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CONTROL OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) WITH *BACILLUS SPHAERICUS* IN MAROUA, CAMEROON

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ABSTRACT. Two strategies were tested to control *Culex quinquefasciatus* with *Bacillus sphaericus* in Maroua (population 130,000), Cameroon. The treatment of all potential breeding sites (27,000) with *B. sphaericus* during the dry season caused up to a 90% reduction in the adult biting rate. Because of the short persistence of *B. sphaericus* and the occurrence of new breeding sites, unacceptable levels of adult biting rates were reached again in 5 months. In the second strategy, two treatments per year of the most productive breeding sites (10,000) stopped the biting rate increase during the rainy season. The results were only partially successful because of variations in *B. sphaericus* toxicity. The first treatment required 1,200 man-days of work vs. 200 for the simplified treatments. The density of breeding sites depends on the rainfall and the presence of a tap-water network. A sustained control program of *Cx. quinquefasciatus* will depend upon the dynamics of the principal breeding sites and an improved formulation of *B. sphaericus*.

INTRODUCTION

Like most tropical countries, Cameroon faces a rapid increase in the urban population, which reached 49% of the total population in 1991 (Schwartz 1992). In Maroua, the capital of the Extreme-North Province, the population almost doubled between 1976 and 1987. Such urbanization is too rapid to allow town planning and public health services to build the necessary infrastructures. This leads to proliferation in many towns of pools filled with stagnant wastewater, providing potential breeding sites for *Culex quinquefasciatus* Say (Horsfall 1955). This mosquito, formerly localized in African ports, has extended its range to all urban areas and even to rural ones. Because of the increasing nuisance and the risk of an extension of the transmission of Bancroftian filariasis (Subra 1980) control of *Cx. quinquefasciatus* is now a priority for public health services. The resistance of this species to most chemical pesticides (Magnin et al. 1988) requires the use of higher dosages and may lead to pollution of the water table. A possible remedy is the use of biological pesticides such as *Bacillus sphaericus* Neide. The efficacy of *B. sphaericus* has been fully studied by many scientists (Anonymous 1985, de Barjac and Sutherland 1990). The harmlessness of *B. sphaericus* to nontarget organisms has been studied as well (Shadduck et al. 1980). The goal of this study was to evaluate the effect of treatments with an aqueous suspension of *B. sphaericus,* strain 2362, on the density of *Cx. quinquefasciatus* in a large city. Results described here (and largely developed in Baldet 1995) include the distribution of the different types of breeding pools in different types of districts, the practical details of the treatments, and the impact of the treatments on the population of *Cx. quinquefasciatus.*

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MATERIALS AND METHODS

Description of the area: The climate of extreme northern Cameroon is the Sudan savannah type, with a dry season from mid-October to mid-April. Average rainfall per year is 800 mm (Olivry 1986). In 1991 rainfall was exceptionally abundant (1,231 mm) (Fig. 1). Monthly relative humidity ranged between 30 and 40% during the drier months and between 70 and 80% during the rainy season. The temperature ranged from 33.6°C in April to 23°C in December.

Maroua covers an area of 2,000 hectares and has a population of 130,000. Three main types of districts were characterized: low-income districts with mud houses rarely connected to electricity or a water supply, high-income districts with cement houses connected to electricity and a water supply, and administrative and industrial districts with cement buildings connected to electricity and a water supply (Table 1). More than 50% of the inhabitants obtain water from street fountains or buy it from carriers on the street, 15–20% are connected to the tap-water network, and 25% have a well. The consumption of water is approximately 15 m^3/month in houses with piped water and 2 m^3 in others. The town is crossed or bounded by rivers that flow intermittently during the rainy season and are dry during most of the year.

Entomological survey: Mosquito density was monitored monthly by human-baited landing collections from February 1991 until January 1994. Collection was performed 2 nights per month at 8 stations from 2000 h on one day to 0600 h the following day. From September 1993 collection was stopped at 2400 h. Results obtained from January 1991 to August 1993 showed the number of females caught from 2000 to 2400 h to reach 30% of the complete night catching. Results from September 1993 until January 1994 were then multiplied by a factor of 3.3 to allow comparison with previous results.

