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Cyt1A from *Bacillus thuringiensis* Restores Toxicity of *Bacillus sphaericus* Against Resistant *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT The 2362 strain of *Bacillus sphaericus*, which produces a binary toxin highly active against *Culex* mosquitoes, has been developed recently as a commercial larvicide. It is being used currently in operational mosquito control programs in several countries including Brazil, France, India, and the United States. Laboratory studies have shown that mosquitoes can develop resistance to *B. sphaericus*, and low levels of resistance have already been reported in field populations in Brazil, France, and India. To develop tools for resistance management, the Cyt1A protein of *Bacillus thuringiensis* subsp. *israelensis* De Barjac was evaluated for its ability to suppress resistance to *B. sphaericus* in a highly resistant population of *Culex quinquefasciatus* Say. A combination of *B. sphaericus* 2362 in a 10:1 ratio with a strain of *B. thuringiensis* subsp. *israelensis* that only produces Cyt1A reduced resistance by >30,000-fold. Resistance was suppressed completely when *B. sphaericus* was combined with purified Cyt1A crystals in a 10:1 ratio. Synergism was observed between the Cyt1A toxin and *B. sphaericus* against the resistant mosquito population and accounted for the marked reduction in resistance. However, no synergism was observed between the toxins against a nonresistant mosquito population. These results indicate that Cyt1A could be useful for managing resistance to *B. sphaericus* 2362 in *Culex* populations, and also provide additional evidence that Cyt1A may synergize toxicity by enhancing the binding to and insertion of toxins into the mosquito microvillar membrane.

KEY WORDS *Culex quinquefasciatus*, *Bacillus sphaericus*, bacterial insecticide, resistance, resistance management

MANY MOSQUITOCIDAL STRAINS of *Bacillus sphaericus* have been evaluated as larvicides (Thiéry and de Barjac 1989, Yap 1990), and one, strain 2362, recently has been marketed in commercial larvicides worldwide for use against *Culex* species developing in polluted waters. The toxicity of *B. sphaericus* 2362 is caused by a small parasporal inclusion produced during sporulation. Studies of this inclusion have shown that it contains a binary toxin consisting of two endotoxin proteins with masses of 52 and 43 kDa, which are, respectively, the binding and toxin domains (Baumann et al. 1991, Charles et al. 1996). After solubilization in the larval midgut, these proteins are cleaved proteolytically to yield peptides of 42 and 39 kDa, which then associate forming the active toxin.

Because *B. sphaericus* 2362 contains only the binary toxin with a single binding domain, the prospects for the development of resistance to *B. sphaericus* larvicides are high. Significant levels of *B. sphaericus* resistance in *Cx. quinquefasciatus* Say have been developed in the laboratory (Georghiou et al. 1992, Rodcharoen and Mulla 1994), and have been reported in field populations in France, Brazil, and India (Sinègre et al. 1994, Rao et al. 1995, Silva-Filha et al. 1995). Although the threat of more widespread *B. sphaericus* resistance is a potentially serious problem, recent studies of another mosquitocidal bacterium, *B. thuringiensis* subsp. *israelensis* De Barjac (BTI) suggest possibilities for managing this resistance.

Bacillus thuringiensis subsp. *israelensis* was discovered in the mid-1970s in Israel (Goldberg and Margalit 1977), and shortly thereafter was shown to be highly effective in controlling the larvae of numerous species of mosquitoes and blackflies, with an LC_{50} of 10–20 ng/ml against fourth instars of many species. This high efficacy quickly led to development of BTI as the active ingredient for commercial bacterial larvicides (Mulla 1990). These larvicides are now used routinely in pest and vector control programs around the world. Despite its intensive use in many control programs, there are no reports of resistance to BTI (Georghiou et al. 1991, Becker and Ludwig 1993).

