

Toxicity of Different Serotypes and Toxins of *Bacillus thuringiensis* to Resistant and Susceptible Indianmeal Moths (Lepidoptera: Pyralidae)¹

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ABSTRACT Fifty-seven isolates of *Bacillus thuringiensis* (BT) known to be toxic to larvae of Indianmeal moths, *Plodia interpunctella* (Hübner), were tested for activity against an Indianmeal moth colony resistant to the HD-1 strain of BT. Twenty-one of the isolates, representing five of the eight serotypes tested, were active against the BT-resistant moths. Fifteen of the isolates, representing serotypes 4a, 4c (*kenyae*), 6 (*entomocidus*), 7 (*atizawai*), 9 (*tolworthi*), and 10 (*darmstadiensis*), had no significant toxicity toward house flies, *Musca domestica* L., indicating that their toxicity toward the BT-resistant Indianmeal moths resulted from differences in the structure, composition, or function of the spore-crystal complex and not from exotoxin contamination. Bioassays confirmed that the Indianmeal moths were resistant to spores and crystals but susceptible to β -exotoxin.

KEY WORDS Insecta, *Bacillus thuringiensis*, Indianmeal moth, insecticide resistance

THE CAPACITY for resistance to *Bacillus thuringiensis* (BT) has recently been discovered in populations of the Indianmeal moth, *Plodia interpunctella* (Hübner) (McGaughey 1985a). The practical significance of this resistance is unknown; however, low levels of resistance and erratic moth control have been observed in farm grain bins treated with BT (McGaughey 1985a,b). The physiological mechanism is also unknown, although the resistance is presumed to be toward the spore and δ -endotoxin complex rather than β -exotoxin, because the selection studies were done using Dipel (Abbott Laboratories, North Chicago, Ill.), a formulation that does not contain β -exotoxin.

We do not know whether the resistance of Indianmeal moth larvae to BT is specific toward the HD-1 isolate used in producing Dipel, or whether it is a more general response toward many or perhaps all BT strains. However, studies of Dulmage (1981) and collaborators have shown wide differences in the activity spectra of different BT isolates toward several pest insect species, including the Indianmeal moth. Moreover, Kinsinger et al. (1980) reported inconsistencies in the responses of three Indianmeal moth colonies toward several different BT isolates. These differential responses toward BT isolates suggest that the resistance phenomenon reported by McGaughey (1985a) could be specific for the HD-1 strain of BT, and that other strains

of BT active on Indianmeal moths might be useful for controlling the resistant insects.

Our study was done to determine whether any of a wide range of BT strains from several different serotypes and active on Indianmeal moths would control the BT-resistant strain. Tests were also done to confirm that the resistance was toward the spore-crystal complex and not to the β -exotoxin.

Materials and Methods

The Indianmeal moth strains used in this study were the resistant and susceptible strains of our colony 343, collected from infested wheat in Oklahoma in 1981. The resistant strain (343R) had been selected for BT resistance by rearing it on diet treated with Dipel at 62.5 mg/kg for 20 to 40 generations at the time of these tests. During this time the response (LD_{50}) of the strain to Dipel was relatively stable at ca. 1,500 mg Dipel per kg of diet. The susceptible strain (343S) had been reared continuously on untreated diet for 40 to 60 generations and its susceptibility (LD_{50}) to dipel was ca. 13 mg/kg. The colonies were reared and tested at 25°C and 60-70% RH on a cracked wheat larval diet as described by McGaughey (1985a).

Fifty-seven BT isolates, in addition to Dipel, were evaluated. These experimental formulations had been tested previously in our laboratory and were known to be active against BT-susceptible Indianmeal moths (unpublished data). They were produced by H. T. Dulmage, Cotton Insects Research Laboratory, Agricultural Research Service, USDA,

¹This paper reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

Brownsville, Tex., by submerged fermentation in medium B-4, a Proflo-based substrate, in shake flasks following procedures described by Dulmage (1971) and Dulmage et al. (1970). The dry formulations consisted of spores, crystals, lactose, insoluble fermentation residues, and in some of the formulations, residues of β -exotoxin. The preparations were designated by HD-number (isolate number in Dulmage's culture collection). The Dipel formulation contained 16,000 international units (IU) per mg.

The experimental isolates were first tested at doses of 50 and 500 mg/kg of diet against the resistant and susceptible insect strains. Stock suspensions containing 5 and 0.5 mg of formulation per ml were prepared for each isolate. These suspensions were added to three replicated 60-g samples of diet at 6 ml/60 g. Each treated sample of diet was divided equally into two glass jars, and each was infested with 50 eggs of the resistant or susceptible Indianmeal moth strains. Mortalities were calculated from the numbers of adults that emerged and were corrected for control mortality (Abbott 1925) in samples of diet treated with water.

