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Synergism between *Bacillus thuringiensis* Spores and Toxins against Resistant and Susceptible Diamondback Moths (*Plutella xylostella*)

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We studied the effects of combinations of *Bacillus thuringiensis* spores and toxins on the mortality of diamondback moth (*Plutella xylostella*) larvae in leaf residue bioassays. Spores of *B. thuringiensis* subsp. *kurstaki* increased the toxicity of crystals of *B. thuringiensis* subsp. *kurstaki* to both resistant and susceptible larvae. For *B. thuringiensis* subsp. *kurstaki*, resistance ratios were 1,200 for a spore-crystal mixture and 56,000 for crystals without spores. Treatment of a spore-crystal formulation of *B. thuringiensis* subsp. *kurstaki* with the antibiotic streptomycin to inhibit spore germination reduced toxicity to resistant larvae but not to susceptible larvae. In contrast, analogous experiments with *B. thuringiensis* subsp. *aizawai* revealed no significant effects of adding spores to crystals or of treating a spore-crystal formulation with streptomycin. Synergism occurred between Cry2A and *B. thuringiensis* subsp. *kurstaki* spores against susceptible larvae and between Cry1C and *B. thuringiensis* subsp. *aizawai* spores against resistant and susceptible larvae. The results show that *B. thuringiensis* toxins combined with spores can be toxic even though the toxins and spores have little or no independent toxicity. Results reported here and previously suggest that, for diamondback moth larvae, the extent of synergism between spores and toxins of *B. thuringiensis* depends on the strain of insect, the type of spore, the set of toxins, the presence of other materials such as formulation ingredients, and the concentrations of spores and toxins.

Insecticides derived from the soil bacterium *Bacillus thuringiensis* are becoming an increasingly important component of ecologically sound pest management (6). Insecticidal crystal proteins from *B. thuringiensis* are extremely toxic to many pests and have been a primary focus of much recent research (6, 7, 12). These toxins, which are produced in crystalline inclusions during sporulation (6), kill insects by binding to and creating pores in midgut membranes (10). The spores from *B. thuringiensis* have received much less attention than the toxins but are known to increase the toxicity of *B. thuringiensis* proteins to some insects (3, 4, 11, 16, 19, 20, 23, 25, 26). For decades, *B. thuringiensis* was used only in conventional applications containing naturally occurring combinations of spores and crystal proteins, as well as other materials (5, 37). Genetic engineering has created transgenic plants and transgenic bacteria that express one or a few toxins from *B. thuringiensis* without spores (7).

As use of *B. thuringiensis* proteins and spore-crystal formulations increases, so too does the likelihood that pests will adapt. Laboratory selection experiments show that many pests can evolve resistance, but so far, the only documented cases of resistance to *B. thuringiensis* in open field populations of insects are in one species, the diamondback moth (8, 18, 31, 32, 38). Knowledge of the role of spores in toxicity to susceptible and resistant strains of diamondback moth may be helpful for understanding and managing pest resistance to *B. thuringiensis.*

Miyasono et al. (22) found that toxin-free spores did not kill larvae, but spores increased the toxicity of *B. thuringiensis* subsp. *kurstaki* crystals to larvae from a susceptible strain of diamondback moth. The interaction observed between these spores and toxins exemplifies synergism, in which the toxicity of a mixture is greater than expected on the basis of the independent toxicity of its components (9, 30). Tang et al. (38) found synergism between *B. thuringiensis* subsp. *kurstaki* spores and each of the three individual Cry1A toxins from *B. thuringiensis* subsp. *kurstaki* against a susceptible strain of diamondback moth but not against a resistant strain from Florida. They also reported that for the susceptible strain and the resistant strain, synergism occurred between *B. thuringiensis* subsp. *kurstaki* spores and Cry1C but not between the spores and Cry2A (38).

