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DOCUMENTATION OF HIGH-LEVEL *BACILLUS SPHAERICUS* 2362 RESISTANCE IN FIELD POPULATIONS OF *CULEX QUINQUEFASCIATUS* BREEDING IN POLLUTED WATER IN THAILAND

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ABSTRACT. *Bacillus sphaericus* (*Bsph*) Neide has been recognized as an effective mosquito larvicide since its discovery 20 years ago. Various strains of this agent, such as 2362, 2297, 1593, and C3-41, have been developed, formulated, and field-evaluated against mosquito larvae in different countries. Their high efficacy in controlling mosquitoes breeding in various habitats, especially those in polluted water, has been documented. However, resistance to *Bsph* has been reported in *Culex pipiens* complex in both laboratory colonies and natural populations. During our field trials on *Bsph* water-dispersible granules (WDG) against natural populations of *Cx. quinquefasciatus* Say in a low-income community, Nonthaburi Province, Thailand, control failure occurred within 4 months after 5 treatments using VectoLex WDG at the dosages of 50–200 mg/m^2. The suspected *Bsph*-resistant *Cx. quinquefasciatus* mosquitoes were collected and colonized in the laboratory, from which the late stage larvae of generations F_{14} through F_{17} were used in bioassays to elucidate the resistance profile. A high level of *Bsph* resistance was documented in this colony as compared with the susceptible mosquitoes from the same area in Thailand or from California, USA. The resistance ratios (RR) at LC_{50}, depending on reference colonies, were 21,100–28,100-fold against *Bsph* WDG or greater than 125,000–200,000-fold against *Bsph* technical-grade material. These *Bsph*-resistant mosquitoes, however, were completely susceptible to *Bacillus thuringiensis* var. *israelensis,* (*Bti*) preparations, LC_{50} ranging from 0.017 ppm for technical material with 7,000 ITU/mg to 0.052 ppm for water-dispersible granules with 3,000 ITU/mg. The RR to mixtures of *Bsph* + *Bti* in this highly *Bsph*-resistant mosquitoes increased steadily upon the increase of *Bsph* ratios in the mixtures from 50, 75, 90, 95 to 99%. The resistance levels to the mixtures with various ratios of *Bsph* and *Bti,* however, were substantially lower than that in *Bsph* alone, suggesting addition of *Bti* to *Bsph* substantially enhanced the mosquitocidal activity (synergism) against these highly *Bsph*-resistant *Cx. quinquefasciatus.* Moderate tolerance to low levels of resistance to *Bsph*/*Bti* recombinant (RR 7.29–12.75 at LC_{50} and 5.15–13.40 at LC_{90}) was also noted in this *Bsph*-resistant population.

KEY WORDS *Culex quinquefasciatus, Bacillus sphaericus, Bacillus thuringiensis* var. *israelensis,* resistance, resistance management

INTRODUCTION

Several *Bacillus sphaericus* (*Bsph*) strains show considerable mosquitocidal properties. Some of these were selected for further development and have been evaluated against larvae of a number of mosquito species (Lacey 1990; Yap 1990; Karch et al. 1992; Hougard et al. 1993; Sinègre et al. 1993; Regis et al. 1995; Mulla et al. 1997, 1999, 2001, 2003; Skovmand and Sanogo 1999; Ali et al. 2000). The most effective strains were commercially developed and employed in mosquito control programs in the 1990s in different countries. However, low to extremely high levels of resistance in natural populations of the *Culex pipiens* complex have been documented to some of these strains in France, India, Brazil, China, and Thailand (Sinègre et al. 1994, Adak et al. 1995, Rao et al. 1995, Silva-Filha et al. 1995, Yuan et al. 2000, Mulla et al. 2003). In France, greater than 50,000-fold resistance was noted after *Bsph* strain 2362 was used for 7 years (Sinègre et al. 1994). In Brazil, application of *Bsph* 2362 for 2 years lowered *Bsph* susceptibility 10-fold (Silva-Filha et al. 1995). In India, after 25 treatments using *Bsph* strain B-101, 150-fold resistance was noted. After 6 generations of laboratory selection of these field-resistant mosquitoes, resistance reached 52,000-fold (Adak et al. 1995). Also in India, 35 treatments using *Bsph* 1593 preparation resulted in 180-fold resistance. Further selection of these resistant mosquitoes using the same *Bsph* strain for 18 generations elevated the resistance level to 31,000-fold (Rao et al. 1995). In China, after *Bsph* C3-41 was used in mosquito control for 8 years, over 20,000-fold resistance was detected in field populations of *Cx. quinquefasciatus* (Yuan et al. 2000). In Thailand, 5 treatments using VectoLex WDG (strain 2362) over 4 months resulted in complete failure of control (Mulla et al. 2003), indicating occurrence of *Bsph* resistance.

