

# Laboratory and field evaluation of an oviposition trap for *Culex quinquefasciatus* (Diptera: Culicidae)

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*An ovitrap (BR-OVT) based on physical and chemical stimuli for attracting gravid Culex quinquefasciatus (Diptera: Culicidae) females was developed and evaluated under laboratory and field conditions. Attractants were assayed using alternative chamber bioassays prior to being used in the BR-OVT oviposition trap. A significant preference of gravid females for sites containing conspecific egg rafts was observed, as a response to the natural oviposition pheromone, as well as for sites treated with the synthetic pheromone erythro-6-acetoxy-5-hexadecanolide. Five- to 20-day old grass infusion was strongly attractive to gravid females for laying eggs. On the other hand, entomopathogenic Bacillus sphaericus (Bs) did not influence the choice of an oviposition site when used in combination with grass infusion and can therefore be used as a larvicide in ovitraps. Results from field trials showed that the BR-OVT with grass infusion and with or without Bs works as a preferred oviposition site for Cx. quinquefasciatus. The BR-OVT was more effective for egg collection when placed indoors and comparison with the number of egg rafts laid in cesspits over 40 days indicates that this very simple ovitrap may be a useful tool for monitoring populations of the most important of the vectors of bancroftian filariasis.*

Key words: *Culex quinquefasciatus* - ovitrap - grass infusion - *Bacillus sphaericus* - oviposition pheromone

*Culex* species are major urban biting nuisances and are vectors of important pathogens including *Wuchereria bancrofti*, the main causative agent of lymphatic filariasis, and arboviruses such as St Louis encephalitis virus and West Nile virus.

The eggs of *Culex quinquefasciatus* Say are laid as floating rafts and each egg has an apical droplet of an oviposition aggregation pheromone (Laurence & Pickett 1982) which tends to concentrate their offspring in high nutrient waste water in urban areas. Their breeding places are typically permanent or semi-permanent sites containing organically enriched and preferably dark polluted water with high microbial activity, found in man-made environments. Gravid females respond to a combination of chemical cues (pheromones and Kairomones) and physical cues (e.g. water reflectance, darkness, and temperature) to locate and recognize suitable oviposition sites. Strategies based on semiochemicals involved in mediating oviposition site location behavior have been used to attract gravid females to artificial breeding sites for trapping and to collect their egg rafts for use in *Cx. quinquefasciatus* surveillance (Reiter 1983, 1986, Millar et al. 1994) and control (Otieno et al. 1988). Fermented infusions of plant material or animal excreta (Bentley & Day 1989, Reisen & Meyer 1990, Lee & Kokas 2004) as well as compounds extracted from grass infusion show strong attractiveness to gravid *Culex* fe-

males (Blackwell et al. 1993, Millar et al. 1994, Mboera et al. 2000). Volatile chemicals produced by some species of fungus and bacteria (Poonam et al. 2002, Geetha et al. 2003) have been shown to be highly active as oviposition attractants for *Cx. quinquefasciatus*.

Egg raft collections can be useful for monitoring population densities of different *Culex* species, seasonal oviposition activity (Madder et al. 1980, Leiser & Beier 1982), for assessing the impact of control actions and for obtaining samples for the study of insecticide resistance and other research purposes (Reiter 1986).

This paper reports the attractiveness for gravid females of a very simple novel ovitrap model using olfactory stimuli and physical cues, evaluated under laboratory and field conditions in a Brazilian city endemic for lymphatic filariasis, with highly favourable conditions for *Cx. quinquefasciatus* breeding throughout the year.

## MATERIALS AND METHODS

Prior to evaluating the field performance of the oviposition trap BR-OVT, the response of local strain *Cx. quinquefasciatus* females to oviposition attractants and to bacterial larvicides was investigated in the laboratory.

*Laboratory assays* - Females of *Cx. quinquefasciatus* Say (Recife strain) were obtained from a colony maintained in the laboratory with an LD 12: 12 h photoperiod at  $27 \pm 2^\circ\text{C}$  at the Centre of Research Aggeu Magalhães-Fiocruz in Recife, Brazil. Larvae were fed on Whiskas® cat food. Adult females (5-6 days old) were bloodfed on chickens. To establish the best time for females to be used in bioassays, 4 groups of 10 females each were introduced, 3 days after their blood-meal, into cages containing 2 cups filled with water from laboratory breeding containers, and left overnight. No oviposition occurred. In a second experiment, 8 groups of 20

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females each were allowed to oviposit under identical conditions, 5 or 8 days after feeding. The average number of egg rafts found the next morning was 9.5 and 11.1 per group, corresponding to 47.5 and 55.6% of the females, respectively. Therefore, the bioassays were restricted to females 8 days subsequent to their blood-meal.