Here we report new evidence that extends our understanding of the role of spores in the toxicity of *B. thuringiensis* toxins. We evaluated the synergism of *B. thuringiensis* toxins with spores from *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai* against a susceptible strain and two resistant strains of diamondback moth from Hawaii. One resistant strain (NO-QA) was extremely resistant to *B. thuringiensis* subsp. *kurstaki* only (33, 36); the other (NO-95) showed significant resistance to *B. thuringiensis* subsp. *aizawai* and Cry1C as well as to *B. thuringiensis* subsp. *kurstaki* (18). In addition to testing for synergism between spores and single toxins as Tang et al. did (38), we tested for synergism between spores and combinations of toxins that occur in crystals from *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai.*

MATERIALS AND METHODS

Insects. All insects were reared on cabbage (32). We studied three strains: LAB-P, NO-QA, and NO-95. The susceptible LAB-P strain had been maintained in the laboratory for >150 generations without exposure to insecticide. Strain NO-QA was derived from a resistant field population (NO) in 1989 and had been selected repeatedly with *B. thuringiensis* subsp. *kurstaki* to increase its resistance (32, 35). Strain NO-95 was derived from the same field population (NO) in 1995 and was reared without exposure to *B. thuringiensis*. NO-95 was highly resistant to *B. thuringiensis* subsp. *kurstaki*, moderately resistant to Cry1C toxin, and slightly resistant to *B. thuringiensis* subsp. *aizawai* (18).

***B. thuringiensis* crystals, formulations, spores, and toxins.** We tested crystals of the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* (Dipel 2X) and the ATCC SD-1372 strain of *B. thuringiensis* subsp. *aizawai* (XenTari) with and without spores. For each *B. thuringiensis* subspecies, a spore-crystal suspension from a pilot scale fermentor was washed in 0.5 M NaCl and then washed in distilled water in a Sorvall model RC5B apparatus at 17,500 × *g* for 45 min at 8 to 10°C. A spore-crystal mixture was separated with 50% Renografin-76 (Squibb). The

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TABLE 1. Effects of spores on toxicity of *B. thuringiensis* crystals to resistant (NO-QA) and susceptible (LAB-P) diamondback moth larvae

Strain	Spores[a]	No. of larvae	Slope (SE)	LC_{50} (95% FL) (mg of protein/liter)	Spore effect ratio[b]	Resistance ratio[c]
B. thuringiensis subsp. *kurstaki*						
LAB-P	+	280	0.6 (0.1)	0.3 (0.1–0.9)		
	−	280	1.0 (0.1)	2.9 (1.7–4.8)	9.7	
NO-QA	+	320	0.8 (0.2)	360 (130–2,600)		1,200
	−	280	0.5 (0.2)	160,000 (8,200–4×10^{10})	450	56,000
B. thuringiensis subsp. *aizawai*						
LAB-P	+	320	1.7 (0.3)	0.9 (0.5–1.3)		
	−	320	1.6 (0.2)	1.3 (0.9–1.9)	1.4	
NO-QA	+	320	1.1 (0.2)	2.9 (1.4–6.8)		3.2
	−	320	1.3 (0.2)	4.4 (2.6–7.6)	1.5	3.4

[a] *B. thuringiensis* subsp. *kurstaki* crystals were tested with (+) and without (−) *B. thuringiensis* subsp. *kurstaki* spores; *B. thuringiensis* subsp. *aizawai* crystals were tested with and without *B. thuringiensis* subsp. *aizawai* spores.
[b] LC_{50} without spores divided by LC_{50} with spores for each strain.
[c] LC_{50} for NO-QA divided by LC_{50} for LAB-P.

material resulting from three passes was lyophilized and used as the spore-crystal mixture. For preparation of a crystal-rich fraction, the pelleted spore-crystal mixture was resuspended in 10 ml of deionized water. This suspension was then layered on 35 ml of 50% Renografin and centrifuged at 25,700 × *g* for 45 min at 8 to 10°C. The pellet from this treatment contained primarily spores. The Renografin column contained the crystal-rich fraction in a broad turbid band. Crystal-rich fractions were aspirated and washed with water three times prior to lyophilization. The resulting crystals contained about 10 spores/μg (dry weight) of the crystal sample. Crystal protein was visualized on a sodium dodecyl sulfate–10% polyacrylamide gel with broad-range molecular mass markers from 200 to 31 kDa. Values were quantified with a Bio-Rad imaging densitometer, model GS-670, versus a bovine serum albumin protein standard (Sigma, St. Louis, Mo.).