Field trials of various *Bsph* formulations against *Cx. quinquefasciatus* have been conducted in Thailand over the past several years (Mulla et al. 1997, 1999, 2001, 2003). In Wat Pikul Community, Nonthaburi Province, Thailand, during September–December 2000, *Bsph* 2362 water-dispersible granules (WDG) were applied 5 times at various dosages (50–200 mg/m^2) to control *Cx. quinquefasciatus*

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larvae breeding in polluted water. The initial treatment at 200 mg/m^2 of the WDG yielded excellent control (over 95%). In further treatments, the level of efficacy went down substantially with no control, even at the high dosage of 200 mg/m^2 on the 5th treatment (Mulla et al. 2003). It was assumed that this population had become resistant to *Bsph* strain 2362. This suspected *Bsph*-resistant population was colonized at the National Institute of Health (NIH), Department of Medical Science, Ministry of Public Health, Thailand, in early 2001 and a subcolony was then established in our laboratory at the University of California at Riverside in late May 2001 for documentation of resistance to *Bsph.*

MATERIALS AND METHODS

Mosquitoes

In order to document the resistance profile in a suspected *Bsph*-resistant population, baseline data of susceptibility in nonresistant populations are essential. Therefore, 2 *Bsph*-susceptible laboratory colonies of *Cx. quinquefasciatus* were utilized in concurrent bioassays. One of them was established in 1999 from a mixture of collections from Midgeville ponds, University of California at Riverside Agricultural Experimental Station, and underground storm drains in Orange County, CA. The other one, collected from Soi Sirichai community, Nonthaburi Province, was initially established at the NIH, Thailand, and a subcolony was established in our laboratory in late November 2001. These two susceptible colonies have not been exposed to *Bsph* preparations since colonization.

Suspected *Bsph*-resistant *Cx. quinquefasciatus* were obtained from a collection at Pikul Community, Nonthaburi Province, Thailand, in January 2001, after a control failure using *Bsph* 2362 WDG was noted. These mosquitoes were colonized initially at the NIH, and a subcolony was established in our laboratory in early May 2001. Generations F_{14} through F_{17} (counted from initial colonization in Thailand) were used in the current studies on resistance-profile elucidation.

Test materials, stock suspensions, and bioassays

Test materials: Test materials used in bioassays were *Bsph* strain 2362 technical-grade material (TM) (ABG-6491, Lot 13-194-W5) and *Bacillus thuringiensis* var. *israelensis* (*Bti*) TM (ABG-6490, Lot 81-634-W5). The potencies for *Bti* TM and *Bsph* TM were 7,000 and 2,000 ITU/mg, respectively. These technical materials were received from Abbott Laboratories (North Chicago, IL) on April 26, 1996. Water-dispersible granules (WDG) of *Bsph* (650 ITU/mg, Lot 72-307-PG) and *Bti* (3,000 ITU/mg, Lot 60-070-BR), received on April 11, 2001, and August 1, 2001, respectively, from Valent Biosciences Corp. (Libertyville, IL) were also tested against the susceptible and *Bsph*-resistant mosquitoes. The test materials also included *Bsph/Bti* recombinant VS063-3 TM, which was received from Valent Biosciences Corp. on July 7, 2001.

