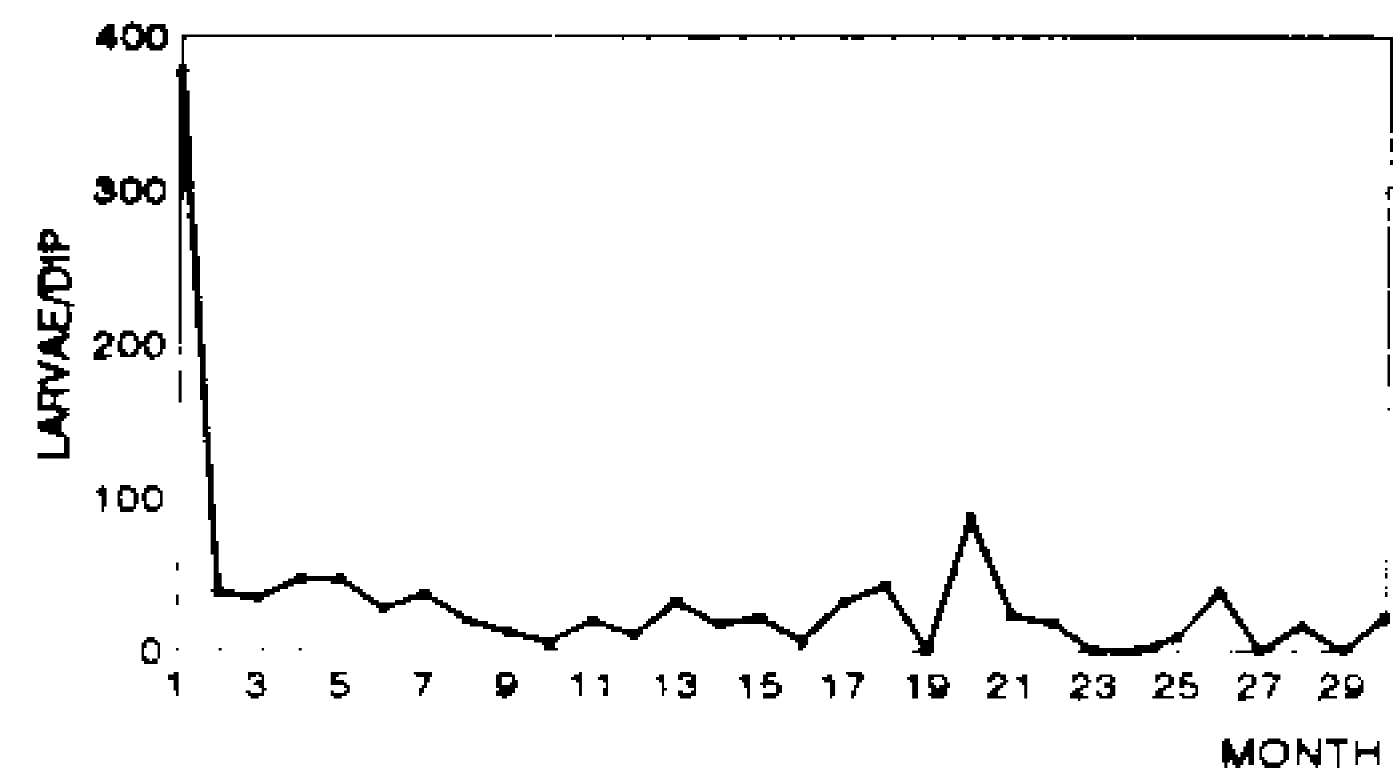

Fig. 4: relative densities of *Culex quinquefasciatus* larvae and pupae estimated from samples collected in 17 breeding sites located in the Evaluation Zone and monitored throughout the treatment period. 1= pretrial density.