Those isolates that appeared active against the resistant insect strain in the preliminary test were retested at 10 doses ranging from 1.95 to 1,000 mg/kg of diet to enable estimation of LD_{50} 's and slopes of the dose-mortality regressions. Stock suspensions containing 10 mg/ml and serial 1:2 dilutions were prepared for each isolate and mixed with 90-g samples of diet at a rate of 9 ml/90 g. Each treated sample was divided equally into four glass jars. Two jars (replicates) were infested with 50 eggs of the resistant Indianmeal moth strain and two with 50 eggs of the susceptible strain. Mortalities were calculated from the numbers of adults that emerged and were corrected for mortality in water-treated controls (Abbott 1925). Mortality data from the replicates were pooled and analyzed by the probit procedure of Finney (1971).

Isolates that were active against resistant Indianmeal moths were also screened for exotoxin contamination using house flies, *Musca domestica* L. Fifty milligrams of formulation was suspended in 72 ml of water and added to 28 g of fly larvae media (Purina; Purina Mills, Inc., St. Louis, Mo.) to yield a dose of 500 mg/kg. Three replicated 100-g samples were prepared and infested with 33 second-instar larvae. Mortalities were calculated from the numbers of adults that emerged and were corrected for mortality in untreated controls (Abbott 1925). A one-way analysis of variance on the arcsin transformed data was used to compare the mortalities for the experimental isolates with the mortality for Dipel.

Sensitivity of the Indianmeal moth strains to β -exotoxin was determined by bioassay against 1.8% w/w thuringiensin (ABG-6162A) obtained from Abbott Laboratories, North Chicago, Ill. This preparation was tested at 10 doses ranging from 0.35 to 180 mg (AI)/kg of diet. A stock suspension containing 1.8 mg (AI)/ml and serial 1:2 dilutions were

prepared and added to diet at 6 ml/60 g. Three replicate samples were treated at each dose and divided equally into two glass jars, each infested with 50 eggs of the resistant or susceptible Indianmeal moth strains. Mortalities were calculated and the data from the replicates were pooled and analyzed in the manner described for the experimental BT isolates.

Sensitivity of the Indianmeal moth strains to spores, crystals, and spore-crystal mixtures of an HD-1 strain isolated from Dipel (Bulla et al. 1977) was determined with similar bioassays. Spores and crystals were separated by renografin-water density gradient centrifugation (Sharpe et al. 1975). The crystals and spores + crystals were tested at 10 doses ranging from 0.78 to 400 mg/kg. Spores were tested at 10 doses ranging from 1.56 to 800 mg/kg. Stock suspensions containing either 3.6 or 7.2 mg/ml and serial 1:2 dilutions were prepared for each and mixed with 90-g samples of diet at a rate of 10 ml/90 g. Each treated sample was divided equally into four glass jars. Each of two jars was infested with 50 eggs of the resistant moth strain and two with 50 eggs of the susceptible strain. Mortalities were calculated and the data from the replicates were pooled and analyzed in the manner described for the experimental BT isolates.

Results and Discussion

The results of the initial comparison of the activity of 57 BT isolates against the resistant and susceptible strains of Indianmeal moths are summarized in Table 1. For most of the isolates it is most informative to compare the mortalities of the two insect strains at the lower dose. The upper dose was usually much higher than required to kill all of the susceptible insects. Dipel, which can be considered the standard for comparing the responses of the two insect strains, killed ca. 30 times as many insects from the susceptible strain as the resistant strain. Among the experimental isolates, the responses of the two insect strains ranged from a difference of about 100-fold to nearly equal. For about one-third (21) of the isolates, the difference in response of the two insect strains was ≤ 2.2 -fold (denoted in Table 1 by †). These active isolates were grouped in only a few serotypes. In serotype 7, subsp. *aizawai*, 10 of 14 isolates were active against the resistant strain; in serotype 9, subsp. *tolworthi*, 3 of 4 isolates were active; in serotype 10, subsp. *darmstadiensis*, 4 of 4 were active; in serotype 6, subsp. *entomocidus*, the single isolate tested was active; and in serotype 4a,4c, subsp. *kenyae*, both isolates tested were active. Conversely, in serotype 1, subsp. *thuringiensis*, only one of 12 isolates was active against the resistant strain. None of the 17 isolates of serotype 3a,3b, subsp. *kurstaki*, or the three isolates of serotype 5a,5b, subsp. *galleriae*, were active. It is not surprising that none of the *kurstaki* isolates was active against the resistant strain, because the strain was selected

