RESIDUAL ACTIVITY OF *BACILLUS THURINGIENSIS* SEROVARS *MEDELLIN* AND *JEGATHESAN* ON *CULEX PIPIENS* AND *AEDES AEGYPTI* LARVAE

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ABSTRACT. *Bacillus thuringiensis* serovar *medellin* strain 163–131 and *Bacillus thuringiensis* serovar *jegathesan* (*B.t.jeg.*) strain 367 are very toxic to mosquito larvae. However, they are 10 times less toxic than *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) to mosquito larvae under laboratory conditions. Lyophilized powders were produced from these strains and their toxicities were compared to that of powder produced from the *B.t.i.* strain. Larvicidal activity was titrated using *Aedes aegypti* (Bora-Bora strain) larvae, with IPS82 powder as the standard. The efficacy of these powders in the field was determined using *Culex pipiens* (Montpellier strain) in Paris, France, and *Ae. aegypti* larvae (French Guiana strain) in Cayenne, French Guiana, in standardized conditions. Residual activity was also assessed in the laboratory, using *Cx. pipiens* (SLAB strain), in Montpellier, France. Any negative effect of direct sunlight, soil, or polluted water on the residual activity of the 3 powders was recorded. Increasing bacterial concentration by a factor of 8 had little effect on the duration of larvicidal activity, except in the presence of polluted water and when substrate was added. All powders had similar initial efficacies against both types of mosquito larvae, in all conditions except water rich in organic matter. *Bacillus thuringiensis* serovar *medellin* had the lowest residual activity, both in the laboratory and in the field, whereas *B.t.jeg.* remained toxic for as long as *B.t.i.*

KEY WORDS   *Aedes aegypti*, *Culex pipiens*, *Bacillus thuringiensis* var. *israelensis*, *Bacillus thuringiensis* serovar *medellin*, *Bacillus thuringiensis* serovar *jegathesan*, residual efficacy, mosquito control

INTRODUCTION

Only *Bacillus thuringiensis* var. *israelensis* (de Barjac) (*B.t.i.*) and *Bacillus sphaericus* (Neide) are currently used in mosquito control programs. Mosquito larvae resistant to *B. sphaericus* products have been reported in various countries (Sinègre et al. 1994, Rao et al. 1994, Silva-Filha et al. 1995), whereas no resistance to *B.t.i.* has occurred, even after 19 years of treatment (Becker and Ludwig 1993, Thiéry et al. 1996). No resistance to *B.t.i.* occurs because it produces 4 toxins that act synergistically. This led us to look for bacteria producing several mosquitocidal toxins different from that of *B.t.i.* to which larvae resistant to *B. sphaericus* would be susceptible. *Bacillus thuringiensis* serovar *medellin* (*B.t.med.*) isolated from Colombia (Orduz et al. 1992) and *Bacillus thuringiensis* serovar *jegathesan* (*B.t.jeg.*) isolated from Malaysia (Seleena et al. 1995) produce at least 7 toxins that are very toxic to mosquito larvae. Both bacteria are about 10 times less active against *Culex* and *Aedes* larvae than *B.t.i.* (Ragni et al. 1996), but have similar larvicidal activity against *Anopheles stephensi* (Liston) to *B.t.i.* in laboratory conditions. Some of the genes encoding these toxins have been characterized and shown to have sequences similar to those

of *B.t.i.* genes (Delécluse et al. 1995, Restrepo et al. 1997, Rosso and Delécluse 1997). These 2 bacteria also contain a cytolytic protein (Cheong and Gill 1997, Thiéry et al. 1997a). The presence of a cytolysin in bacterial strains may overcome the resistance of resistant insects or prevent development of resistance (Federici and Bauer 1997, Wirth et al. 1997, Thiéry et al. 1998). Hence, it was worthwhile checking the persistence of the toxicity of these 2 strains to see whether they fulfilled the requirements for further development. This study determined the residual activity of these 2 bacteria towards *Aedes aegypti* (L.) (French Guiana strain) and *Culex pipiens* L. (Montpellier or SLAB strains) in outdoor and indoor containers. The effects of various factors, such as sunlight, substrate, polluted water, temperature, and bacterial concentration were recorded.

MATERIALS AND METHODS

Mosquito strains: Generations of *Ae. aegypti* (Bora-Bora strain) and *Cx. pipiens* (Montpellier and SLAB strains) have been reared for more than 20 years at 26 ± 1°C, 14:10 h (light:dark) photoperiod, and 80% relative humidity. Larvae of *Ae. aegypti* (French Guiana strain) were sampled from several areas in French Guiana and were reared at 28 ± 2°C and 80 ± 10% relative humidity for 2 years.