To compare the larval population dynamics before the first treatment versus after the treatments,

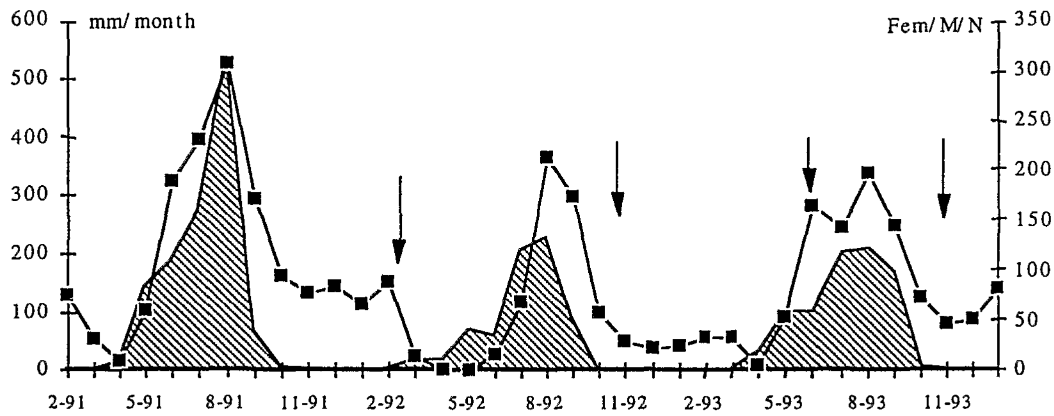

Fig. 1. Rainfall and variations in the biting rate during treatment with *Bacillus sphaericus* of breeding sites in Maroua. February 1991–January 1994.

a sample of 33 potential breeding sites (called "sentinel breeding sites"), located in the different districts and representative of almost all types available for *Cx. quinquefasciatus,* were chosen in February 1991 and surveyed during the complete study. Monthly dipping was done to evaluate the density of preimaginal populations of *Cx. quinquefasciatus* until January 1994. Results are expressed as the percentage of positive breeding sites and as a density index ranging from 0 to 3 (0 larvae per 3 dippings = 0; 1–5 = 1; 5–20 = 2; more than 20 = 3). After the first treatment the survey was completed by the study of those breeding sites that were the most difficult to control. These sites had been treated during the exhaustive treatment but with less satisfactory results. They included breeding sites with very high larval density, or with water flow, as well as every breeding site located in streets. There were more than 2,500 such sites, which were surveyed by dipping every month. Breeding sites were considered positive if old larvae or pupae of *Cx. quinquefasciatus* were found. If only 1st- or 2nd-stage larvae were found, a 2nd survey was done 48 h later.

Treatments: Before each treatment, the local broadcast station informed the population of Maroua daily of the control campaign. Using 1/5,000 scale maps, the town was divided into 19 districts grouping 122 zones. Each zone (generally <20 ha) was treated by one team in 1–3 days.

The exhaustive treatment (February–March 1992) began 2 months before the end of the dry season, that is, when the density of *Culex* was at its lowest level. Two to 8 teams with 6 people each and using a vehicle completed the treatment of the entire town in 40 days. All premises were searched for breeding sites and inhabitants were asked about any potential breeding sites, which were sprayed using a manual sprayer and marked with a cross. Posttreatment verification that all buildings and breeding sites were sprayed was done using these marks. Three lots (1,315 liters) of a 22% aqueous suspension of *B. sphaericus* were used to treat the entire town at a dosage of 10 g of *B. sphaericus* per square meter of breeding site.

For the simplified treatments (November 1992, June 1993, and November 1993) potential breeding sites were searched for only in buildings connected to the tap-water network (dwellings, administrative buildings, shops etc.) and in the streets. Eight teams of 2 people from the urban health service using motorcycles to travel completed the simplified spray treatments according to the protocol of the first treatment. For each treatment a new suspension of *B. sphaericus* was furnished by the producer.

