

## Resistance to *Bacillus thuringiensis* by the Indian Meal Moth, *Plodia interpunctella*: Comparison of Midgut Proteinases from Susceptible and Resistant Larvae

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Midgut homogenates from susceptible and resistant strains of the Indian meal moth, *Plodia interpunctella*, were compared for their ability to activate the entomocidal parasporal crystal protein from *Bacillus thuringiensis*. The properties of midgut proteinases from both types of larvae were also examined. Electrophoretic patterns of crystal protein from *B. thuringiensis* subspecies *kurstaki* (HD-1) and *aizawai* (HD-133 and HD-144) were virtually unchanged following digestion by either type of midgut homogenate. Changes in pH (9.5 to 11.5) or midgut homogenate concentration during digestion failed to substantially alter protein electrophoretic patterns of *B. thuringiensis* HD-1 crystal toxin. In vitro toxicity of crystal protein activated by either type of midgut preparation was equal toward cultured insect cells from either *Manduca sexta* or *Choristoneura fumiferana*. Electrophoresis of midgut extracts in polyacrylamide gels containing gelatin as substrate also yielded matching mobility patterns of proteinases from both types of midguts. Quantitation of midgut proteolytic activity using tritiated casein as a substrate revealed variation between midgut preparations, but no statistically significant differences between proteolytic activities from susceptible and resistant Indian meal moth larvae. Inhibition studies indicated that a trypsin-like proteinase with maximal activity at pH 10 is a major constituent of Indian meal moth midguts. The results demonstrated that midguts from susceptible and resistant strains of *P. interpunctella* are similar both in their ability to activate *B. thuringiensis* protoxin and in their proteolytic activity.

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### INTRODUCTION

Resistance to the biological insecticide, *Bacillus thuringiensis*, has been demonstrated in several populations of the Indian meal moth, *Plodia interpunctella* (McGaughey, 1985a, b). Other instances of resistance have occurred in the tobacco budworm, *Heliothis virescens* (Stone et al., 1989), and in the almond moth, *Cadra cautella* (McGaughey and Beeman, 1988). In all three cases, the resistant larvae develop

normally and do not deviate in growth rate, size, or appearance when challenged with the bacterium at levels toxic to susceptible strains. The mechanism of resistance is unknown and is the subject of the current investigation.

The entomocidal parasporal crystal of *B. thuringiensis* is acted upon by the digestive fluids of the alimentary canal when ingested by a susceptible larva. Specifically, the alkaline pH and the proteolytic activity of the fluid contained in the midgut of susceptible larvae are required for the solubilization and activation of toxic protein from intact crystals (Andrews et al., 1985; Whiteley and Schnepf, 1986). Once activated, the toxin interacts with the membrane of the midgut epithelial cells, which consequently

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swell, lyse, and cause the eventual death of the insect (Endo and Nishiitsutsuji-Uwo, 1980).

Since proteolysis plays a key role in the activation of the protoxin, we speculated that the observed resistance might result from a change in the level or type of proteinases responsible for protoxin activation in the gut of the resistant insects. To test this hypothesis, the toxicity of *B. thuringiensis* crystals activated by midguts from susceptible and resistant strains was determined using an insect cell bioassay. Products from midgut fluid digestion of crystals from several bacterial strains were analyzed by polyacrylamide gel electrophoresis. Also, electrophoretic and enzymatic analyses of midgut proteinases were performed and the major proteolytic activity of *Plodia* was partially characterized.

## MATERIALS AND METHODS

### Organisms

The origin and cultivation of Indian meal moth colonies 343-S (susceptible) and 343-R (resistant) have been described previously (McGaughey and Beeman, 1988). Colonies were maintained on a standard diet based on cracked wheat and supplements (McGaughey, 1985b), and exhibited a 140-fold difference in susceptibility to Dipel (Abbott Laboratories, North Chicago, Illinois) at the time these experiments were initiated (McGaughey and Johnson, 1987). Midguts from third and fourth instars were excised and stored briefly at 4°C. Accumulated tissue from five larvae was homogenized by hand in a tissue grinder with 0.1 ml of buffer (20 mM Tris-HCl, pH 7.8) or deionized water at 4°C until a uniform dispersion of the contents was achieved. The supernatant was collected following centrifugation and frozen at -60°C until further use.