Bacterial strains: *Bacillus thuringiensis* serovar *medellin* strain 163–131, *B.t.jeg.* strain 367, and *B.t.i.* strain 1884 were obtained from the International Entomopathogenic *Bacillus* Center Collection held by the Unité de Bactéries Entomopathogènes at Institut Pasteur, Paris, France.

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Production of lyophilized powders: Bacillus thuringiensis serovar *medellin* strain 163–131, *B.t.jeg.* strain 367, and *B.t.i.* strain 1884 were grown in 16 liters of medium (UG, de Barjac and Lecadet 1976) in a draft-tube fermenter (Biolafitte LSL Biolafitte SA, Niort, France) for 48 h at 30°C. Bacteria were lyzed, collected by centrifugation, filtered through a Filtron® (Ball Filtron Laboratories, St. Germain en Laye, France) cassette (Omega open channel 0.16 μm), and centrifuged. Spores and crystal pellets were then freeze-dried and stored at 4°C. A *B.t.i.* microgranule experimental formulation ABG 6462 was kindly provided by Abbott Laboratories, Chicago, Illinois, and was used in French Guiana as a control. The protein content of each lyophilized powder was assessed by Bradford assay (Bradford 1976), and sodium dodecyl sulfate–polyacrylamide gel electrophoresis, as described by Delécluse et al. (1988).

Laboratory bioassays: The larvicidal activity of each of the 3 lyophilized powders and the microgranule formulation was titrated against the reference lyophilized powder IPS82 on *Ae. aegypti* larvae (Bora-Bora strain), and was checked against *Ae. aegypti* larvae (French Guiana strain) and *Cx. pipiens* (Montpellier and SLAB strains). Bioassays were performed using 2 groups of 25 4th-stage larvae in plastic cups filled with 150 ml of bacterial suspension as described by de Barjac and Larget-Théry (1984). Each product was assayed at 6 dilutions. Two cups containing 150 ml each of deionized water were used as controls. Bioassays were performed in triplicate. Larval mortality was recorded after 24 h. Median lethal concentrations (LC_{50}s) and 90% lethal concentrations (LC_{90}s) were estimated using a log-probit program (created by E. Frachon, Institut Pasteur, Paris, France) on a Macintosh computer. The LC_{50} values are expressed as means ± standard error (SE).

Outdoor experiments: Outdoor experiments were performed in containers filled with 5 liters of water and a 2-cm depth of sterile sand in Paris or with 8 liters of water with no substrate in Cayenne, French Guiana. The water was approximately 25 cm deep. A net cover prevented mosquito colonization. At Cayenne, for each concentration of each product, 2 containers were placed in sunny conditions and 2 were placed in the shade. In Paris, all containers, 2 for each product concentration, were placed in normal daylight. The concentration of each product used corresponded to 4 times the LC_{90} against *Cx. pipiens* in Paris and 2, 4, and 8 times the LC_{90} against *Ae. aegypti* (French Guiana strain) as determined in laboratory bioassays. In each experiment, control containers were placed in the same conditions. One hundred 3rd-stage *Cx. pipiens* (Montpellier strain) in Paris or 50 *Ae. aegypti* (French Guiana strain) larvae in Cayenne were added to each container when all previously added mosquito larvae were dead. Temperature and larval mortality were recorded 24 h after the introduction

of larvae and every 2 days. Therafter, the cadavers were left in the containers. New batches of larvae were added to establish the residual activity of each concentration. The containers were removed when less than 50% mortality was recorded.

Indoor experiments: Similar experiments were performed indoors at Montpellier, France, in an aquarium filled with 10 liters of water. The concentration of product used corresponded to 0.5, 1, 2, 4, 8, or 16 times the LC_{90} on *Cx. pipiens* (SLAB strain), depending on the factors involved (soil and polluted water). Three control aquaria were used in each experiment. One hundred 3rd-stage larvae were added to each aquarium. Temperature and larval mortality were recorded 24 h after the larvae were introduced. The cadavers were left in the aquarium and surviving larvae were removed before new larvae were introduced. The volume of water was adjusted if necessary. The effects of 2 cm of soil, bacterial concentration, and polluted water on residual activity of the 3 powders were determined. Two experiments were performed using polluted water. The aquaria were filled with 8 liters of tap water plus 2 liters of polluted water, or with 10 liters of very polluted water sampled directly from a water treatment plant (Lattes village, France). In both cases, physical and chemical characteristics were determined on the 1st day of the experiment by a calibration protocol using a portable WTW MultiLine P4 F/set3 apparatus from WTW France (Limonest, France) to measure pH, dissolved O_2 concentration, conductivity, salinity, gaseous O_2 concentration, and saturated O_2 concentration. The concentrations (mg/liter) of nitrate, sulfate, and ammonium, and the total water hardness (calcium and magnesium ions) were evaluated as described (Rodier 1966).

