Impact of Slow-Release *Bacillus sphaericus* Granules on Mosquito Populations Followed in a Tropical Urban Environment

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ABSTRACT A floating, slow-release, granular formulation of *Bacillus sphaericus* (Neide) was used to control mosquito larvae in two suburban areas of two tropical cities: Ouagadougou and Bobo-Dioulasso, Burkina Faso. A circular area of 2 km^2, diameter 1,600 m, was treated in each city using a similar, smaller area 1 km away as an untreated control. Mosquito captures were made in houses in four concentric circles, from the periphery to the center; each circle was 50 m in width. Mosquitoes were captured in CDC light traps or from human landings. More than 95% of the mosquitoes were *Culex quinquefasciatus* (Say) (Diptera: Culicidae). The human landing catches provided twice as many mosquitoes as did the CDC traps/night/house. The treatments resulted in important reductions relative to the control area and to preintervention captures. The reduction was more prominent in the inner circle (up to 90%) than in the outer circle (50–70%), presumably because of the impact of immigrating mosquitoes from nontreated breeding sites around the intervention area. This effect was more pronounced for light trap catches than from human landings. The impact of treatment was also measured as the mean ratio of mosquito density in the two outer circles to that of the two inner circles. This ratio was ~1:1 before the intervention and reached 1:0.43 during the intervention. This comparison does not depend on the assumption that, in the absence of intervention, the mosquito population development in the two areas would have been identical, but does depend on the homogeneity of the intervention area. The study showed that it is possible to organize mosquito control in a tropical, urban environment by forming and rapidly training teams of young people to carry out the mosquito control mostly using a biopesticide that can be applied without any tools except an iron bar to lift lids on some cesspits.

KEY WORDS *Bacillus sphaericus*, *Culex quinquefasciatus*, mosquito control, larvicide

The dominant mosquito populations in larger, tropical cities live in manmade habitats. In West Africa, these are (1) waste water in septic tanks and poorly managed drainage systems that host year-round larvae of *Culex quinquefasciatus* (Say) (Diptera: Culicidae), (2) holes along dirt roads created by digging or by heavy vehicles resulting in puddles that fill with water in the rainy season and at this period host larvae of *Anopheles gambiae* (Giles) (Diptera: Culicidae); (3) irrigated periurban agricultural land with both species, and (4) storage containers for water and tins, jars, and all sorts of containers that fill with rain in the rainy season and become breeding areas for *Aedes aegypti* Linné (Diptera: Culicidae). Because of the rapid growth of the outer part of the cities without proper drainage and roads and insufficient treatment systems for sullage water, the former two species are provided with very favorable conditions, resulting in high population densities. In West Africa, *An. gambiae* is the vector of malaria and—to a limited extent—filariasis, but the more common *Cx. quinquefasciatus* is the main source of nuisance (Baldet et al. 2000, Samuelsen et al. 2004).

This mosquito is often found to be difficult to control because of insecticide resistance (Chandre et al. 1997, 1998) and is thus not controlled with impregnated bed nets. Ouedraogo et al. (2006) showed that *Cx. quinquefasciatus* in Bobo-Dioulasso was highly resistant to dichloro-diphenyl-trichlorethane (DDT) and pyrethroids and slightly to organophosphates but not to the biological larvicide *Bacillus sphaericus* (Neide). The use of this bacterium has the further advantage of being nontoxic to nontarget organisms including humans and thus causes no substantial health hazards during application. People can be trained in a short time for the proper use of this larvicide.

Successful programs have shown efficacy of operational treatment with *B. sphaericus* against *Cx. quinquefasciatus* (Barbazan et al. 1997), but at high costs.

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Small-scale field trials (Skovmand and Bauduin 1997, Skovmand and Sanogo 1999) showed that a floating, slow-release granule could control *Cx. quinquefasciatus* larvae in septic tanks for 3 wk, thus potentially reducing costs of applications. Two demonstration trials were established in the two larger cities of Burkina Faso, West Africa. In 1999, the larvicide was used as the only control method in Bobo-Dioulasso. Larvicide treatments were carried out by five two-person teams recruited locally, trained, and supervised by experienced technicians. In 2000, the use of bacterial larvicide was supplemented with several programs of environmental management in a smaller part of the test area in Ouagadougou and supplemented with tests of new types of cesspits in Bobo-Dioulasso to gain experience of costs of such interventions. The cost of all operations was established and compared with the spending of the inhabitants on mosquito control before and during the control operation. Entomological data were obtained from human landing and catches in light traps in the test and control areas. Socio-anthropological data were obtained from interviews using a questionnaire and in-depth interviews with groups, and the results are reported elsewhere (Samuelsen et al. 2004; L. Toe, O. S., T. B. and H. S., personal communication). The entomological results of the two larvicide campaigns are presented here.

Materials and Methods

Test Areas. The test areas were selected in the two largest cities of Burkina Faso: Bobo-Dioulasso in the south and Ouagadougou in the middle of the country; both are located in the savannah tropical region. The rainy season starts between April and May in the former and about a month later in the latter. The average rainfall in Bobo-Dioulasso is 1,200 mm compared with 800 mm in Ouagadougou. Duration is normally ≈6 mo in Bobo and 4 mo in Ouagadougou. In the 2 study yr, the rainy season started in April–May 1999 and the total rainfall was 1,070 mm in Bobo-Dioulasso, but was late in 2000, and the rainfall was 700 mm in Ouagadougou. At the beginning of the rainy season, rain water is absorbed rapidly, but later in the season, it fills primitive septic holes in front of houses, low areas in and along roads, and partly blocked drainage channels and creates breeding sites for *Cx. quinquefasciatus* and *An. gambiae.* The major breeding sites for *Cx. quinquefasciatus* are deep cesspools in front of houses with access to tap water, when these tanks are open or only partly covered.