*B. thuringiensis* was grown on YEG medium (Johnson, 1987), and spores and crystals were separated by density gradient centrifugation in sodium bromide (Ang and Nickerson, 1978). Purified crystals were

dissolved in 0.0135 N NaOH (pH 12.0) for 4 hr at room temperature with stirring. The dissolved protein was dialyzed overnight at 4°C against 20 mM Tris-HCl, pH 8.5, and 50 mM KCl. Proteolytic activation of protoxin protein was achieved by the addition of 0.5%  $\alpha$ -chymotrypsin (bovine pancreas, Sigma Chemical Co., St. Louis, Missouri) per milligram of protoxin protein in solution or by 0.01 to 0.02 ml of midgut homogenate (containing 0.01 to 0.04 mg protein), followed by incubation at 30°C for 2 hr.

### Protein Determination

Total protein was determined using the bicinchoninic acid assay at room temperature (Smith et al., 1985). Absorbances at 562 nm of 1:10 dilutions of the larval gut homogenates were compared with a standard curve constructed using bovine serum albumin as the standard protein. All assays were performed in duplicate.

### Electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Laemmli (1970) using a 9% separating slab gel unless otherwise noted. All samples were heated at 100°C for 2 min in the presence of 2% SDS and 5% 2-mercaptoethanol (2-ME). A 5% stacking gel was used to align sample proteins before entering the separating gel. Gels were maintained at 15°C during electrophoresis in a Buchler Poly-slab apparatus and were silver-stained (Sigma) following completion of the run.

Gels containing gelatin (0.1%) as substrate (both nondenaturing and SDS) (modified from Heussen and Dowdle, 1980) were also used for analyzing proteinases present in larval gut homogenates of *P. interpunctella*. Nondenaturing electrophoresis was conducted in 7.5 and 10% acrylamide gels using the method of Davis (1964). SDS-PAGE was performed as above, except that 2-ME was omitted to allow protein disulfide bonds to remain intact. After electrophoresis, SDS gels were washed briefly in 2.5%

(w/v) Triton X-100 to remove the SDS and incubated overnight in 0.1 M Tris-HCl, pH 7.8, at 37°C. Nondenaturing gels were transferred directly to Tris buffer for overnight incubation. The gels were then stained with Coomassie brilliant blue R-250 and destained briefly with methanol:acetic acid:water (40:10:50) followed by several hours in 5% acetic acid. Proteolytic activity was revealed as a zone of clearing in a dark blue background. Molecular weights were estimated by comparison to the relative mobilities of prestained molecular weight markers (Bethesda Research Laboratories).

#### *Insect Cell Cytotoxicity*

In vitro toxicity of *B. thuringiensis* crystal protein was bioassayed using cultured tissue cells from the spruce budworm, *Choristoneura fumiferana*, and the tobacco hornworm, *Manduca sexta* (Johnson and Davidson, 1984). The bioassay consisted of the measurement of ATP in treated cells using the luciferin-luciferase firefly enzyme complex. LC<sub>50</sub> values were determined by a probit analysis procedure from the Statistical Analysis System (SAS Institute, 1982).

#### *Midgut Proteinase Activity*

Quantitative assays for the level of proteolytic activity were performed using [<sup>3</sup>H]casein as the substrate (Murdock et al., 1987). For this assay bovine α-casein was labeled by reductive methylation using formaldehyde in the presence of [<sup>3</sup>H]NaBH<sub>4</sub> (Amersham/Searle, Des Plaines, Illinois). The labeled protein was separated from low-molecular-weight radioactive by-products by gel filtration chromatography on a short Sephadex G-25 desalting column. The radiolabeled casein was diluted to yield about 400,000 cpm · ml<sup>-1</sup> and the protein concentration adjusted to 2 mg · ml<sup>-1</sup> with unlabeled casein. The assay was performed by incubating a solution containing 0.05 ml of [<sup>3</sup>H]casein, 0.04 ml of

buffer, and 0.01 ml of the gut homogenate at 37°C for 20 min. The reaction was stopped by addition of 0.1 ml of 10% trichloroacetic acid. After standing on ice for about 20 min, the samples were centrifuged at 14,000 rpm for 5 min. An aliquot of the supernatant (0.175 ml) was pipetted into a scintillation vial containing 5 ml of cocktail and counted.