Toxicity of the B. sphaericus*:* The 7 lots of *B. sphaericus* used during the treatments were tested in the laboratory upon their arrival in Cameroon. Tests were performed on 3rd- and 4th-stage larvae of the Maroua strain of *Cx. quinquefasciatus* collected before the first treatment. A test included 5 concentrations (10^{-5} mg/liter to 1 mg/liter), 2 or 3 trials (3 × 25 larvae each), and a control. The median lethal concentration (LD_{50}) was compared to reference strain SPH 88 and results are given in international toxic units (ITUs). If mortality in the control was between 5 and 20%, the results were corrected according to Abbott's formula (Abbott 1925). If control mortality exceeded 20%, the test was eliminated.

RESULTS

Types of breeding sites (Table 1):

(i) Cesspools (total = 7,604). These structures collected wastewater and were either crudely dug pits or cemented, open or closed. Capacity ranged from liters to several cubic meters. Before the simplified treatments, a hole (1-cm diam) was made in the center of the heaviest cement lids to make the spraying easier.

(ii) Inspection chambers (total = 3,220). Sewage pipes converged to these structures, which were connected to cesspools by a drain. The drains were

Table 1. Number and type of potential breeding sites sprayed during the exhaustive treatment with *Bacillus sphaericus* in Maroua, February–March 1992. Results are given by districts.

	No. of buildings		Type of breeding sites per 100 buildings					
District (type[1])	Total	Per ha	Tapwater	Cesspools[2]	Latrines	Dips	Puddles	Others
IND (A)	148	0.5	56.8	282.4	18.9	6.8	33.8	26.4
Dja. ADM (A)	290	1.4	55.9	298.3	12.8	6.6	12.4	25.5
Missing. (L&A)	166	2.5	27.7	66.3	32.5	5.4	22.3	12.0
ADM (A)	291	2.6	34.7	244.0	66.7	12.7	28.2	12.0
Koutbao (L&A)	126	6.2	37.3	62.7	31.0	4.0	0.0	0.8
Djarengol (H&L)	1,026	6.6	27.5	107.4	33.5	0.8	21.0	31.5
Dougoy Sara (L)	104	7.4	9.6	50.0	99.0	7.7	12.5	1.0
Dougoy (L&H)	3,055	8.2	17.7	64.2	85.7	2.7	14.4	14.3
Ouro-Tch. (L)	526	9.1	0.6	21.5	16.3	0.2	18.8	17.3
SIC (H)	64	11.2	85.9	240.6	1.6	3.1	21.9	3.1
Monad. (L&A)	245	15.8	30.2	82.0	68.6	1.2	26.5	15.5
Baouliwol (L)	319	15.9	11.9	22.9	14.4	0.0	15.0	12.2
Domayo (L)	3,764	18.5	19.9	55.4	51.4	2.8	12.6	12.3
Toupouri (L)	719	19.3	0.8	7.5	29.9	3.6	12.8	16.0
Doualare (L)	1,698	21.8	5.0	17.8	47.0	0.1	14.7	9.2
Founange (L)	1,416	22.0	20.6	65.8	112.2	2.0	26.6	8.9
Zokok (L)	1,718	25.1	23.2	49.7	64.9	6.4	12.3	11.1
Lopere (L)	1,693	26.8	17.0	39.2	76.0	3.2	15.2	12.6
Patching. (L)	1,011	33.4	1.6	9.4	29.4	2.0	9.2	15.5
Total	18,379	9.5[3]	3,276	10,824	10,951	530	2,853	2,521

[1] Type of districts: L, low-income; H, high-income; A, administrative and industrial; L&A, mixed habitat.
[2] Cesspools, including inspection-chambers.
[3] Mean density.

often clogged and the chambers had accumulated several liters of water. In Table 1 these sites are grouped with the cesspools.

(iii) Pit latrines (total = 10,951). These sites contained water when they were used also as showers. Most of the pit latrines were treated also when they were dry according to the inhabitants' request.

(iv) Medium to large depressions (total = 530). These sites occurred in the road or were dug as open drains to dispose of wastewater (dips in Table 1). The depressions ranged from a few meters to more than 100 m in length.