Preparation of stock suspensions: Stock suspensions (1%) of the TM or WDG formulations were prepared in distilled water by vigorously shaking 0.2 g of the powder in 20 ml of distilled water in screw-cap glass vials. Serial dilutions (10×) were prepared in distilled water and aliquots of appropriate dilutions were used in bioassays. For mixtures of *Bsph* and *Bti* TM (1:1) or WDG in various ratios (*Bsph*:*Bti* = 1:1, 3:1, 9:1, 19:1, and 99:1), appropriate amounts of *Bti* and *Bsph* TM or WDG were weighed, mixed, and suspended in 20 ml of distilled water and then serially diluted in distilled water as above.

Bioassays: Bioassays were conducted by placing 20 late 3rd or early 4th stages in 116-ml waxed-paper cups each containing 100 ml of distilled water. One drop of larval food (2 g ground-up rabbit pellets in 20 ml distilled water) was added per cup. The cups were treated with a range of concentrations of *Bsph* or *Bti* preparations, or mixtures of both or *Bsph/Bti* recombinant. Four to 5 concentrations were used in each bioassay to yield mortality ranging from 10 to 95% during the test period. Each concentration in each test was replicated 3–4 times. The treated larvae were held at 27–29°C ambient temperature. Larval mortality was assessed at 24 h for *Bti* or 48 h for *Bsph,* the recombinant or the mixtures of *Bsph* and *Bti.* Moribund larvae were counted as dead. Bioassays against late 3rd or early 4th stages from susceptible *Cx. quinquefasciatus* colonies (Thailand and California) were run concurrently, with each bioassay using the procedures described above, and were used as baselines to calculate resistance ratios (RR) in resistant mosquitoes. Bioassay cups with various concentrations of suspensions, and any leftovers of stock suspensions of *Bsph/Bti* recombinant, were treated by utilizing 10% bleach for 24 h before disposal (Zahiri et al. 2004).

Data analysis

The bioassay data were subjected to probit regression analysis using POLO-PC (LeOra Software 1987) to obtain LC_{50}, LC_{90}, their 95% confidence intervals (CIs), the slope values of the dose–response regression lines, and data heterogeneity (χ^2/df), a parameter for data-quality control. The RR at the LC_{50} and LC_{90} were calculated based on the LC_{50} and LC_{90} values of instars from susceptible *Cx. quinquefasciatus* colonies from Thailand and California.

Table 1. Activity of *Bsph, Bti, Bsph/Bti* recombinant technical materials and WDG formulations alone or in mixture against *Bsph*-susceptible *Culex quinquefasciatus* from California and Nonthaburi, Thailand.