**Bioassays** - Oviposition bioassays were carried out at  $27 \pm 2^\circ\text{C}$  and 12L: 12 D cycle in muslin-covered wooden framed small cages ( $30 \times 22 \times 20$  cm) or large cages ( $115 \times 82 \times 64.5$  cm). Gravid females were offered the choice between 2 oviposition sites (glass cups or traps), placed into the cage on opposite sides: one test and one control. The assays were conducted between 18:00 and 08:00 h. Egg rafts deposited in each cup were counted at the end of the assay.

**Egg raft pheromone** - Test cups contained 250 ml dechlorinated tap water plus 5 egg rafts within 24 h after oviposition. Control cups contained 250 ml dechlorinated tap water plus 5 egg rafts at the same age, but washed with a solvent (hexane) to override any visual stimulus that could influence the choice of the oviposition site. A total of 18 repetitions of this experiment were carried out, with one gravid female per replicate (to prevent any subsequent oviposition from changing the initial number of rafts, a factor that could have biased the results).

**Synthetic oviposition pheromone (SOP)** - A sample of racemic erythro-6-acetoxy-5-hexadecanolide (obtained from Dr Jocelyn Millar, University of California, Riverside, CA, US) was tested in doses ranging from 0.03 to 1.56  $\mu\text{g}$ . According to the dose to be tested, 2 to 100  $\mu\text{l}$  of a SOP solution in hexane was applied with a Hamilton syringe to a filter paper disc (2 cm diameter). The disc was placed on a polystyrene disc. Next, the arrangement was positioned on the surface of the water where it remained floating. Equal volumes (2, 10, 25, 50 or 100  $\mu\text{l}$ ) of hexane were applied to the control disc using the same procedure. The numbers of egg rafts laid in the treatment and control cups were counted the next day. Twelve replicate cages with 20 gravid females each were used.

**The oviposition trap (BR-OVT)** - An oviposition trap model was developed associating physical and chemical factors to attract gravid *Culex* females for oviposition. The trap BR-OVT (Intellectual Property: PI 0201899-3) is composed of a simple black plastic box ( $13 \times 35 \times 24$  cm) with a central window ( $16 \times 9$  cm) on the top surface, not only allowing females to access the oviposition site, but also facilitating the perception of chemical cues emanating from within the apparatus. A black bowl, 21 cm in diameter and 3.50 cm high, filled with 800 ml of tap water with an oviposition attractant was placed in the bottom of the box (Fig. 1). The black coloured box and bowl should provide an environment with low light levels and assures an internal environment protected from the wind. One side of the box can be opened to permit the bowl to be moved in and out.

**Grass infusion (GI) trials** - Infusions of *Eleusine indica* (Poaceae) were tested to select the best result of infusion to be used to attract female mosquitoes. The

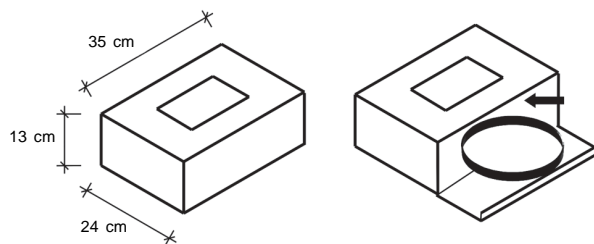


Fig. 1: schematic diagram of the BR-OVT oviposition trap.

grass infusion (GI) was prepared by adding 30 g of fresh grass to 2 l of tap water and incubated at  $27^\circ \pm 2^\circ\text{C}$  for 5, 10, 15, and 20 days in storage bottles. The bioassays were conducted in a large cage using groups of 200 gravid females. Two BR-OVT, filled with either GI or tap water, were placed on the floor of the cage at 18:00 h and left overnight. The experiment was repeated 6 times using the 15-day-old GI (the best result) to study the attractiveness of the trap with GI as compared to water.