(v) Small puddles (total = 2,853). These sites collected wastewater, with many created by water leaks in pipes. The puddles were treated if they were still full of water 48 h after the first survey.

(vi) Other sites (total = 2,521). Other sites included wells that were abandoned or contained nonpotable water (346 treated out of 1,159 full of water); "canaris" (water storage jars; *ca.* 20 liters) not in use; and plastic or metal tanks, drums, cans, tires, and pails were also treated.

According to the district, the mean number of potential breeding sites per house varied from 0.6 to 3. Because of their quantity, their capacity, their high probability of containing water (more than 55% from April to August) and their frequent colonization by *Cx. quinquefasciatus* (more than 45% from August to October) cesspools were the most important category of breeding site. Considering the town as a whole, the mean density was 5.6 cesspools/ha. The occurrence of these breeding sites was correlated (Spearman coefficient = 0.90; $P <$ 0.004) with the density of the tap-water network in the 19 districts (Fig. 2).

Natural variations in the density of Cx. quinquefasciatus *(Fig. 1):* In 1991 the mosquito density varied according to the rainfall. At the start of the rainy season the number of females collected per man per night (♀♀/m/n) increased 20-fold from April to June, before peaking in August. The density decreased in September as did the rainfall.

Density of *Cx. quinquefasciatus* also varied according to the districts, ranging from 0 ♀♀/m/n (station 3) to 32 (station 8) in April (lowest monthly density) and from 150 (station 1) to 560 (station 7) in August (highest monthly density). Rainfall contributed heavily to filling the depressions; 32.8% contained water in April 1992 vs. 83.4% in August. For the traditional cesspools inside habitations, this figure was 44% vs. 77%. On the other hand, the cemented cesspools were relatively independent of the rainfall; 55.9% were filled in April vs. 60.3% in August. Rainfall had a differential effect on the population of *Culex* in the different types of districts. In the administrative and high-income districts the biting rate was only 4–10 times as high in the rainy season as in the dry season vs. 18–261 times in the low-income districts.

The results of the sampling of 33 sentinel breeding sites were consistent with the results of the catches. At the onset of the rainy season, the number of positive breeding sites increased (16 in April vs. 24 in July), as did larval density (1.6 in April vs. 2.5 in July). These indicators decreased after August.

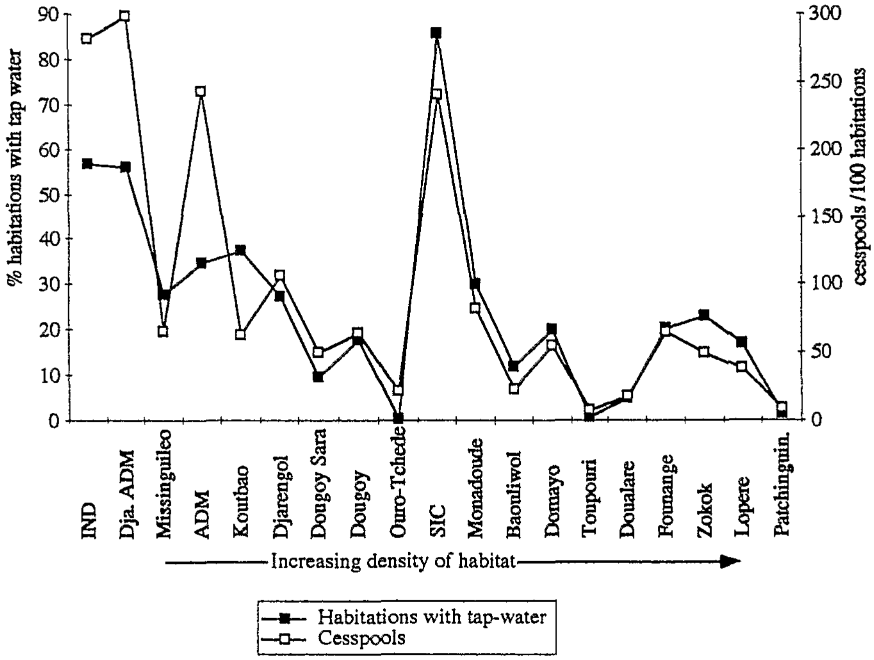

Fig. 2. Relation between the percentage of habitations with tap water in the different districts of Maroua and the number of cesspools.