RESULTS

Production and titration of lyophilized powders

The 3 strains 163–131, 367, and 1884 were cultured in a fermenter and final whole culture (FWC) for each strain was tested against *Ae. aegypti* larvae (Bora-Bora strain) and the bacteria were then harvested and lyophilized. The LC_{50}s, approximately 1 × 10^{-4}, 3 × 10^{-5}, and 1 × 10^{-6} dilutions of the FWC of *B.t.jeg.*, *B.t. med.*, and *B.t.i.*, respectively, were similar to those for FWCs grown in flasks (data not shown). Each pellet from the 16-liter culture yielded approximately 50–60 g of powder. The *B.t.jeg.*, *B.t.med.*, and *B.t.i.* powders contained 100, 190, and 250 μg protein/mg, respectively. The protein profiles of the powders were similar to the common profile of these toxic proteins, as described by Ragni et al (1996): 7 major proteins, 3 major and 5 minor ones, and 4 major proteins in *B.t.jeg.*, *B.t.med.*, and *B.t.i.*, respectively (data not shown). The larvicidal activity of the 3 powders was evaluated using all the mosquito species in-

volved in the experiments and also against *Ae. aegypti* (Bora-Bora strain) for titration (Table 1). The microgranule formulation ABG 6462 (CL_{50} = 0.046 ± 0.008 mg/liter and CL_{90} = 0.078 ± 0.011 mg/liter) was also titrated against IPS82. The titer of ABG 6462 was 6,520 ± 1,952 International Toxic Units (ITU)/mg against *Ae. aegypti* (Bora-Bora strain). The LC_{90} of ABG 6462 (0.053 ± 0.002 mg/liter) against *Ae. aegypti* (French Guiana strain) was used to determine its concentrations in outdoor experiments. The lethal concentrations of *B.t.i.* powder were 10–20 times lower than those of *B.t.med.* and *B.t.jeg.* against *Ae. aegypti,* and were 5–8 times lower against *Cx. pipiens. Bacillus thuringiensis* serovar *medellin* and *B.t.jeg.* were more toxic against *Cx. pipiens* strains than against *Ae. aegypti* strains, as previously described by Ragni et al. (1996). The *Ae. aegypti* larvae from French Guiana were slightly less susceptible than those from Bora-Bora, which were taken from long-established populations. In contrast, the SLAB strain of *Cx. pipiens* was twice as susceptible as the Montpellier strain. The titer of *B.t.i.* powder was 4, 13, and 30 times higher than those of the microgranule product, and *B.t.med.* and *B.t.jeg.* powders, respectively.

Outdoor experiments

Bioassays were used to determine the concentrations to be used in containers for *Cx. pipiens* and *Ae. aegypti* larvae. At Cayenne, powders were added to containers at final concentrations of 2, 4, or 8 times the LC_{90} of each strain. This corresponded to 0.07, 0.14, and 0.28 mg/liter for *B.t.i.*; 0.8, 1.6, and 3.2 mg/liter for *B.t.med.*; 2, 4, and 8 mg/liter for *B.t.jeg.*; and 0.1, 0.2, and 0.4 mg/liter for the microgranule formulation. In the shade, air temperatures during the day were approximately 30°C and the temperature of the water was 28–30°C. In direct sunlight, air temperatures reached 40°C at midday and water temperatures reached 32–35°C. All powders were 100% effective in both sun and shade. The days on which more than 90% or less than 50% larval mortality was observed were recorded (Fig. 1). Residual efficacy was not affected by the concentration applied, except for the lowest concentration of *B.t.jeg.* More than 90% mortality was reported for 14 days with *B.t.med.,* 25 days with *B.t.jeg.,* 34 days with *B.t.i.* powders, and 31 days with ABG microgranules in the shade. All powders gave more than 90% mortality for 8–11 days if containers were subjected to direct sunlight. Fifty percent mortality was reported after 25 days with *B.t.med.,* 31 days with *B.t.jeg.,* and 38 days with *B.t.i.* powders, respectively, in the shade and only after 8–11 days with *B.t.med.,* 13–22 days with *B.t.jeg.,* and 22 days with *B.t.i.* powders under sun exposure.

In Paris, powders were added to containers, in duplicate, at a single concentration (4 times the LC_{90} of each powder): 0.1, 0.4, and 0.56 mg/liter

Table 1. Larvicidal activity of lyophilized powders from *Bacillus thuringiensis* var. *israelensis, Bacillus thuringiensis* serovar *jegathesan,* and *Bacillus thuringiensis* serovar *medellin* against *Aedes aegypti* and *Culex pipiens* larvae.[1]