The intervention areas of 2 km^2 in the two cities were selected in newly constructed districts containing no large unoccupied areas and no ponds or agricultural land. All roads were dirt roads, and only a few cemented channels were found. In general, roads were not drained. Some houses had cemented cesspits, but most houses had just a hole or even just a depression in the soil outside the house where waste water from showers and the kitchen accumulated. These were permanently filled with water in the rainy season but remained dry in the dry season. Deeper cesspits had water with mosquito larvae year round. A control area was selected ≈1 km from the intervention area, which was 0.04 km^2, rectangular, and of similar type and structure. The distance between control and intervention areas was sufficient to ensure little impact on the mosquito population in the control area from the treatments in the intervention areas because <10% of *Cx. quinquefasciatus* will fly this distance in their lifetime in an urban area (Subra 1972). The intervention areas were circular, with a diameter of 1,600 m. Impact of the interventions was followed in four concentric circles as indicated in Fig. 1. Each circle was divided into blocks of 5,400 m^2 (from the outer circle and inward 45, 32, 18, and 6 blocks/circle, respectively), and 12, 8, 4, and 4 of these blocks were chosen randomly for measurements. The control area was divided in eight blocks, four of which were randomly selected for the study. Within each of these blocks, the house at the northern corner was chosen, but if it was uninhabited, the neighboring house was substituted. The same rooms were used throughout the study for measuring treatment impact and were inhabited by single, young men.

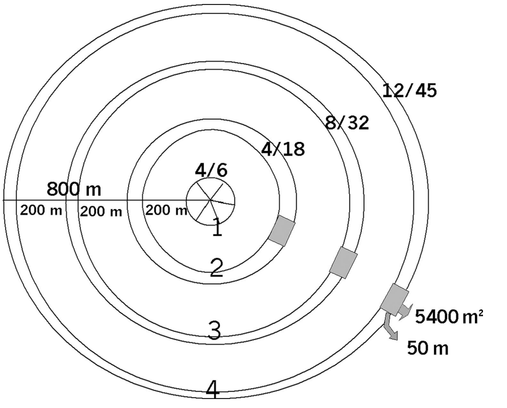

Fig. 1. Diagram of the intervention area. The central circle (1) was divided in six blocks from which four were selected for sampling as indicated 4/6. The next circle (2) was divided in 18 blocks from which 4 were selected, as indicated 4/18, etc.

All houses and suspected breeding places were mapped by GPS in the rainy season of the year before the intervention by the entomologists and transferred to a digitized card (Environmental Research Institute. 1992–1998; scale: 1:2,000). Breeding sites were listed on tables delivered to the treatment teams together with the map of their working area. All houses were given an identification code that was used for data registration. Address and description of the houses (description of buildings, breeding sites, water access, electricity, and composition of the family) were noted and registered according to the identification code.

Mosquito Sampling. Data collection started each year at the end of May, 1 mo before the intervention began. In Bobo-Dioulasso in 1999, this was 1 mo into the rainy season, and there were many breeding sites. In Ouagadougou in 2000, this was at the start of the rainy season, and there were few breeding sites.

Table 1. Type and no. of counting sites per week

Position	Circle 1		Circle 2		Circle 3		Circle 4		Control		Total	
	CDC	HL	CDC	HL	CDC	HL	CDC	HL	CDC	HL	CDC	HL
Mode												
Night 1	1	1	1	1	2	1	3	1	1	1	8	5
Night 2	1	1	1	1	2	1	3	1	1	1	8	5
Night 3	1	1	1	1	2	1	3	1	1	1	8	5
Night 4	1	1	1	1	2	1	3	1	1	1	8	5
Total	4	4	4	4	8	4	12	4	4	4	32	20

No. sites used per night in weeks of observation. For the two inner circles, both types of counting were established for all sites; for the two outer circles, human landing (HL) counts were only established for some of the houses and were rotated between counting weeks.

Adult mosquitoes were collected from 1800 to 0600 hours inside the selected houses in the intervention area and in the nearby untreated control area. Catches were obtained by positioning untreated bed nets (donated by Vestergaard-Frandsen, Kolding, Denmark) in the room used for trapping and a CDC trap or a mosquito catcher was positioned beside the net as described by Lines et al. 1991. Only one person slept under the bed net. A CDC trap (CDC Miniature Light Trap; John W Hock Co., Gainesville, FL) was suspended at the level of the head of the sleeping person, 50 cm from the net. Traps were emptied at midnight and again in the morning. Mosquitoes collected at midnight were stored in an icebox. Alternatively, a technician sat on a chair at the same site and collected mosquitoes from dusk till midnight, when he was replaced by a second technician until the morning. These informed volunteers were provided free and rapid treatment when suspected clinical signs of malaria were seen according to World Health Organization (WHO)-recommended regimen on the basis of fever and detectable *P. falciparum* parasitemia.