Impact of the treatments (Fig. 1): In March 1992 the number of female *Culex* collected showed a 93.7% reduction compared to March 1991. A very low density persisted through the beginning of the rainy season; with more than 90% reduction for all catching sites in May and June and a 78.1% reduction in July. In August and September 1992, results reached a level similar to the one recorded in 1991.

In 1992, the percentage of positive sentinel breeding sites was 3.8% in April, 43.4% in June, and 53.6% in September as compared to 57.1%, 72.4%, and 67.7% in 1991. During the 3 months following the treatment, the mean density in the positive breeding sites was 1.5 in 1992 versus 2.1 in 1991.

In April 1992, the survey of the 2,500 "difficult-to-control" breeding sites was initiated. The percentage of positive sites among those with water was 3.5% in April, 21.2% in June, and 43.7% in September (Fig. 3). The efficacy of the treatment varied according to the type of breeding site. In April, a few weeks after the end of the treatment, the percentage of positive sites was less than 10% in each type except in the puddles linked to water

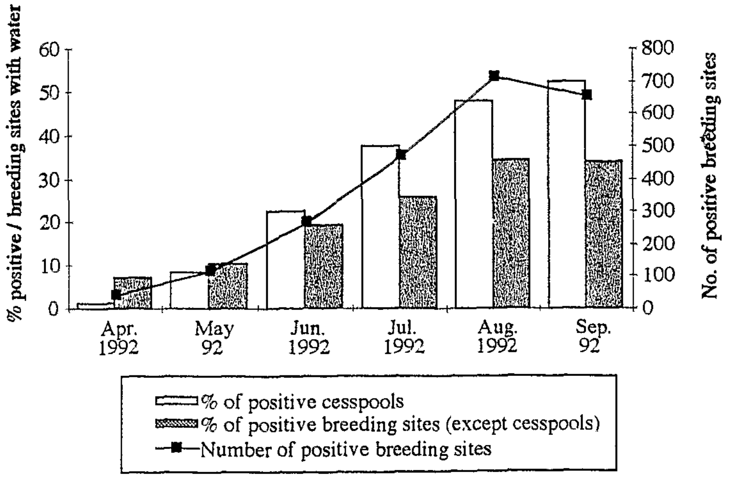

Fig. 3. Survey of 2,500 difficult-to-control breeding sites after the exhaustive treatment in Maroua, February–March 1992.

Table 2. Toxicity of the *Bacillus sphaericus* formulations at the time of their receipt in Maroua.[1]

Date of receipt (trials)	LD_{50} mg/liter	LD_{95} mg/liter	ITU/mg vs. SPH 88
Feb. 1992 (2)	0.0058 S	0.0647	305
March 1992 (2)	0.0032 S	0.0365	553
Oct. 1992 (3)	0.015 NS	0.07	118
April 1993 (3)	0.013 S	0.0837	136
June 1993 (3)	0.0124 S	0.0706	143
June 1993 (2)	0.0069 S	0.0388	256
Oct. 1993 (2)	0.0498 NS	0.277	169

[1] LD_{50}, lethal dose for 50% of the larvae; LD_{95}, lethal dose for 95% of the larvae; S, χ^2, significant (5%); NS χ^2, not significant (5%).

Table 3. Comparison of the cost of the different larvicidal treatments with *Bacillus sphaericus* performed in Maroua from February 1992 to June 1993.

Treatment	Feb. 1992	Nov. 1992	June 1993	Nov. 1993
Houses visited	18,279	2,815	4,009	3,962
Breeding sites treated	27,679	7,722	10,556	8,808
Man days of work	1,200	200	208	208
Liters of *B. sphaericus*	1,315	800	960	640[1]

[1] For this treatment, we did not have enough *B. sphaericus*; some peripheral districts not connected to the water network were only partially treated.

running away from the public stand pipes (17.6%) and in depressions (22.5%).

