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Lack of Cross-Resistance to Cry19A from *Bacillus thuringiensis* subsp. *jegathesan* in *Culex quinquefasciatus* (Diptera: Culicidae) Resistant to Cry Toxins from *Bacillus thuringiensis* subsp. *israelensis*

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***Culex quinquefasciatus* mosquitoes with high levels of resistance to single or multiple toxins from *Bacillus thuringiensis* subsp. *israelensis* were tested for cross-resistance to the *Bacillus thuringiensis* subsp. *jegathesan* polypeptide Cry19A. No cross-resistance was detected in mosquitoes that had been selected with the Cry11A, Cry4A and Cry4B, or Cry4A, Cry4B, Cry11A, and CytA toxins. A low but statistically significant level of cross-resistance, three to fourfold, was detected in the colony selected with Cry4A, Cry4B, and Cry11A. This cross-resistance was similar to that previously detected with *B. thuringiensis* subsp. *jegathesan* in the same colony. These data help explain the toxicity of *B. thuringiensis* subsp. *jegathesan* against the resistant colonies and indicate that the Cry19A polypeptide might be useful in managing resistance and/or as a component of synthetic combinations of mosquitocidal toxins.**

Commercial products for the biological control of mosquitoes are based upon two entomopathogenic bacterial species, *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*. Products derived from these two bacteria are used worldwide to control mosquito and blackfly larvae. Because few alternative materials are available, insecticide resistance would seriously limit the usefulness of these two products. In fact, field resistance to *B. sphaericus* has recently been reported in *Culex pipiens* complex in France (12), India (8), Brazil (11), and China (17). To date there are no reported cases of field resistance to *B. thuringiensis* subsp. *israelensis*. However, laboratory selections using cloned *B. thuringiensis* subsp. *israelensis* toxins have shown that resistance can develop and is inversely correlated with the number of toxins used in the selection (3).

The use of alternative bacterial strains is one approach to managing resistance to bacterial insecticides. Although there are other mosquitocidal strains of *B. thuringiensis* and *B. sphaericus*, most lack the high activity of *B. thuringiensis* subsp. *israelensis* (7, 13). Additionally, synthetic combinations of mosquitocidal toxins from different microbial strains could be engineered to increase toxicity and to expand the host range of microbial insecticides. Ideally, the latter approach would utilize mosquitocidal toxins that differ in structure and mode of action, which combined into a recombinant bacterium may help prevent or significantly delay the development of insecticide resistance.

Bacillus thuringiensis subsp. *jegathesan* is a potential alternative to *B. thuringiensis* subsp. *israelensis* because it is as toxic to *Anopheles stephensi* as *B. thuringiensis* subsp. *israelensis* and is only slightly less toxic to *Aedes aegypti* and *C. pipiens* (10). Furthermore, insecticidal crystals from *B. thuringiensis* subsp. *jegathesan* contain seven major polypeptides, with molecular masses of 80, 70 to 72, 65, 37, 26, and 16 kDa, which are not related to those produced in *B. thuringiensis* subsp. *israelensis* (7). Such complex toxin mixtures may be beneficial in delaying the onset of resistance, as previously observed with *B. thuringiensis* subsp. *israelensis* (3).

The potential of *B. thuringiensis* subsp. *jegathesan*, or any of its component toxins, to be incorporated into a resistance management program depends upon the degree of cross-resistance to *B. thuringiensis* subsp. *israelensis* and, especially, upon the degree of cross-resistance between the component endotoxins. It has previously been shown that high levels of resistance to the Cry toxins in *B. thuringiensis* subsp. *israelensis* generally did not confer significant levels of cross-resistance to *B. thuringiensis* subsp. *jegathesan* (14). However, significant levels of cross-resistance were detected in the strains resistant to Cry11B, a highly toxic polypeptide produced by *B. thuringiensis* subsp. *jegathesan* (14). Cry11B is not the major polypeptide of *B. thuringiensis* subsp. *jegathesan*; therefore, other polypeptides in the native crystal are probably involved in toxicity.

