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Effects of Cry1F and Cry34Ab1/35Ab1 on storage pests

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ABSTRACT

Two crystalline protoxins from *Bacillus thuringiensis* (Bt), Cry1Fa1 and Cry34Ab1/Cry35Ab1 (Cry1F, Cry34/35Ab1), were evaluated for efficacy against lepidopteran and coleopteran storage pests. Cry1F was tested against the lepidopterans *Sitotroga cerealella* (Angoumois grain moth) and colonies of *Plodia interpunctella* (Indian mealmoth) that are susceptible or resistant to Bt Cry1Ab and Cry1Ac toxins, Bt subspecies *entomocidus*, and the commercial formulation Dipel®. Cry1F was also tested against the coleopterans *Cryptolestes pusillus* (flat grain beetle) and *Tribolium castaneum* (red flour beetle). Cry34/35Ab1 was tested against *S. cerealella*, *C. pusillus*, and *T. castaneum*, and against additional coleopteran storage pests, including *Tenebrio molitor* (yellow mealworm), *Trogoderma variabile* (warehouse beetle), *Oryzaephilus surinamensis* (sawtoothed grain beetle), *Rhyzopertha dominica* (lesser grain borer), and *Sitophilus oryzae* (rice weevil). Strains of Bt-susceptible or -resistant *P. interpunctella* generally were more sensitive to Cry1A protoxin or toxin than either Cry1F protoxin or Dipel. Despite difficulties with the bioassay of *S. cerealella* larvae, the data suggest that Cry1F and Cry34/35Ab1 caused increased larval mortality, and a developmental delay was observed and no pupae emerged with 0.9% Cry1F. Neither Cry1F nor the corn rootworm-active toxin Cry34/35Ab1 significantly affected the biological parameters of the coleopteran species evaluated.

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1. Introduction

Storage pests cause economic losses to stored grain and grain products worldwide. For example, in 2008 22.6 million bushels of corn and 4.3 million bushels of wheat were harvested in the U.S., with receipts of $64 billion (http://www.nass.usda.gov). Post-harvest losses due to insect pests, estimated at 5–10% (Cuperus, 1995), would have contributed to losses of $3.2–6.4 billion for that year alone. In developing countries, the losses can be much higher (Haines, 2000).

The arsenal of traditional synthetic chemical controls used by the cereal industry is rapidly being depleted because of increased regulatory constraints and insect resistance. Most of the current grain protectants are organophosphates, under scrutiny by the Environmental Protection Agency (EPA) because of the 1996 Food Quality Protection Act. Methyl bromide, a fumigant used for stored products and milling structures, was banned under the Clean Air Act and the Montreal Protocol, with a few exceptions noted in exemptions by the EPA. Other fumigants, such as sulfuryl fluoride, must be custom applied and are not generally considered compatible with IPM. In addition, many fumigants carry significant human health risks and are regulated by exposure and ventilation restrictions.

Because of all of these constraints, new insecticidal treatments are needed for integrated pest management (IPM) of raw grains, mills, and food storage facilities. We seek treatments that are effective against target pests, safe to the environment and non-target organisms (including insect predators/parasites), while also minimizing the development of resistant insect populations. The insecticidal toxins from the common soil bacterium *Bacillus thuringiensis* Berliner (Bt) are the most successful and widely used biopesticides to date and provide many of these use and safety attributes.

Transgenic corn hybrids expressing the insecticidal crystal (Cry) protein Cry1Ab from Bt have been available for commercial planting since the mid 1990's. Transgenic grain and processed grain fractions were found to be less susceptible to attack and damage by lepidopteran stored-grain pests (Sedlacek et al., 2001). However, resistance to Bt was first noted in a major stored grain pest, *Plodia interpunctella* (Hübner) (Indian mealmoth) (McGaughey, 1985), and resistance to Cry1A toxins has been observed in many lepidopteran insects (Ferré and Van Rie, 2002). Therefore, there is a need to evaluate and understand the spectrum and efficacy of novel Bt

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proteins against stored-grain insects to predict and prolong the efficacy of transgenic grain to reduce damage by storage pests.

Cry1F (Event TC1507) was commercially available in hybrid corn in the United States in 2003 under the trade name Herculex™[1] I *Insect Protection*. This product controls several destructive foliar and kernel feeding Lepidoptera. Cry1F transgenic corn negatively impacted the growth and development of both Bt-susceptible and -resistant populations of *P. interpunctella* selected with Dipel[1] (http://www.reeis.usda.gov/web/crisprojectpages/190198.html). Cry34/35Ab1 (Herculex® RW *Rootworm Protection*) are Bt proteins expressed simultaneously in corn to protect against damage to the roots by the larvae of the coleopteran western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) (Moellenbeck et al., 2001). Neither Cry1F nor Cry34/35Ab1 has been widely evaluated for the potential to control stored-grain insects. The research detailed here provides some insight into the activity of these proteins for the management of destructive coleopteran and lepidopteran stored-grain insects.