Materials and potency	LC_{50} (ppm) (95% confidence limits [CL])	LC_{90} (ppm) (95% CL)	Slope	χ^2/df
	California strain			
Bsph TM (2,000 ITU/mg)	0.005 (0.004–0.006)	0.029 (0.019–0.062)	1.7 ± 0.24	0.96
Bsph WDG (650 ITU/mg)	0.003 (0.002–0.004)	0.022 (0.014–0.052)	1.4 ± 0.24	0.45
Bsph TM + *Bti* TM (1:1)	0.007 (0.005–0.008)	0.023 (0.017–0.035)	2.4 ± 0.26	0.65
Bsph WDG + *Bti* WDG (1:1)	0.009 (0.005–0.017)	0.044 (0.022–0.253)	1.8 ± 0.20	1.17
Bsph WDG + *Bti* WDG (3:1)	0.011 (0.009–0.013)	0.042 (0.031–0.063)	2.2 ± 0.21	0.94
Bsph WDG + *Bti* WDG (9:1)	0.005 (0.003–0.006)	0.033 (0.022–0.060)	1.5 ± 0.20	0.54
Bsph WDG + *Bti* WDG (19:1)	0.005 (0.002–0.010)	0.028 (0.013–0.262)	1.8 ± 0.22	1.26
Bsph WDG + *Bti* WDG (99:1)	0.004 (0.003–0.006)	0.019 (0.015–0.032)	1.9 ± 0.19	0.45
Bsph/Bti recombinant TM	0.004 (0.003–0.005)	0.010 (0.008–0.018)	3.1 ± 0.52	0.54
Bti TM (7,000 ITU/mg)	0.013 (0.011–0.015)	0.033 (0.027–0.044)	3.3 ± 0.38	0.52
Bti WDG (3,000 ITU/mg)	0.028 (0.018–0.044)	0.059 (0.039–0.188)	4.0 ± 0.40	1.70
	Thailand strain			
Bsph TM (2,000 ITU/mg)	0.008 (0.005–0.010)	0.041 (0.030–0.065)	1.8 ± 0.24	0.45
Bsph WDG (650 ITU/mg)	0.004 (0.003–0.005)	0.020 (0.014–0.030)	1.7 ± 0.17	1.35
Bsph TM + *Bti* TM (1:1)	0.015 (0.008–0.027)	0.048 (0.026–0.171)	2.5 ± 0.24	1.26
Bsph WDG + *Bti* WDG (1:1)	0.014 (0.007–0.028)	0.051 (0.026–0.277)	2.3 ± 0.22	1.51
Bsph WDG + *Bti* WDG (3:1)	0.011 (0.008–0.014)	0.068 (0.045–0.122)	1.6 ± 0.18	0.44
Bsph WDG + *Bti* WDG (9:1)	0.011 (0.008–0.014)	0.059 (0.041–0.100)	1.7 ± 0.18	0.51
Bsph WDG + *Bti* WDG (19:1)	0.008 (0.003–0.019)	0.037 (0.016–0.754)	1.9 ± 0.21	1.91
Bsph WDG + *Bti* WDG (99:1)	0.004 (0.003–0.006)	0.021 (0.013–0.030)	2.1 ± 0.27	0.29
Bsph/Bti recombinant TM	0.007 (0.005–0.008)	0.026 (0.019–0.041)	2.2 ± 0.24	0.56
Bti TM (7,000 ITU/mg)	0.019 (0.017–0.0220	0.044 (0.037–0.057)	3.5 ± 0.36	0.57
Bti WDG (3,000 ITU/mg)	0.066 (0.048–0.088)	0.110 (0.084–0.323)	5.8 ± 0.70	1.66

RESULTS AND DISCUSSION

Two *Bsph*-susceptible colonies from California and Thailand provided reliable baseline information for elucidation of resistance profile in the suspected *Bsph*-resistant *Cx. quinquefasciatus* population from Thailand. The susceptible population originating from collections in southern California has not been exposed to *Bsph* preparations either before or after colonization. *Culex quinquefasciatus* populations in southern California have the potential of development of low to moderate levels of resistance (Rodchareon and Mulla 1994, Zahiri et al. 2002). This laboratory colony exhibited high susceptibility to all materials tested alone or in various combinations (Table 1, Fig. 1). Among the 11 materials tested, the LC_{50} range was 0.003–0.005 ppm and LC_{90} was 0.022–0.029 ppm for *Bsph* alone. For the mixtures of *Bsph* and *Bti* as well as *Bsph/Bti* recombinant, the levels of LC_{50} were within 0.004–0.011 ppm, LC_{90} within 0.010–0.044 ppm. The levels of LC_{50} were 0.013–0.028 ppm, LC_{90} 0.033–0.059 ppm for *Bti* alone. The LC values were generally greater in *Bti* than in *Bsph,* indicating higher susceptibility to the latter. Upon increase of percentage of *Bsph* in *Bsph/Bti* mixtures from 50 to 99%, the LC values decreased progressively toward those of *Bsph* alone. The activity of *Bsph/Bti* recombinant was more or less like those of *Bsph* alone and the *Bsph/Bti* mixtures with low percentages of *Bti* (1–10%), i.e., *Bsph*:*Bti* = 99:1, 19:1, and 9:1. The slope values were greater (steeper dosage–response lines) in *Bti* preparations or combinations containing *Bti* as compared with *Bsph* alone (Table 1), indicating the population was more homogeneous in response to *Bti* than to *Bsph.* The heterogeneity values of the data represented by χ^2/df were 0.45–1.7, indicating that the dose–response data fit the probit model well, as most of the values were close to or less than 1 (LeOra Software 1987).