The gene for the 65-kDa protein from *B. thuringiensis* subsp. *jegathesan, cry19A,* has been isolated, sequenced, and expressed (9). The resulting polypeptide is toxic to *C. pipiens,* demonstrates a 50% lethal concentration (LC_{50}) of 0.187 μg/ml with purified inclusions against fourth instars, but shows no activity against *A. aegypti* (9). Because Cry19A has no significant similarity to known *B. thuringiensis* toxins, it belongs to a novel class of δ-endotoxins (9). The lack of similarity of Cry19A to other mosquitocidal Cry toxins suggests that it may be useful in resistance management if no cross-resistance to other Cry toxins is present. Here we report that *Culex quinquefasciatus,* with high levels of resistance to Cry toxins from *B. thuringiensis* subsp. *israelensis*, exhibits little or no cross-resistance to Cry19A.

Five colonies of *C. quinquefasciatus* were used in this study. These colonies were *Cq*Syn90, a parental reference colony that is susceptible to microbial insecticides, and four highly resis-

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TABLE 1. Lethal concentrations of Cry19A from *B. thuringiensis* subsp. *jegathesan* for *C. quinquefasciatus* strains that are susceptible or resistant to single or multiple toxins of *B. thuringiensis* subsp. *israelensis*

Strain	LC_{50} (μg/ml)	Fiducial limits	LC_{95} (μg/ml)	Fiducial limits	Slope	RR at:	
						LC_{50}	LC_{95}
*Cq*Syn90	0.978	0.829–1.15	10.1	7.49–14.8	1.6	1.0	1.0
*Cq*4D	1.97	1.66–2.32	21.1	17.9–34.7	1.5	2.0	2.4
*Cq*4AB	1.47	1.21–1.77	26.6	18.8–40.9	1.3	1.5	2.6
*Cq*4ABD	2.98	1.97–4.55	37.8	15.4–96.4	1.5	3.0	3.7
*Cq*4ABDCytA	1.15	0.852–1.57	9.92	5.42–19.0	1.7	1.2	0.98

tant colonies that were derived from *Cq*Syn90 by selection with strains of *B. thuringiensis* that produce single or multiple *B. thuringiensis* subsp. *israelensis* toxins (3). The mosquito colonies and their levels of resistance at concentrations that kill 95% of the population (resistance ratio, [RR]) are *Cq*4D, selected with Cry11A (formerly Cry4D) (RR > 7,000); *Cq*4AB, selected with Cry4A and Cry4B (RR, 290); *Cq*4ABD, selected with Cry4A, Cry4B, and Cry11A (RR, 949); and *Cq*4ABDCytA, selected with the wild-type preparation of *B. thuringiensis* subsp. *israelensis* (RR, 12.7) (14). These colonies have been maintained under laboratory selection pressure since 1990.

Cry19A was cloned into *B. thuringiensis* subsp. *thuringiensis* SPL407 as described by Rosso and Delécluse (9). *B. thuringiensis* cells were grown in HCT medium (5) with shaking at 30°C until cell lysis. Spores and crystals were harvested and washed once in 1 M NaCl and twice in cold, double-distilled water. Lyophilized spore-crystal powders were used for all bioassays because of the large amount of material required for these tests.

Stock suspensions of the lyophilized spore-crystal powder were prepared in 125-ml flasks with distilled water. Approximately 25 glass beads were added to the flask, and the suspension was agitated for 5 min with a vortex mixer. Tenfold serial dilutions were prepared. Stock suspensions were prepared monthly, and dilutions were made weekly. Stocks and dilutions were stored at −20°C when not in use.