The lack of resistance to BTI apparently is caused by its complex of mosquitocidal proteins, which are synthesized during sporulation and assembled into separate inclusions enveloped together to form a spherical parasporal body. Four major proteins have been identified in this parasporal body; Cyt1A (27 kDa), Cry4A (134 kDa), Cry4B (128 kDa), and Cry11A (66 kDa). Studies have shown that the broad activity spectrum and acute toxicity of BTI are caused by syner-

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gistic interactions between Cyt1A and the Cry proteins, and among the Cry proteins (Wu and Chang 1985, Poncet et al. 1995, Ibarra and Federici 1986, Crickmore et al. 1995). Of greater relevance to the management of *B. sphaericus* resistance are more recent studies in which it has been shown that Cyt1A delays the development of BTI resistance in *Cx. quinquefasciatus* (Georghiou and Wirth 1997), and it can suppress resistance levels of several hundred fold to Cry4 and Cry11A when combined with these endotoxins (Wirth et al. 1997).

The capacity of Cyt1A to markedly suppress resistance to the Cry4 and Cry11 endotoxins of BTI indicated that it might have a similar effect on *B. sphaericus* resistance when combined with *B. sphaericus* preparations. In anticipation of field populations of mosquitoes developing high levels of resistance to commercial preparations of *B. sphaericus*, we undertook a study to test this hypothesis. In the current study, working with a *Cx. quinquefasciatus* population at least 30,000 times resistant to *B. sphaericus* 2362, the strain used in commercial formulations, we show that combining Cyt1A with *B. sphaericus* completely suppressed resistance.

Materials and Methods

Bacterial Strains and Toxins. Toxin preparations used in this study were lyophilized powders of lysed cultures of *B. sphaericus* 2362 and a recombinant strain of *B. thuringiensis* subsp. *israelensis* that only produces Cyt1Aa (Wu and Federici 1993). These powders contained the spore and the crystal (=parasporal body) along with cell debris and media solids resulting from lyophilization. The three specifc powders tested were as follows: (1) *B. sphaericus* strain 2362, obtained as a technical powder of the wild-type strain from Abbott Laboratories (North Chicago, IL); (2) Cyt1Aa, the recombinant strain of BTI noted above; and (3) BTI 4Q7, an acrystalliferous strain of this subspecies that does not produce any endotoxins. This strain was obtained from the *Bacillus* Stock Center (The Ohio State University) and used as one of the controls. Lyophilized powders of purified Cyt1A crystals (Wu and Federici 1993) also were used in this study.

Toxin Powder Production and Storage. Bacterial strains producing the various toxins were grown on solid or liquid media as described previously (Wirth et al. 1997, Park et al. 1998). The sporulated cells were washed in distilled water, sedimented, and the resultant pellet was lyophilized. For mosquito selections and bioassays, stock suspensions of the powders were prepared in distilled water and homogenized with the aid of ≈25 glass beads. Stocks were prepared monthly and 10 times serial dilutions were prepared weekly. All stocks and dilutions were frozen at −20°C when not in use.

Mosquito Strains. The following two strains of *Cx. quinquefasciatus* were used: BS-R, a strain resistant to *B. sphaericus* 2362, and Syn-P, an unselected, nonresistant strain. BS-R has been selected with *B. sphaericus* 2362 since 1992 (Georghiou et al. 1992) and routinely survives 48 h of exposure to 1,000 μg/ml, a concentration 149,000 times higher than the concentration that kills 50% of Syn-P, the sensitive reference strain. Syn-P is a "synthetic" population of *Cx. quinquefasciatus* derived from larval populations collected in 1995 from three different geographic areas in southern California. This colony has been maintained in the laboratory without exposure to *B. sphaericus.*

Selection and Bioassay Procedures. As noted above, the BS-R strain has been maintained under selection pressure with *B. sphaericus* 2362 since 1992. Selection consisted of exposing groups of ≈1,000 early fourth-instars to concentrations of *B. sphaericus* ranging between 100 and 120 μg/ml in enameled metal pans in ≈1 liter of deionized water for 48–96 h. Average mortality of the larvae under selection was 10% or less per selection, and the survivors were used to continue the colony.

