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IMPACT OF TREATMENTS WITH *BACILLUS SPHAERICUS* ON *ANOPHELES* POPULATIONS AND THE TRANSMISSION OF MALARIA IN MAROUA, A LARGE CITY IN A SAVANNAH REGION OF CAMEROON

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ABSTRACT. Simultaneously with a control of breeding sites primarily for *Culex quinquefasciatus* and secondarily for anophelines with *Bacillus sphaericus* in the town of Maroua (120,000 inhabitants) in North Cameroon, a survey of anopheline populations and of transmission rates of malaria was performed. Monthly night catches in 8 districts of the town emphasized the relation between the biting rate by *Anopheles* in the districts and two main factors. One factor was the distance of a district from the breeding sites, i.e., natural flooded areas along the periphery of the town or artificial breeding sites (ditches, puddles) filled with rain water during the rainy season and with water from the water network throughout the year. The second factor was the density of the habitation that reduced dispersal of female mosquitoes from the breeding sites and the risk for inhabitants to be injected because of scattered bites. The treatment with *B. sphaericus* was followed by a delay (2 months) in the beginning of the transmission period and a decrease in the incidence of malaria cases studied in a health facility of the town. It thus seems to be possible to reduce malaria transmission by applying *B. sphaericus* to the breeding sites, but this requires a good knowledge of the location and dynamics of breeding sites and an improved formulation of the pesticide.

KEY WORDS Biological control, *Bacillus sphaericus,* malaria, vectors, urban, Cameroon

INTRODUCTION

During the last 10 years, studies on the prevalence of malaria have been carried out in North Cameroon (Couprie et al. 1985; Josse et al. 1987a, 1987b), but few recent data are available on the vectors of malaria in this area. Moreover, these studies on malaria concern mostly areas surrounding rice plantations or dams and seldom the situation prevailing in towns. Nevertheless, the rapid urbanization in developing countries (more than 5% per year in most of the African countries, 10% per year in Maroua from 1976 to 1987) increases the part of the population threatened by malaria in urban environments, where it is characterized by a low transmission, a low immunity, but frequent severe cases concerning every age (Anonymous 1995).

To evaluate the efficacy of a large-scale use of the biological pesticide *Bacillus sphaericus* against *Culex quinquefasciatus,* treatments were performed in every potential breeding site of Maroua, a large city in North Cameroon (Baldet 1995, Barbazan et al. 1997). An entomological survey of mosquito populations was performed before, during, and after the treatments. Because most of the breeding sites for *Anopheles* appeared likely to be colonized by *Culex* larvae, they were also sprayed. Because *Bacillus sphaericus* is efficacious against *Anopheles* larvae (Davidson 1985), the impact of the treatment on the *Anopheles* density and on the transmission of malaria was studied. The continuance of anopheline species despite the increasing urbanization is also discussed.

MATERIALS AND METHODS

Description of the area: The climate of the extreme north of Cameroon is of the Sudanese type, with a dry season from October to April. Average rainfall is 800 mm per year (Olivry 1986), but during 1991, it reached 1,231 mm (Fig. 1). Relative humidity is between 30% and 40% during the drier months and between 70% and 80% during the rainy season. The monthly average temperature ranges from 23°C in December to 33.6°C in April. The town is crossed or bounded by rivers superficially dry most of the year, but there are irrigated crops along them, with market gardens or orchards, made possible by the proximity of the phreatic sheet. Farms on the periphery have cotton and millet as their main crops.

The population of Maroua is 120,000 inhabitants. The surface area of the town is 2,000 ha (Fig. 2). There is a great diversity in the types of habitations. They can be of traditional type (i.e., made of earth and rarely connected to the electricity and water supply network) in the densely populated districts of the center of the town or in its rural periphery. In the administrative, industrial, commercial, or high-income districts, most of the habitations are made of cement and are connected to the electricity and water supply network.