#### *pH Optimum*

The optimum pH for [<sup>3</sup>H]casein hydrolytic activity was determined using a universal buffer containing phosphoric, acetic, and boric acids formulated to provide constant ionic strength at all pH values (Frugoni, 1957). A [<sup>3</sup>H]casein stock solution was diluted in the buffer of appropriate pH and the proteinase assays were conducted as described above.

#### *Inhibition Assays*

Proteinase inhibitors obtained from Sigma were antipain, diisopropylphosphorofluoridate (DFP), L-epoxy-succinyl-leucylamino-(4-guanidino)-butane (E-64), ethylenediaminetetraacetic acid (EDTA), leupeptin, soybean trypsin inhibitor (STI), tosyl-lysine chloromethylketone (TLCK), and tosyl-phenylalanine chloromethylketone (TPCK). Each inhibitor was dissolved in either water or methanol at 10× the desired concentration. Inhibitor solution (0.01 ml) was added to 0.08 ml of pH 10 buffer and 0.01 ml of the larval gut homogenate. This mixture was vortexed and preincubated 10 min at 37°C. Remaining proteolytic activity was assayed at pH 10 using 0.01-ml aliquots of the preincubation mixture. Calculations of percentage inhibition were based on the appropriate control samples preincubated with either water or methanol.

## RESULTS

#### *Larval Toxicity*

The toxicity of purified crystals of *B. thuringiensis* subsp. *kurstaki* HD-1 separately

and in combination with spores was established for susceptible and resistant strains of *P. interpunctella* in an earlier study (McGaughey and Johnson, 1987). The difference in LD<sub>50</sub> for crystal toxicity toward the two types of larvae was 28-fold. When we compared this work with new data using activated toxin in which purified crystals were dissolved and activated with  $\alpha$ -chymotrypsin before administration to the larvae, we were surprised to observe a dramatic increase in the difference between LD<sub>50</sub> values for susceptible and resistant larvae (Table 1). The susceptible strain (343-S) exhibited increased sensitivity to chymotrypsin-activated toxin when compared with intact crystals, whereas the resistant strain (343-R) was slightly less sensitive. The variation in sensitivity represented a 253-fold difference in LD<sub>50</sub> values between susceptible and resistant *Plodia* strains when chymotrypsin-activated crystal protein was used, as compared to the previously mentioned 28-fold difference for intact crystals. These data suggested that the method of protoxin activation (either catalyzed by a purified proteinase such as chymotrypsin or by proteinases present in midgut extracts) could cause a substantial change in the toxic larval response.

#### Cytotoxicity

An alternative technique for measuring

crystal protein toxicity after activation by midgut extracts is by insect tissue cell bioassay (Johnson and Davidson, 1984). This method is more direct (toxin protein remains unaltered during administration) and is relatively specific for certain lepidopteran tissue cells. Unactivated protoxin is normally 10-fold less toxic to cultured insect cells than a comparable solution following treatment with chymotrypsin (see Materials and Methods). The release of activated crystal protein by protoxin proteolysis is essential for maximal toxicity toward insect cell lines. In Table 2, chymotrypsin activation of soluble protoxin resulted in approximately 3- and 17-fold greater toxicity toward cell lines of the spruce budworm and tobacco hornworm, respectively. Likewise, protoxin activation by midgut fluids from susceptible and resistant larvae also resulted in similar increases in toxicity toward both cell lines. Minor variations were evident between the level of toxin activation for the two midgut preparations when compared with chymotrypsin. However, the level of cytotoxicity with either crystal preparation treated with susceptible or resistant larval midgut proteinases was uniform, regardless of the cell type used for bioassay.