Table 1. Mortality of susceptible (S) and resistant (R) strains of Indianmeal moths in diet treated at two doses with isolates of eight serotypes of *B. thuringiensis*

Isolate	Corrected % mortality ^a				S/R ratio ^b
	50 mg/kg		500 mg/kg		
	S	R	S	R	
Serotype 1 (<i>thuringiensis</i>)					
HD-2	93	5	100	100	18.6
HD-26	85	12	100	100	7.1
†HD-59	98	68	100	100	1.4
HD-96	52	6	100	22	8.7
HD-120	88	11	100	26	8.0
HD-260	94	14	100	72	6.7
HD-264	38	10	99	11	3.8
HD-271	78	8	100	24	9.8
HD-288	100	7	100	99	>14.3
HD-290	96	10	100	100	9.6
HD-300	28	3	100	9	9.3
HD-309	74	3	100	100	24.7
Serotype 3a,3b (<i>kurstaki</i>)					
Dipel	93	3	100	14	31.0
HD-1	99	9	100	39	11.0
HD-73	81	8	100	24	10.1
HD-87	98	1	100	92	98.0
HD-164	88	7	100	29	12.6
HD-191	84	3	100	13	28.0
HD-203	94	1	100	36	94.0
HD-231	96	5	100	31	19.2
HD-244	85	8	100	35	10.6
HD-263	100	5	100	28	>20.0
HD-267	84	1	100	13	84.0
HD-269	98	2	100	45	49.0
HD-270	99	5	100	46	19.8
HD-304	98	12	100	29	8.2
HD-306	20	51	91	28	(3.2)
HD-332	74	1	100	100	74.0
HD-336	19	2	89	3	(29.7)
HD-337	100	1	100	56	>100.0
Serotype 4a,4c (<i>kenyae</i>)					
†HD-291	13	8	67	85	1.6
†HD-293	91	74	100	100	1.2
Serotype 5a,5b (<i>galleriae</i>)					
HD-359	37	1	94	33	(2.8)
HD-360	6	0	91	11	(8.3)
HD-232	93	0	100	40	>93.0
Serotype 6 (<i>entomocidus</i>)					
†HD-198	77	68	99	98	1.1
Serotype 7 (<i>aizawai</i>)					
†HD-52	94	73	100	99	1.3
†HD-122	33	2	95	77	(1.2)
†HD-112	88	52	100	96	1.7
HD-128	81	17	100	78	4.8
†HD-133	96	68	100	100	1.4
†HD-134	92	49	100	99	1.9
†HD-137	91	56	100	98	1.6
HD-144	68	0	99	21	(4.7)
HD-248	65	5	100	43	13.0
†HD-249	100	65	100	100	(1.5)
HD-255	12	0	93	22	(4.2)
†HD-274	99	60	100	100	1.6
†HD-282	94	42	100	99	2.2
†HD-283	92	41	100	100	2.2
Serotype 9 (<i>tolworthi</i>)					
†HD-124	44	55	88	94	0.8
†HD-125	31	34	97	92	0.9
HD-285	94	6	100	31	15.7
†HD-301	100	51	100	100	2.0

Table 1. Continued

Isolate	Corrected % mortality ^a				S/R ratio ^b
	50 mg/kg		500 mg/kg		
	S	R	S	R	
Serotype 10 (<i>darmstadtensis</i>)					
†HD-146	8	9	99	99	1.0
†HD-147	24	29	100	100	0.8
†HD-199	25	21	100	100	1.2
†HD-499	10	4	100	99	(1.0)

^a Mortalities calculated from the numbers of adults that emerged, corrected for mortality in water-treated controls (Abbott 1925). Values are averages from three replicated samples, each infested with 50 insects.

^b Calculated from mortalities at 50 mg/kg rate; values in () calculated from mortalities at 500 mg/kg rate. Isolates with ratio of ≤ 2.2 are denoted by †.

with Dipel, which is produced from a member of this subspecies.

These 21 isolates active against both the resistant and susceptible insects were retested using a series of doses against each Indianmeal moth strain. The LD₅₀'s and slopes of the dose-mortality regressions for these bioassays are presented in Table 2. The data confirm the results of the preliminary test. All of the isolates were much more effective against the resistant strain than would be expected based upon the 140-fold difference in LD₅₀ of the two strains toward Dipel. Ten of the isolates produced LD₅₀'s in the two strains that differed <2-fold, for 17 isolates the difference was <5-fold, and for all the isolates the difference was <10-fold. One of the isolates, HD-291, was more active against the resistant strain than the susceptible one; however, its activity level against both strains was very low.