We used two commercial spore-crystal formulations: Dipel 2X (lot no. 73; Abbott Laboratories, North Chicago, Ill.) from the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* and XenTari (lot no. 71; Abbott Laboratories) from *B. thuringiensis* subsp. *aizawai*, both in wettable powder. Using previously described methods (38), we obtained spores of *B. thuringiensis* subspp. *kurstaki* and *aizawai* from cultures of our samples of Dipel 2X and XenTari, respectively. The purity of spore preparations was determined by counting spores and crystals under a microscope, which indicated preparations with >99.5% spores and <0.5% crystals. For brevity, we refer to spore preparations as spores. We also used liquid formulations of Cry1Ab (MYX03604) and Cry1C (MYX833-4C1) expressed in and encapsulated by transgenic *Pseudomonas fluorescens* (Mycogen, San Diego, Calif.). Cry2A protein was obtained by overexpressing the *cry2A* gene from the NRD-12 strain of *B. thuringiensis* subsp. *kurstaki* in *Escherichia coli* (24).

Bioassays. One week after eggs were placed on cabbage plants, larvae were used for leaf residue bioassays (17). All materials tested were diluted with distilled water containing 0.2% Triton AG-98 (a surfactant; Rohm & Haas Co., Philadelphia, Pa.). Ten larvae were placed on each treated leaf disk (equal to one replicate). After 2 days, untreated fresh cabbage leaves were provided. Mortality was recorded 5 days after bioassays were started. Bioassays and rearing were conducted at 28°C with a photoperiod of 14 h of light and 10 h of dark.

Effects of spores on the toxicity of *B. thuringiensis* crystals. We tested crystals of *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai* with and without spores against susceptible LAB-P larvae and resistant NO-QA larvae. In each bioassay, we used a series of six or seven concentrations of *B. thuringiensis* and a control without *B. thuringiensis* against each diamondback moth colony. Each concentration was replicated four times.

Effects of spores and streptomycin. We used the antibiotic streptomycin (Sigma) to block germination of spores in spore-crystal formulations of *B. thuringiensis*. Inhibition of germination of *B. thuringiensis* spores by streptomycin has been demonstrated in several studies (2, 13, 14). To determine an appropriate concentration of streptomycin, we tested streptomycin at 0 (control), 10, 100, and 1,000 mg/liter against susceptible (LAB-P) larvae with and without spores from *B. thuringiensis* subsp. *kurstaki* (50 mg/liter). We then tested streptomycin at 0 (control) and 100 mg/liter against resistant (NO-QA) larvae with and without spores (50 mg/liter). We also tested combinations of streptomycin and Cry1Ab against LAB-P to determine if streptomycin affects the toxicity of Cry1Ab. The treatments were as follows: control, streptomycin (100 mg/liter) alone, Cry1Ab (10 mg/liter) alone, and streptomycin (100 mg/liter) plus Cry1Ab (10 mg/liter). Each treatment was replicated four times.

Effects of streptomycin on the toxicity of *B. thuringiensis* spore-crystal formulations. We tested spore-crystal formulations of *B. thuringiensis* subsp. *kurstaki* (Dipel 2X) and *B. thuringiensis* subsp. *aizawai* (XenTari) against susceptible LAB-P larvae and resistant larvae (NO-QA versus Dipel 2X and NO-95 versus XenTari). In each bioassay, we used a series of five concentrations of *B. thuringiensis* and a control without *B. thuringiensis* against each diamondback moth colony. All concentrations of *B. thuringiensis* were tested with and without streptomycin (100 mg per liter). Each treatment was tested in four replicate experiments on each of two separate dates.

Interactions between spores and single *B. thuringiensis* toxins. We tested Cry2A and spores from *B. thuringiensis* subsp. *kurstaki* alone and in combinations against susceptible LAB-P larvae. The concentrations for Cry2A were 0, 10, and 100 mg/liter. The concentrations for spores were 0, 1, and 10 mg/liter. We tested Cry1C and spores from *B. thuringiensis* subsp. *aizawai* against both LAB-P larvae, which are susceptible, and NO-95 larvae, which are resistant to Cry1C and *B. thuringiensis* subsp. *aizawai* (18). Concentrations of Cry1C were 0, 0.05, and 0.5 ml/liter. Concentrations of the spores were 0, 1, and 10 mg/liter. In each experiment, all possible combinations between spores and Cry2A or Cry1C were replicated four times.