The susceptible population from Soi Sirichai Community, Nonthaburi Province, Thailand, showed a similar response to all test materials as the California susceptible population (Table 1, Fig. 1). The strain was considered to be equally susceptible, even though values of LC_{50} and LC_{90} were slightly greater than those for the California susceptible colony. These slightly greater values of LC_{50} and LC_{90} (lower susceptibility) might indicate a higher potential for *Bsph*-resistance development in Thailand susceptible populations than in the California susceptible population. The difference in response patterns to *Bti* and *Bsph* preparations as indicated by slopes of dosage–response lines was similar in the two susceptible populations, i.e., the population was more homogeneous in response to *Bti* than to *Bsph.* High-quality data and good fitness of the probit model were justified by low data heterogeneity value (χ^2/df), ranging from 0.29 to 1.91 (Table 1).

In Wat Pikul Community, Nonthaburi Province,

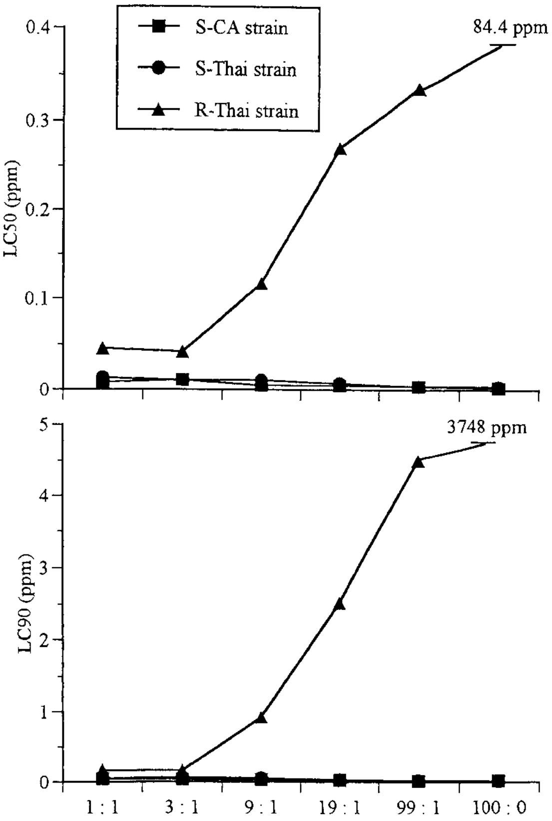

Fig. 1. Activity of *Bsph* and *Bti* WDG formulations in various mixtures against *Bsph* susceptible (California and Thailand), and resistant (Thailand) *Culex quinquefasciatus*.

Thailand, during September–December 2000, *Bsph* 2362 WDG was applied 5 times at various dosages, ranging from 50 to 200 mg/m^2 to control immature *Cx. quinquefasciatus*. Progressively declining performance of *Bsph* WDG was noted during the treatments (Mulla et al. 2003). At the end of these field trials, mosquitoes were collected and colonized. After colonization, there was no more exposure to *Bsph* preparations in this *Cx. quinquefasciatus* colony, and bioassays with the various products and mixtures were carried out.

Mosquitoes from Wat Pikul Community showed extremely high levels of resistance against *Bsph* 2362 preparations. Compared with susceptible ones from California or Thailand, resistance was >200,000- or >125,000-fold to TM, and 28,100- or 21,100-fold to WDG at LC_{50}, respectively. At LC_{90}, the extent of resistance, however, was not measurable to TM, as the larval mortality was not more than 60% at 5,000 ppm (data not in tables). The resistance was 187,400- or 170,400-fold to WDG at LC_{90}, based on susceptible California or Thailand colonies, respectively (Tables 2 and 3, Figs. 1 and 2). The levels of resistance of this population to *Bsph* were much higher than those reported from France (Sinègre et al. 1994), India (Adak et al. 1995, Rao et al. 1995), Brazil (Silva-Filha et al. 1995), and China (Yuan et al. 2002).