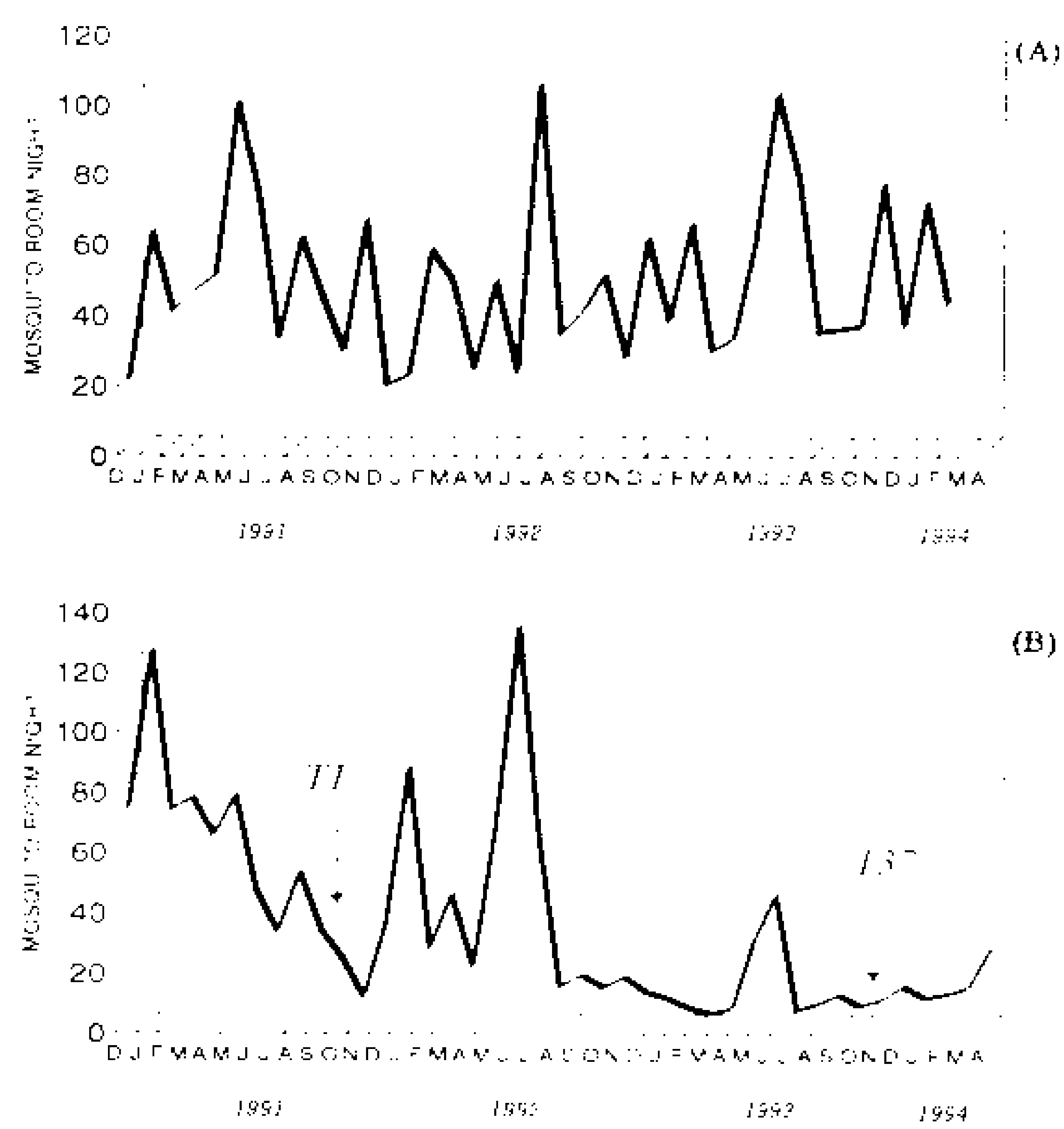

Fig 5: average number of adult mosquito per room per night, caught in 9 stations distributed within the Check Area (A) and in 12 stations located in the Operational Area, Evaluation Zone (B), using CDC miniature light traps.

ing for six months during which it rained continuously. An important impact on adult mosquito population was obtained thereafter and densities have been kept at low levels for 20 months, including four months after the interruption of the treatment. During this period, only a moderate peack was observed, in July 1993.

C. quinquefasciatus population from the OA has shown a decreased susceptibility to *B. sphaericus* after it had been exposed to that bacterium for 24 months. The same populations presented however a slightly increased susceptibility to *B. thuringiensis* serovar *israelensis* (Silva-Filha et al. submited). This indicate the need for strategies to manage the risk of *Culex* resistance to bioinsecticides.

CONCLUDING REMARKS

The ideal scenario for mosquito proliferation - poverty, continuous immigration of human population resulting in disordered urbanization, associated with high rainfall, temperatures and humidity throughout the year - is typical of several areas in Recife, like Coque where a large number of highly productive *Culex* breeding sites results in adult densities as high as 60-120 mosquito/room/night. The local sewerage system existing in that area is in fact the major mosquito source.