While this experiment demonstrated a high incidence of activity among different isolates toward the resistant Indianmeal moths, it provided no indication of the toxin(s) responsible. In Table 3 the responses of the two Indianmeal moth strains toward several BT toxins are summarized. The BT-resistant strain is obviously resistant to all components of the HD-1 spore- δ -endotoxin complex—spores, crystals, and spore-crystal mixture. However, no resistance is evident toward β -exotoxin. Thus, if β -exotoxin was present in any of the experimental isolates, it could be responsible for the activity of those isolates toward the resistant insect strain.

Because we recognized that some of the effective isolates in this study belonged to serotypes capable of producing β -exotoxin (deBarjac et al. 1966), we tested each of the 21 isolates for the presence of exotoxins using a house fly bioassay. Three of the isolates apparently contained large amounts of exotoxin because they produced 65–100% mortality of the house flies (Table 2). These were isolate HD-59 (serotype 1, *thuringiensis*), and isolates HD-147

Table 2. Dose-response of susceptible (S) and resistant (R) strains of Indianmeal moths and response of house flies to isolates of seven serotypes of *B. thuringiensis*

Isolate	IMM strain	<i>n</i> ^a	Slope ± SE	LD ₅₀ (95% FL) (mg/kg)	R/S ratio ^b	% house fly mortality at 500 mg/kg ^c
Serotype 1 (<i>thuringiensis</i>)						
HD-59	S	600	3.1 ± 0.5	8.8 (6.1-12.5)	10.1	100**
	R	500	5.4 ± 0.5	88.7 (81.9-96.2)		
Serotype 3a,3b (<i>kurstaki</i>)						
Dipel	S	2,400	2.4 ± 0.1	11.8 (11.0-12.6)	141.4	12
	R	3,500	1.1 ± 0.2	1,668.5 (953.0-3,858.6)		
Serotype 4a,4c (<i>kenyae</i>)						
HD-291	S	600	1.5 ± 0.1	902.2 (700.1-1,267.3)	0.5	1
	R	1,000	1.1 ± 0.2	493.5 (211.3-3,025.9)		
HD-293	S	700	2.2 ± 0.1	13.3 (11.7-15.1)	1.8	1
	R	900	1.8 ± 0.1	24.7 (21.5-28.3)		
Serotype 6 (<i>entomocidus</i>)						
HD-198	S	700	2.5 ± 0.2	29.1 (25.8-32.7)	1.2	0
	R	1,000	1.7 ± 0.1	34.0 (26.8-43.0)		
Serotype 7 (<i>aizawai</i>)						
HD-52	S	1,000	1.8 ± 0.2	52.4 (36.6-75.1)	1.5	16
	R	800	2.3 ± 0.2	81.1 (61.1-107.4)		
HD-112	S	800	2.2 ± 0.1	21.1 (18.6-23.9)	2.4	0
	R	800	2.3 ± 0.2	51.2 (39.5-66.0)		
HD-122	S	900	1.6 ± 0.1	52.1 (39.9-67.9)	1.2	28*
	R	900	2.1 ± 0.1	65.0 (57.1-74.0)		
HD-133	S	600	2.9 ± 0.5	5.5 (3.5-8.1)	5.2	0
	R	900	2.5 ± 0.2	28.8 (25.6-32.4)		
HD-134	S	700	2.3 ± 0.2	14.0 (10.5-18.6)	3.1	34**
	R	700	2.1 ± 0.2	43.9 (32.6-59.9)		
HD-137	S	900	2.3 ± 0.3	9.0 (6.1-13.0)	2.9	7
	R	800	1.8 ± 0.2	26.3 (18.4-37.7)		
HD-249	S	800	2.6 ± 0.3	12.7 (9.3-17.4)	3.5	2
	R	1,000	2.1 ± 0.3	44.3 (31.0-63.2)		
HD-274	S	800	2.6 ± 0.3	14.5 (11.3-18.5)	3.2	13
	R	800	2.9 ± 0.2	47.2 (42.3-52.7)		
HD-282	S	800	2.2 ± 0.1	16.4 (14.5-18.6)	2.2	16
	R	1,000	2.2 ± 0.2	36.6 (27.6-48.6)		
HD-283	S	800	2.3 ± 0.1	12.2 (10.8-13.8)	2.9	5
	R	1,000	1.7 ± 0.2	35.4 (23.2-53.5)		
Serotype 9 (<i>tolworthi</i>)						
HD-124	S	1,000	1.5 ± 0.2	49.7 (30.0-82.8)	1.3	3
	R	1,000	1.6 ± 0.1	63.7 (47.9-85.0)		
HD-125	S	900	1.6 ± 0.2	49.8 (30.1-93.3)	1.3	0
	R	800	1.5 ± 0.2	64.9 (38.8-105.0)		
HD-301	S	700	2.6 ± 0.3	6.2 (4.6-8.0)	9.4	20
	R	800	2.2 ± 0.2	58.4 (44.8-75.6)		
Serotype 10 (<i>darmstadiensis</i>)						
HD-146	S	1,000	2.0 ± 0.5	124.1 (54.8-305.1)	1.3	4
	R	700	2.9 ± 0.2	167.2 (150.0-186.5)		
HD-147	S	600	2.7 ± 0.5	9.2 (5.4-15.5)	8.1	78**
	R	900	2.0 ± 0.7	74.3 (10.6-1,180.8)		
HD-199	S	900	1.6 ± 0.4	97.6 (37.6-412.9)	1.4	30*
	R	600	4.0 ± 0.3	132.2 (120.4-145.0)		
HD-499	S	900	2.1 ± 0.7	111.6 (42.7-401.0)	1.2	65**
	R	700	3.0 ± 0.8	130.2 (60.8-303.5)		