The use of larvicides in ovitraps is necessary to avoid them becoming a breeding place. Prior to using entomopathogenic bacteria as larvicides in the BR-OVT, the oviposition response of mosquito females to the presence of *Bacillus sphaericus* (*Bs*) and *B. thuringiensis israelensis* (*Bti*) was investigated. For this, a series of oviposition assays in a two-choice chamber using *Bs* whole fermented culture (1000 ppm, 2000 ppm or 3000 ppm) and a product based on *Bti*, VectoBac-CG<sup>®</sup> (0.45 mg/l) added either to tap water or to GI, tested against their respective controls, was conducted in large cages. Each experiment, using batches of 50 or 100 gravid females was carried out in 6 to 8 replicates. The assay protocol was the same as described above.

**Field tests** - Experiments were conducted in three districts of Olinda, a coastal city near to Recife, as well as in a district of Recife, Northeast Brazil. The city of Recife and its surrounding urban area, with about 3.5 million inhabitants, is the main endemic area for lymphatic filariasis in Brazil. High temperatures, heavy rainfall, and unplanned urban development with poor sanitation are responsible for high mosquito population densities throughout the year (Regis et al 1995). *Cx. quinquefasciatus* is the main mosquito species in this area, followed by the dengue vector *Aedes aegypti*. The districts of Nova Olinda, Peixinhos, and Vila Popular in Olinda and Caçote in Recife were selected for field tests.

The efficacy of the BR-OVT in attracting gravid females for oviposition was compared to that of a black bowl (21 cm diameter and 3.5 cm high), both filled with 800 ml of GI. They were placed 1 m apart from each other in a house located in the Vila Popular district of Olinda, on the ground, one pair indoors and one pair outdoors. Twelve repetitions were carried out within a period of 30 days. The experiment was set up at 18:00 h and left overnight and the position of the trap and bowl alternated daily. Egg rafts were recovered and counted every day.

To assess the attractiveness of the BR-OVT with GI compared to the BR-OVT containing tap water alone, under field conditions, an experiment was carried out simultaneously in 3 districts of Olinda, placing one pair of traps in one household per district. The BR-OVTs were placed 1 m apart from each other on the floor of a room at 18:00 h and left overnight. At 08:00 h the egg rafts laid in each BR-OVT were removed and counted. The experiment ran for 12 days and the positions of the treatment and control traps were alternated daily. Trap bowls were filled with either GI or water at the beginning and were not changed throughout the experimental period. Egg rafts were recovered and counted every day.

The number of egg rafts collected in the BR-OVT was compared to the number of females caught in a CDC gravid trap (model 1712 by John Hock Company). Grass infusion was used in both traps. The traps were placed in a household in the Vila Popular district of Olinda, 1 m apart from each other on the floor of a room at 18:00 h and left overnight. Each morning at 08:00 h the egg rafts laid and the females caught in the traps were removed and counted. This experiment ran for 60 days.

To evaluate the performance of the BR-OVT (with GI and *Bs*) according to its placement, traps were set indoors and/or outdoors alternatively or simultaneously in 11 premises located in Caçote, a district of Recife. One trap per house was set up in the backyard for 40 days and subsequently within rooms in the same households for an equal time. In a second trial, 2 BR-OVTs were placed simultaneously one indoors and the other outdoors in the same premises. A product based on *Bs*, Vectolex<sup>®</sup>, was applied to the traps at a concentration of 0.5 mg/l and egg rafts laid were recovered and counted every 2 days. The outdoor traps were always set up close to a cesspit from which samples were taken to count the egg rafts. On each occasion, 9 samples were taken using a 300 ml dipper, 2 samples from each side and one from the centre of the cesspit.

**Data analysis** - The difference between treatments and control in each experiment was analyzed using the Wilcoxon signed rank test or Friedman (Siegel & Castellan 1988). Values of  $p \leq 0.05$  (two-tailed) were considered significant. The final value of  $N$  might be subject to some variation due to the number of replications when the Wilcoxon test is manually applied, according to the normal procedures. This test does not take into account paired data when both have identical ranks, reducing the  $N$  of the sample (Siegel & Castellan 1988). The oviposition activity index (OAI) was estimated, according to Kramer and Mulla (1979). Index values lay within the range of +1 to -1, and, as suggested by Hwang et al. (1982) positive values of +0.3 and above indicate that the material is an attractant in a broad sense, while negative values of -0.3 and below indicate repellency.