Larvae[2]	B. t. israelensis		B. t. medellin		B. t. jegathesan	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Cx. pipiens (Montpellier strain)	0.0095 ± 0.0015	0.024 ± 0.010	0.059 ± 0.029	0.10 ± 0.01	0.044 ± 0.0085	0.14 ± 0.064
Cx. pipiens (SLAB strain)	0.0038 ± 0.0003	0.0097 ± 0.0011	0.033 ± 0.002	0.064 ± 0.005	0.021 ± 0.002	0.047 ± 0.004
Ae. aegypti (French Guiana strain)	0.014 ± 0.0045	0.035 ± 0.012	0.16 ± 0.017	0.40 ± 0.10	0.31 ± 0.06	0.97 ± 0.041
Ae. aegypti (Bora-Bora strain)	0.006 ± 0.001	0.013 ± 0.005	0.086 ± 0.010	0.20 ± 0.04	0.20 ± 0.04	0.80 ± 0.47
Titer[3]	24,857 ± 857		1,809 ± 559		812 ± 312	

[1] LC_{50}, median lethal concentration, LC_{50}s (mg/liter) are means ± SE of 3 experiments; LC_{90}, 90% lethal concentration.
[2] Old 3rd- or young 4th-instar larvae.
[3] Titers are expressed in international toxic units (ITU/mg) of powder using IPS82 (15,000 ITU/mg) as the reference for titration against *Ae. aegypti* (Bora-Bora strain).
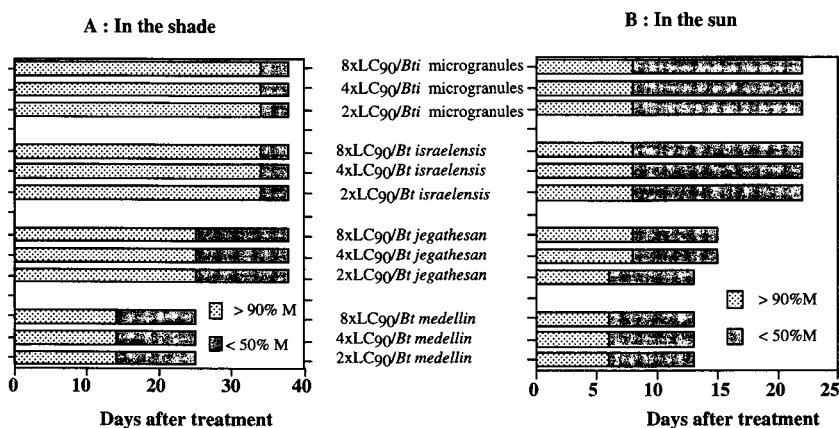
A : In the shade

B : In the sun

8xLC$_{90}$/Bti microgranules
4xLC$_{90}$/Bti microgranules
2xLC$_{90}$/Bti microgranules

8xLC$_{90}$/Bt israelensis
4xLC$_{90}$/Bt israelensis
2xLC$_{90}$/Bt israelensis

8xLC$_{90}$/Bt jegathesan
4xLC$_{90}$/Bt jegathesan
2xLC$_{90}$/Bt jegathesan

8xLC$_{90}$/Bt medellin
4xLC$_{90}$/Bt medellin
2xLC$_{90}$/Bt medellin

> 90% M
< 50% M

Days after treatment

Fig. 1. Outdoor experiments to determine the residual activity against *Aedes aegypti* larvae of 3 lyophilized powders from *Bacillus thuringiensis* serovar *medellin*, *Bacillus thuringiensis* serovar *jegathesan*, and *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and a microgranule formulation of *B.t.i.*, ABG 6462, applied at 3 concentrations. (A) In the shade; (B) in the sun. The spotted bars represent days on which at least 90% mortality was recorded and the gray bars correspond to the 1st days on which mortality below 50% was observed.

for *B.t.i.*, *B.t.med.*, and *B.t.jeg.*, respectively. Temperatures of water were from 14 to 26°C. The powders were all initially 100% effective, with or without the addition of sand (Fig. 2). If no sand was added, activity (90% mortality) lasted twice as long as in the presence of sand, for all powders other than *B.t.med.* Fifty percent mortality was observed after 8–16 days, with *B.t.i.* and *B.t.jeg.* powders being the most persistent. Sand reduced the residual activity of the 3 powders.

Indoor experiments

Two series of experiments were performed in Montpellier to determine the effects of soil alone