Early-fourth instars from the five mosquito colonies were concurrently tested with Cry19A spore-crystal powder using the same stocks and dilutions. Thus, differences in sensitivity to Cry19A among the five mosquito colonies would be primarily due to their selection regimen and their resulting resistance spectrum. Twenty larvae were placed in 237-ml plastic cups in 100 ml of deionized water. Ten to 12 different concentrations of Cry19A suspension, causing mortality between 2 and 98% after 24 h, were used. Bioassays were replicated on five different days. All data were analyzed with a probit program (2; version 3.3, Praxeme, Saint Georges d'Orques, France). Lethal concentrations with overlapping fiducial limits were not considered to be significantly different. RRs were calculated relative to the lethal concentrations for the susceptible parental strain, *Cq*Syn90.

Cry19A had LC_{50}s and LC_{95}s of 0.978 and 10.1 μg/ml, respectively, for the synthetic parental colony *Cq*Syn90 (Table 1). The LC_{50}s for three of the four resistant colonies, *Cq*4D, *Cq*4AB, and *Cq*4ABDCytA, were 1.97, 1.47, and 1.15 μg/ml. These values, as well as their associated LC_{95}s, were not significantly different from those of the parental reference colony, *Cq*Syn90. RRs at the LC_{50} and LC_{95} for these three resistant colonies ranged from 0.98 to 2.6. However, for colony *Cq*4ABD the LC_{50} and LC_{95} were 2.98 and 37.8 μg/ml and the RRs were 3.0 and 3.7, respectively. These values were statistically different from those for *Cq*Syn90, and the LC_{50}, but not the LC_{95}, was statistically different from those for *Cq*4AB and *Cq*4ABDCytA.

The data above indicate that Cry19A was equally toxic to a susceptible colony of *C. quinquefasciatus* and to three highly resistant colonies derived from that susceptible colony by laboratory selection pressure with combinations of *B. thuringiensis* subsp. *israelensis* toxins (3). For fourth colony, *Cq*4ABD, the lethal concentrations were significantly higher. Because these tests were performed concurrently, differences in susceptibility reflect differences in the resistance spectrums of the colonies. Interestingly, in previous tests the *Cq*4ABD colony showed a similar, and statistically significant, level of cross-resistance to *B. thuringiensis* subsp. *jegathesan,* 3.9-fold at the LC_{50} (14).

Previous tests revealed much higher levels of cross-resistance, from 53.1 to 567 at the LC_{95}, when these same five colonies were tested with Cry11B, another component toxin of *B. thuringiensis* subsp. *jegathesan* (14). Therefore, it is probable that the high toxicity of *B. thuringiensis* subsp. *jegathesan,* particularly toward the resistant colonies, is due in part to the toxicity of Cry19A. Cry19A is less toxic, 13.8- and 22.9-fold less toxic at the LC_{50} and LC_{95}, respectively, than the wild-type inclusions of *B. thuringiensis* subsp. *jegathesan*. Therefore, it seems likely that other polypeptides in *B. thuringiensis* subsp. *jegathesan* also contribute to toxicity toward the resistant colonies, possibly due to additive or synergistic interactions. Synergistic interactions are known to play an important role in the toxicity of *B. thuringiensis* subsp. *israelensis* (1, 4, 6, 16), and they have been demonstrated to delay the development of resistance to this material (3). Furthermore, synergism between *B. thuringiensis* subsp. *israelensis* Cry and Cyt toxins has been found to suppress high levels of Cry resistance (15). Because *B. thuringiensis* subsp. *jegathesan* also possesses a mixture of Cry and Cyt toxins, such interactions would not be unexpected. However, the contribution of other component toxins to the toxicity of *B. thuringiensis* subsp. *jegathesan,* and the interactions among these toxins, must be investigated to confirm this hypothesis.

The lack of biologically significant levels of cross-resistance to Cry19A in mosquitoes that possess high levels of resistance and cross-resistance to other mosquitocidal Cry toxins helps explain the toxicity of *B. thuringiensis* subsp. *jegathesan* toward the resistant colonies. These results also indicate that the Cry19A polypeptide may be useful as a component toxin in synthetic combinations intended for resistance management. Further, the unique characteristics of Cry19A, which placed it in a new class of δ-endotoxins, may provide insight into toxin characteristics that are important for mosquitocidal activity.

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