## RESULTS

**Response of females to the oviposition pheromone** - Bioassay results showed significant preference of gravid females for cups containing 5 recently laid unwashed egg rafts, compared to those with egg rafts recently laid and washed with hexane. The cups containing unwashed

egg rafts received 16 out of a total of 18 egg rafts laid during the assay ( $n = 18, k = 2, p = 0.02$ ).

Significantly more egg rafts were laid in the cup treated with SOP than in the control, except for the highest SOP dose. The cups treated with doses ranging from 0.03 to 0.78 mg received similar levels of oviposition: 70 to 76% of the total number of egg rafts deposited in treatment and control ( $p \leq 0.05$ , in all cases). However, when 1.56 mg of SOP was added to the test cup, 91.5% of the egg rafts were deposited in the control cup ( $n = 6, T = 21, p < 0.05$ ). The OAI was negative, suggesting a repellent effect of this high dose (Fig. 2).

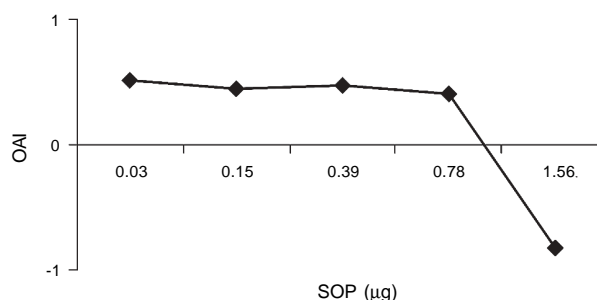


Fig. 2: oviposition activity indices (OAI) calculated for the response of *Culex quinquefasciatus* gravid females to different concentrations of synthetic oviposition pheromone (SOP) in selected chamber bioassays.

**Response of females to entomopathogenic bacteria** - Cups containing water treated with *Bs* at concentrations of 2000 ppm or 3000 ppm received more egg rafts than the control: 79.8% compared with 20.2% and 72.1% compared with 27.9% respectively. At a lower concentration (1000 ppm) no difference was observed between the percentage of egg rafts laid in the treated water (57.7%) and the control (42.3%,  $p > 0.05$ ). GI received significantly more egg rafts than *Bs* (2000 ppm) added to water ( $p < 0.05$ ) (Table) but the assays comparing *Bs* plus GI and GI alone showed no preference for either option ( $p > 0.05$ ). Similar results were obtained from the assays using *Bti* added to water compared with water or GI. However, when *Bti* was applied to GI, the cup containing GI only received more egg rafts ( $p < 0.02$ ) (Table).

**Response of females to GI in the BR-OVT** - To select the optimal number of days of infusion to be used in the trap, 5, 10, 15, and 20-day old infusions were assayed one by one against tap water as a control. The results showed that all attracted more than 80% of the females for oviposition as compared to their respective controls, the OAI ranging from 0.63 to 0.75. However the number of egg rafts laid in the BR-OVT containing 15 day-infusion was higher than in the other assays: 131 egg rafts against 47, 36, and 108 respectively for 5, 10, and 20 days of infusion.

TABLE

Response of gravid females to *Bacillus sphaericus* (*Bs*) (fermented culture at 2000 ppm) and to *B. thuringiensis israelensis* (*Bti*) (VectoBac CG 0.45 g/l) combined with either tap water or grass infusion (GI) regarding the choice of an oviposition site

Assay	Females/Assay	Number of egg rafts laid											
		<i>Bs</i> Water		<i>Bs</i> GI		<i>Bs</i> +GI GI		<i>Bti</i> Water		<i>Bti</i> GI		<i>Bti</i> +GI GI	
1	100	27	6	24	72	37	35	45	5	25	45	4	45
2	100	40	8	22	80	38	30	17	3	34	32	4	40
3	100	37	6	8	84	33	38	22	19	8	85	19	26
4	100	53	12	14	80	17	36	12	8	1	24	11	32
5	100	73	26	0	80	5	30	32	4	0	95	9	20
6	100	79	20	4	90	48	52	39	10	6	64	4	28
7	50	-	-	-	-	-	-	45	2	0	78 <sup>a</sup>	16	34
8	50	-	-	-	-	-	-	46	0	4	94 <sup>a</sup>	5	45
Total		309	78	72	486	178	221	258	51	78	517	72	270
%		79.8	20.2	12.9	87.1	44.6	55.4	83.5	16.5	13.1	86.9	21.1	78.9
OAI		0.6		-0.7		-0.1		0.7		-0.7		-0.6	

a: 100 females per assay.