B.t.i. without sand / with sand

B.t. jegathesan without sand / with sand

> 90% M
< 50% M

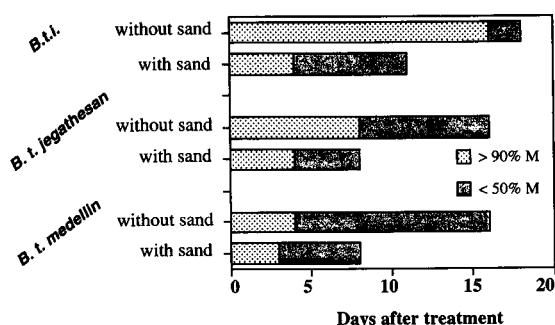*B.t. medellin* without sand / with sand

Days after treatment

Fig. 2. Outdoor experiments to determine the residual activity against *Culex pipiens* (Montpellier strain) of 3 lyophilized powders from *Bacillus thuringiensis* serovar *medellin*, *Bacillus thuringiensis* serovar *jegathesan*, and *Bacillus thuringiensis* var. *israelensis* applied at one concentration corresponding to 4 times the 90% lethal concentration of each powder. The effects of the presence or absence of sand at the bottom of the container were compared. The spotted bars represent days on which at least 90% mortality was recorded and the gray bars correspond to the 1st day on which mortality below 50% was observed.

and of soil and polluted water, on the residual activity of the 3 powders against *Cx. pipiens* (SLAB strain). If no soil was added, aquaria were filled with 0.5–4 times the LC$_{90}$ of each powder. If soil was added, the concentrations used were 2–16 times the LC$_{90}$. Temperature was 23 to 25°C, and all powders were initially 100% effective (Figs. 3A, 3B). If no substrate was added, the residual activity of all concentrations used persisted for a long time, with 42–58 days of mortality over 90% (Fig. 3A). In the presence of soil, more than 90% mortality was observed after 3 days with *B.t.med.* powder and after 7–18 days with *B.t.i.* and *B.t.jeg.* according to the concentrations used. Less than 50% mortality was observed after 4 and 7 days with the lowest and highest concentrations of *B.t.med.* powder, respectively (Fig. 3B). *Bacillus thuringiensis* var. *israelensis* and *B.t.jeg.* powders still gave 50% mortality after 21 days with 4 times the LC$_{90}$ of each powder, and after 28 days at their highest concentrations.

In a 2nd series of experiments, aquaria containing soil were filled with 20 or 100% polluted water sampled from a water treatment plant. The physical and chemical characteristics of the water in the aquaria determined on the 1st day of the experiment were: pH 7.13 (20% polluted water) and 7.69 (100% polluted water); −7 (20%) and −40 mV (100%) dissolved O$_2$; 1,152 (20%) and 1,499 μS/cm (100%) conductivity; 0.4 (20%) and 0.6 mg/liter (100%) salt; 1.06 (20%) and 0.04 mg/liter (100%) O$_2$ concentration; 13.6 (20%) and 0.4% (100%) saturated O$_2$; 0.32 (20%) and 0.4 mg/liter (100%) NO$_2$; 45 (20%) and 70 mg/liter (100%) SO$_4$; 21 (20%) and 58 mg/liter (100%) ammonium; 5.1 (20%) and 26.7 mg/liter (100%) organic alkaline matter; 6.7 (20%) and 37.5 mg/liter (100%) organic acid matter; 35.6 (20%) and 39.6 (100%)
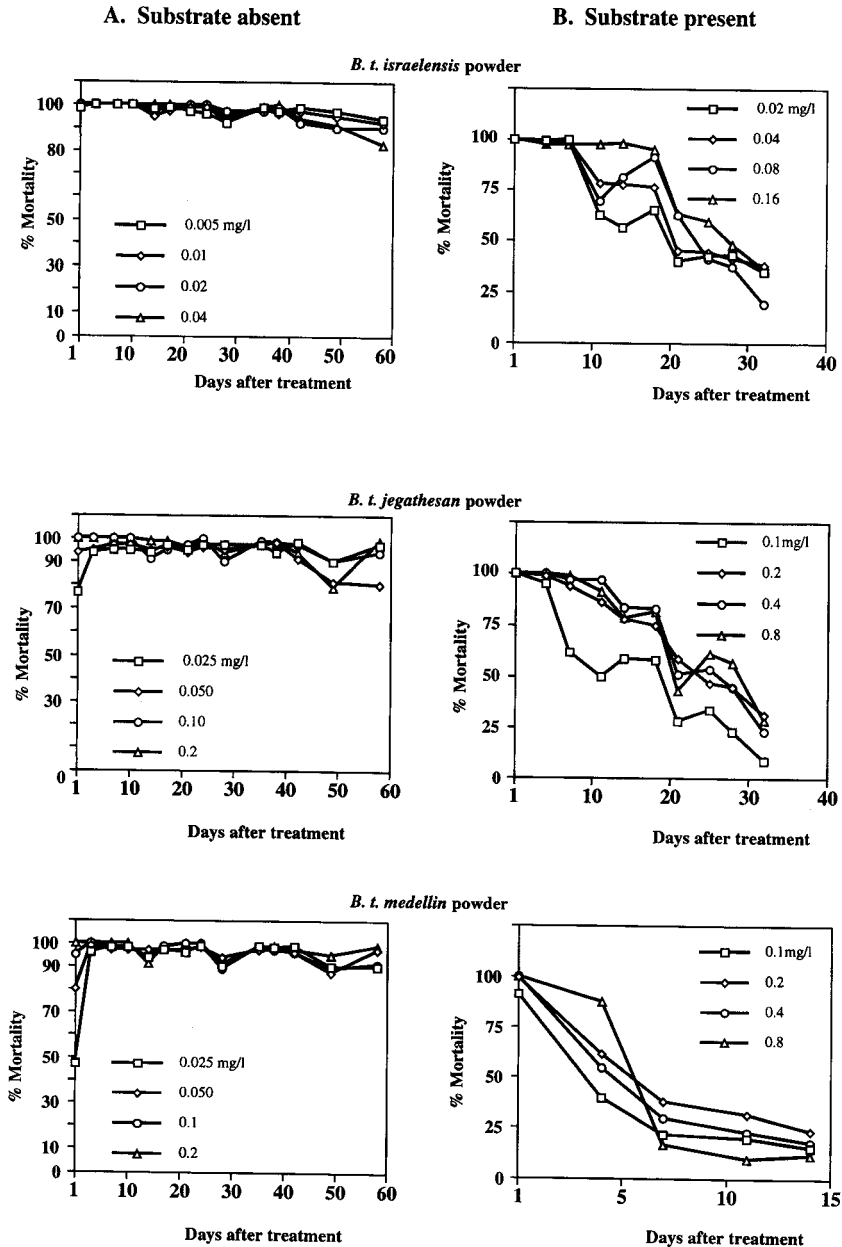
A. Substrate absent
B. Substrate present