Many data on the population of Maroua were obtained during the 1991 treatment period when ev-

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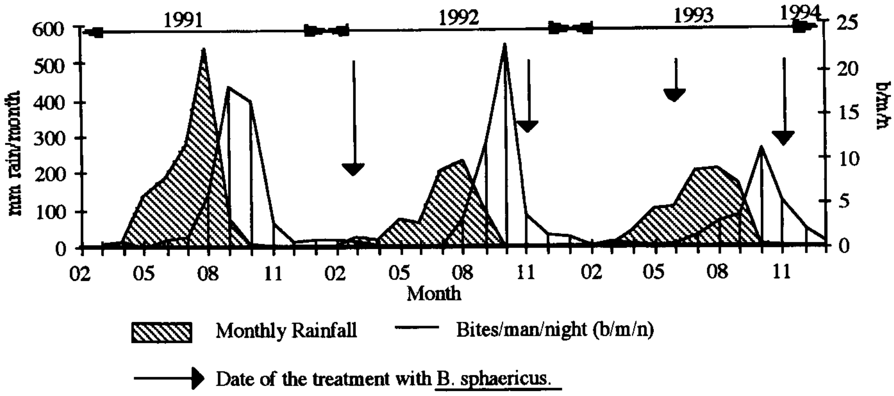

Fig. 1. Rainfall and monthly aggressivity of the *Anopheles* mosquitoes in the eight catching stations, Maroua, February 1991–January 1994.

ery habitation was visited. The average density was 9.5 habitations per hectare. Density ranged from less than 3 habitations per hectare, in some administrative and industrial districts, to more than 25 habitations per hectare in the most highly inhabited districts in the center of the town. Habitations include the following: dwellings, offices, shops, factories, hotels, administration buildings, and so on. The average occupation rate was 7 inhabitants per dwelling.

Entomological survey February 1991–January 1994: An entomological study was performed to follow the fluctuations of the *Culex* and *Anopheles* population densities. Eight teams of 2 men each completed 2 night catches per month from 2000 h to 0600 h in 8 districts of Maroua. The collecting stations were distributed in districts representative of the different types of urbanization in the town. Their locations appear in Fig. 2 and their descriptions in Table 1.

The parity of the females was determined by examination of the ovaries (Detinova 1963). Salivary glands were examined for sporozoites.

From September 1993 to January 1994, catching was stopped at 0000 h, and the mosquitoes were not dissected. From February 1991 to August 1993, the number of females caught between 2000 h and 0000 h was 33% of the complete night. From September 1993, to allow comparison with results of complete nights, the results are multiplied by 3.

Dispersal study: The same type of catching was performed in 16 stations located in the south of Maroua during 10 days in June 1993 to follow the dispersal of females. The area for this experiment included districts with low, medium, and high density of habitation, extending at increasing distances from a large breeding site area.

No effort was made to specifically locate breeding sites for *Anopheles*. Data on the principal types of breeding sites and their locations were obtained during the general survey of all types of water collections made during the treatment of the town.

Spraying of the potential breeding site: Between mid-February and the end of March 1992, every habitation (18,379) and every street in the town were surveyed. The 27,679 collections of water found (positive or negative for larvae) were sprayed with a suspension of *B. sphaericus* strain 2362 at the dosage of 10 g/m^2. Three simplified treatments concerning only the potential breeding sites in the streets or in the houses connected to the water network were performed in November 1992, June 1993, and November 1993. A different batch of *B. sphaericus* was used for each treatment.

Incidence of malaria: A retrospective study on the incidence of malaria from February 1991 to January 1993 was carried out by census of the number of patients daily (presumptively) diagnosed with malaria. The Dispensaire de Dougoy, a health facility in Maroua, was chosen for this study because it receives people coming from all districts of the town. Diagnosis was established at the arrival of the patients according to clinical examination.

RESULTS

By species: From February 1991 to January 1992, 1,481 *Anopheles* spp. were caught, 1,438 from February 1992 to January 1993 and 806 from February 1993 to January 1994, of which 85.7% were of the *Anopheles gambiae* complex (*An. gambiae s.s.* and *An. arabiensis* can be found in North Cameroon; Gillies and Coetzee 1987, Robert et al. 1992), 8.9% *An. pharoensis,* and 5.2% *An. funestus. Anopheles squamosus, An. coustani,* and *An. rufipes* together reached 0.2% of the females caught. *Anopheles gambiae* was usually more abundant than the other species, but *An. pharoensis* reached its peak density 1 month before *An. gambiae* (and *An. funestus*) and increased slightly during the dry season, December–February, after the decrease of *An. gambiae* and *An. funestus.*