Mosquitoes collected manually were stored individually in tubes and stored in bags that were changed every hour. Data were collected every second week by rotating traps and human catchers in a way that all sites were measured with CDC traps but not all sites were used for landing catches every trapping week. Sites not used in 1 wk were used in the following period 2 wk later or again 2 wk later in the two outer circles (Table 1). This method allowed us to compare the two methods for trapping efficacy and sex and species composition comparing data per circle and week, but with expected larger variation in the data for human landing rates in the two outer circles because of fewer trapping points per week.

Mosquitoes were determined to species, and 25 *Culex* females per circle were taken for ovarian dissection to determine their parity (Detinova 1962).

Treatments. Every 2 wk, the teams treated all identified breeding sites and crossed them off the tables and added and treated unidentified breeding sites that may have arisen since the initial mapping. Rain water channels and rain-filled puddles were inspected weekly and treated when larvae were found. The sustained release granular formulation of *B. sphaericus* (Skovmand and Bauduin 1997) was used for the treatment of septic tanks, whereas a fluid concentrate (Spherimos; Novo Nordisk Biokontrol, Bagsvard, Denmark) was used in 1999 and a water suspendable micro-granule Vectolex (Valent Bioscience) was used in 2000 for weekly treatments of rain-filled puddles. A weekly treatment was needed because of the very temporary presence of these *An. gambiae* breeding sites. Granules were applied by hand, whereas the diluted flowable and suspended microgranules were applied with dosage-calibrated back sprayers. The doses were 3 g/m^2 of the granule (20% Technical powder), 3 g/m^2 of the fluid concentrate (80 ITU/mg), and 0.5 g/m^2 of the microgranule (100% technical powder).

Ten important breeding sites were selected without revealing their identity to the treatment teams and followed every second week by a technician for a survey of team efficiency. All mapped or nonmapped breeding sites were inspected by one entomologist and a technician in August in both intervention years. This showed some extra breeding sites that were included in the treatment.

In October 1999 (Bobo-Dioulasso), several treatment teams did not have enough products to treat all breeding sites, and the last two treatments were incomplete. In August 2000 (Ouagadougou), it was found that several breeding sites had not been treated every 2 wk of the start of the treatment campaign, but just once a month or not at all (revealed by the inhabitants and by sampling). The treatment teams who were at fault were therefore instructed to treat all breeding sites every week using Vectolex, and a closer inspection of their work was arranged.

Statistics and Analysis. Analysis of variance (ANOVA) for repeated measurements was used to compare circles over the season and intervention areas to control areas, analyzing data from CDC or human landing catches independently. Data per trap and night were log-transformed for normalization, and the means per circle per fortnight were back-transformed to provide geometric means for the figures. To measure impact as a function of distance from the center of the circular intervention area, the density per circle was first compared by ANOVA, and when found significant, a correlation analysis was carried out each month with the circle number as the independent parameter and the density per circle as the dependent parameter. A significant slope indicates a combined effect of treatment and immigration of mosquitoes from the periphery. A simpler analysis was also applied comparing the ratio of mosquitoes caught in the two inner circles to that of the two outer circles.

To obtain data for graphic presentation, the mean number of mosquitoes per night per circle was calculated by back-transformation of log-mean values and shown as histograms as function of time, where each column represents the mean value per capture week. For simplicity, effect was calculated for circles 1 and 2 together and circles 3 and 4 together and displayed below the histograms. The percent reduction achieved was calculated on the basis of the three preintervention data and corrected for the changes in the control area as follows: we calculated the weekly ratio between mean captures in the circles (1 + 2 and 3 + 4) to that in the control area for the preintervention dataset. The mean value of these three ratios was used as a correction factor between capture data in the intervention area and the natural changes in the untreated control area and thus expressed the relative effect of the treatment for each week.

Results

Bobo-Dioulasso, 1999. The intervention area of 2 km^2 contained 2,400 compounds or 1,200/km^2 compared with 1,700/km^2 in the control area. Furthermore, more people lived in each compound in the control area than in the intervention area: 14.8 versus 8 per compound, giving a population of 9,600 and 25,100 inhabitants/km^2, respectively. Although the two areas were close to each other in the same part of the city and appeared quite homogenous, the control area included part of a former village and was thus older, although rebuilt.

The intervention zone contained [1200–1,500 open or covered cesspits that were treated and 400 closed (concrete lids cemented to the tank, no access to mosquitos) cesspits that were not treated. Twelve concrete channels were treated when they contained stagnant water. These sites were the *Cx. quinquefasciatus* breeding sites. Around 1,000 pit latrines were monitored, but mostly contained only fly larvae. Occasionally, some were flooded with rain and then treated. On average, the intervention zone contained 60 cesspits and 1 cemented channel to treat for every 100 compounds, whereas the control zone had 68 cesspits to treat and no cemented channels. Eighty-three to 139 rain-filled water puddles were treated against *An. gambiae* larvae.

Mosquito populations were followed from May to November by CDC traps and man-landing captures. The *Cx. quinquefasciatus* population peaked the week after the intervention started in the intervention area, whereas it continued to increase for 8 wk in the control area. The mosquito population was already higher in the control zone than in the intervention zone before intervention whether measured by CDC traps or man-landing catches (Fig. 2A and B, top graphs). This is probably linked to the higher human density in the control area. The impact of the intervention was not immediate, but was delayed 2–4 wk from the start of the intervention. A significant decrease was obtained in the treatment area from August when a few undiscovered sites, plus a blocked and very productive road side channel beside a peripheral road, were included in the treatment program. The effect of including this blocked channel was at first most pronounced in the outer circle 4.