For bioassays, groups of 20 early fourth instars were exposed to a range of concentrations of the lyophilized spore/crystal powders in 100 ml of deionized water held in 237-ml plastic cups. Seven to nine different concentrations of the powders, which yielded mortality between 2 and 98% after 48 h, were replicated on five different days. For the bioassays in which different combinations of Cyt1A and *B. sphaericus* 2362 were tested, different ratios of these toxins were based on the weights of the lyophilized powders of the bacterial strain.

Because the quantity of purified Cyt1A crystals was limited, bioassays with this powder used 10 early fourth instars held in 10 ml of deionized water in 30-ml plastic cups and replicated on 2–3 different days. Bioassays combining *B. sphaericus* 2362 technical powder and Cyt1A purified crystals at a 10:1 ratio (10 parts *B. sphaericus* 2362: one part Cyt1A crystal) were based on the weights of the lyophilized powders of *B. sphaericus* 2362 and Cyt1A.

All data were subjected to probit analysis (Finney 1971) using a program for the PC (Raymond et al. 1995). Dose–response values with overlapping fiducial limits were not considered significantly different. Resistance ratios were calculated by dividing the respective lethal concentration value for the BS-R strain by that of the Syn-P strain. Resistance ratios whose fiducial limits contained the number one were not considered significant.

Evaluation of Synergism. Synergistic interactions between *B. sphaericus* 2362 and Cyt1A were evaluated using the method of Tabashnik (1992). Theoretical lethal concentration values for the different mixtures of Cyt1A and *B. sphaericus* 2362 were calculated from the weighted harmonic means of the individual values for these toxins. Because the *B. sphaericus* 2362 powder was not toxic to the BS-R strain at any of the concentrations tested, the calculation of the theoretical toxicity of a combination of Cyt1A and *B. sphaericus* 2362 was based on the toxicity and proportion of Cyt1A alone for this strain. The synergism factor, defined as the ratio of the theoretical lethal concentration value to the observed lethal concentration value, was determined for combinations of *B. spha-*

Table 1. Toxicity of *B. sphaericus* (strain 2362) technical powder, Cyt1A crystal/spore powder from *B. t.* subsp. *israelensis*, and various combinations of *B. sphaericus* and Cyt1A against susceptible (Syn-P) and *B. sphaericus* resistant (BS-R) *C. quinquefasciatus*