2. Materials and methods

2.1. Insect colonies and bioassays

Insects were obtained from stock cultures maintained at the Grain Marketing and Production Research Center in Manhattan KS and included: the Angoumois grain moth, *Sitrotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae); the red flour beetle, *Tribolium castaneum* (Herbst) and yellow mealworm, *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae); the Indian mealmoth, *Plodia interpunctella* (Lepidoptera: Pyralidae); the warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae); the flat grain beetle, *Cryptolestes pusillus* (Schoenherr) (Coleoptera: Laemophloeidae); the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae); the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae); and the rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae). *P. interpunctella* larvae were reared on a cracked wheat diet. *S. oryzae* and *R. dominica* were reared on hard red winter wheat. *T. castaneum* and *T. molitor* were reared on 95% wheat flour mixed with 5% brewer's yeast; the diet for *T. molitor* was mixed 1:1 with rolled oats. *O. surinamensis* and *C. pusillus* were reared on 90% rolled oats, 5% brewer's yeast, and 5% wheat germ. *T. variabile* were reared on 50% rolled oats and 50% powdered dog food.

Insects that were tested with Cry1F included *S. cerealella*, *T. castaneum*, *C. pusillus*, and several colonies of *P. interpunctella*. *P. interpunctella* colonies included those that are susceptible (designated RC688 and EP) and resistant (designated RC688-HD198 and EP-Dpl500) to Bt toxins and formulations. The resistant colony RC688-HD198 was selected from the parental RC688 colony with Bt subspecies *entomocidus* (HD198), resulting in approximately 100-fold resistance to the selection toxins (Oppert et al., 2000). EP-Dpl500 was selected from the parental EP, with 500 mg Dipel (Bt subspecies *kurstaki* HD-1) per kg diet. This colony was not evaluated for resistance levels to the selection toxins, but we have successfully selected individuals from EP-Dpl500 with up to 10,000 mg/kg Dipel (unpublished data). Both Bt-resistant *P. interpunctella* colonies were maintained in the laboratory on the selection diet.

Insects tested with Cry34/35Ab1 included *C. pusillus*, *O. surinamensis*, *R. dominica*, *S. cerealella*, *S. oryzae*, *T. castaneum*, *T. molitor*, and *T. variabile*.

[1] HERCULEX is a trademark of Dow AgroSciences LLC; DIPEL is a trademark of Valent Biosciences Corporation.

2.2. Toxins and formulations

Our Dipel source was a wettable powder formulation of Dipel 2X (formerly Abbott Laboratories, now distributed by Valent USA, Walnut Creek, CA). Cry1Ab was obtained from an *Escherichia coli* ECE54 (Cry1Ab) stock culture provided by the *Bacilllus* Genetic Stock Center, The Ohio State University, Columbus, OH, according to a recommended protocol (Zeigler, 1999) and as previously described (Li et al., 2005). Cry1Ab protoxin was trypsin-activated and purified as previously described (Li et al., 2004b); activated Cry1Ab toxin was used in bioassays. Cry1Ac was obtained from Bt subspecies *kurstaki* HD-73 as previously described (Oppert et al., 1997); Cry1Ac protoxin was used in the bioassay. Cry34/35Ab1 and Cry1F protoxins were supplied as powders by Dow AgroSciences (DAS).

For bioassays, Cry1Ab and Cry1Ac were estimated from previous bioassays (Oppert et al., 1997; Li et al., 2004b). DAS Bt proteins (10 μg per well) were evaluated in 10% Bis–Tris gels using MES buffer during electrophoresis (Invitrogen, Carlsbad, CA). Gels were Coomassie-stained, and the amount of protein in each sample was estimated by densitometry (Fig. 1). Cry1F protoxin was estimated as 60% of the powder dry weight; the binary toxin Cry34/35Ab1 was estimated to be essentially 100% of the powder. These estimations were used in toxin dosage calculations for bioassays.