The resistance level against *Bsph* TM (2,000 ITU/mg) was much higher than against WDG (650 ITU/mg), suggesting that *Bsph* WDG, a formulated material, was more active in killing *Bsph*-resistant mosquitoes than the TM (Table 3). The RRs based on California susceptible *Cx. quinquefasciatus,* as expected, were generally higher in most cases than those based on Thailand susceptible ones (Table 3, Fig. 2), as the former were slightly more susceptible than the latter to the same materials tested (Table 1). Overall, the slope values of dosage–response lines in *Bsph* materials tested were small in resistant mosquitoes (Table 2), even smaller than in susceptible ones (Table 1), indicating that this *Bsph*-resistant population is highly heterogeneous. This heterogeneity in response to *Bsph* could lead even to a higher level of resistance upon further selection.

The mixtures of *Bsph* and *Bti* (1:1 using TM and WDG, 3:1 using WDG) exhibited high levels of activity in killing both *Bsph*-resistant and susceptible larvae, even though *Bsph*-resistant mosquitoes

Table 2. Activity of *Bsph, Bti, Bsph/Bti* recombinant technical materials and WDG formulations alone or in mixture against *Bsph* 2362-resistant *Culex quinquefasciatus* from Wat Pikul Community, Nonthaburi, Thailand.

Materials and potency	LC_{50} (ppm) (95% confidence limits [CL])	LC_{90} (ppm) (95% CL)	Slope	χ^2/df
Bsph TM (2,000 ITU/mg)	>1,000	—	—	—
Bsph WDG (650 ITU/mg)	84.4 (63.0–114.3)	3748 (1706–13036)	0.8 ± 0.09	0.55
Bsph TM + *Bti* TM (1:1)	0.017 (0.013–0.020)	0.049 (0.039–0.071)	2.8 ± 0.43	0.65
Bsph WDG + *Bti* WDG (1:1)	0.046 (0.034–0.059)	0.165 (0.112–0.406)	2.3 ± 0.26	1.54
Bsph WDG + *Bti* WDG (3:1)	0.042 (0.033–0.050)	0.162 (0.120–0.269)	2.2 ± 0.34	0.87
Bsph WDG + *Bti* WDG (9:1)	0.117 (0.065–0.196)	0.924 (0.484–2.902)	1.4 ± 0.14	1.30
Bsph WDG + *Bti* WDG (19:1)	0.271 (0.152–0.505)	2.523 (1.119–13.173)	1.3 ± 0.14	1.35
Bsph WDG + *Bti* WDG (99:1)	0.337 (0.164–0.614)	4.509 (2.206–13.941)	1.1 ± 0.08	1.73
Bsph/Bti recombinant TM	0.051 (0.044–0.060)	0.134 (0.108–0.179)	3.1 ± 0.30	0.53
Bti TM (7,000 ITU/mg)	0.017 (0.014–0.019)	0.046 (0.037–0.062)	2.9 ± 0.32	1.00
Bti WDG (3,000 ITU/mg)	0.052 (0.035–0.067)	0.081 (0.064–0.170)	6.7 ± 0.74	1.91

Table 3. Resistance ratio of *Bsph* 2362 resistant *Culex quinquefasciatus* (Wat Pikul Community, Nonthaburi, Thailand) to various test materials and mixtures.