Toxin(s)	Strain	No.	LC_{50} (μg/ml) (fiducial limits)	LC_{95} (μg/ml) (fiducial limits)	Slope (± SE)	χ^2	Resistance ratio at		SF	
							LC_{50}(FL)	LC_{95}(FL)	LC_{50}	LC_{95}
B. sphaericus (strain 2362)										
	Syn-P	1,100	0.00671 (0.0055–0.0082)	0.466 (0.300–0.790)	0.89 (0.045)	13.1	1.0	1.0		
	BS-R	600	No mortality at 1,000 μg/ml				≈149,000			
Cyt1A										
	Syn-P	600	11.7 (10.2–13.4)	59.8 (47.7–79.7)	2.3 (0.16)	7.3	1.0	1.0		
	BS-R	700	32.5 (28.3–37.6)	222 (172–304)	2.0 (0.12)	4.1	2.7 (2.3–3.3)	3.7 (2.6–5.3)		
B. sphaericus + Cyt1A (10:1)[a]										
	Syn-P	900	0.0288 (0.0163–0.0508)	0.0422 (0.162–1.23)	1.4 (0.21)	22.8	1.0	1.0	0.26	1.2
	BS-R	800	2.47 (1.46–4.20)	36.6 (14.0–97.4)	1.4 (0.17)	25.4	85.8 (56.8–129)	82.9 (39–174)	132	61
B. sphaericus + Cyt1A (5:1)										
	Syn-P	700	0.0274 (0.0232–0.0322)	0.278 (0.209–0.397)	1.6 (0.10)	2.4	1.0	1.0	0.29	2.0
	BS-R	1,000	1.23 (1.05–1.43)	9.58 (7.49–12.9)	1.8 (0.11)	12.5	45.0 (38.1–53.2)	34.4 (25.2–46.9)	155.9	136.8
B. sphaericus + Cyt1A(3:1)										
	Syn-P	800	0.0147 (0.0086–0.0354)	0.652 (0.177–2.48)	1.0 (0.12)	27.1	1.0	1.0	0.6	1.0
	BS-R	600	1.99 (180–2.22)	7.17 (5.87–9.31)	2.9 (0.22)	6.0	297 (255–347)	15.4 (10.9–1.7)	65	124
B. sphaericus + Cyt1A(1:1)										
	Syn-P	1,000	0.0381 (0.0323–0.0449)	0.464 (0.348–0.655)	1.5 (0.08)	10.1	1.0	1.0	0.35	2.0
	BS-R	1,000	0.735 (0.632–0.853)	6.49 (5.06–8.73)	1.7 (0.09)	5.8	19.3 (16.5–22.5)	14.0 (10.5–18.7)	88	69
B. sphaericus + Cyt1A (1:3)										
	Syn-P	900	0.234 (0.191–0.287)	7.54 (5.00–12.5)	1.1 (0.06)	11.5	1.0	1.0	0.11	0.24
	BS-R	900	1.71 (1.45–2.00)	18.4 (14.1–25.5)	1.6 (0.09)	6.5	7.3 (6.3–8.5)	2.4 (1.8–3.2)	25	16
B. sphaericus + CytA (1:5)										
	Syn-P	1,000	0.189 (0.149–0.236)	6.74 (4.66–10.6)	1.1 (0.06)	13.5	1.0	1.0	0.21	0.39
	BS-R	900	1.56 (1.34–1.81)	11.8 (9.23–15.9)	1.9 (0.11)	8.9	8.2 (6.9–9.6)	1.8 (1.3–2.3)	25.3	23.0
B. sphaericus + Cyt1A (1:10)										
	Syn-P	900	1.06 (0.859–1.29)	25.9 (18.1–40.1)	1.2 (0.07)	4.8	1.0	1.0	0.10	0.17
	BS-R	900	4.72 (4.12–5.38)	24.6 (19.8–32.0)	2.3 (0.15)	13.0	4.4 (3.7–5.2)	1.0 (0.69–1.3)	7.7	10.0

SF, synergism factor.

[a] Ratios in brackets represent the relative proportion of *B. sphaericus* technical powder to cyt1A spore/crystal powder (Bs:CytA). All ratios were based on the weight of each respective powder.

ericus 2362 and the Cyt1A strain as well as for combinations of *B. sphaericus* 2362 and purified Cyt1A crystals. When the ratio was >1, the toxin interaction was considered synergistic because toxicity exceeded the value predicted from individual additive toxicity. When the ratio was <1, the interaction was considered antagonistic, whereas a ratio of one indicated that the values were additive.

Results

In the bioassays to determine toxin baseline values under standard conditions against the resistant and sensitive mosquito strains, no mortality resulted from exposure of BS-R, the resistant strain of *Cx. quinquefasciatus*, to 1,000 μg/ml of *B. sphaericus* 2362 (Table 1). This concentration was 149,000 times higher than the LC_{50} (0.0067 μg/ml) obtained against Syn-P, the sensitive strain. When the bioassays were carried out in 10 ml of water with 10 larvae per cup rather than 20 larvae in 100 ml, no mortality was obtained against BS-R, but the the toxicity of BS 2362 was lower (LC_{50}, 0.032 μg/ml) against Syn-P (Table 2; Fig. 1). Increasing larval density has been previously shown to require lower amounts of BTI toxin to induce the same level of mortality observed at lower densities (Aly et al. 1988). The estimated difference in the sensitivity of BS-R and Syn-P using the smaller bioassay system was 31,000 times (Table 2).