2.3. Insect bioassays

For *P. interpunctella*, the bioassay diet consisted of disks from a flattened cereal mixture, as previously described (Herrero et al., 2001). Diet disks were treated with different doses by applying approximately 5 μl of solution to the disk using a micropipettor, allowing solutions to completely absorb into the disk. Dilutions were made of Dipel, Cry1Ac or Cry1F protoxin suspensions, or Cry1Ab toxin and control (water), $n = 16$, in triplicate. Treated diet disks were placed in 16-well black assay trays (Bio-Serv, Frenchtown, NJ), and eggs were added to each well (16 individuals per dose). Trays were covered with perforated adhesive plastic sheets and incubated at 28°C and 75% relative humidity (RH) in darkness. Results were reported as the LD_{50} in μg of toxin per 4 mm (15 mg) diet disk, with 95% confidence intervals.

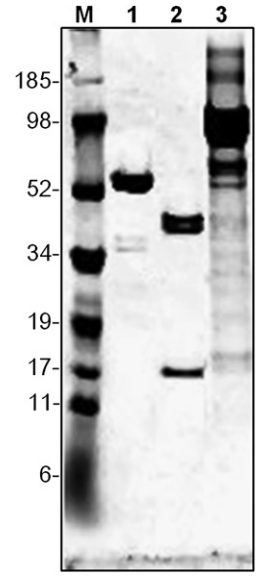

Fig. 1. Coomassie-stained SDS-PAGE of DAS toxin preparations used in bioassays of storage pests. Lane 1, Cry1Ab toxin; lane 2, Cry 34/35; lane 3, Cry1F protoxin; M: multimark molecular mass markers (Invitrogen).

For bioassays with *C. pusillus*, *T. castaneum*, *O. surinamensis*, *T. molitor*, and *T. variabile*, protoxin powders were mixed with a diet consisting of 20% glucose, 20% wheat germ, 30% amylopectin, and 30% brewer's yeast at a dose of 1%. The diet was equilibrated at 28°C and 75% RH before infestation with insects. Aliquots of 3–15 mg of treated or control diets were added to individual 0.2 ml microcentrifuge tubes, and eggs or neonates ($n = 5–15$) were added. Each species was monitored for various biological parameters, as indicated in Tables 2 and 3.

R. dominica and *S. cerealella* feed internally on wheat. For bioassays, 0.5–1% protoxin was mixed with 20% glucose, 20% wheat germ, 30% amylopectin, and 30% brewer's yeast, and 6–15 mg of treated or control diet was compacted to the bottom of 0.2 ml microcentrifuge tubes and equilibrated as previously described. Individual eggs ($n = 8–15$) were added to each tube, and developmental time and adult emergence were monitored.

For *S. oryzae*, the diet consisted of 80% corn starch, 10% casein, 7.5% brewer's yeast, 2.5% wheat germ, and less than 1% additional additives (corn oil, cholesterol, methyl *p*-hydroxybenzoate, sorbic acid); treated diets also contained 1% Cry34/35Ab1. To individual 0.2 ml microcentrifuge tubes, 30 mg of compacted treated or control diet was added and equilibrated at 28°C and 75% RH Individual eggs ($n = 10–11$) were added to each tube, and adult emergence was monitored.

2.4. Statistical analyses

Means were compared by Fully Factorial MANOVA with diet treatment as the factor and data on individual larval weight as the dependent variable. Statistical significance was determined by the Tukey HSD multiple comparison test (Tukey, 1951). For insect bioassays with multiple doses, POLO-PC was used for probit analyses (Robertson et al., 1980). The Fisher exact test was used to test for significant differences among mortalities ($n = 16$).

3. Results

The efficacy of Cry1F and Cry34/35Ab1 was evaluated in a number of stored-products pests. Various parameters were measured to determine the effects of toxins on different lepidopteran and coleopteran pests, such as mortality, growth inhibition, and/or development time. Appropriate parameters were selected for each stored-grain pest based on the feeding behavior and basic biology of each insect, i.e., some insects are amenable to weighing, whereas others are internal feeders and/or more sensitive to handling. While all experiments were replicated, a few species were characterized using only a single successful bioassay.

3.1. Bioassays with Dipel and Cry protoxins/toxins

Cry1F protoxin was tested with Bt-susceptible and -resistant *P. interpunctella* colonies, and responses were compared to bioassays with Cry1Ab toxin and Cry1Ac protoxin, as well the formulation Dipel (Table 1). Bt-susceptible *P. interpunctella* colonies included RC688 and EP, and Bt-resistant colonies were RC688-HD198 and EP-Dpl500, selected with Bt subsps. *entomocidus* HD198 and *kurstaki* HD1 (Dipel), respectively. There are some differences in the crystal (Cry) proteins found in the Bt formulations used in the selection experiments. HD198 contains Cry1Aa, Cry1Ab, Cry1C, and Cry1D toxins (Zeigler, 1999); Dipel is a formulation of HD1, which contains Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ia, Cry2Aa, and Cry2Ab toxins (Crickmore et al., 2009). Therefore, unique toxins in each formulation may result in distinct responses and select for a different resistance mechanism(s).