By season (Fig. 1): Considering the complete study (3 years), 91.3% of the *Anopheles* spp. were

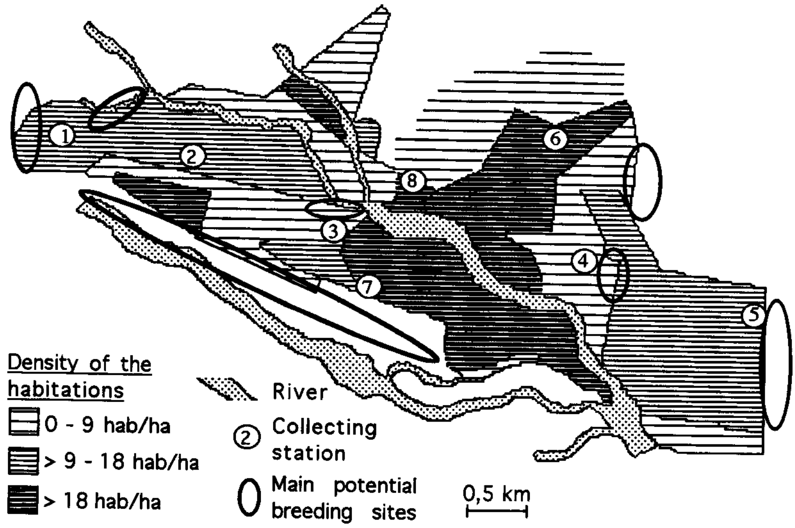

Fig. 2. Plan of Maroua, density of the habitations, collecting stations, and main potential breeding sites for *Anopheles*.

caught during the rainy season (6 months, May–October). In 1991, according to the district, the increase in the biting rate began 1–4 months after the beginning of the rainy season and the decrease 1–2 months after its end. In stations 2, 3, 6, and 8, there were 8 months with less than 1 female caught per man per night.

By district (Table 2): During the 1st year, the biting rate was high (2,500 bites per year) in stations 4, 5, and 7; moderate (around 1,000) in stations 1, 2, 3, and 6; and low in station 8. According to the stations, the treatments induced different changes in the biting rate. In 1992–1993, the highest decrease was obtained in stations 2, 6, and 8, the most central stations.

Dispersal study (Fig. 3): The 16 stations studied in this experiment were in an area where the density of habitation ranged from 0 habitations per hectare near the breeding site to more than 20 in the center of the town. The biting rate decreased from 31.8 bites per man per 10 days near the breeding sites to 29.5 at 100 m away and 3 at 200 m away. From 300 to 600 m from the breeding sites, the density of the habitations was >12 habitations per hectare; the biting rate ranged from 2 to 5.5 bites per man per 10 days.

Dissections: The average parity rates were 65.9% in 1991 and 81.8% in 1992. The rate was low during the first half of the rainy season (42.1% in July 1991, 48.3% in July 1992) and increased progressively until parity reached 76.2% in October 1991 and 83.0% in October 1992.

Sporozoites were found in the salivary glands of 14 anophelines. The percentages of positive anophelines were 0.5% in 1991 (1,146 dissected) and 1.1% in 1992 (476 dissected). The rates of inoculation (number of infective bites per human per year) were 8.8 in 1991 and 14.3 in 1992. Twelve infected anophelines were found during the rainy season (in each station except station 8). The 2 found during the dry season (December) were in station 4.

Breeding sites: During the dry season, larvae were mostly found in puddles, persisting in the drying-up rivers (until January) or ditches filled by the water frequently escaping from the water network. During the rainy season, the most productive breeding sites were located in peripheral flooded areas,

Table 1. Location and description of the collection stations within Maroua, February 1991–January 1994.

Station	Location	Type of habitat	Density (habitations/ha)
1	Intermediary	Traditional	9.8
2	Central	Traditional/high income	6.6
3	Central	Administrative/high income	4.9
4	Central	Administrative	2.9
5	Peripheral	Traditional/high income	6.7
6	Intermediary	Traditional	23.3
7	Peripheral	Traditional/high income	14.3
8	Intermediary	Traditional	21.9

Table 2. Number of *Anopheles* spp. caught per man per year, by station, February 1991–January 1994.