The Cyt1A bacterial strain was slightly less toxic to the BS-R strain (LC_{50}, 32.5 μg/ml) than to Syn-P (LC_{50}, 11.7 μg/ml) in the standard bioassay system (Table 1). However, in the tests using Cyt1A crystals in the smaller bioassay system, no difference in sen-

Table 2. Toxicity of *B. sphaericus* (strain 2362), Cyt1A inclusions, and the combination of *B. sphaericus* and Cyt1A inclusions at a 10:1 ratio, against susceptible and resistant *C. quinquefasciatus*

Toxin(s)	Strains	No.	LC_{50} (fiducial limits) (μg/ml)	LC_{95} (fiducial limits) (μg/ml)	Slope ($\pm$ SE)	χ^2	Resistance ratio at LC_{50}	Resistance ratio at LC_{95}	SF LC_{50}	SF LC_{95}
Cyt1A inclusions										
	Syn-P	130	20.3 (15.1–27.8)	119 (71.2–306)	2.1 (0.35)	4.4	1.0	1.0		
	BS-R	170	20.0 (14.8–26.8)	138 (84.8–314)	1.9 (0.28)	2.2	1.0	1.1		
B. sphaericus (strain 2362)										
	Syn-P	280	0.0322 (0.0215–0.0490)	1.63 (0.725–5.46)	1.0 (0.10)	9.4	1.0	1.0		
	BS-R	120	No mortality at 1,000 μg/ml				≈31,000			
B. sphaericus (strain 2362) + Cyt1A inclusions (10:1)										
	Syn-P	280	0.0108 (0.00540–0.0184)	2.37 (0.716–21.5)	0.7 (0.11)	7.0	1.0	1.0	3.3	0.76
	BS-R	140	0.173 (0.0910–0.322)	4.96 (1.56–96.4)	1.1 (0.26)	3.5	15.9 (11.5–22.1)	2.1 (0.82–5.3)	1,511	278
Spore powder with no inclusions										
	Syn-P		No mortality at 1,000 μg/ml							
	BS-R		No mortality at 1,000 μg/ml							

SF, synergism factor.

sitivity (LC_{50}s, ≈20 μg/ml) was observed between BS-R and Syn-P (Table 2).

Adding Cyt1A to the *B. sphaericus* 2362 preparations restored most of its toxicity against the resistant BS-R strain. A *B. sphaericus* 2362 ratio to Cyt1A of 10:1 was highly toxic to both the resistant and sensitive mosquito strains. Toxicity levels for this combination were higher against Syn-P than BS-R, with LC_{95} values of 0.442 and 36.6 μg/ml, respectively, and a resistance ratio (LC_{95}) of 82.9 for BS-R (Table 1). The 5:1 ratio was more toxic toward Syn-P and BS-R, and the resistance ratio at the LC_{95} level was reduced to 34.4-fold. At a ratio of 3:1 *B. sphaericus* 2362:Cyt1A, the mixture was again significantly more toxic to BS-R (LC_{50}, 1.99 μg/ml), and the resistance ratio decreased to 15.4-fold at the LC_{95} level (Table 1). Toxicity at a 1:1 ratio against BS-R was not significantly different from that of the 3:1 ratio. Overall, as the proportion of *B. sphaericus* 2362 to Cyt1A was increased, the toxicity increased toward both the resistant and sensitive mosquito strains. However, the resistance ratios at the LC_{95} values for BS-R declined to insignificant levels for ratios of 1:3, 1:5, and 1:10, in which Cyt1A was the principle component (Table 1)

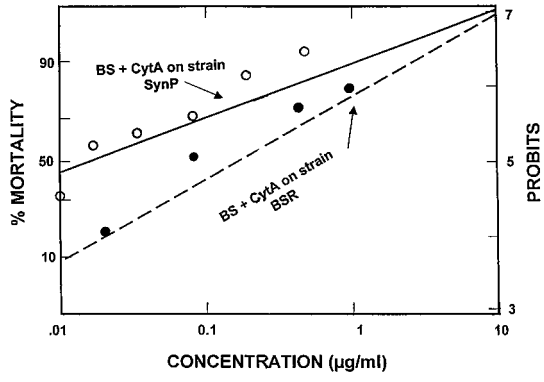

Fig. 1. Toxicity of *B. sphaericus* technical powder combined with purified Cyt1A inclusions at a 10:1 ratio based on weight, against the susceptible laboratory reference strain Syn-P and the *B. sphaericus* resistant strain BS-R. Lines represent the predicted dose-response lines for each strain. Actual data points are shown as circles.