Materials and potency	Based on susceptible California strain at LC_{50}	Based on susceptible California strain at LC_{90}	Based on susceptible Thailand strain at LC_{50}	Based on susceptible Thailand strain at LC_{90}
Bsph TM (2,000 ITU/mg)	>200,000.0	—	>125,000.0	—
Bsph WDG (650 ITU/mg)	28,100.0	170,400.0	21,100.0	187,400.0
Bsph TM + *Bti* TM (1:1)	2.4	2.1	1.1	1.0
Bsph WDG + *Bti* WDG (1:1)	5.1	3.8	3.3	3.2
Bsph WDG + *Bgi* WDG (3:1)	3.8	3.9	3.8	2.4
Bsph WDG + *Bgi* WDG (9:1)	23.4	28.0	10.6	15.7
Bsph WDG + *Bti* WDG (19:1)	54.2	90.1	33.9	68.2
Bsph WDG + *Bgi* WDG (99:1)	84.3	237.3	84.3	214.7
Bsph/Bgi recombinant TM	12.8	13.4	7.3	5.2
Bti TM (7,000 ITU/mg)	1.3	1.4	0.9	1.1
Bti WDG (3,000 ITU/mg)	1.9	1.4	0.8	0.7

showed some low level of tolerance to the mixtures tested (RR, 3.3–5.1-fold at LC_{50}, 2.4–3.9-fold at LC_{90}, depending on reference colonies) (Tables 2 and 3, Figs. 1 and 2). Evaluation of different ratios of mixtures yielded interesting information, as the ratio of *Bsph* WDG in *Bsph/Bti* mixtures increased from 3:1 to 9:1, 19:1, or 99:1. The activity of the mixtures declined progressively in killing *Bsph*-resistant mosquitoes (Table 2, Fig. 1) with an increase in *Bsph* and a decrease in *Bti* contents. Correspondingly, the RR to *Bsph/Bti* mixtures in *Bsph*-resistant mosquitoes progressively increased when the ratio of *Bsph* increased in the mixtures (Table 3, Fig. 2). *Bsph/Bti* WDG mixtures containing as low as 1% of *Bti* (*Bsph*:*Bti* = 99:1) lowered RR to *Bsph* alone from 21,100-fold to 84.3-fold at LC_{50} (Table 3, Fig. 2), where a strong synergism between *Bsph* and *Bti* was indicated. The lowered resistance levels to various *Bsph/Bti* WDG mixtures as compared with that to *Bsph* WDG alone were attributable to the presence of *Bti* toxins, especially Cyt1A toxin. Cyt1A can restore toxicity of *Bsph* against resistant *Cx. quinquefasciatus* (Wirth et al. 2000), where a combination of *Bsph* 2362 in a 10:1 ratio with *Bti*, which only produces Cyt1A toxin, reduced resistance by >30,000-fold. Resistance was completely suppressed when *Bsph* was combined with purified Cyt1A crystals in a 10:1 ratio. Cyt1A of *Bti* may synergize *Bsph* toxicity by enhancing the binding to and insertion of toxins into microvillar membrane in the gut of mosquito larvae (Wirth et al. 2000).

A low to moderate level of resistance to *Bsph/Bti* recombinant was noted in the highly *Bsph*-resistant mosquitoes as compared with susceptible colonies (RR, 7.3–12.8-fold at LC_{50}, 5.2–13.4-fold