Station	Density (habitations/ha)	*Anopheles* caught/man/year 1991–1992 (1,232 mm rain)	1992–1993 (699 mm rain)	1993–1994 (827 mm rain)
1	9.8	1,193	803	1,363
2	6.6	720	248	640
3	4.9	758	945	848
4	2.9	2,588	3,075	800
5	6.7	2,460	2,370	1,235
6	23.3	1,275	248	145
7	14.3	2,633	3,135	1,126
8	21.9	323	83	293

along the edges of the rivers, and in the noncemented ditches trenched along the roads and filled by the rain.

Prevalence of malaria (Fig. 4): In 1991, there was a significant increase from July to November in the percentage of patients diagnosed as having malaria. The consultations attributed to malaria reached more than 50% of the total vs. 20–35% during the rest of the year. The increase in the number of patients consulting at the dispensary during the rainy season was mostly due to patients diagnosed as having malaria. This increase in the incidence of malaria was much smaller during the 1992 rainy season and showed a rapid decrease 1–2 months after the 1993 treatment in June.

DISCUSSION

Urbanization and density of Anopheles *population:* The pattern of malaria transmission in urban environments is mainly characterized by the following factors: 1) the density of breeding sites is low, 2) there is a gradient in the rate of transmission according to the distance from the breeding sites, frequently located in the periphery of the towns, and 3) the scattering of bites in a great number of people reduces the risk for the inhabitants to receive an infective bite (Trape et al. 1992).

Many reasons could explain the scarcity of breeding sites in towns. Extensive swampy areas and the risk of inundations were probably the reasons for not constructing a greater number of dwellings there. Most of the natural stretches of water disappeared either because of the need to recover space for buildings or for public health reasons. Drain networks were frequently built to eliminate rain and waste water. Finally, the few remaining stretches were often too polluted to allow *Anopheles* larvae to survive.

Trape et al. (1992) studied the impact of the distance from the breeding sites on malaria transmission in Dakar, where large breeding sites (marshland) persist in the center of the town. By performing pyrethrum spray collections at increasing distance (0–910 m) from the breeding sites, they observed an anopheline gradient from 414 female *An. arabiensis* collected to 21. An indirect fluorescent immunoassay (with *Plasmodium falciparum*) on blood samples from school children in the study area showed from 83% (0 m) to 27% (910 m) positive results, and an estimate of the entomologic inoculation rate ranged from 0.382 (0 m) to 0.014

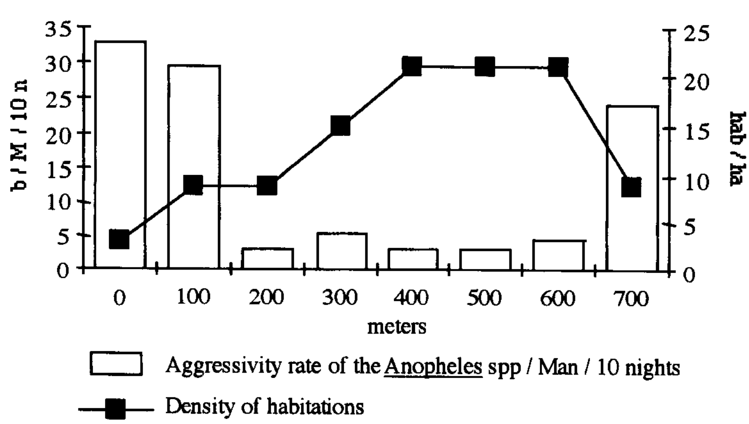

Fig. 3. Aggressivity rate of the *Anopheles* spp. and density of the habitat at increasing distance from the main breeding sites, Maroua.

