Histopathology and the lethal effect of Cry proteins and strains of *Bacillus thuringiensis* Berliner in *Spodoptera frugiperda* J.E. Smith Caterpillars (Lepidoptera, Noctuidae)

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Abstract

Among the phytophagous insects which attack crops, the fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera, Noctuidae) is particularly harmful in the initial growth phase of rice plants. As a potential means of controlling this pest, and considering that the entomopathogen *Bacillus thuringiensis* Berliner demonstrates toxicity due to synthesis of the Cry protein, the present study was undertaken to evaluate this toxic effect of *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73 on *S. frugiperda*. The following method was used. Both bacterial strains were evaluated in vitro in 1st instar *S. frugiperda* caterpillars, by means of histopathological assays. The Cry1Ab and Cry1Ac proteins, codified by the respective strains of *B. thuringiensis,* were evaluated in vivo by bioassays of 1st instar *S. frugiperda* caterpillars in order to determine the Mean Lethal Concentration (LC_{50}). The results of the histopathological analysis of the midget of *S. frugiperda* caterpillars demonstrate that treatment with the *B. thuringiensis thuringiensis* strain was more efficient, because the degradations of the microvilosities started 9 hours after treatment application (HAT), while in the *B. thuringiensis kurstaki* the same effect was noticed only after 12 HAT. Toxicity data of the Cry1Ab and Cry1Ac proteins presented for the target-species LC_{50} levels of 9.29 and 1.79 $\mu g.cm^{-2}$ respectively. The strains and proteins synthesised by *B. thuringiensis thuringiensis* and *B. thuringiensis kurstaki* are effective in controlling *S. frugiperda*, and may be used to produce new biopesticides or the genes may be utilised in the genetic transformation of *Oryza sativa* L.

Keywords: Bacillus thuringiensis, bioassays, histopathology, Cry proteins, *Spodoptera frugiperda*.

Histopatologia e efeito letal de cepas e proteínas Cry de *Bacillus thuringiensis* Berliner para lagartas de *Spodoptera frugiperda* J.E. Smith (Lepidoptera, Noctuidae)

Resumo

Entre os insetos fitófagos que atacam as culturas, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera, Noctuidae) destaca-se como uma praga polífaga que causa prejuízos na fase inicial da cultura do arroz. No seu controle, o entomopatógeno *Bacillus thuringiensis* Berliner revela-se tóxico devido à síntese de proteínas Cry. Nesse contexto, o objetivo deste trabalho foi avaliar a toxicidade das cepas e proteínas Cry de *B. thuringiensis thuringiensis* 407 (pH 408) e *B. thuringiensis kurstaki* HD-73 sobre *S. frugiperda*. As duas cepas bacterianas foram avaliadas, in vitro, em lagartas de 1º instar de *S. frugiperda*, através de ensaios de histopatologia. As proteínas Cry1Ab e Cry1Ac, codificadas pelas respectivas cepas de *B. thuringiensis*, foram avaliadas in vivo, através de bioensaios com lagartas de 1º instar de *S. frugiperda* para determinação da Concentração Letal Média (CL_{50}). Os resultados da análise histopatológica do intestino médio das lagartas *S. frugiperda* mostram que o tratamento com a cepa *B. thuringiensis thuringiensis* foi mais eficiente e a degradação das microvilosidade iniciou-se 9 horas após a aplicação dos tratamentos (HAT). Para *B. thuringiensis kurstaki*, o mesmo efeito foi observado, 12 HAT. Os dados de toxicidade das proteínas de Cry1Ab e Cry1Ac revelaram para a espécie-alvo uma CL_{50} de 9,29 e 1,79 $\mu g.cm^{-2}$, respectivamente. As cepas e proteínas sintetizadas por *B. thuringiensis thuringiensis* e *B. thuringiensis kurstaki* são eficientes no controle de *S. frugiperda*, e poderão ser usadas na produção de novos biopesticidas ou a utilização dos genes na transformação genética de *Oryza sativa* L.

Palavras-chave: Bacillus thuringiensis, bioensaios, histopatologia, proteínas Cry, *Spodoptera frugiperda*.

1. Introduction

Agricultural production has evolved, and scientific progress has laid the foundation for various technologies leading to increased agricultural production. Despite such progress, insects still destroy cultivated plants, many times jeopardising the production process (Gallo et al., 2002). Attacks by phytophagous pests cause significant decreases in the productivity of corn and irrigated rice crops. Among the phytophagous insects that attack these cultures, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera, Noctuidae) is considered one of the most harmful pests (Grützmacher et al., 2000) because it feeds on the young plants and, in irrigated rice plantations before the crops are flooded, it can reach high population levels and totally destroy the plants (Martins and Botton, 1998).

In the State of Rio Grande do Sul in Brazil, infestation by this pest is a serious concern for the producers who react by using preventative chemical pesticides that may create resistance to the synthetic molecules of the pesticides, and cause various environmental problems. As these environmental problems increase, researchers look for alternative methods of pest control capable of reducing the harmful effects of the chemical products, by rationalising the applications (Carlini and Grossi-de-Sá, 2002).