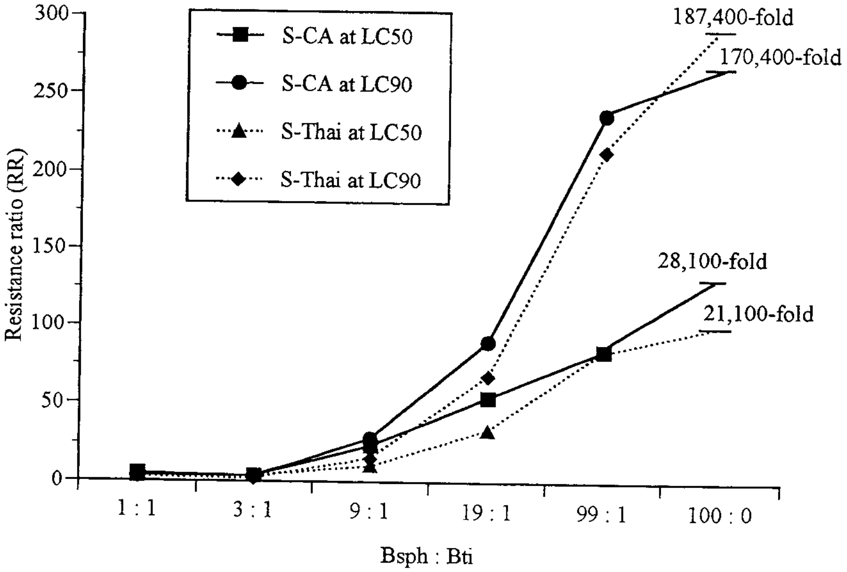

Fig. 2. Resistance ratio of *Bsph*-resistant *Culex quinquefasciatus* (Thailand) to various mixtures of *Bsph* and *Bti* WDG formulations, based on California and Thailand susceptible populations.

at LC_{90}, depending on reference colonies) (Tables 2 and 3). The activity of *Bsph*/*Bti* recombinant was similar to the *Bsph*/*Bti* mixtures containing 10–25% of *Bti* (*Bsph*:*Bti* = 9:1 to 3:1) in combating *Bsph*-resistant *Cx. quinquefasciatus* (Tables 2 and 3).

The Thailand *Bsph*-resistant mosquitoes, however, were highly susceptible to *Bti* TM and WDG (Table 2); the susceptibility levels were comparable with *Bsph*-susceptible mosquitoes from Thailand and California (Table 1), indicating these *Bsph*-resistant mosquitoes could be easily controlled by using *Bti* preparations alone or as a mixture of the two, as was evidenced in the field (Mulla et al. 2003). The lack of cross-resistance between *Bti* and *Bsph* was also true in laboratory-selected *Bsph*-resistant *Cx. quinquefasciatus* (Rodcharoen and Mulla 1996, Zahiri et al. 2002), where the resistance levels were much lower.

As in susceptible colonies, the slope values were greater (steeper dose–response lines) in *Bti* preparations or combinations containing *Bti* as compared with *Bsph* alone (Tables 1 and 2), indicating the population was more homogeneous in response to *Bti* than to *Bsph*. The data heterogeneity values represented by χ^2/df were 0.53–1.91, indicating the dosage–response data fit the probit model well, as in susceptible colonies (Table 1).

It has been found that *Bti* used as substitute or *Bsph* and *Bti* in mixture or rotation could be used to reverse previously established *Bsph* resistance (Zahiri et al. 2002) and that *Bti* and *Bsph* in mixture could be an effective way to prevent or delay occurrence of *Bsph* resistance (Zahiri and Mulla 2003). The highly *Bsph*-resistant *Cx. quinquefasciatus* population from Thailand seems to have a different genetic makeup from those from California, as the former became highly resistant to *Bsph* within a short period of time after 5 treatments. Previous studies in our laboratory indicated that *Cx. quinquefasciatus* from southern California was able to develop low to moderate levels of resistance as a response to selection pressure in the laboratory (Rodcharoen and Mulla 1994, Zahiri et al. 2002, Zahiri and Mulla 2003), and, so far, no noticeable resistance has been detected in some field *Cx. quinquefasciatus* populations in southern California, where *Bsph* formulations have been applied for several years now (Su et al. 2001). Management of *Bsph* resistance (reversion and prevention/delay) in tropical *Culex* populations with high risk of resistance development would be desirable, but challenging. Is the resistance management strategy, such as utilization of rotation and mixtures of *Bsph* and *Bti* (Zahiri et al. 2002, Zahiri and Mulla 2003), applicable to tropical *Culex* populations? In what ratios should the two agents be mixed to combat previously established resistance or preclude occurrence of emerging *Bsph* resistance? *Bsph*/*Bti* mixtures in the ratios of 1:1 or 3:1 could be possible candidates in future studies on resistance management, as these mixtures did show high activity in killing both *Bsph*-resistant and -susceptible mosquitoes. More importantly, the initial and residual activity of the mixtures as compared with *Bsph* alone needs to be elucidated under field conditions.

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