Overall, *P. interpunctella* was more sensitive to Cry1Ab toxin or Cry1Ac protoxin than to either Cry1F protoxin or Dipel. RC688 larvae were 2.5 to 10-fold more susceptible to Cry1Ab toxin and Cry1Ac protoxin than EP larvae. RC688 larvae were 169-fold less susceptible to Dipel and 1038-fold less susceptible to Cry1F than Cry1Ab toxin and Cry1Ac protoxin; EP larvae were 60–240-fold less susceptible to Dipel and 192–765-fold less susceptible to Cry1F than Cry1Ab toxin and Cry1Ac protoxin.

Although Bt-resistant RC688-HD198 were 10.1-fold resistant to Dipel, they were more resistant to individual toxins, displaying 66.0-fold resistance to Cry1Ab toxin and 237-fold resistance to Cry1Ac protoxin. Although Cry1Ac was not in the selection formulation (Bt subsp. *entomocidus* HD198), the fact that RC688-HD198 was more resistant to protoxin than toxin can be explained by the loss of a major trypsin-like activity critical to Cry protoxin activation in RC688-HD198 larvae (Oppert et al., 1994, 1996, 1997). While stunting of RC688-HD198 insects was observed at Cry1F protoxin doses >50 μg, mortality was less than 40% up to the highest dose tested, 700 μg, and a resistance ratio was not determined. RC688-HD198 insects were most susceptible to Dipel (10.1-fold), possibly due to additional protoxins in HD1 not found in HD198 (Cry1Ia, Cry2Aa, Cry2Ab), and suggesting that at least some of these unique toxins may be activated with enzymes different from those involved in Cry1A protoxin activation.

In contrast, the *P. interpunctella* colony EP-Dpl500, selected with Dipel, was more resistant to Dipel than Cry1Ab toxin and Cry1F protoxin. While approximately 60% of EP-Dpl500 insects died at 60 μg of Dipel, higher doses resulted in lower mortality, suggesting behavioral avoidance. Of the individual Cry proteins, the colony was most resistant to Cry1Ac, similar to RC688-HD198. However, the resistance ratio was lowest with Cry1F protoxin (18-fold).

Cry1F was also tested at 1% with *C. pusillus* and *T. castaneum* (Table 2). With *C. pusillus*, the development period was similar for the control and treatment, and no mortality was observed. With *T. castaneum*, the larval weights were similar in each replicate, and mortality was not significantly different in the control or treated larvae. Therefore, Cry1F had no effect on the coleopteran species tested.

There were significant problems encountered in *S. cerealella* bioassays, and no method or diet was judged satisfactory for routine screening of compounds with this insect species. The mortality in the Cry1F treatments was not significant (according to the Fisher exact test), but this may have been due to the relatively high control mortality and the small number of larvae per treatment (12–15) (Table 2). Treatment with 0.90% Cry 1F caused a significant delay in development, and no pupae developed. Although promising, further work is needed to determine whether Cry1F has activity in *S. cerealella*.

3.2. Bioassays of Cry34/35Ab1

Cry34/35Ab1 was tested at 0.9 or 1% with larvae of *R. dominica*, *O. surinamensis*, *C. pusillus*, *T. castaneum*, *T. molitor*, *S. oryzae*, *T. variabile*, and *S. cerealella* (Table 3). In assessing the effect of Cry 34/35 on weight, mortality, and/or development time of these larvae, there were no overall reproducible and statistically significant differences observed between control and treated coleopteran larvae. In one trial with *T. castaneum*, there was significant larval mortality ($P = 0.08$) and no male survivors when larvae were fed 1% Cry34/35Ab1, however, this was not reproduced in the second trial. With *O. surinamensis* and *S. oryzae* there were problems with control mortality, but the data suggested that Cry34/35Ab1 affected neither mortality nor development time. Therefore, Cry34/35Ab1 had no reproducible effects on the coleopteran species tested. Significant larval mortality occurred with 0.90% Cry34/35Ab1 in

Table 1
Effect of toxin crystals Cry1Ab, Cry1Ac, and Cry1F, as well as the formulation Dipel®, on *Plodia interpunctella* colonies, as measured by the LC_{50} (μg per 15 mg diet disk) and resistance ratios (RR), with 95% confidence intervals in parentheses.