The ability of the BR-OVT to attract gravid females for oviposition was first evaluated in the laboratory, offering 2 options to 6 groups of 200 gravid females per cage: a BR-OVT containing 15 day-GI and a BR-OVT with tap water only. The results showed a high preference for the BR-OVT + GI: 946 (92.1%, OAI = + 0.8) egg rafts compared with 81 laid in the BR-OVT + tap water ( $n = 6$ ,  $T = 21$ ,  $p < 0.05$ ).

**Field tests** - Comparison of the efficacy of BR-OVTs with black bowls, both filled with GI, showed that, indoors, females laid 43 (81.1%) out of 53 egg-rafts in the BR-OVT ( $n = 12$ ;  $T = 78$ ;  $p < 0.001$ ), while, in the backyard, the BR-OVT received 20 (86.9%) of the total of 23 egg rafts.

Field experiments comparing GI to tap water in the BR-OVT confirmed that the ovitrap with infusion is more attractive to *Cx. quinquefasciatus* gravid females than the ovitrap with tap water. During this experiment, which ran for 12 days, the total number of egg rafts found in the 2 traps placed in each house ranged from 15 (Vila Popular district) to 36 (Nova Olinda district) to 48 (Peixinhos district) and the percentages of oviposition in the GI-treated BR-OVT in each district were, respectively 75% ( $n = 6$ ,  $T = 21$ ,  $p < 0.05$ ), 73.9% ( $n = 11$ ,  $T = 66$ ,  $p < 0.001$ ), and 79.1% ( $n = 10$ ,  $T = 55$ ,  $p < 0.01$ ) (Fig. 3).

The attractiveness of the BR-OVT for gravid females was compared to that of the CDC gravid trap, both placed simultaneously in the same premises, by counting the egg rafts laid in the first and the females caught in the second trap over 2 months. The results showed that 51 egg rafts were laid in the BR-OVT, while 20 females were caught in the CDC gravid trap ( $n = 12$ ,  $T = 63.5$ ,  $p < 0.064$ ).

When the performance of the BR-OVT was evaluated in the backyard of the premises (September-October 2004) and subsequently indoors (October-November 2004), significantly more egg rafts were deposited in the traps located indoors (760) (Fig. 4) than out of doors (186) (Fig. 5). When two traps were used simul-

taneously in the same premises, a total of 1144 egg rafts were found in the traps, of which 673 egg rafts were laid indoors and 471 outdoors, this difference was not statistically significant ( $p > 0.05$ ) (Fig. 6).

No statistically significant differences were found between the number of egg rafts laid in the traps placed inside the premises and that found in the samples taken from the cesspit ( $p > 0.05$ ) in the experiment where one trap per premise was used (Fig. 4). However, the traps set up close to the cesspit received significantly fewer egg rafts than those counted in the samples from the cesspit (Fig. 5). When 2 traps per premise were used (inside and outside the house), the sum of egg rafts laid (1144) over 40 days was similar to that observed in the samples from the cesspits (1373) (Fig. 6).

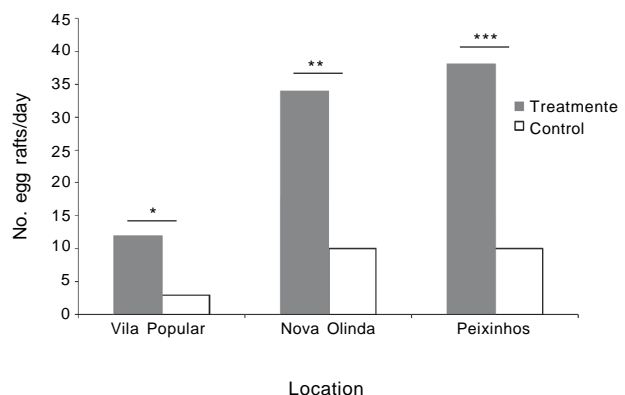


Fig. 3: number of egg rafts laid per day in the BR-OVT ovitrap with grass infusion or tap water at Olinda city, Brazil, during 30 days (September, 2000). Wilcoxon signed rank test (two-tailed); \*  $p \leq 0.05$  ( $n = 6$ ,  $T = 21$ ), \*\*  $p \leq 0.01$  ( $n = 10$ ,  $T = 55$ ), \*\*\*  $p \leq 0.001$  ( $n = 11$ ,  $T = 66$ ).