^a Two replicate samples were tested at each dose using 50 insects/sample. Data were pooled (100 insects per dose) for probit analysis. Six replicates were done for Dipel, for a total of 300 insects per dose.

^b R/S ratio = LD₅₀ resistant strain ÷ LD₅₀ susceptible strain.

^c Mortalities calculated from the numbers of adults that emerged, corrected for mortality in water-treated controls (Abbott 1925). Values are averages from three replicates (nine for Dipel), each infested with 33 larvae. Means that are significantly different from the mean for Dipel are denoted by * (*P* < 0.05) or ** (*P* < 0.01).

Table 3. Dose-response of BT resistant and susceptible Indianmeal moths to β -exotoxin (thuringiensin) and to HD-1 spores, crystals, and spore + crystal mixture

Fraction	Resistant			Susceptible		
	n^a	Slope \pm SE	LD ₅₀ (95% FL) (mg/kg)	n^a	Slope \pm SE	LD ₅₀ (95% FL) (mg/kg)
Spores	500	2.0 \pm 0.6	292.2 (55.2-)	700	2.7 \pm 0.2	4.3 (3.8-4.8)
Crystals	700	1.9 \pm 0.3	126.6 (86.4-203.7)	700	2.7 \pm 0.2	4.6 (4.1-5.1)
Spores + crystals	1,000	1.4 \pm 0.2	83.3 (50.7-156.0)	500	3.4 \pm 0.6	1.4 (0.9-2.0)
Thuringiensin (ABG-6162A)	750	3.3 \pm 0.8	1.4 (0.6-3.0)	750	3.4 \pm 0.2	1.2 (1.1-1.3)

^a Two replicate samples (three for thuringiensin) were tested at each dose using 50 insects per sample. Data were pooled for probit analysis.

and HD-499 (serotype 10, *darmstadiensis*). Three others, HD-122 and 134 (serotype 7, *aizawai*) and HD-199 (serotype 9, *tolworthi*) produced lower house fly mortalities, but significantly greater mortality than Dipel. The heat stability of the exotoxin found in these six isolates is unknown since the samples were not heat treated before assay. For the remaining 15 isolates, representing serotypes 4a,4c (*kenyae*), 6 (*entomocidus*), 7 (*aizawai*), 9 (*tolworthi*), and 10 (*darmstadiensis*), house fly mortality was zero or not significantly different from that caused by an equal dose of Dipel, indicating that exotoxin was absent or present in only small amounts. Eleven of these 15 exotoxin-free isolates belonged to serotypes 7 (*aizawai*) and 9 (*tolworthi*), and most of them exhibited very good activity (i.e., had low LD₅₀'s) toward the resistant Indianmeal moths.

These data indicate that the Indianmeal moth resistance is specific toward unique constituents of the HD-1 type spore-crystal complex rather than general toward all BT crystal types. The activity of these isolates toward the resistant Indianmeal moth strain apparently arises from differences in the structure, composition, or function of constituents of their spore-crystal (δ -endotoxin) complex. Studies of the biochemical properties of these isolates in conjunction with studies of physiological changes in the gut of resistant larvae could elucidate the mechanisms of BT toxicity and resistance in Indianmeal moths and other insect species. Some of these isolates may also prove useful for controlling Indianmeal moth strains that are resistant to the HD-1 formulations of BT.

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