Before the intervention, the density was on two occasions higher in the center circle than in the other circles, but there was no significant decline or increase in density from the circle to the periphery. The ratio of the mean number of mosquitoes caught in CDC traps per house in the two inner circles to that of the two outer circles was 1:1 before treatment, declined to 1:0.43 during treatment, and increased again after treatment (Table 2). The same ratios cannot be calculated for the human landings because not all sites were included in the weekly counts for the two outer circles.

The mosquito density changes in the intervention zone were compared with that of the control zone (Fig. 2A and B, bottom graphs). The mean from the two outer circles and for the two inner circles are corrected for the changes in the mosquito population in the control zone. The overall impact of treatment was more pronounced when measured by human landing catches than by CDC traps and developed somewhat differently. For the inner circles, the reduction achieved was ≈90% as measured by both methods, but the reduction achieved was only 50% for the outer circle (circle 4) measured by CDC traps compared with 70–80% measured by human landing catches. In the control area, a large increase in population was found with the CDC trap after the end of the intervention, but not with human landing catches. Because the reduction achieved is calculated relative to the control area, this results in two different outcomes for the two methods of estimating population changes. In general, the human landing catches gave two to four times more female mosquitoes per house than the light traps, and the variations were larger.

Dissections of female mosquitoes of *Cx. quinquefasciatus* showed an initial large number of nulliparous and thus a low percent of parous (Fig. 3A and B). An increase in the number of parous (older) mosquitoes was found in the intervention zone as well as in the control zone at the start of the intervention until the beginning of August, followed by a decline and a second less pronounced peak of older females from the middle of September to the end of October. The parous rate peaked at very high values in some circles, because of very small numbers of nulliparous females found in the circle in periods when the number of parous were high. The very high peaks were not simultaneous for females caught in CDC traps (Fig. 3A) and in human landings (Fig. 3B). The females caught by CDC traps or human landing had generally the same parous rate, but the second peak of parous females was less pronounced for females caught in traps than on humans.

All female *An. gambiae* caught were dissected. From the start of the intervention, the percent of parous to nulliparous was initially higher in the inner circle (circle 1) than in the outer circle (Fig. 4), but the