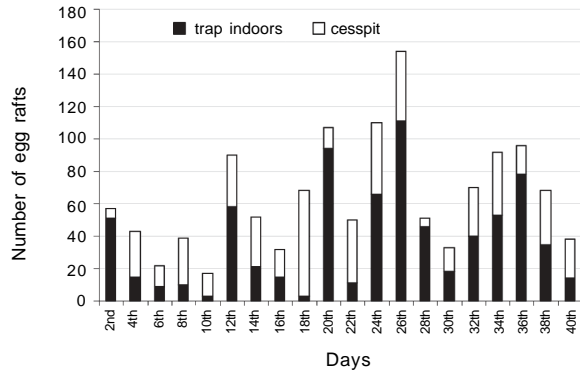


Fig. 4: number of egg rafts collected in 11 BR-OVTs placed inside premises (one trap per premise) in the Caçote district of Recife, compared to the number of egg rafts found in the cesspit in the backyard of the premises (9 samples per cesspit). September-October 2004. Wilcoxon signed rank test (two-tailed);  $p > 0.05$  ( $n = 20$ ,  $T = 56$ ).

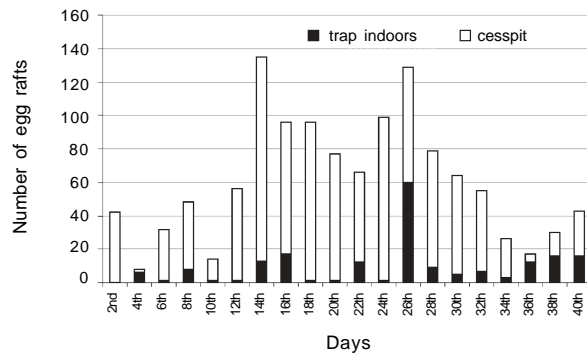


Fig. 5: number of egg rafts collected in 11 BR-OVTs placed outside premises (one trap per premise) in the Caçote district of Recife compared to the number of egg rafts found in the cesspit in the backyard of the premises (9 samples per cesspit). October-November 2004. Wilcoxon signed rank test (two-tailed);  $p < 0.001$  ( $n = 20$ ,  $T = 4$ ).

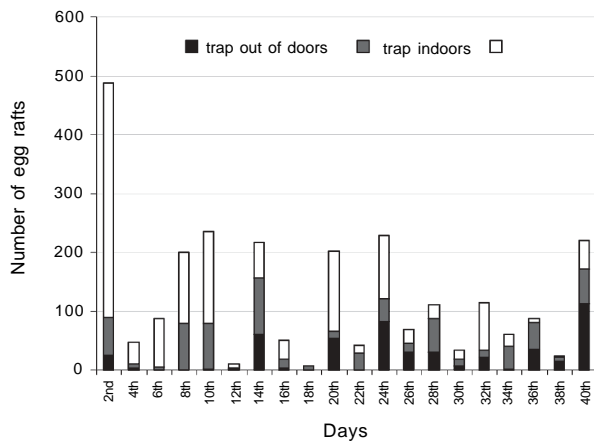


Fig. 6: number of egg rafts collected in 11 BR-OVT placed inside and 11 outside premises (2 traps per premise) in the Caçote district of Recife compared to the number of egg rafts found in the cesspit in the backyard of the premises (9 samples per cesspit). June-September 2004. Friedman signed rank test;  $p > 0.05$ .

## DISCUSSION

The evaluation of chemical cues in the choice of an oviposition site using 2 choice bioassays showed that the Recife strain of *Cx. quinquefasciatus* produces and responds to the oviposition pheromone, as with other populations of this species (Bruno & Laurence 1979, Isoe et al. 1995), confirming that oviposition site selection is influenced by the pheromone emanating from apical droplets on the eggs, as previously reported by Laurence and Pickett (1982). This information is important for egg or adult trapping purpose because the first egg rafts laid will contribute to increasing the ovitrap's attractiveness thereafter. At most of the doses tested, gravid females also responded to the synthetic oviposition pheromone, corroborating previous observations regarding *Cx. quinquefasciatus* (Laurence & Pickett 1982). However, the highest dose (1.56 µg) assayed gave a negative OAI (− 0.82), indicating a repellent effect. One might suppose that a very high concentration of pheromone could be perceived by a female as an inappropriate area for oviposition with possible competition for resources by the larvae already present.