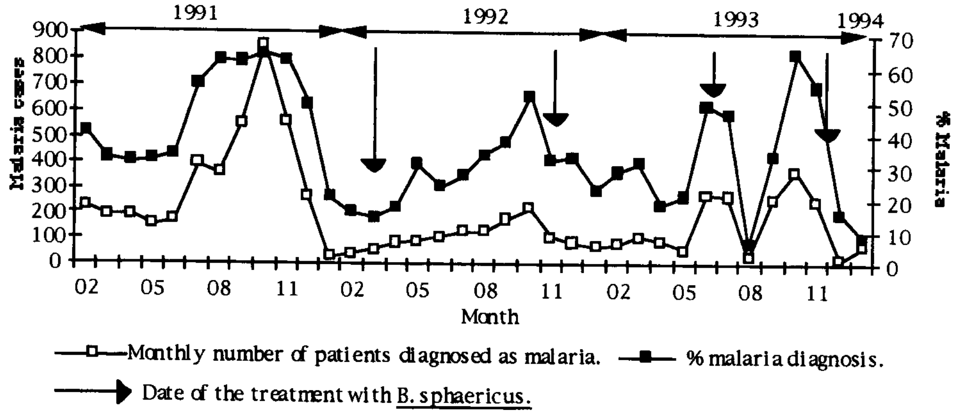

Fig. 4. Monthly number of patients and percentage of malaria diagnosed in a dispensary of Maroua, February 1991–January 1994.

(910 m) infective bites per human per year. Carnevale et al. (1993), in a review, reported a decreasing gradient in the rate of transmission from the periphery to the center in Bobo-Dioulasso, Ouagadougou, and Pikine. In towns, a high density of inhabitants allows *Anopheles* females to take their blood meal without having to fly far from their breeding sites. The numerous physical obstacles to the flight of mosquitoes provided by buildings and other city structures also contribute to reducing the dispersal.

To interpret the results obtained in 1991, before the treatment, the following factors are important. 1) Despite Maroua being a very flat town (only 20 m between the higher and lower parts) and therefore unfavorable to the discharge of the rain water, few natural breeding sites persist in the urbanized area. However, the level of *Anopheles* aggressivity reached in the central or intermediary stations cannot be explained only by the dispersion of females from natural peripheral breeding sites; it also involves artificial breeding sites, supplied with rain and tap water, that persist during the dry season. This situation was found in station 4, where water escaping from the network caused the persistence of overflooded areas throughout the year and created a source of *Anopheles* that caused a relatively high level of biting activity. In this station, the dry season results were more than 12% of the total caught during the year vs. only 6% in the 7 other stations. 2) There was a general decrease (except at stations 4 and 8) in the number of *Anopheles* caught from the periphery of the town (2,500 bites per human per year in stations 5 and 7), through the intermediary districts (1,200 bites per human per year in stations 1 and 6), to the center (700 bites per human per year in stations 2 and 3). The high rate of aggressivity at station 4, despite its central location, was due to a low density of inhabitants and the proximity of a permanent artificial breeding site. The low rate of aggressivity at station 8 was due to a high density of inhabitants and the proximity of a mountain area unfavorable to supporting breeding sites for *Anopheles*. The dispersal study confirmed that general model. 3) The human population density in the 8 stations ranged from 20 inhabitants per hectare to more than 160. This variation in density of the population induced a differential scattering of bites. An evaluation of the impact of this factor can be obtained by calculating the density of bites per surface unit (hectare): bites/hectare/year = bites/human/year × number of inhabitants/hectare.

The use of this index induces changes in the apparent density of the *Anopheles* populations in the different districts (Table 3). At the stations far from flooded areas (station 6 and, to a lesser degree, station 8), the number of bites per human was low, whereas the number of bites per hectare was high. In these districts, *Anopheles* could find enough artificial breeding sites to maintain themselves at a high density, even if the level of transmission (and

Table 3. Range of the different stations according to the density of human population, the number of bites per human per year, and the number of bites per hectare per year.[1]

	Station							
	1	2	3	4	5	6	7	8
Range/human population	5	3	2	1	4	8	6	7
Range/bites/human/year	5	3	4	7	6	2	8	1
Range/bites/hectare/year	5	1	2	4	7	6	8	3

[1] Density increases from range 1 to 8.

of malaria prevalence) was low because of a high density of inhabitants. In these districts, the breeding sites were mainly puddles in the ditches and along the water network. We also observed this impact of the density of the habitat on the apparent density of *Anopheles* populations in the dispersal study, just as entering the area with a high density of habitat induced a dramatic decrease in the number of bites per human.