In December 1992 and 1993, 1 month after the treatment, the percentage of positive sites among the 33 sentinel breeding sites decreased to 24% and 33.3%, respectively, versus 56% and 47.1% before the treatment. In 1991 this index showed a slight increase from 44.4% to 54.2%. The percentage of positive sites among the 2,500 difficult-to-control breeding sites was halved. The decrease in these two indices was limited to December. On the other hand, we did not observe a significant decrease in the number of females caught.

In 1993, the June treatment was followed by a decrease in the density of *Cx. quinquefasciatus* from 164 to 142 in July. The impact was greater in the districts with a higher density of tap-water connections. However, this decrease was very short in duration and in August the population began to rise again.

The results of tests on the formulations of *B. sphaericus* are given in Table 2. The ITU value of the formulations used during the first treatment differed significantly ($P = 0.05$) from those used during the next ones, except for June 1993 (256 ITU). This lower efficacy was confirmed by the survey of breeding sites after the simplified treatments, where recolonization occurred in less than 10 days.

The cost of the exhaustive treatment was near 50 CFA Francs (<0.2 U.S. $) per inhabitant, excluding the cost of the pesticide. During the first treatment, 20–25 potential breeding sites were treated per man per day versus more than 50 during the third treatment (Table 3). This difference was mainly due to an easier access to the breeding sites during the simplified treatments, as they were in the street or in specific houses; greater mobility of the teams (motorcycles); and the preparation of the campaign (drilling holes in the cement lids covering cesspools) and a more accurate cartography of the districts, which reduced travel. On the other hand, as most of the breeding sites treated during the simplified treatments were larger, the quantity of *B. sphaericus* used was relatively higher. In the districts without connection to the tap-water network 200 houses could be surveyed in 6 h. In the administrative and high-income districts, some houses required more than 1 h per house.

DISCUSSION

Types of breeding sites: The problem in controlling *Cx. quinquefasciatus* in Maroua (and in other urban tropical environments) was not in the detailed localization of breeding sites, as they were large, situated close to houses, roughly uniform in a given district, and frequently in the cesspools and sewer network. In addition, Maroua has several characteristics favorable to treatment: the sewer network is not extensive and most of the inhabitants traditionally use cesspools, built outside houses where their treatment was easier; the inhabitants' cooperation was excellent, especially after the first treatment had obvious results; and, because Maroua is isolated in a savannah area, external reinvasions are very unlikely. Moreover, very few natural breeding sites (i.e., not related to human activity) persist during the dry season.

The number of potential breeding sites can be correlated with the consumption of water (and the disposal of wastewater), as water consumption was higher in the houses connected to the main water network than in those where water was supplied through street fountains or wells, or purchased from carriers on the street. The decay of the network, with leaks causing hundreds of puddles, also contributed to creating many potential breeding sites. Consequently, after the dry season, the increase in the population of *Cx. quinquefasciatus* was more dependent on rainfall in the low-income districts without domestic water connections than in the high-income districts.

The same relation has been observed in many African urban areas. Our estimate of approximately 5 cesspools per hectare is similar to the one recorded by Subra et al. (1970) in a town in a savannah area in West Africa. In Kenyan towns (Subra 1982), the presence of many septic tanks maintains a high population of *Cx. quinquefasciatus* for the entire year, especially in the high-income districts, where 49% of the habitations have one breeding site versus 19% in low-income districts. In Abidjan (Coulibaly et al. 1992), a survey of the distribution of breeding sites within the districts showed a preponderance of cesspools and depressions (the sites most frequently infested by *Cx. quinquefasciatus*) in the more urbanized districts. In Yaounde (southern Cameroon), which has a more rainy climate and a more extensive water network than Maroua, the number of positive breeding sites was also found to be dependent upon the density and type of habitat (Barbazan 1985) but relatively independent of the amount of rainfall (Hougard et al. 1993).