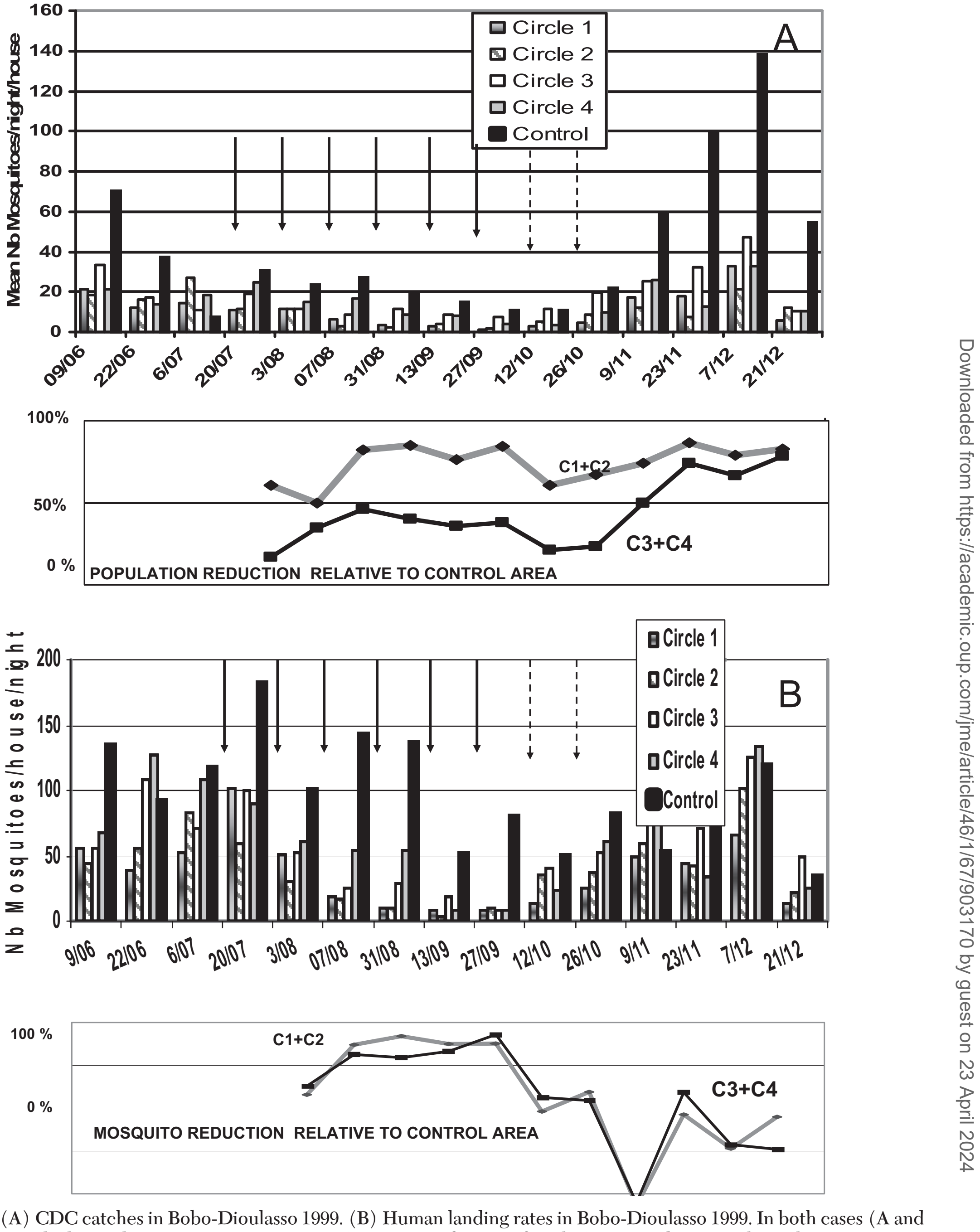

Fig. 2. (A) CDC catches in Bobo-Dioulasso 1999. (B) Human landing rates in Bobo-Dioulasso 1999. In both cases (A and B), the top graph shows the mean mosquito counts (*Cx. quinquefasciatus* females) per week per circle in the intervention area and in the control area. The arrows indicate larvicide treatments. The two last treatments were incomplete (stippled arrows). The bottom graph shows the impact of the intervention relative to the untreated area (control). The curve marked C1 + C2 measures the mean impact on the two inner circles, and the curve marked C3 + C4 that on the two outer circles.

difference over the period was not significant and was not significantly different from that of the control zone.

Ouagadougou, 2000. The intervention area had a smaller human population per square kilometer than the control area, 8,800 and 19,800 inhabitants/km^2, respectively, because of a less intensive use of land. Number of inhabitants per compound was the same, around five per compound, and the number of *Cx. quinquefasciatus* breeding sites per 100 houses was similar, 107 and 116 for intervention and control areas, respectively.

Table 2. Ratios between the two inner circles was compared with that in the two outer circles before, during, and after treatment

Mosquito count (CDC traps)	Week no.	City	Ratio of outer circles/inner circles
Before intervention	1–3	Bobo-Dioulasso	1.02 ± 0.12[a]
During intervention	4–12		0.46 ± 0.08[b]
After intervention	13–17		0.67 ± 0.11[c]
Before intervention	1–4	Ouagadougou	1.31 ± 0.23[a]
During intervention	5–13		0.45 ± 0.14[b]
After intervention	14–17		0.40 ± 0.23[b]

There was an impact of treatments in both towns when mean no. mosquitoes (*Cx quinquefasciatus* females) caught by CDC traps in the two inner circles was compared with that in the two outer circles before, during, and after treatment, here indicated as ratios between these numbers. In the right column, for each city data items sharing the same superscript letter, data do not differ significantly.

The captured mosquito populations consisted mostly of *Cx. quinquefasciatus* (26,000) followed by *An. gambiae* (120). Data from human landing rates showed that the *Cx. quinquefasciatus* females increased in numbers until a week after the treatment and started to decline, except in the center circle (circle 1), where they increased for another 2 wk (Fig. 5B, top graph). The population increased again when the treatment stopped. The number of females caught in CDC traps were at the same level in the control zone as in the intervention zone before the intervention (Fig. 5A, top graph), but after the treatment started, it was consistently higher and declined less than it did in the intervention zone. One month after the treatment stopped, the density in the intervention zone increased, whereas it remained low in the control zone.

Calculations on the ratio of the CDC catches between the two outer and the two inner circles showed that the ratio rose to 1.9 before the intervention and declined to 0 at the end of the treatment period. It increased again after the intervention stopped (Table 2). The ratio thus describes an increased effect of treatment in inner circles compared with outer circles. Human-landing numbers showed the same general characteristic, but the numbers caught per night were higher, and the scatter was larger.

Discussion

Treatment with a floating, slow-release *B. sphaericus* larvicide on a large scale (2 km^2) in an urban environment resulted in a marked but delayed reduction of mosquito density (Figs. 2, 4, and 5). In Bobo-Dioulasso, the treatment achieved a reduction of up to 90% in the inner circles and 50–65% in the outer circles depending on the capture method when the