In spite of that difficult scenario, the control measures used have been able to keep *Culex* population at a low density level for at least 20 months.

B. sphaericus showed good efficacy providing excellent initial control even when used in very polluted water. At least a month of control persistence was obtained in most breeding site types. However structural characteristics of the major breeding site type, the inspection boxes, were not propitious to control persistence for long period, leading to the need for frequent applications. Maintenance of concrete covers seems to be the only viable measure to avoid mosquito breeding in that site type. This and others physical control measures, such as polystyrene bead layers applied to cess pits, can be implemented at the beginning of a control program in order to reduce the number of places to be treated with *B. sphaericus*. Biological control using larvivorous fishes in lagoons, for example, is also recommended once *Gambusia* was found breeding in some water collections in Recife.

Based on both rainfall regime and preimaginal population dynamic, September is the best time to start a mosquito control program in Recife. Treatments with *B. sphaericus* must be repeated monthly, covering as many places as possible during the first six months in order to reach a significant decrease of adult mosquito population before the heavy rainy season.

Because of the presence of predators of *C. quinquefasciatus* larvae among the natural fauna found associated with *Culex* in several breeding sites, the use of non selective insecticides could result, besides other problems, in an significant increase of *Culex* population after a control program. The use of a material harmless for non target organisms is particularly important in towns like Recife, where waters from domestic use, channels and rivers communicate and aquatic animals collected in urban areas are an usual source of food for humans. Field trials have shown that the use of *B. sphaericus* does not disturb the natural equilibrium among the various populations of the natural fauna (Mulla et al. 1984, Karch et al. 1990).

We strongly support the biological control with *B. sphaericus* as the best method currently available for *C. quinquefasciatus* control to be used in those places that could not be eliminated by mean of physical measures or environmental management.

Some other data gathered during this pilot project, like the dynamic of preimaginal population in different types of sites, the dispersal of *C. quinquefasciatus* population in urban areas, the susceptibility of *Culex* population after two years of treatment with *B. sphaericus* and the impact of such treatments on non target species, vector infection and infective rates as well as community participation on mosquito control, are to be analyzed and will be published sooner.

ACKNOWLEDGMENTS

To Prof. H de Barjac, International Entomopathogenic Bacillus Centre/Institut Pasteur, Paris, for supply of *B. sphaericus* SPH88 reference powder; to field team from FNS (National Health Foundation) for field works; to Ana Lúcia and R Pimentel for helping in samples analysis and to Wainer Souza and Tiago Lapa for helping in statistical analysis.

REFERENCES

Azevedo R, Dobbin JE 1952. Filariose (*Wuchereria bancrofti*) no grupo residencial do IAPB no bairro dos Afogados (Recife). *Publicações Avulsas do Instituto Aggeu Magalhães I:* 157-162.

De Barjac H, Southerland DJ 1990. *Bacterial control of mosquitoes and blackflies.* Unwin Hyman Ltda, London, xix+349 pp.

Kalfon A, Larget-Thiéry I, Charles JF, de Barjac H 1983. Growth, sporulation and larvicidal activity of *Bacillus sphaericus. Europ J Appl Microbiol Biotechnol 18:* 168-173.

Karch S, Monteny N, Julien JL, Sinègre G, Coz J 1990. Control of *Culex pipiens* by *Bacillus sphaericus* and role of nontarget arthropods in its recycling. *J Amer Mosq Control Assoc* 6: 47-54.

Mulla MS, Darwazer HA, Davidson EW, Dulmaghe HT, Singer S 1984. Larvicidal activity and field efficaccy of *Bacillus sphaericus* strains agains mosquitoes larvae and their safety to nontarget organism. *Mosq News 44:* 336-342.

Rachou RG, Villela AM, Cruz AE, Carvalho G 1956. A filariose bacroftiana em Recife (Pernambuco): Resultado de um inquérito realizado em 1954-1955. *Rev Bras Malariol D Trop 8:* 359-367.

Reiter P 1978. Expanded polystyrene balls: an ideia for mosquito control. *Ann Trop Med Parasitol 72:* 595-596.

Reiter P 1985. A field trial of expanded polystyrene balls for the control of *Culex* mosquitoes in pit latrines. *J Amer Mosq Control Assoc 1*: 519-524.

Rios EM, Silva-Filha MHNL 1992. *Bacillus sphaericus* local production for large scale trials. *Anais Simpósio de Controle Biológico 3:* 166 EMBRAPA-CNPDA, SP.