Data analysis. For each bioassay of *B. thuringiensis* crystals with or without spores and of spore-crystal formulations with or without streptomycin, we used probit analysis (28, 34) to estimate 50% lethal concentrations (LC_{50}s). LC_{50}s from two strains were considered significantly different if their 95% fiducial limits did not overlap. Resistance ratios were calculated as the LC_{50} for a resistant strain divided by the LC_{50} for LAB-P. For each strain, spore effect ratios were calculated as the LC_{50} of *B. thuringiensis* without spores (crystals without spores or spore-crystal formulation with streptomycin) divided by the corresponding LC_{50} of *B. thuringiensis* with spores. Mortality data from bioassays with *B. thuringiensis* spores and toxins were analyzed by χ^2 tests (9, 21, 38) to evaluate synergism between spores and single toxins. The *G* test (29) was used to test for effects of spores alone and toxins alone on larval mortality.

RESULTS

Effects of spores on toxicity of *B. thuringiensis* crystals. Combinations of spores and crystals from *B. thuringiensis* subsp. *kurstaki* were significantly more toxic than *B. thuringiensis* subsp. *kurstaki* crystals to susceptible and resistant diamondback moth larvae (Table 1). In tests with *B. thuringiensis* subsp. *kurstaki* crystals, spores reduced the LC_{50} by 10-fold against susceptible LAB-P larvae and by 450-fold against resistant NO-QA larvae (Table 1). The highest concentration of *B. thuringiensis* subsp. *kurstaki* crystals without spores, which was 1,000 mg of protein/liter, killed only 22.5% of NO-QA larvae. The ratios of the LC_{50} for NO-QA to that for LAB-P were 1,200 with the *B. thuringiensis* subsp. *kurstaki* spore-crystal mixture and 56,000 with *B. thuringiensis* subsp. *kurstaki* crystals without spores (Table 1).

Spores from *B. thuringiensis* subsp. *aizawai* had little or no effect on the toxicity of crystals of *B. thuringiensis* subsp. *aizawai*. No significant difference in LC_{50}s between paired treatments with and without *B. thuringiensis* subsp. *aizawai* spores occurred for susceptible or resistant larvae (Table 1). In each of the two paired comparisons, the LC_{50} with spores was only slightly lower than those without spores (range for spore effect ratio, 1.4 to 1.5) (Table 1). The ratio of the LC_{50} for NO-QA

TABLE 2. Effects of streptomycin on toxicity of *B. thuringiensis* subsp. *kurstaki* spores to resistant (NO-QA) and susceptible (LAB-P) diamondback moth larvae

Strain[a]	Streptomycin (mg/liter)	Mortality (%) with indicated concn (mg/liter) of spores	
		0	50
LAB-P	0	2.5	70.0
	10	0	20.0
	100	0	2.5
	1,000	0	2.5
NO-QA	0	0	0
	100	0	0

[a] Forty larvae were tested for each treatment.

to that for LAB-P was about 3 for *B. thuringiensis* subsp. *aizawai* crystals with or without spores (Table 1).

Effects of spores and streptomycin. Fifty milligrams of *B. thuringiensis* subsp. *kurstaki* spores per liter killed 70% of susceptible LAB-P larvae but did not kill any NO-QA larvae (Table 2). Streptomycin significantly reduced mortality of LAB-P larvae caused by spores (in the *G* test, the *G* statistic calculated with Williams' correction [29] for the significant toxicity of each component tested alone was 67.6, the *df* was 3, and *P* was <0.001 [29]). Streptomycin at concentrations of 100 or 1,000 mg/liter eliminated the toxicity of spores. Streptomycin alone did not kill larvae (Table 2) and did not affect the toxicity of Cry1Ab toxin against LAB-P larvae. Cry1Ab (1 ml/liter) caused 97.5% mortality to LAB-P larvae with or without streptomycin (100 mg/liter).

Effects of streptomycin on the toxicity of *B. thuringiensis* spore-crystal formulations. Streptomycin significantly reduced the toxicity of Dipel 2X, a spore-crystal formulation of *B. thuringiensis* subsp. *kurstaki*, to the resistant NO-QA strain but not to the susceptible LAB-P strain (Table 3). For NO-QA, the LC_{50} of Dipel 2X was tripled by adding streptomycin (Table 3). The ratios of the LC_{50} of Dipel 2X for NO-QA to that for LAB-P were 78 without streptomycin and 190 with streptomycin (Table 3).