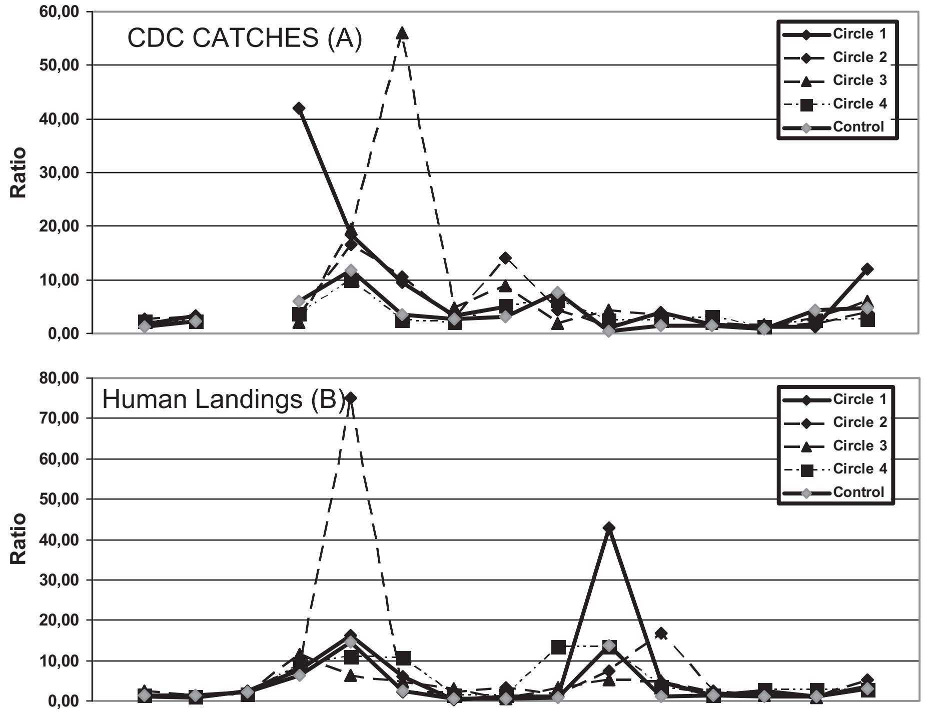

Fig. 3. Ratio of parous to nulliparous *Cx. quinquefasciatus* females in the capture per circle in the intervention area and in the control area, CDC traps (A), and human landings (B); data from Bobo-Dioulasso 1999. There are nearly always more parous than nulliparous, and there is a decrease in nulliparous at the onset of the intervention, but this was also found in the untreated area (control).

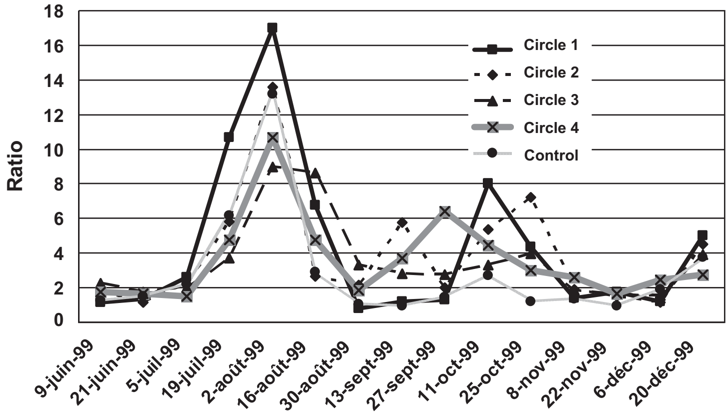

Fig. 4. Dissections of *An. gambiae* females (caught by CDC traps and human landings together). Ratios of parous to nulliparous per circle in the intervention area and in the control area; data from Bobo-Dioulasso 1999. As for *Cx. quinquefasciatus*, there was a decrease in the number of nulliparous females at the same time as an increase in parous females in July and August, resulting in a peak in the ratio. Except for this period, there was no systematic variation of the ratio, and it was not significantly higher with respect to the inner and outer circle and control area.

impact was corrected for the changes in the control area as is usually done. It can be seen from Fig. 2A and B that the difference in evaluation by the two methods is partly explained by difference in the population estimates in the control zone. On the contrary, the ratio of inner and outer circles does not depend on the development in the control area and gives perhaps a better estimate of the effect of the intervention (Table 1). The mosquito populations recovered again on cessation of treatments, and the human landing rate indicates that this happened before the ending of the treatment. This may be explained by the incomplete treatment during the last two rounds because of lack of granules.

A more consistent effect was found in Ouagadougou where the treatment started earlier compared with the start of the mosquito season as seen in Fig. 5. The impact was up to 90% for several months, and as for Bobo-Dioulasso, was larger in the inner circles than in the outer circles. At the end of our measurements in December, the number caught in light traps became very low, and the relative control effects are therefore uncertain.

Treatment with *B. sphaericus* had no impact late on fourth-instar larvae or pupae because these do not ingest. The duration of these two stages together is ≈5 d. In measurements of the adult population, it also takes some extra time before a decline in recruitment is manifested in the population. Accordingly, it is not surprising that impact of the interventions in the 2 yr were not seen the week after the treatment but at the next measurements 3 wk after treatments, as seen in the histograms (Figs. 2 and 5). Similarly, when important breeding sites were discovered by close field inspections in late August in both years and treated, the effect was not seen before the middle of September. The full impact of the larvicide is therefore not seen in the middle of September, and this delay was linked to the recruitment of young people without experience except for our initial training. However, the campaign also showed that, within a season, these young people could learn to handle the treatments consistently because the application of a biolarvicide is not difficult.

Some treatment groups exhausted their stock of granules before the end of the season. The reason was that they had used the granules only, since it is so much easier to apply than the products made for back sprayer application. These teams therefore had to treat their breeding sites weekly for the last month, because the sprayable products have a shorter residual effect (Skovmand and Sanogo 1999). Obviously, the slow release granule is not only more economic to use because of longer treatment intervals, but it was also more popular among the people paid to carry out the larval control.

The initial higher mosquito density in the control zones was caused by a higher density of people and more breeding sites per square kilometer. A decline in mosquito density was seen in the middle of the rainy season, especially in the southern city of Bobo-Dioulasso. A similar decline was found in Maroua in a nonintervention year and was ascribed to dilution of the soapy, dirty water in the most productive breeding sites, making these cesspits less suitable for *Cx. quinquefasciatus* and more suitable for other *Culex* species (Barbazan et al. 1997).