P. interpunctella colony	Cry1Ab toxin	Cry1Ac protoxin	Cry1F protoxin	Dipel
RC688	0.008 (0.003–0.015)	0.008 (0.002–0.021)	8.30 (5.70–13.2)	1.35 (0.36–2.45)
RC688-HD198	0.51 (0.21–0.92)	1.90 (1.12–3.16)	>700	13.6 (7.45–21.0)
Resistance ratio	66.0 (31.0–140)	237 (82.1–686)	UD[a]	10.1 (4.22–24.2)
EP	0.02 (0.01–0.03)	0.08 (0.05–0.12)	15.3 (7.31–25.4)	4.80 (3.60–10.3)
EP-Dpl500	2.37 (0.96-3.57)	>250	280 (161–831)	UD
Resistance ratio	124 (66.0–230)	UD	18.0 (8.00–44.0)	UD

[a] UD = unable to determine because of insufficient mortality at the highest dose tested.

bioassays with *S. cerealella* ($P = 0.03$), but there was no delay in pupation in survivors (data not shown).

4. Discussion

With the loss of fumigants and sprays due to resistance or increasing regulatory scrutiny, the development of new control products for storage pests is critical. The efficacy of Cry1A toxins against lepidopterans, including major moth pests in stored products, has been well-documented (reviewed in Ferré and Van Rie, 2002). However, the ecology of storage pests, found in confined spaces, suggests a propensity for resistance. In fact, the first reported case of resistance to Bt products was from *P. interpunctella* collected from farm storage bins (McGaughey, 1985). In the present study, the resistant colony EP-Dpl500 was selected from insects collected in seed storage with a history of control failure with Bt products. The parental colony, EP, is approximately 2–10-fold less susceptible to Cry1A toxins and Dipel than RC688, a Bt-susceptible colony that has been reared in the laboratory since 1988. The potential for resistance development to Bt in lepidopteran storage pests coupled with the low efficacy of Bt products in many coleopterans present major hurdles for the effective use of Bt to control storage pests.

In the present study, Cry1F protoxin was less active than Cry1Ab toxin or Cry1Ac protoxin against Bt-susceptible *P. interpunctella* in most cases. However, Cry1F transgenic corn expressing full-length protoxin was demonstrated to affect the development and survival of Bt-susceptible and Dipel-resistant Kentucky and Kansas colonies of *P. interpunctella* (http://www.reeis.usda.gov/web/crisprojectpages/190198.html). It is possible that transgenic grain can be more potent to *P. interpunctella* because of intrinsic corn proteins, such as enzyme inhibitors. Alternatively, geographically distinct populations of *P. interpunctella* may have differences in Cry toxin response. More studies are needed to determine if the intrinsic potency of Cry1F combined with adequate expression in grain produces useful levels of activity against lepidopteran stored-product pests. In this study, cross resistance to Cry1F was detected in the two populations of *P. interpunctella* resistant to Dipel and Cry1Ab, and so Cry1F protoxin may be unsuitable for use against Bt-resistant *P. interpunctella*. It is less clear, and it was not evaluated in these studies, whether combinations of Bt or other insect control proteins can delay the onset of resistance in susceptible populations of *P. interpunctella* or other lepidopteran stored-grain pests.

Proteinase-mediated resistance in Bt-resistant insects was first described in RC688-HD198 (Oppert et al., 1994, 1996, 1997). Resistance also was determined to be receptor-mediated in this strain, although the difference in Cry1Ab toxin binding affinity was much more dramatic in a Dipel-selected strain from the same colony (Herrero et al., 2001). In the previous study, RC688 and RC688-HD198 insects were 42- and 446-fold more susceptible to Cry1Ab toxin than protoxin. While these colonies were less susceptible to Cry1F protoxin than either Cry1Ab toxin or Cry1Ac protoxin, we did not evaluate activated Cry1F toxin, and we speculate that activated toxin may be more potent toward these insects.

The EP-Dpl500 colony was selected as a "high-level resistance" *P. interpunctella* colony. Resistance to Cry1Ab toxin in a previously characterized high-level resistance colony selected with Dipel was associated with a loss of receptor affinity for the toxin (Van Rie et al., 1990). Our hypothesis has been that colonies such as EP-Dpl500 demonstrating high-level resistance to Bt control products have major receptor alterations that contribute to resistance (Herrero et al., 2001). As previously noted, proteinase-mediated resistance has been associated with relatively lower (<250-fold) resistance levels (Oppert et al., 1997; Li et al., 2004a; Karumbaiah et al., 2007), and so the extreme lack of sensitivity to Cry1A protoxin/toxin and Cry1F protoxin toxin in EP-Dpl500 suggests a target site resistance mechanism.