B. t. israelensis powder

B. t. jegathesan powder

B. t. medellin powder

Fig. 3. Residual activity against *Culex pipiens* (SLAB strain) of the 3 lyophilized powders from *Bacillus thuringiensis* serovar *medellin, Bacillus thuringiensis* serovar *jegathesan,* and *Bacillus thuringiensis* var. *israelensis* applied at 4 concentrations. (A) No substrate added, concentrations represent 0.5, 1, 2, and 4 times the 90% lethal concentration (LC_{90}) of each powder. (B) Soil added, concentrations represent 2, 4, 8, and 16 times the LC_{90} of each powder.

total water hardness (°F); 108.3 (20%) and 308.7 (100%) pollution index (equivalent to (10 × ammonium) + organic acid matter/2). Powders were added to the aquaria at concentrations 2–16 times the LC_{90}. All powders were initially 100% effective in 20% polluted water (Fig. 4A), except for the lowest concentration of *B.t.med.* Ninety percent mortality was recorded after 4–8 days for *B.t.i.* and *B.t.jeg.* Residual activity inducing more than 50%

mortality lasted for 4–7 days with *B.t.med.,* depending on the concentration. Fifty percent mortality was recorded for 15–21 days with *B.t.i.* and *B.t.jeg.,* except with the lowest concentration of *B.t.i.* Mean water temperature was 24.9 ± 1.6 (11:00 a.m.) and 25.9 ± 1.6 (11:00 p.m.).

Based on these preliminary results, only *B.t.i.* and *B.t.jeg.* powders were tested in 100% polluted water. Mean 100% polluted water temperature was