Subra (1972) showed by mark-recapture studies of young *Cx. quinquefasciatus* females that 90% do not disperse >800 m from the point of release within their lifetime. In our study, we chose a circular intervention area with a radius of 800 m and would therefore expect that immigration would be significant at the periphery of the area and insignificant in the center, thus giving a declining mosquito density from the periphery toward the center. This pattern was only approximately found both years in the intervention periods. There was, in general, no significant correlation between

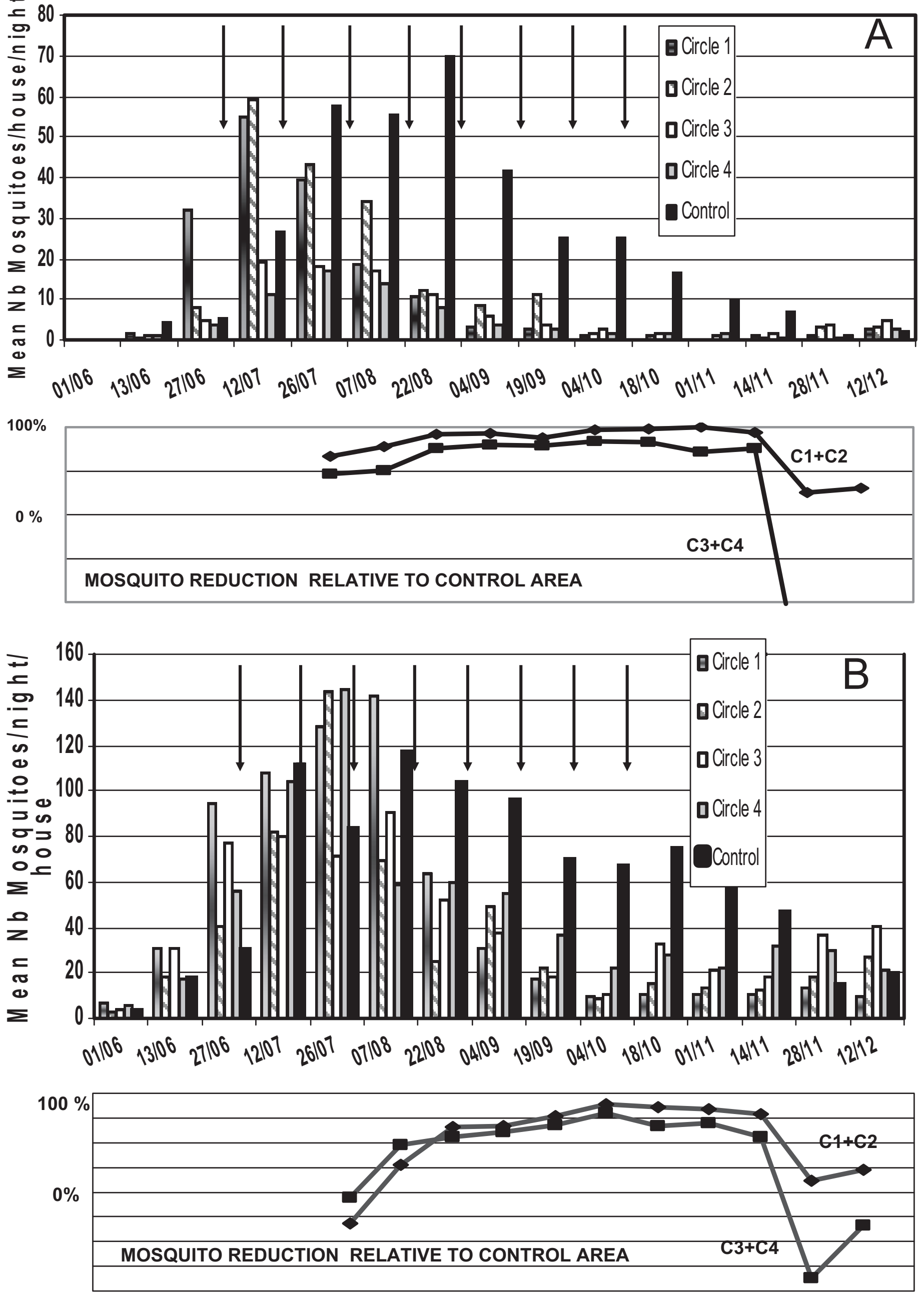

Fig. 5. (A) CDC catches in Ouagadougou 2000. There was a decrease in the number of mosquitoes caught (*Cx. quinquefasciatus* females) per circle in the intervention zone after the treatment started, whereas the population in the untreated area (control) continued to increase. There was a slight recovery of density in the intervention zone after treatment was stopped, but not in the control area, where density continued to decline because of the onset of the dry season. Below is shown the summed reduction of mosquitoes relative to the control area for circles 1 + 2 and circles 3 + 4. It is seen that, after treatment, the control effect is higher in the inner circles (1 and 2) than in the outer circles. (B) Human landing rates in Ouagadougou 2000. The graphics show the same as in A, but the number of mosquitoes caught (*Cx. quinquefasciatus* females) by night is higher. The bottom graph shows that the control effect seems to be slower when measured by human landings than when measured from light trappings.

circle distance from the center and mosquito densities, but the simplified ratio of the sum of the two inner circles to the two outer circles showed a ratio above 1 (Bobo-Dioulasso 1999) or ~1 (Ouagadougou 2000) before intervention and ~0.5 during the intervention (Table 2). In 1999, the inclusion of a peripheral breeding site in the treatment zone from the beginning of September resulted in an immediate decline in the count at the periphery and in all counts 2 wk later. This may show that the impact was initially local and then equalized by mosquito random migration. In the second year in Ouagadougou, a very productive breeding site was found between the second and third circles, and the treatment of this breeding site resulted in a decline in the center areas compared with the periphery (Fig. 2; Table 2). These events showed that mosquitoes disperse from very productive breeding sites to larger areas.