In cross-resistant Cry1A/Cry1F *Plutella xylostella* (L.), a model was proposed whereby Cry1A toxins share a common midgut binding site with Cry1F (Ballester et al., 1999). This may also be true for *Ostrinia nubilalis* (Hübner), in which a Cry1Ab-resistant colony was demonstrated to have low-level resistance (<5-fold) to Cry1F (Siqueira et al., 2004). However, the high-affinity receptor for Cry1A toxins in *Heliothis virescens* Fab., a midgut cadherin, was not a receptor for Cry1F

Table 2
Effect of Cry1F on the larval weight and mortality of *Tribolium castaneum*, developmental time and mortality of *Cryptolestes pusillus*, and pupation and mortality of *Sitotroga cerealella*. Number of larvae (n) in parentheses. Data in the same column followed by the same letter are not statistically different as determined by the Tukey HSD multiple comparison (Tukey, 1951).

Insect Species	Trial	Dose (%)	Larval weight (mg)	Larval Mortality (%)	Developmental time (d)[b]	Pupation (%)[c]
C. pusillu	–	0	n/a[d]	0	23.7 ± 0.4 (15) a	n/a
		1		0	24.1 ± 0.4 (10) a	
T. castaneum	1	0	1.25 ± 0.08 (14) a	0	n/a	n/a
		1	1.19 ± 0.15 (10) a	0		
	2	0	1.41 ± 0.07 (9) a	0		
		1	1.27 ± 0.12 (9) a	10		
S. cerealella	–	0	n/a	29	n/a	70
		0.5		67		25
		0.9		62		0[e]

[b] Egg to adult.
[c] Percentage of survivors on day 36 after egg hatch.
[d] n/a, Data not available.
[e] Significantly different at $P = 0.03$ using the Fisher exact test.

Table 3
Effect of Cry34/35Ab1 on the larval weight and/or mortality, developmental time, and/or adult female and male weight of *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *C. pusillus*, *T. castaneum*, *Tenebrio molitor*, *Sitophilus oryzae*, *Trogoderma variabile*, and *S. cerealella*. Number of individuals (n) in parentheses. Significantly different data within trials are identified by superscripts described in the footnotes.

Insect Species	Trial	Dose (%)	Larval weight (mg)	Larval Mortality (%)	Developmental time (d)[a]	Adult female weight (mg)	Adult male weight (mg)
R. dominica	1	0	n/a[b]	0	26.4 ± 0.42 (8)	n/a	n/a
		1		0	27.3 ± 0.40 (8)		
O. surinamensis	1	0	n/a	38	23.4 ± 0.61 (5)	0.568 (1)	0.503 ± 0.020 (4)
		1		0	24.5 ± 0.20 (6)	0.558 ± 0.021 (4)	0.526 (1)
	2	0		13	19.4 ± 0.60 (7)	n/a	n/a
		1		29	19.8 ± 0.70 (5)		
C. pusillus	1	0	n/a	0	25.8 ± 0.43 (10)	0.242 ± 0.008 (4)	0.260 ± 0.009 (5)
		1		0	26.2 ± 0.51 (10)	0.240 ± 0.014 (5)	0.251 ± 0.006 (5)
	2	0		0	23.7 ± 0.40 (15)	n/a	n/a
		1		0	23.6 ± 0.41 (9)		
T. castaneum	1	0	1.82 ± 0.07 (9)[c]	0	33.3 ± 0.40 (9)	1.69 ± 0.04 (4)	1.70 ± 0.02 (5)
		1	1.96 ± 0.10 (5)	44[d]	32.6 ± 0.20 (5)	1.76 ± 0.03 (5)	0
	2	0	1.25 ± 0.08 (14)[e]	0	n/a	n/a	n/a
		1	1.16 ± 0.09 (10)	0			
T. molitor	1	0	3.69 ± 0.16 (11)[f]	8	n/a	n/a	n/a
		1	3.21 ± 0.47 (10)	22			
S. oryzae	1	0	n/a	33	n/a	n/a	n/a
		1		44			
	2	0		60			
		1		40			
T. variabile	1	0	2.80 ± 0.20 (5)[g]	0	n/a	n/a	n/a
		1	2.90 ± 0.30 (5)	0			
S. cerealella	1	0	n/a	29	n/a	n/a	n/a
		0.9		73[h]			

[a] Egg to adult.
[b] n/a, Data not available.
[c] Larval weight day-17 post egg hatch.
[d] Significantly different at $P = 0.08$ using the Fisher exact test.
[e] Larval weight day-11 post egg hatch.
[f] Larval weight day-23 post egg hatch.
[g] Larval weight day-21 post egg hatch.
[h] Significantly different at $P = 0.03$ using the Fisher exact test.