#### Electrophoresis of Midgut Digests of *Parasporal* Crystals

Electrophoretic patterns of crystal pro-

TABLE 1  
DOSE-RESPONSE OF SUSCEPTIBLE AND RESISTANT INDIAN MEAL MOTH LARVAE TO CRYSTALS AND DISSOLVED CRYSTAL PROTEIN OF *Bacillus thuringiensis*<sup>a</sup>

Fraction	Susceptible larvae		Resistant larvae		Ratio <sup>b</sup> R/S
	LD <sub>50</sub> (mg/kg)	Slope $\pm$ SE	LD <sub>50</sub> (mg/kg)	Slope $\pm$ SE	
Crystals <sup>c</sup>	4.6 (4.1-5.1)	2.7 $\pm$ 0.2	126.6 (86.4-203.7)	1.9 $\pm$ 0.3	28
Activated toxin	0.8 (0.6-1.0)	2.4 $\pm$ 0.3	207.1 <sup>d</sup>	3.5 $\pm$ 0.8	253

<sup>a</sup> Two replicate samples were tested at each dose using 50 insects per sample. Data were pooled for probit analysis. Bioassay conducted according to McGaughey and Johnson (1987). Values in parentheses are 95% confidence intervals.

<sup>b</sup> Ratio of LD<sub>50</sub> of resistant to susceptible larvae.

<sup>c</sup> From McGaughey and Johnson (1987).

<sup>d</sup> CI could not be calculated.

TABLE 2  
EFFECT OF SUSCEPTIBLE AND RESISTANT INDIAN MEAL MOTH LARVAL MIDGUT FLUIDS ON THE INSECT CELL CYTOTOXICITY OF CRYSTAL PROTEIN FROM *Bacillus thuringiensis* (HD-1)

Treatment	Toxicity (LC <sub>50</sub> ) <sup>a</sup>	
	Spruce budworm ( <i>Choristoneura fumiferana</i> )	Tobacco hornworm ( <i>Manduca sexta</i> )
Soluble protoxin	25.7 ± 6.7 (38) <sup>b</sup>	3.5 ± 2.0 (6)
Toxin activation by		
Chymotrypsin	9.8 ± 3.7 (100)	0.2 ± 0.2 (100)
Susceptible gut fluid	4.8 ± 1.8 (204)	0.9 ± 0.4 (22)
Resistant gut fluid	4.0 ± 0.8 (245)	1.0 ± 0.4 (20)

<sup>a</sup> μg toxin protein · ml<sup>-1</sup>. Mean values ± SE, n = 4.

<sup>b</sup> Values in parentheses are percentage toxicity relative to chymotrypsin-activated toxin.

tein that was digested by midgut fluid extracted from susceptible and resistant larvae were very similar (Fig. 1). When the amount of midgut fluid incubated with protoxin was varied, a gradation of proteolysis was visible ranging from complete activation to virtually none. The pattern of protein released during protoxin activation was unaltered when midgut fluids from either susceptible or resistant larvae were used. Specific proteolytic cleavage of the 130-kDa protoxin appeared consistent between

susceptible and resistant midgut homogenates. However, the quantity of activated toxin generated with a molecular weight of approximately 60–62 kDa did not appear to increase proportionally with extensive protoxin degradation, suggesting that the protoxin contributed little additional activated toxin upon proteolysis. Although the quantity of crystal protein applied to each sample well was constant, large sample volumes may have contributed to more diffuse protein bands in some of the lanes.