The BR-OVT received more egg rafts than controls when filled with 15-day old GI in the laboratory (OAI = + 0.82) as well as under field conditions. It is known that the ability of GI to attract gravid female mosquitoes is variable over time, according to changes in the composition of infusions, as a consequence of the dynamics of microbial fermentation (Hazard et al. 1967, Millar et al. 1992, Sant'Ana et al. 2006). Although in this study the 15-day infusion showed the best outcome, as revealed by the largest number of rafts deposited in the test cup, all infusions tested, from 5 to 20 days old were, to varying degrees, significantly more attractive than water. This observation justified use of GI in the new ovitrap for prolonged periods without changing it. This was only made possible by the addition of a larvicide, removing the risk of transforming the ovitrap into a breeding place for larvae hatched from egg rafts laid there. The entomopathogenic bacteria assayed showed themselves to be an appropriate larvicide for use in oviposition traps for *Culex*, since they did not have a repellent effect on gravid females. Furthermore, it was observed that both *Bs* and *Bti* attracted and/or stimulated more oviposition than water, confirming the results obtained by Poonam et al. (2002) when testing culture filtrates of these bacteria for attractancy to gravid females of *Cx. quinquefasciatus*. On the other hand, according to our results, the addition of *Bs* to GI did not alter the attractiveness of the site. This is not true, however, for *Bti*, the presence of which, in combination with GI, under laboratory conditions, attracted fewer females to oviposit as compared to GI alone. By contrast, in a field study using a combination of *Bti* and grass infusion in ovitraps for *Aedes aegypti*, Santos et al. (2003) concluded that *Bti* appears not to interfere in the choice of trap for oviposition by gravid females.

The fact that the BR-OVT received many more egg rafts than a simple black bowl, tested under identical conditions, irrespective of their location indoors or out-

doors, clearly shows that the dark and wind-protected microenvironment created by the black box of the trap enhances its attractiveness for gravid females.

Based on the data from these preliminary experiments, the field performance of the BR-OVT was evaluated using a combination of GI and *Bs*. Besides being considered the best option as a larvicide against *Cx. quinquefasciatus* (Regis et al. 2001) this bacterium has proved to be compatible for use in combination with infusion in ovitraps. It is important to emphasize that in field experiments the number of egg rafts deposited in the ovitrap will depend on the local mosquito population density and structure, particularly the proportion of gravid females locally available during the experiment. The population size of *Cx. quinquefasciatus*, which is an *r*-strategist, shows marked natural fluctuations over time. Therefore it is not surprising that the number of egg rafts found in the BR-OVT during the field trial carried out in Olinda, varied markedly among the districts studied and within the same house at different times. Nevertheless, the BR-OVT with GI received significantly more egg rafts than the control in all experiments.

The results observed in the Caçote district of Recife demonstrate that the BR-OVT can collect more eggs inside houses than when installed in a backyard close to a cesspit, the main type of breeding site for *Cx. quinquefasciatus* in Recife (Regis et al. 1995, Silva-Filha et al. 2001). There are at least 2 reasons for this: on the one hand, this anthropophilic mosquito shows a preference for biting and resting inside houses, where water collections rich in organic material, their preferred oviposition sites, are infrequent and therefore there are rarely any other oviposition sites competing with the trap; on the other hand, in the backyard the cesspit constitutes a strong competitor for the trap.

The sensitivity of the BR-OVT as a tool for monitoring mosquito population densities was demonstrated by comparing the number of egg rafts collected in it with that found simultaneously in cesspits or with the number of females caught in the CDC-gravid trap. The BR-OVT does not require electricity and is cheap, robust, and easy to manipulate. Using a larvicide in the trap makes it unnecessary to remove eggs on a daily basis. This is of practical interest because it guarantees the safety of the ovitrap and extends the intervals between inspections, reducing the operational costs of programs. In combination with oviposition attractants and a larvicide, it is a potential candidate for use in surveillance as well as for collecting population samples for research purposes and perhaps as a control method in which large numbers of egg rafts would be laid into the traps where the larvae would not survive.

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