With XenTari, a spore-crystal formulation of *B. thuringiensis* subsp. *aizawai*, no significant differences in LC_{50}s occurred between paired treatments with and without streptomycin for either the LAB-P or the NO-95 strain. The ratios of the LC_{50} for NO-95 to that for LAB-P were about 5 with streptomycin and 6 without streptomycin (Table 3).

Interactions between spores and single toxins. Synergism was found in all three spore-toxin combination experiments (Table 4). However, synergism between spores and toxins depended on the concentrations of spores and toxins tested. Ten of 12 spore-toxin combinations in the three experiments showed significant synergism between spores and toxins (Table 4).

Synergism between *B. thuringiensis* subsp. *kurstaki* spores and Cry2A occurred in three of the four spore-toxin combinations against LAB-P larvae. The preparation of *B. thuringiensis* subsp. *kurstaki* spores alone caused significant mortality of susceptible LAB-P larvae; Cry2A alone did not cause significant mortality at either concentration tested (Table 4). Synergism occurred between *B. thuringiensis* subsp. *aizawai* spores and Cry1C toxin for all four combinations against LAB-P larvae and for three combinations against NO-95 larvae. *B. thuringiensis* subsp. *aizawai* spores alone did not cause significant mortality of either LAB-P or NO-95 larvae. Cry1C at the higher concentration caused significant mortality to both LAB-P and NO-95 (Table 4).

DISCUSSION

Results from this study provide additional examples of synergism between spores and toxins of *B. thuringiensis*, but they also confirm that such synergism is far from universal. Along with the results of previous work (38), our results suggest that, for diamondback moth larvae, the extent of synergism between spores and toxins of *B. thuringiensis* depends on the strain of insect, the type of spore, the set of toxins, the presence of other materials such as formulation ingredients, and the concentrations of spores and toxins.

The observed toxicity of spore preparations to larvae apparently was caused by synergism between spores and the small amount of toxin ($<0.5\%$) in the spore preparations. Previous results showed that toxin-free spores are not toxic (16, 22). We found that streptomycin eliminated toxicity of the spore preparation (Table 2) but did not reduce the toxicity of Cry1Ab to susceptible larvae. In our study, the synergistic effects of *B. thuringiensis* subsp. *kurstaki* spores against a resistant strain of diamondback moth were equal to or greater than those against a susceptible strain (Tables 1 and 3). These results contrast with previous findings that synergism occurred between spores of *B. thuringiensis* subsp. *kurstaki* and individual Cry1A toxins

TABLE 3. Effects of streptomycin on toxicity of spore-crystal formulations of *B. thuringiensis* to resistant (NO-QA or NO-95) and susceptible (LAB-P) diamondback moth larvae

Strain	Streptomycin[a]	No. of larvae	Slope (SE)	LC_{50} (95% FL) (mg [AI]/liter)	Spore effect ratio[b]	Resistance ratio[c]
B. thuringiensis subsp. *kurstaki*						
LAB-P	−	480	1.3 (0.2)	1.6 (0.9–2.4)		
	+	480	1.6 (0.2)	1.9 (1.3–2.6)	1.2	
NO-QA	−	480	1.8 (0.2)	120 (80–180)		78
	+	480	2.3 (0.4)	360 (260–520)	2.9	190
B. thuringiensis subsp. *aizawai*						
LAB-P	−	480	1.3 (0.2)	4.6 (2.8–6.7)		
	+	480	1.6 (0.2)	8.8 (5.7–13)	1.9	
NO-95	−	480	1.4 (0.1)	28 (21–37)		6.2
	+	480	1.4 (0.2)	47 (31–72)	1.7	5.4

[a] −, no streptomycin; +, 100 mg of steptocycin per liter.
[b] LC_{50} with streptomycin divided by LC_{50} without streptomycin for each colony.
[c] LC_{50} for NO-QA or NO-95 divided by LC_{50} for LAB-P.