Calculation of the synergism factor for these combinations revealed significant synergism between Cyt1A and *B. sphaericus* 2362 against the BS-R strain, but not against Syn-P (Table 1). Synergism factor values ranged from 10 to 137 at the LC_{95} level for BS-R. The highest levels of synergism were observed in the combinations in which Cyt1A was present in the lowest proportion (10:1, 5:1, 3:1). These combinations were antagonistic toward Syn-P at the LC_{95} level at ratios 1:10, 1:5, and 1:3, and additive or mildly synergistic at ratios of 1:1, 3:1, 5:1, and 10:1 (i.e., where *B. sphaericus* became the predominant component [Table 1]).

Bioassays using *B. sphaericus* 2362 combined with the purified Cyt1A crystals at a ratio of 10:1 demonstrated that this combination was highly toxic to both BS-R (LC_{95}, 4.96 μg/ml) and Syn-P (LC_{95}, 2.37 μg/ml). Although the BS-R strain was slightly less sensitive to the mixture, the toxicity values were not significantly different (Table 2; Fig. 1). Importantly, no resistance was detected against the BS-R strain with this combination, which had a high synergism factor value of 278 (Table 2).

Discussion

Combining Cyt1A with BS 2362 restored the toxicity of the latter against a highly resistant strain of *Cx. quinquefasciatus.* Moreover, we were able to completely restore toxicity with sublethal concentrations of Cyt1A crystals, and therefore suppress resistance to *B. sphaericus* in the BS-R mosquito strain. In contrast

to the high level of activity observed against the resistant mosquito population, little or no enhanced activity resulted with these same mixtures against the nonresistant reference strain, Syn-P.

The ability of Cyt1A at low concentrations to restore high toxicity to *B. sphaericus* 2362 against resistant mosquitoes has practical implications for control of *Culex* populations and provides insight into its mode of action. Bacterial larvicides based on *B. sphaericus* are used in several countries and resistance in field populations of *Cx. quinquefasciatus* has already been reported in France (Sinègre et al. 1994), Brazil (Silva-Filha et al. 1995), and India (Rao et al. 1995). The results of our experiments indicate that adding Cyt1A at a ratio as low as 1:10 to *B. sphaericus* larvicides should be able to restore most of the toxicity against even highly resistant populations of *Cx. quinquefasciatus.* Therefore, Cyt1A provides a practical tool for managing *B. sphaericus* resistance. Furthermore, adding a small quantity of Cyt1A to *B. sphaericus* preparations possibly may delay resistance in mosquito populations in which it has not already developed. Precedence for this is found in studies on the effect of Cyt1A on the development of resistance to BTI in *Cx. quinquefasciatus,* in which strong evidence was provided that Cyt1A delayed resistance to the Cry11A, and Cry4A and 4B proteins (Georghiou and Wirth 1997).

Aside from the current study, it has been shown recently that Cyt1Ab from *B. thuringiensis* subsp. *medellin* can suppress resistance to *B. sphaericus* 2297, a mosquitocidal strain of this bacterium that produces a large toxin crystal, in *Cx. pipiens* (Thiéry et al. 1998). However, Cyt1Ab suppression of resistance to *B. sphaericus* 2297 was not nearly as effective as Cyt1A suppression of resistance shown here to *B. sphaericus* 2362. The reduced capacity of Cyt1Ab to suppress resistance to *B. sphaericus* 2297 may be caused by the five-fold lower toxicity of this Cyt toxin to *Cx. pipiens* in comparison to Cyt1A (Thiéry et al. 1997) or to differences between the two strains of *B. sphaericus.*