A. In polluted water (20%), with substrate

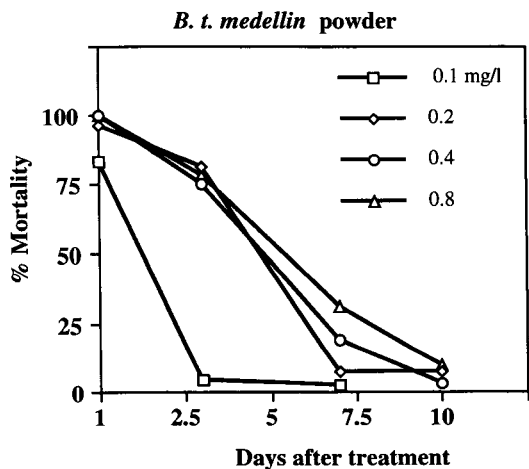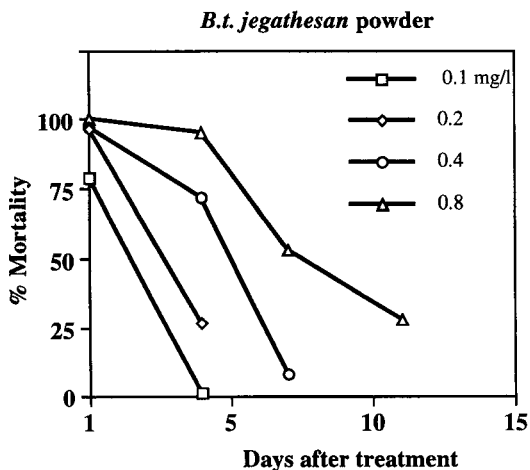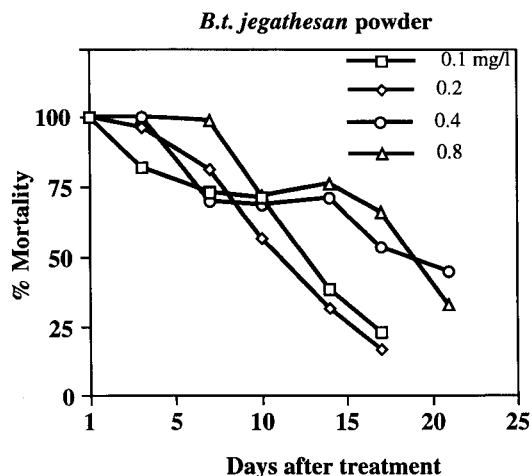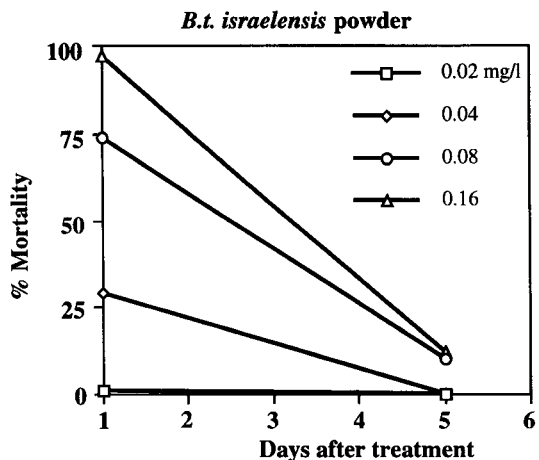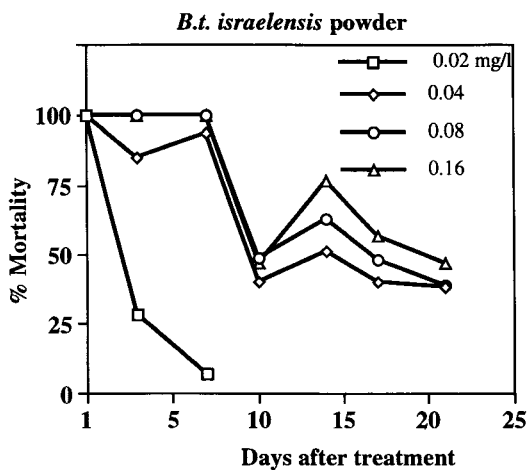**B. In polluted water (100%), with substrate**

Fig. 4. Residual activity against *Culex pipiens* (SLAB strain) of the 3 lyophilized powders from *Bacillus thuringiensis* serovar *medellin, Bacillus thuringiensis* serovar *jegathesan,* and *Bacillus thuringiensis* var. *israelensis* applied at 4 concentrations. Soil was added to all aquaria; concentrations represent 2, 4, 8, and 16 times the 90% lethal concentration of each powder. (A) Aquaria filled with 80% tap water and 20% polluted water. (B) Aquaria filled with 100% polluted water.

22.3 ± 1.1°C (10:00 a.m.) and 21.6 ± 1.1°C (11:00 a.m.). Initial efficacy depended on the concentration used (Fig. 4B). The 3 highest concentrations of *B.t.jeg.* powder and the highest concentration of *B.t.i.* caused more than 90% mortality after 24 h of larval exposure. No residual activity was observed with *B.t.i.*, whereas 50% mortality was observed for 4–7 days with *B.t.jeg.* powder.

DISCUSSION

The aim of this study was to determine the residual activities of *B.t.med.* and *B.t.jeg.* powders and to compare them with that of *B.t.i.* against *Ae. aegypti* and *Cx. pipiens* larvae. *Bacillus thuringiensis* var. *israelensis* was more active than *B.t.med.* and *B.t.jeg.* against *Ae. aegypti* and to a lesser extent against *Cx. pipiens* larvae, as previously described (Ragni et al. 1996). The 2 *Cx. pipiens* populations (SLAB and Montpellier strains) that originated from the same source differed in susceptibility to these 3 strains after years of being reared in different conditions. The SLAB population was more susceptible than the Montpellier population. This was also the case for *B. sphaericus* strains (Thiéry et al. 1997b). *Aedes aegypti* from Cayenne was slightly less susceptible than the Bora-Bora population. This is unusual because mosquitoes sampled from the natural environment are usually more susceptible than reared mosquitoes. We compared the titers of *B.t.i.*, *B.t.med.*, and *B.t.jeg.* powders and their protein contents. The *B.t.i.* powder was 13 times and 30 times more active than the *B.t.med.* and *B.t.jeg.* powders, respectively. This suggests that the *B.t.i.* powder should contain 13–30 times more active ingredient, whereas it contained only 1.3–2.5 times more protein. Thus, 1 μg of *B.t.i.* protein killed roughly 10 times more *Aedes* larvae than did 1 μg of the other 2 powders. However, the titration of *Bacillus thuringiensis* products is different from that of *B.t.i.* against the IPS82 standard. These products contain different toxins, so the principle of standardization cannot be strictly respected.