TABLE 4. Independent and joint toxicity of *B. thuringiensis* spores and single toxins to diamondback moth larvae[a]

Test	Concn[b] of:		Observed mortality (%)	G_{adj}[c]	Expected mortality (%)	χ^{2d}
	Spores	Toxin				
LAB-P vs *B. thuringiensis* subsp. *kurstaki* spores and Cry2A toxin	1	0	29.4	6.29*		
	10	0	41.2	11.37***		
	0	10	3.1	0.09		
	0	100	8.8	0.74		
	1	10	44.1		31.6	7.35**
	1	100	35.5		35.6	0.0003
	10	10	58.8		43.0	5.81*
	10	100	76.5		46.4	19.53**
LAB-P vs *B. thuringiensis* subsp. *aizawai* spores and Cry1C toxin	1	0	10.3	2.88		
	10	0	7.7	1.86		
	0	0.05	5.1	0.98		
	0	0.5	42.7	22.05***		
	1	0.05	59.0		14.9	130.52***
	1	0.5	87.2		48.6	30.66***
	10	0.05	59.0		12.4	175.13***
	10	0.5	94.9		47.1	48.51***
NO-95 vs *B. thuringiensis* subsp. *aizawai* spores and Cry1C toxin	1	0	2.6	0.32		
	10	0	5.1	0.98		
	0	0.05	0	0.002		
	0	0.5	15.4	5.23*		
	1	0.05	10.3		2.6	22.80***
	1	0.5	22.4		17.6	1.31
	10	0.05	30.8		5.1	129.51***
	10	0.5	41.0		19.7	23.03***

[a] A total of 40 larvae were tested for each treatment. Observed mortality was adjusted for control mortality. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[b] Concentration units were milligrams of spores per liter, milligrams of Cry2A protein per liter, and milliliters of the liquid formulation of Cry1C per liter.
[c] G statistic calculated with Williams' correction (29) for the significant toxicity of each component tested alone.
[d] χ^2 analysis (21, 38) for synergism between spores and toxin.

against a susceptible strain of diamondback moth but not against a resistant strain (38).

Our finding of significant synergism between *B. thuringiensis* subsp. *aizawai* spores and Cry1C, but not between *B. thuringiensis* subsp. *aizawai* spores and crystals, suggests that synergism between spores and one toxin contained in a mixture does not necessarily indicate synergism between spores and the mixture. Interactions among toxins in the mixture, including toxin-toxin synergism or antagonism (1, 15, 27, 30), might interfere with or mask spore-toxin synergism. Against diamondback moth larvae resistant to *B. thuringiensis* subsp. *kurstaki*, Tang et al. (38) found no synergism between *B. thuringiensis* subsp. *kurstaki* spores and single Cry1A toxins, whereas we found synergism between *B. thuringiensis* subsp. *kurstaki* spores and either crystals or a spore-crystal formulation containing a mixture of Cry1A and Cry2 toxins. This difference suggests that lack of synergism between spores and the individual toxins in a mixture does not preclude synergism between spores and the mixture.

B. thuringiensis subsp. *kurstaki* spores synergized Cry2A against susceptible larvae in three of four combinations of spore-toxin concentrations that we tested; Tang et al. (38) found no significant synergism between *B. thuringiensis* subsp. *kurstaki* spores and Cry2A against either susceptible or resistant larvae. These differences may be related to differences in the strains of insects studied, preparations of spores and toxins, concentrations and ratios of spores and toxins, or experimental methods. Differences between analytical approaches (i.e., testing by comparison of LC_{50}s versus testing with single concentrations) in their power to detect statistically significant synergism might also have contributed to observed variation in synergism between and within studies.

Our results show that for resistant and susceptible diamondback moth larvae, the effects of adding *B. thuringiensis* subsp. *kurstaki* spores to crystals of *B. thuringiensis* subsp. *kurstaki* (Table 1) were greater than those of treating a spore-crystal formulation of *B. thuringiensis* subsp. *kurstaki* with the antibiotic streptomycin (Table 2). These results suggest that ingredients in the formulation that are absent from crystals may reduce the synergistic effects of spores. An alternative explanation is that streptomycin did not completely eliminate effects of spores in the spore-crystal formulation, and thereby this approach reduced the apparent extent of synergism. Perhaps the streptomycin prevented germination of spores but did not completely block delivery of synergistic compounds associated with spores.

We conclude that in some, but not all, cases, spores increased mortality by interacting with individual *B. thuringiensis* toxins, naturally occurring mixtures of *B. thuringiensis* toxins in crystals, and *B. thuringiensis* formulations. It is tempting to assume that the absence of spores in *B. thuringiensis* toxin-expressing transgenic plants and transgenic bacteria will accelerate evolution of pest resistance. Direct experimental evaluations will be needed to test this hypothesis.

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