If *Cx. quinquefasciatus* dispersal is slow and random, as indicated by Subra (1972), an effective treatment of a circular area should result not only in a lower density in the middle but also in an older population in the middle than in the periphery. Using the ratio of nulliparous to parous females, this ratio should decline from the periphery to the middle, which it did not (Figs. 2 and 5, bottom graphs). This may be explained in two ways: (1) most mosquitoes found in each circle were of very local origin mixed with fewer immigrants from neighboring zones, the latter establishing the decline in density toward the center, or (2) female mosquito migration over larger distances is dominated by nulliparous individuals. Because the population in the inner circles was on average one half of that in the outer circles during the effective treatment period (September to November), one would still expect an impact on the parous ratio unless the migrants were very young mosquitoes. This was not found, and it is suggested that the immigrants were mostly younger mosquitoes that may fly a larger distance, whereas older females only fly short distances. Data on *Cx. pipiens fatigans* (now *Cx. pipiens quinquefasciatus*) marked with ^{32}P indicated an age-dependent dispersal pattern (Lindquist et al. 1967). The principle is explained by Service (1997) as a result of a memorized home range between feeding and oviposition site, meaning that once a female has found a host locality and a nearby breeding site, she will tend to mostly move between these two sites.

Dissections of *An. gambiae* females showed relatively more older females in the inner circle (circle 1) than in the outer circle after the intervention started (Fig. 4), but the changes in the control zone and in the circles were not significantly different: ~1.8 times more parous than nonparous before the intervention, increasing to 4 (control zone and outer circles) to 5–6 for inner circles during the intervention and again decreasing to ~2 times more parous in the postintervention period. Only 125 females were caught by both capture methods, which may explain the lack of significance despite interesting tendencies.

Comparing a treated zone to an untreated zone or to the year before is of doubtful validity. The control area needs to be sufficiently far from the treated area so that migration between the two can be ignored. However, local environment and, in our case, urban development should be very similar. In Bobo-Dioulasso in 1999, the two districts appeared similar, but a closer study showed that the nonintervention area was more densely populated and thus had more breeding sites per square kilometer, because breeding sites in urban environments are all manmade and sustained. Furthermore, seasonal variation of the mosquito population may induce declines or increase of population that not dependent on the treatment. These effects are seen in the bottom graphs in Figs. 2 and 5, where control effects are calculated relative to population development in the nonintervention area. The advantage of a large circular area where mosquito density is followed in concentric circles is that a decline toward the middle is a proof of effect independent of all these variations provided that the decline was not already present before the intervention. In our studies, density was initially higher at the center or the same as at the periphery, and the change of densities in the outer circles to the inner circles provided the proof of treatment effect in addition to the comparisons of density before and after treatment and in the control areas.

The ratio of CDC-trapped mosquitoes to human landing catches was ≈1:2 and sometimes 1:4, but slightly density dependent. Lines et al. (1991) found a ratio near 2:3 for three species compounded: *Cx. quinquefasciatus*, *An. gambiae*, and *An. funestus*, the latter presenting only a minor fraction of the total. These comparisons are analyzed more closely in a separate paper (unpublished data). In our tests with human landing catches, there were two persons in the room, whereas with CDC traps, there was only one. That may explain at least partly the higher numbers caught with human landings. In general, the two methods showed the same patterns of treatment effect as seen by comparing Fig. 2A to Fig. 2B for the tests in Bobo-Dioulasso and Fig. 5A to Fig. 5B for Ouagadougou.

The catches also showed more variation in the human landings than for light traps. The larger variation around means is partly caused by a more limited number of counting sites for human landing counts than for CDC counts, but it may also depend on the two different catch principles. Mosquitoes caught on humans are there to bite, and biting is a complex behavior influenced by several factors such as age, nutritional status, and weather, whereas mosquitoes trapped in light traps are probably more related to flight activity and escape from shelter. In a study on houseflies caught in light traps, Skovmand and Mourier (1986) showed that sugar feeding, protein feeding, and age determined the percentage of female flies caught.

In conclusion, the program showed that the vast majority of mosquitoes caught in the rainy season in periurban areas in Burkina Faso were *Cx. quinquefasciatus*, followed by *An. gambiae.* Treatment of breeding sites reduced the *Cx. quinquefasciatus* population on average by 80% and by >90% in the central areas of the treatments. In a separate publication, Samuelsen et al. (2004) showed that the inhabitants were satisfied with the larvicide treatments and reduced their own expenditures on adult mosquito control >50%. In a further publication, we will show that the treatment applied here, including all costs for the products and payments, was much less expensive per household than what the households spent themselves in the less intense season of mosquito control at the beginning of the rainy season. The work cited above showed that, when people were asked for the main problem with mosquitoes before the intervention, most people reported that nuisance problems were their major concern. During the intervention that especially reduced the *Cx. quinquefasciatus* density, people changed priority and reported they were mostly concerned about the disease aspect. It has often been found that people are motivated by the nuisance problems to use bed nets and not by the disease aspect (Van Bortel et al. 1996). In personal communications, many of these authors have expressed that *Culex* control should not be encouraged because that might result in decreased use of bed nets and thus result in more malaria. Our study seem to indicate that opposite may be true: after reducing the nuisance aspect, people will focus on the disease aspect and therefore may be more likely to use bed nets when the nuisance problem is reduced at the beginning of the dry season when malaria transmission is still high. It is therefore desirable that malaria campaigns in urban environment include larvicide control against *Cx. quinquefasciatus* to reduce the nuisance problem that otherwise have higher priority to people than malaria.

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