(Jurat-Fuentes and Adang, 2006). Furthermore, stacked transgenic cotton expressing both Cry1Ac and Cry1F (Phytogen 440W) was demonstrated to be effective in controlling heliothines (Siebert et al., 2008). Our data suggest that *P. interpunctella* has a response to Cry1A and Cry1F that is similar to *P. xylostella* and *O. nubilalis*.

Less is known about the molecular mechanism of toxicity of Cry34/35Ab1, but midgut sections from intoxicated *D. virgifera vergifera* were similar to those of lepidopteran insects intoxicated with Cry1A toxins, suggesting a similar mechanism (Moellenbeck et al., 2001). The sequences of Cry34 and Cry35 proteins are widely distributed in the environment (Schnepf et al., 2005). However, the toxicity of these toxins is dependent on each other, and they apparently target a narrow range of coleopterans, as the only susceptible insect to date is the rootworm.

Bioassays that examine host range are critical for Bt toxins to gain the greatest utility from toxins that are already being commercialized. Cry1F has only been commercialized for the control of foliar feeding lepidopteran pests and so lack of efficacy against coleopteran stored-product pests was not surprising. Cry34/Cry35 has been commercialized for the control of a narrow range of root-feeding *Diabrotica* spp. larvae, western, northern and Mexican corn rootworm. Based on this known specificity, it is expected that efficacy against stored-product pests from different coleopteran families would be unlikely, in agreement with our observations.

Collectively, these results highlight the high degree of primary target selectivity associated with both Cry1F and Cry34/35Ab1, and suggest that the potential for problems associated with toxicity in nontarget species is minimal. The effects of transgenic products on nontarget species have been a focus of researchers in the development of new insect control strategies. These data are important in the evaluation of transgenic products by regulatory agencies.

The utility for controlling stored-product insects with these Bt products is expected to be low based on the results of these studies. However, growth inhibition is an important feature in demonstrating toxicity. In the absence of acute effects, similar and related (or modified) toxins can be evaluated for an increase in activity, especially those that may be truncated to be partially or fully activated. Our observations that Cry1F caused larval stunting in *P. interpunctella* and mortality of *S. cerealella* larvae indicate that more studies may be warranted for these storage pests. Apparent activity of Cry34/35Ab1 against *S. cerealella* was not expected based on the known spectrum of activity (Coleoptera only) of this binary protein and warrants additional investigation to confirm the finding. Ideally, toxin combinations effective against both lepidopteran and coleopteran storage pests could be developed into granular or spray formulations for surface treatments or engineered into cereals to provide protection from damaging cereal pests.

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References

Ballester, V., Granero, F., Tabashnik, B.E., Malvar, T., Ferré, J., 1999. Integrative model for binding of *Bacillus thuringiensis* toxins in susceptible and resistant larvae of the diamondback moth (*Plutella xylostella*). Applied and Environmental Microbiology 65, 1413–1419.

Crickmore, N., Zeigler, D.R., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J., Bravo, A., Dean, D.H., 2009. *Bacillus thuringiensis* Toxin Nomenclature. http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt.

Cuperus, G., 1995. Why stored product integrated pest management is needed. In: Stored Product Management. Cooperative Extension Service, Oklahoma State University, p. 199. Circular Number E-912.

Ferré, J., Van Rie, J., 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. Annual Review of Entomology 47, 501–533.

Haines, C.P., 2000. IPM for food storage in developing countries: 20th Century aspirations for the 21st Century. Crop Protection 19, 825–830.

Herrero, S., Oppert, B., Ferré, J., 2001. Different mechanisms of resistance to *Bacillus thuringiensis* in the Indianmeal moth. Applied and Environmental Microbiology 67, 1085–1089.

Jurat-Fuentes, J.L., Adang, M.J., 2006. The *Heliothis virescens* cadherin protein expressed in *Drosophila* S2 cells functions as a receptor for *Bacillus thuringiensis* Cry1A, but not Cry1Fa toxins. Biochemistry 45, 9688–9695.

Karumbaiah, L., Oppert, B., Jurat-Fuentes, J.L., Adang, M.J., 2007. Analysis of midgut proteinases from *Bacillus thuringiensis*-susceptible and -resistant *Heliothis virescens* (Lepidoptera: Noctuidae). Comparative Biochemistry and Physiology 146B, 139–146.

Li, H., Oppert, B., Higgins, R.A., Huang, F., Zhu, K.Y., Buschman, L.L., 2004a. Comparative analysis of proteinase activities of *Bacillus thuringiensis*-resistant and -susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae). Insect Biochemistry and Molecular Biology 34, 753–762.