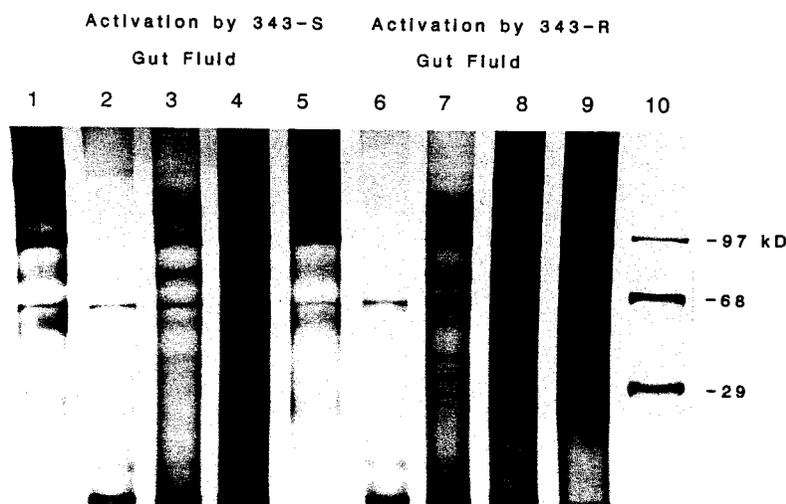


FIG. 1. SDS-PAGE of *Bacillus thuringiensis* HD-1 crystal protein activated with varying amounts of midgut fluid extracts from Indian meal moth larvae. Lane 1: protoxin; lanes 2-5: activation with susceptible larval midgut fluid (1, 0.1, 0.01, and 0.001 μg of fluid protein); lanes 6-9: activation with resistant larval midgut fluid (1, 0.1, 0.01, and 0.001 μg of fluid protein); lane 10: molecular weight standards (kDa).

### Comparison of *B. thuringiensis* Subspecies

Of two strains of *B. thuringiensis* subsp. *aizawai*, HD-133 can overcome the resistance in *P. interpunctella* 343-R and is effective toward larvae from this strain, while HD-144 is not as toxic (McGaughey and Johnson, 1987). Electrophoretic profiles of crystal proteins from these two *B. thuringiensis* strains activated by midgut fluid extracted from either susceptible or resistant Indian meal moth larvae are compared with *B. thuringiensis* subsp. *kurstaki* HD-1 in Figure 2. Unactivated protoxin from each species is also shown. Activated peptides produced by susceptible and resistant larval midgut fluids were uniform within species, but each subspecies contained unique toxin proteins. Although not readily apparent in Figure 2, HD-144 lacks a 130-kDa protein that is present in HD-133 (Han, McGaughey, Johnson, and Aronson, unpublished data) and may be responsible for the difference in activity between HD-133 and HD-144 toward resistant *Plodia* larvae. However, there appeared to be no substantial difference in the polyacrylamide gel

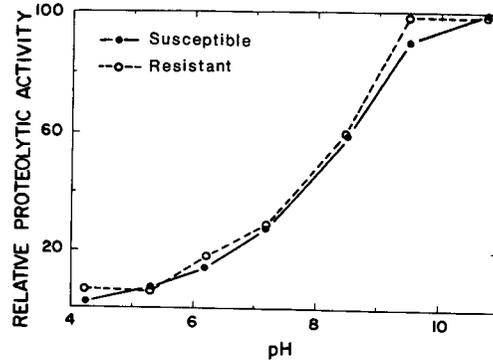


FIG. 3. Effect of pH on proteolytic activity in larval gut homogenates of *Bacillus thuringiensis* susceptible and resistant *Plodia interpunctella*. Aliquots of gut homogenates were assayed using [ $^3$ H]casein dissolved in universal buffer adjusted to the appropriate pH. Each point is the average of two determinations.

protein profile after activation of HD-133 and HD-144 protoxins by midgut proteinases from either susceptible or resistant larvae.