Impact of the treatments: The comparison of results after a treatment with those in 1991 at the same period is approximate because of the variations in the rainfall. However, comparison of the level of the biting rate in 1991 with those before the treatments showed that the density and the dynamics of the mosquito population were similar. Because the goal of the study was to evaluate the impact of a simultaneous treatment of the entire town, it was impossible to maintain an area untreated.

The impact of the exhaustive treatment on the *Culex* population was very rapid and significant, as the biting rate decreased up to 99% in less than 1 month and stayed below 2 ♀♀/m/n for 3–4 months in 7 stations. The resurgence of the population of *Cx. quinquefasciatus* after the treatment was also very rapid. In 1992, between April and August, among the 2,500 potential breeding sites surveyed monthly, the number with water multiplied only by 1.4, whereas the number with larvae increased 13-fold and the biting rate increased 150-fold. The resurgence of the mosquito population was enhanced by several simultaneous factors such as: the increase in the longevity of the imagoes and the range of their flight during the rainy season (Horsfall 1955); the appearance of many new breeding sites after the treatment (created by the inhabitants or filled by the rain; very few had been overlooked by the teams); and the rapid return to positive of many breeding sites (within 1–2 wk) after their treatment because of a continual flow of water that may have diluted or carried away the *B. sphaericus* and/or because of sunlight reducing the persistence of the *B. sphaericus* (Mulligan et al. 1980). Sinègre et al. (1993) found that the larval mortality was still 100% 47 days after a treatment with *B. sphaericus* in closed tanks versus 30% after 17 days in tanks exposed to the sun. In our experiment *B. sphaericus* persisted only 2–6 wk in a sample of breeding sites sprayed in March, although *B. sphaericus* can persist 5–12 wk in tropical environments (Nicolas et al. 1987, Hougard et al. 1993). The very long period with low levels of *Culex* obtained after spraying was mainly a result of an initial sharp decrease in the *Culex* population throughout the town. The continued impact of the *B. sphaericus* was observed through the end of May.

These factors made the resurgence of the population of mosquitoes explosive and simultaneous in all districts. In 1991, the potential breeding sites were more progressively reactivated at the end of the dry season and the population grew more slowly.

The treatment done in June 1993 had some effect on the population of *Culex* as the number of females caught decreased in July whereas the numbers would have continued to increase in the absence of a treatment. Temporary control of the *Culex* population during the rainy season is possible, but treatments must be repeated at least once a month to maintain a low biting rate. The treatments performed during the beginning of the dry season (November) had a limited impact because of the low toxicity of the *B. sphaericus* formulations used.

The expense of the first treatment in terms of personnel and vehicles was not very suitable to most tropical areas. The following treatments implemented by local teams were more cost-effective and participation by the inhabitants was higher. In most tropical towns in Africa it is not possible to maintain such continuous spraying as is done in southern France, where breeding sites exposed to the sun are sprayed at 9-day intervals for several months of each year (Sinègre et al. 1993). The experimental strategy tested by Hougard et al. (1993) in some districts of Yaounde (southern Cameroon) was better adapted to tropical environments: Six to 8 sprays were done only during the dry season—the season having high densities of *Culex*.

Laboratory tests (and field results) showed a wide range (1–5) of variation in the toxicity of the different suspensions of *B. sphaericus*. This could be due to storing the *B. sphaericus* too long or to changes in the conditions of formulation. Recent studies (Silva-Filha et al. 1995) show that a strategy for mosquito control with *B. sphaericus* must also take into account the risk of resistance if many treatments are done.

The *B. sphaericus* strain 2362 is competitive with chemical pesticides for the control of *Cx. quinquefasciatus*. The harmlessness of *B. sphaericus* to nontarget organisms is a definite advantage, especially in urban environments. However, the formulation has to be improved, particularly in regard to sensitivity to sunlight, persistence in polluted water, and recycling of this agent. Also, use of *B. sphaericus* has to be integrated into a global strategy, including community participation to eliminate some breeding sites (clogged puddles, poorly closed cesspools, etc.) and other specific control tactics such as mechanical ones using polystyrene beads (Maxwell et al. 1990).

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