Li, H., González-Cabrera, J., Oppert, B., Ferré, J., Higgins, R.A., Buschman, L.L., Zhu, K. Y., Huang, F., 2004b. Binding analyses of Cry1Ab and Cry1Ac with membrane vesicles from *Bacillus thuringiensis*-resistant and -susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae). Biochemical and Biophysical Research Communications 323, 52–57.

Li, H., Oppert, B., Zhu, K.Y., Higgins, R.A., Huang, F., Buschman, L.L., 2005. Susceptibility of Dipel-resistant and -susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae) to individual *Bacillus thuringiensis* protoxins and toxins. Journal of Economic Entomology 98, 1333–1340.

McGaughey, W.H., 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. Science 229, 193–195.

Moellenbeck, D.J., Peters, M.L., Bing, J.W., Rouse, J.R., Higgins, L.S., Sims, L., Nevshemal, T., Marshall, L., Ellis, R.T., Bystrak, P.G., et al., 2001. Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nature Biotechnology 19, 668–672.

Oppert, B., Kramer, K.J., Johnson, D.E., MacIntosh, S.C., McGaughey, W.H., 1994. Altered protoxin activation by midgut enzymes from a *Bacillus thuringiensis* resistant strain of *Plodia interpunctella*. Biochemical and Biophysical Research Communications 198, 940–947.

Oppert, B., Kramer, K.J., Johnson, D., Upton, S.J., McGaughey, W.H., 1996. Luminal proteinases from *Plodia interpunctella* and the hydrolysis of *Bacillus thuringiensis* CryIA(c) protoxin. Insect Biochemistry and Molecular Biology 26, 571–583.

Oppert, B., Kramer, K.J., Beeman, R.W., Johnson, D., McGaughey, W.H., 1997. Proteinase-mediated resistance to *Bacillus thuringiensis* insecticidal toxins. Journal of Biological Chemistry 272, 23473–23476.

Oppert, B., Hammel, R., Throne, J.E., Kramer, K.J., 2000. Fitness costs of resistance to *Bacillus thuringiensis* in the Indianmeal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). Entomologia Experimentalis et Applicata 96, 281–287.

Robertson, J.L., Russell, R.M., Savin, N.E., 1980. POLO: a user's guide to Probit Or LOgit analysis. General Technical Report PSW-038. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Berkeley, CA, 15 pp.

Schnepf, H.E., Lee, S., Dojillo, J., Burmeister, P., Fencil, K., Morera, L., Nygaard, L., Narva, K.E., Wolt, J.D., 2005. Characterization of Cry34/Cry35 binary insecticidal proteins from diverse *Bacillus thuringiensis* strain collections. Applied and Environmental Microbiology 71, 1765–1774.

Sedlacek, J.D., Komaravalli, S.R., Hanley, A.M., Price, B.D., Davis, P.M., 2001. Life history attributes of Indian meal moth (Lepidoptera: Pyralidae) and Angoumois grain moth (Lepidoptera: Gelechiidae) reared on transgenic corn kernels. Journal of Economic Entomology 94, 586–592.

Siebert, M.W., Nolting, S., Leonard, B.R., Braxton, L.B., All, J.N., Van Duyn, J.W., Bradley, J.R., Bacheler, J., Huckaba, R.M., 2008. Efficacy of transgenic cotton expressing Cry1Ac and Cry1F insecticidal protein against heliothines (Lepidoptera: Noctuidae). Journal of Economic Entomology 101, 1950–1959.

Siqueira, H.A., Moellenbeck, D., Spencer, T., Siegfried, B.D., 2004. Cross-resistance of Cry1Ab-selected *Ostrinia nubilalis* (Lepidoptera: Crambidae) to *Bacillus thuringiensis* delta-endotoxins. Journal of Economic Entomology 97, 1049–1057.

Tukey, J.W., 1951. Quick and dirty methods in statistics, part II: simple analysis for standard designs. In: Proceedings of the 5th Annual Convention. American Society for Quality Control, pp. 189–197.

Van Rie, J., McGaughey, W.H., Johnson, D.E., Barnett, B.D., Mellaert, H.V., 1990. Mechanism of insect resistance to the microbiol insecticide *Bacillus thuringiensis*. Science 247, 72–74.

Zeigler, D.R., 1999. *Bacillus thuringiensis* and *Bacillus cereus*. In: Bacillus Genetic Stock Center Catalog of Strains, vol. 2. The Ohio State University, Columbus, OH. seventh ed.