Just how Cyt1A restores the toxicity of *B. sphaericus* 2362 is unknown. However, previous studies of the mechanism of resistance in our BS-R strain of *Cx. quinquefasciatus* and the binding properties of Cyt1A suggest that Cyt1A assists binding and insertion of the toxin into the microvillar membrane. Our resistant strain of *Cx. quinquefasciatus* has no functional receptor for the *B. sphaericus* 2362 toxin (Nielsen-LeRoux et al. 1995), and therefore it cannot bind effectively to the midgut microvilli. Studies of Cyt1A have shown that it perturbs membranes by binding to the lipid portion (Butko et al. 1996, 1997), and that it also binds to Cry toxins (Ibarra and Federici 1986). Moreover, in the presence of the BTI Cry toxins, Cyt1A binds to the microvilli of cells in the gastric caeca and posterior midgut of mosquito larvae (Ravoahangimalala et al. 1993, Ravoahangimalala and Charles 1995). These observations suggest several mechanisms for restoring *B. sphaericus* toxicity. The Cyt1A and *B. sphaericus* toxins may bind together after dissolution, and then insert into the membrane as a complex caused by the lipophilic properties of Cyt1A. Another possibility is that Cyt1A may first bind to the membrane, after which the *B. sphaericus* toxin binds to Cyt1A and inserts into the membrane. Finally, Cyt1A may permeate the membrane causing lesions that allow the *B. sphaericus* toxin to gain access to the original target.

The synergism we obtained with the combinations of Cyt1A and *B. sphaericus* 2362 also provides additional evidence that Cyt1A enhances toxicity by assisting other protein toxins in binding to the mosquito microvillar membrane, especially those that do not bind efficiently. In previous studies we demonstrated that Cyt1A can synergize Cry4 and Cry11 toxins from mosquitocidal strains of *B. thuringiensis* against resistant mosquitoes (Wirth et al. 1997, 1998). However, synergism in nonresistant mosquitoes was observed only with the Cry4 and Cry11A toxins of BTI, not with the Cry11B toxin from *B. thuringiensis* subsp. *jegathesan,* which is much more toxic than Cry11A. A similar pattern of synergism was observed in the current study wherein Cyt1A synergized the toxicity of *B. sphaericus* 2362 against the resistant BS-R strain, but not against the sensitive Syn-P strain. The implication of these results, in conjunction with those obtained in the previous studies cited above, is that toxins that are highly toxic or have a high binding affinity, such as Cry11B or the *B. sphaericus* 2362 binary toxin, gain little or no value from assisted binding by Cyt1A. But when the toxin receptors are modified or lost through resistance, the ability of Cyt1A to bind to and perturb the microvillar membrane restores the capacity of these toxins to insert into the membrane and exert toxicity. As both the Cyt1A and *B. sphaericus* toxins dissolve in the mosquito midgut lumen, they may associate immediately after dissolution in the lumen as well as at the microvillar membrane surface. An implication of these results is that Cyt1A, and possibly other Cyt proteins, may extend the insecticidal spectrum of nonCyt protein toxins to other insect species.

The observation that both Cyt1Aa from BTI and Cyt1Ab from *B. thuringiensis* subsp. *medellin* can reduce resistance to *B. sphaericus,* as well as the ability of Cyt1A to suppress resistance to other mosquitocidal *B. thuringiensis* strains, indicates that other mosquitocidal cytolytic toxins also may prove useful in resistance management. The Cyt toxin group now contains several different toxins including Cyt1Aa and Cyt2Ba from BTI (Waalwijk et al. 1985; Guerchicoff et al. 1997), Cyt2Aa from *B. thuringiensis* subsp. *kyushuensis* (Koni and Ellar 1994), Cyt1Ab1 from *B. thuringiensis* subsp. *medellin* (Thiéry et al. 1997), and Cyt2Bb from *B. thuringiensis* subsp. *jegathesan* (Cheong and Gill 1997). As these Cyt proteins vary in their toxicity to mosquitoes, they may find different roles in managing resistance to *B. thuringiensis* and *B. sphaericus* in mosquito populations.

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