Malaria incidence in Maroua: According to the 1991 results in the dispensary, the malaria incidence showed seasonal variations. The incidence was low during most of the dry season and increased quickly 3 months (July) after the beginning of the rains, simultaneously with the increase in the biting rate. Malaria incidence reached its maximum only 2 months later (September), at the end of the rainy season, and remained high for 6 months (until December), 2 months after the decrease in the biting rate. Variations in the incidence appeared to be linked not only to the number of bites but also to other factors. 1) Rain induces an increase in the atmospheric humidity that is favorable to the survival and the flight of the females and results in a higher probability of completing the sporogonical cycle as soon as the beginning of the rainy season. In Maroua, the percentage of parous females increased from June to October. 2) In North Cameroon, *P. falciparum* represents approximately 90% of the *Plasmodium* found (Languillon 1957; Couprie et al. 1985; Josse et al. 1987a, 1987b). Because the life span of *P. falciparum* in humans is less than 12 months, the prevalence of malaria and of immunity decreases during the dry season. This is consistent with a study performed in Maroua in April 1986 (end of the dry season), where the plasmodic index was relatively low, i.e., 7.3%, whereas the prevalence of the antibodies was 53.9% (Josse et al. 1987b). Moreover, because *P. falciparum* is rapidly virulent, the first febrile episodes can occur only a few days after the beginning of the transmission period.

Effect of the treatments with B. sphaericus*:* Because they were not the main goal of this experiment, only a part of the potential breeding sites for the *Anopheles* were sprayed: the puddles along the streets due to the rain or to the water running over from the water network or the part of those overflowable areas that were near the houses. In 1992, this seems to have been sufficient to delay the increase in the biting rate, which occurred 5 months after the beginning of the rainy season vs. 3 months the preceding year (rain began at the same period although it was not as extensive as in 1991). The sharp increase in the incidence of malaria cases in the dispensary observed during the 1991 rainy season did not occur in 1992, and the monthly number of malaria cases reached only 223 in October 1992 vs. 858 in 1991.

In 1993 (treatment in June), the increase in the *Anopheles* population began in June, as in 1991, but at a more gradual rate. The incidence of malaria increased rapidly until it stabilized in July and then began decreasing in August, i.e., 1 and 2 months, respectively, after the treatment of breeding sites with *B. sphaericus.* In the stations where the artificial breeding sites were preponderant, the treatments had more effect because those breeding sites were systematically sprayed. At station 6 in 1991, for example, where a high number of bites were recorded despite the absence of natural flooded areas, the treatments with *B. sphaericus* of the permanent artificial breeding site close to the catching station resulted in a dramatic decrease of the biting rate. On the other hand, large natural breeding sites in the periphery of the town were only partially sprayed, resulting in a maintenance of biting rates at high levels.

During the dry season, in December 1992 and 1993, the impact of the treatments was difficult to assess because the biting rate for *Anopheles* normally decreases at that time of the year.

Conclusion: In an urban environment in Cameroon, partial larvicidal treatments against *Anopheles* seem to have had an impact on the transmission of malaria. In Panaji City, Kumar et al. (1994), by spraying the main breeding sites (natural and artificial) for *Anopheles stephensi* with *B. sphaericus* (Strain 101, 1 g/m^2), obtained a dramatic decrease in the number of positive breeding sites and in *Anopheles* densities. The World Health Organization includes the spray of breeding sites in its recommendations for the control of malaria when they are easy to locate and to treat (Anonymous 1993), which is frequently the case in towns. Moreover, in urban environments, its harmlessness to nontarget organisms (Shadduck et al. 1980) makes *B. sphaericus* preferable to using a residual organic chemical pesticide to spray breeding sites for *Anopheles* because of the risk of contaminating the phreatic sheet. Meanwhile, the use of *B. sphaericus* against *Anopheles* should require a precise knowledge of the location and dynamics of the breeding sites. As these sites are almost fully exposed to the sun, a formulation that protects the spores and toxins from ultraviolet light (Mulligan et al. 1980) could improve the performance (Skovmand and Bauduin 1997).

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REFERENCES CITED

Anonymous. 1993. Mise en oeuvre de la stratégie mondiale de lutte antipaludique. OMS, Ser. Rapp. Tech. 839.

Anonymous. 1995. Lutte contre les vecteurs du paludisme et autres maladies transmises par des moustiques. OMS, Ser. Rapp. Tech. 857.

Baldet, T. 1995. Etude comparative de deux stratégies de lutte contre *Culex quinquefasciatus* Say, 1823 par *Bacillus sphaericus* Neide, 1904 dans la ville de Maroua (Nord-Cameroun). Thèse. Université Montpellier II, Montpellier, France.