### Proteolytic Activity and pH of *P. interpunctella* Midguts

The effect of pH on the activity of *P. interpunctella* gut proteinases was mea-

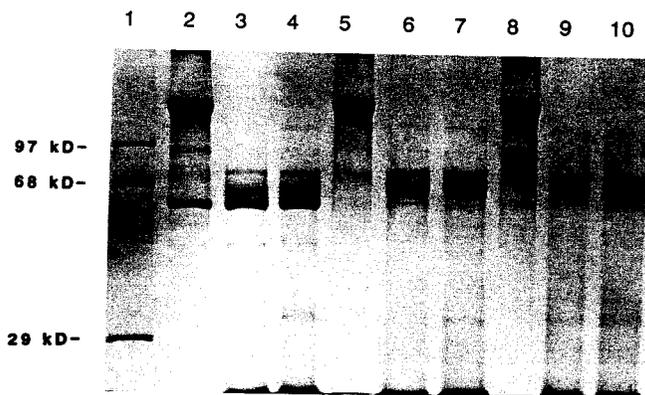


FIG. 2. SDS-PAGE of protoxin activation profiles from various serotypes of *Bacillus thuringiensis* using midgut fluid from susceptible and resistant Indian meal moth larvae. Acrylamide (8%); stained with Coomassie blue R-250. Lane 1: molecular weight standards (kDa). Lanes 2, 3, and 4: *B. thuringiensis* subsp. *kurstaki* HD-1 (2: protoxin; 3: susceptible midgut fluid activated; 4: resistant midgut fluid activated); lanes 5, 6, and 7: *B. thuringiensis* subsp. *aizawai* HD-133 (5: protoxin; 6: susceptible; 7: resistant); lanes 8, 9, and 10: *B. thuringiensis* subsp. *aizawai* HD-144 (8: protoxin; 9: susceptible; 10: resistant). Approximately 10  $\mu$ g of protein applied per well.

sured by two methods using both susceptible and resistant midgut homogenates. Varying the buffer pH from 9.5 to 11.5 during protoxin activation by larval midgut fluid had no effect on the electrophoretic protein patterns, whether fluid from susceptible or resistant larvae were used (gels not shown). When analyzed using radiolabeled casein as substrate, the gut proteinases from both larval strains responded similarly to changing pH, with the highest activity measured in the alkaline range up to pH 10.8 (Fig. 3).

Quantitative measurement of midgut proteolytic activity was performed with [<sup>3</sup>H]casein hydrolytic assays using susceptible and resistant strains of *P. interpunctella* at two different pH values (Table 3). Both protein content and proteolytic activity were measured using midgut homogenate tissue from pooled groups of susceptible and resistant larvae. No significant differences were observed in either total protein or proteolytic activity of the larval gut homogenates from the susceptible and resistant strains at either pH, although total protein and proteolytic activity were higher at pH 10 than at pH 7.8.

*Electrophoresis of Midgut Proteinases in the Presence of Gelatin*

Electrophoresis of gut homogenates using SDS-PAGE in the presence of gelatin revealed little or no difference in the mobilities of the major proteolytic enzymes from the susceptible and resistant strains (gels

not shown). Major activities in both midgut preparations were associated with proteins of approximate molecular weights 20 and 40 kDa. Minor bands of activity corresponding to apparent molecular weights as high as 200 kDa were also observed.

*Inhibitor Effects*

To characterize further the proteolytic activity present in the larval gut homogenates of *P. interpunctella*, we examined the effects of a series of proteinase inhibitors on [<sup>3</sup>H]casein hydrolysis (Table 4). The serine protease inhibitor DFP and soybean trypsin inhibitor were potent inhibitors of the *P. interpunctella* gut proteinases with estimated IC<sub>50</sub> values (concentration of inhibitor that produces 50% maximum inhibition, extrapolated from Table 4) of approximately 10<sup>-4</sup> and 10<sup>-5</sup> M, respectively. Other inhibitors effective against trypsin, such as TLCK, leupeptin, and antipain, were also quite effective toward the *Plodia* proteinases. The chymotrypsin inhibitor, TPCK, a sulfhydryl protease inhibitor, E-64, and a metalloprotease inhibitor, EDTA, had less effect on the larval gut proteolytic activity even at high concentrations, indicating that these types of proteinases are not major enzymes in *Plodia* midguts.

DISCUSSION

Although the initial electrophoretic analyses of midgut proteinases from susceptible and resistant larvae of *P. interpunctella* suggested that proteolytic activity might be

TABLE 3  
LARVAL GUT PROTEINASE ACTIVITY IN *Bacillus thuringiensis* SUSCEPTIBLE AND RESISTANT STRAINS OF *Plodia interpunctella*<sup>a</sup>

Strain	pH of assay	Net CPM released	Protein (mg · ml <sup>-1</sup> )	Net CPM released per milligram protein
Susceptible	7.8	1256 ± 128	1.3 ± 0.1	960 ± 193
Resistant	7.8	1139 ± 117	1.1 ± 0.1	1125 ± 179
Susceptible	10.0	2899 ± 408	1.4 ± 0.1	2340 ± 285
Resistant	10.0	2458 ± 422	1.5 ± 0.1	1849 ± 200

<sup>a</sup> [<sup>3</sup>H]casein used as substrate. Mean values ± SE, n = 5. Assays conducted at pH 10 in 0.1 M CAPS and at pH 7.8 in 0.1 M Tris-HCl.