The *B.t.i.* powder was approximately 4–6 times more active than the other 2 powders against *Culex* populations. However, depending on protein content, 1 μg of *B.t.i.* killed 5 times more *Culex* than did *B.t.med.* but only twice as many as *B.t.jeg.* in laboratory bioassays. The *B.t.jeg.* proteins had a strong effect on *Culex* larvae, probably due to the 80-kDa protein that, alone, was as toxic as the wild-type strain towards *Culex* larvae (Delécluse et al. 1995).

Under seminatural conditions at Cayenne, the difference in activity of *B.t.i.* and *B.t.jeg.* against *Ae. aegypti* was not as large as that in the laboratory. *Bacillus thuringiensis* serovar *jegathesan* was as effective in the shade as *B.t.i.* In contrast, although *B.t.med.* was more active than *B.t.jeg.* in the laboratory, it had the lowest residual activity. Tem-

peratures as high as 30 or 40°C had no clear effect on the residual activity of any of the powders, whereas, in the sun, the toxins were presumably degraded by ultraviolet (UV) light, resulting in a significant decrease in the residual efficacy of all powders. The effects of UV light on bacterial larvicidal efficacy are not clear. Some studies have suggested that the UV kills the spores but does not affect the toxic proteins (Burke et al. 1983), and that UV-treated preparation of *B.t.i.* could be used in mosquito control (Engler et al. 1980). Other studies have suggested that the UV in sunlight prevents *B.t.i.* toxicity (Ignoffo et al. 1981). In both conditions, shade and sun, the microgranule formulation showed the same residual activity as the *B.t.i.* powder, although its titer was 4 times lower. Thus, formulation improved the efficacy of the microgranule product.

We compared the residual activity of the 3 powders in Paris, with no substrate, with those in Cayenne. The mosquito populations were different, but *B.t.i.* residual activity in the sun was higher in Paris than in tropical areas. The other 2 powders behaved similarly in both environments, although *B.t.jeg.* was toxic for longer than *B.t.med.* This demonstrated the stronger effect of UV light on *B.t.i.* toxicity in tropical areas than in temperate climates.

After the addition of substrate, a large decrease occurred in residual activity outdoors in Paris and indoors in Montpellier. This was due to the adsorption of bacteria to sediment and particulate matter (Ramoska et al. 1982, Silapanuntakul et al. 1983, Margalit and Bobroglo 1984, Sheeran and Fisher 1992). The residual activity of the 3 powders in aquaria with substrate lasted twice as long indoors as outdoors. Without substrate, the difference was more significant, with residual activity lasting 4–10 times longer indoors than outdoors. A large difference was also observed with *B. sphaericus* powders (Thiéry et al. 1997b). Differences in concentrations up to a factor of 8 significantly affected the persistence of toxicity if substrate was present, and had a clear effect in polluted water. *Bacillus thuringiensis* serovar *medellin* was less persistent indoors if substrate was added and was also less active in 20% polluted water. It was clearly the least efficient of the 3 powders. The polluted water contained a large amount of organic matter and probably proteolytically degraded *B.t.med.* toxins, whereas *B.t.i.* and *B.t.jeg.* toxins were less affected. In 100% polluted water, 0.16 mg/liter (16 times the LC_{90} concentration) of *B.t.i.* caused similar levels of mortality to 0.2 mg/liter (4 times the LC_{90} concentration) of *B.t.jeg.* Thus, *B.t.jeg.* powder is effective in polluted water, with its toxins apparently less sensitive to proteinase degradation. Only *B.t.jeg.* powder still had residual efficacy after 4 and 7 days. In the case of larval resistance to *B. sphaericus*, the only current alternative for maintaining biological mosquito control is to rotate *B. sphaericus* with *B.t.i.* products, as it is now done in the south of France (Jul-

lien et al. 1998). *Bacillus thuringiensis* serovar *jegathesan* was as least as effective as *B.t.i.* in 100% polluted water, although it was 5 times less active against *Cx. pipiens* larvae in laboratory conditions.

We have shown in this study that *B.t.med.* strain 163–131, although highly mosquitocidal in laboratory conditions, had less residual activity than *B.t.jeg.* strain 367. *Bacillus thuringiensis* serovar *jegathesan* had strong residual activity in polluted water, and was at least as effective as *B.t.i.* Thus, because this strain contains several toxins, and produces large crystals, it is a potential candidate for further development for use in urban mosquito control.

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