Barbazan, P., T, Baldet, F. Darriet, H. Escaffre, D. Haman Djoda and J. M. Hougard. 1997. Control of *Culex quinquefasciatus* (Diptera, Culicidae) with *Bacillus sphaericus* in Maroua, Cameroon. J. Am. Mosq. Control Assoc. 13(3):263–269.

Carnevale, P., V. Robert, G. Le Goff, E. Fondjo, L. Manga, P. Gazin and M. Akogbeto. 1993. Données entomologiques sur le paludisme urbain en Afrique tropicale. Cah. Santé 3:239–245.

Couprie, B., Y. Claudot, A. Same Ekobo, H. Issoufa, H. Léger-Debruyne, J. Tribouley and C. Ripert. 1985. Etude épidémiologique du paludisme dans les régions rizicoles de Yagoua et Maga (Nord-Cameroun). Bull. Soc. Pathol. Exot. 78:191–204.

Davidson, E. W. 1985. *Bacillus sphaericus* as a microbial control agent for mosquito larvae, pp. 213–226. *In:* M. Laird and J. Miles (eds.). Mosquito control methodologies, Volume 2. Academic Press, London.

Detinova, T. S. 1963. Méthodes à appliquer pour classer par groupe d'âge les diptères présentant une importance médicale. OMS, Ser. Monogr. 47.

Gillies, M. T. and M. Coetzee. 1987. A supplement to the *Anophelinae* of Africa south of the Sahara (Afrotropical region). Publ. S. Afr. Inst. Med. Res. 55.

Josse, R., B. Josseran, M. Audibert, M. Merlin, A. Combe, M. F. Sauneron, S. Adjidji, B. Mondet, J. Y. Le Hesran, D. Kouka-Bemba, J. Tribouley and R. Ripert. 1987a. Paludométrie et variations saisonnières du paludisme dans la région du projet rizicole de Maga (Nord-Cameroun) et dans la région limitrophe. Cah. ORSTOM, Ser. Entomol. Med. Parasitol. No. Spécial:63–71.

Josse, R., M. Merlin, R. De Backer, B. Josseran, A. Kollo, M. Moussa, D. Sale, M. C. Drevet, H. Georges, A. Blanc, J. Combe, J. Tribouley and C. Ripert. 1987b. A propos d'une enquête paludométrique menée en saison sèche à Maroua, Province de l'Extrême-Nord (République du Cameroun). Bull. Liais. Doc. OCEAC, 80, Mai–Juin 1987:39–47.

Kumar, A., V. P. Sharma, P. K. Sumodan, D. Thavashelam and R. H. Kamat. 1994. Malaria control utilizing *Bacillus sphaericus* against *Anopheles stephensi* in Panaji, Goa. J. Am. Mosq. Control Assoc. 10:534–539.

Languillon, J. 1957. Carte épidémiologique du paludisme au Cameroun. Bull. Soc. Pathol. Exot. 50:585–600.

Mulligan, F. S., C. H. Schaeffer and T. Miura. 1980. Efficacy and persistence of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) against mosquitoes under laboratory and field conditions. J. Econ. Entomol. 73: 684–688.

Olivry, J. C. 1986. Fleuves et rivières du Cameroun. Monogr. Hydrol. ORSTOM 9:1–733.

Robert, V., P. Gazin, V. Ouedraogo and P. Carnevale. 1986. Le paludisme urbain à Bobo-Dioulasso (Burkina Faso). 1. Etude entomologique de la transmission. Cah. ORSTOM Ser. Entomol. Med. Parasitol. 24:121–128.

Shadduck, J. A., S. Singer and S. Lause. 1980. Lack of mammalian pathogenicity of entomocidal isolates of *Bacillus sphaericus*. Environ. Entomol. 9:403–407.

Skovmand, O. and S. Bauduin. 1997. Efficacy of a granular formulation of *Bacillus sphaericus* against *Culex quinquefasciatus* and *Anopheles gambiae* in West African countries. J. Vector Ecol. 22(1):43–51.

Trape, J. F., E. Lefebvre-Zante, F. Legros, G. Ndiaye, H. Benganali, P. Druilhe and G. Salem. 1992. Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. Am. J. Trop. Med. Hyg. 47:181–189.