TABLE 4  
EFFECT OF POTENTIAL PROTEINASE INHIBITORS ON  
[<sup>3</sup>H]CASEIN DIGESTION BY LARVAL GUT  
HOMOGENATES OF *Plodia interpunctella*

Inhibitor	Concentration (M)	Percentage inhibition
DFP	$1 \times 10^{-3}$	$96 \pm 1$
TLCK	$1 \times 10^{-2}$	$82 \pm 2$
TPCK	$1 \times 10^{-2}$	$32 \pm 0$
STI	$3 \times 10^{-5}$	$60 \pm 1$
E-64	$1 \times 10^{-3}$	$20 \pm 7$
Leupeptin	$2 \times 10^{-4}$	$78 \pm 2$
Antipain	$1 \times 10^{-3}$	$85 \pm 2$
EDTA	$1 \times 10^{-2}$	$1 \pm 1$

a resistance factor, additional studies revealed that there was little or no difference in activity discernable in polyacrylamide gel enzyme patterns or when [<sup>3</sup>H]casein was used as a substrate. While there was reasonably good agreement among individual larvae taken from a single group, gut proteolytic activity varied considerably from one cohort of larvae to the next. Other factors, such as optimum pH for proteolytic activity, gut pH, and osmotic balance (data not shown) of the gut homogenates, were also found to be similar in both susceptible and resistant strains. Some of the variation among groups of larvae may have resulted from the lack of good staging cues. Due to their small size, the maturity of larvae selected for these experiments was difficult to judge and the developmental time of each strain within the third and fourth stadia may have differed slightly.

Until recently, digestive proteinases of phytophagous insects were considered to be either trypsin-like or chymotrypsin-like (Law et al., 1977; Applebaum, 1985). However, investigation of the gut protease of the cowpea weevil, *Callosobruchus maculatus*, revealed a cysteine proteinase to be the major form (Gatehouse et al., 1985; Kitch and Murdock, 1986). Murdock et al. (1987) suggested that cysteine proteinases are used generally as digestive enzymes by the Coleoptera. In Lepidoptera, Baker (1986) concluded that the major digestive

protease in the larval gut of *P. interpunctella* was trypsin-like, based on chromogenic substrate hydrolysis. Results from this study support that conclusion. Strong inhibition by DFP, TLCK, antipain, leupeptin, and STI, but not by E-64, EDTA, or TPCK indicates the gut proteolytic activity in *Plodia* to be predominately that of a proteinase with an active site serine and histidine and with a substrate specificity similar to that of trypsin. Another general property of lepidopteran midgut proteinases is that maximal activity occurs at pH values equal to or greater than 10, which has now been demonstrated in *P. interpunctella* (this study), *H. zea* (Klocke and Chan, 1982), *Spodoptera litera* (Ahmad et al., 1976), and *Ostrinia nubilalis* (Houseman et al., 1989).

Many factors may be involved in the mechanism of insect resistance to *B. thuringiensis*. We have identified one resistant *Plodia* strain whose midgut proteinases are similar to the susceptible strain, indicating that proteinases do not play a major role in the resistance mechanism. However, midgut proteinases from different insect species have been reported to influence *B. thuringiensis* toxin specificity (Haider et al., 1986), and other naturally resistant insect larvae may possess proteinases that either inactivate or incompletely activate the parasporal crystal. Alternatively, the dramatic change observed in *Plodia* sensitivity to  $\delta$ -endotoxin could be the direct result of changes in the midgut tissue itself, leading to an alteration of the specific binding of toxic proteins. There is evidence for the presence of specific binding sites for the toxin in brush-border membranes of certain insect midguts (Hofmann et al., 1988a, b). These receptor sites appear similar to specific toxin-binding sites located on the surface of insect cells grown in culture (Knowles and Ellar, 1986; Haider and Ellar, 1987). These toxin-binding proteins or their abundance may undergo alteration during the development of resistance, such that those which occur in resistant strains no longer bind or recognize toxin with the

same proficiency as those present in susceptible strains. These possibilities are currently under investigation.

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