In the last fifty years, one of the alternatives to biological control that has achieved success in controlling various insect pests on a commercial scale is the use of the entomopathogen *Bacillus thuringiensis* (Schnepf et al., 1998).

Bacteria of the *Bacillus* genus have great potential use as biological control agents because they remain viable for long storage periods (Alves, 1998). *B. thuringiensis* is a Gram-positive bacterium that produces crystal inclusions of proteins during the sporulation made up of insecticidal Cry proteins (Van Rie et al., 1990; Hofmann et al., 1998). These crystals, when ingested by susceptible insects are dissolved under alkaline conditions in the midgut, and then broken into smaller fragments by proteases (Tojo and Aizawa, 1983; Schnepf et al., 1998; Shao et al., 1998; Bravo et al., 2007). Binding occurs due to the association of the activated toxin molecules with specific proteins located in the microvilosities of the epithelial cells in the midgut (Schwartz et al., 1997; Masson et al., 1999; De Maagd et al., 2003). The formation of a pre-pore oligomeric structure facilitates the insertion of the Cry proteins inside the membrane of the columnar cells and is important for the toxicity of the toxin. The formation of the structure has been demonstrated for the toxins, Cry1Aa, Cry1Ab, Cry1Ca, Cry1Da, Cry1Ea, Cry1Fa e Cry3, and the formation of the oligomeric structure of the toxins correlated with that of the pore (Bravo et al., 2007). Subsequently, the pore is formed (Masson et al, 1999) and the ion flux through that pore leads to cell lysis and the consequent death of the susceptible pest organisms (Schnepf et al.,1998; Monnerat and Bravo, 2000). When the toxin dosage fails to kill the insect, its cells are substituted allowing normal feeding to proceed and the recuperation of development of the insect (Spies and Spence, 1995). The midgut of the Lepidoptera is composed of pseudostratified epithelium, formed by three kinds of cells - columnar, globular (Cioffi, 1979) and regenerative -located on the base, between the columnar and the globular cells. These cells demonstrate mitosis activity before each change (Baldwin and Hakim, 1991; Engelhard et al., 1991).

Cry proteins are coded by different genes, whose classification is based on the similarity of aminoacid sequences (Crickmore et al., 1998). More than 350 Cry proteins genes have been cloned and sequenced in recent years from different strains of *B. thuringiensis* (Crickmore et al., 2009). However, the proteins of the Cry 1, Cry 2 and Cry 9 classes (Bravo et al., 1998) demonstrate the broadest insecticidal spectrum against the Lepidoptera order.

Various Cry toxins evaluated in recent studies may be active in *S. frugiperda*, for example: Buntin (2008), testing transgenic corn, and expressing the Cry1Ab and Cry1F proteins of *B. thuringiensis* in *S. frugiperda* and *Helicoverpa zea* Boddie, 1850, concluded that, while both reduced the infestation of the tested insects, Cry1F was more efficient – this result was also obtained by Siebert et al. (2008). Lima et al. (2008), utilizing the Cry2Ab protein, found a CL_{50} of 3.45 $\mu g.mL^{-1}$ for larvae of the 2º ínstar of *S. frugiperda*. Sivasupramaniam et al. (2008), testing transgenic cotton and expressing the Cry1Ac and Cry2Ab2 proteins, found that cotton plants expressing Cry1Ac isolation and those expressing both Cry1Ac and Cry2Ab2 were toxic for *Heliothis virescens* Fabicius, 1777, and *S. frugiperda*. The no-choice tests resulted in mortality levels of 20-69% in *S. frugiperda*.

In this context, the objective of this work is to assess the toxicity of the strains and Cry proteins of *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73 against the *S. frugiperda* armyworm.

2. Material and Methods

2.1. Insects

Spodoptera frugiperda caterpillars were collected in irrigated rice fields in Cachoeirinha/Rio Grande do Sul, Brazil by the Instituto Riograndense do Arroz – IRGA, in October 2004, and identified by Ms. Jaime Vargas de Oliveira. They were maintained in the laboratory, and fed with Poitout and Bues's diet (Poitout and Bues, 1970). The biological cycle was developed under controlled conditions (25 °C, 12-hour photophase and 70% Relative Humidity), in the Insect Breeding Room, Center 2, at the University of the Vale do Rio do Sinos - UNISINOS.

2.2. Bacillus thuringiensis

The *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73 strains were provided by the International Entomopathogenic Bacillus Centre, Pasteur Institute, Paris (France). The strains were grown in a standard glycolic media, at 180 rpm and 28 °C, for 48 hours (Debarjac and Lecadet, 1976). Subsequently, the bacterial suspension was centrifuged at 5000 rpm, and the cell count was made in a Neubauer Chamber

with an optical microscope, and in a concentration of 1.10^{10} cells.mL^{-1}.

In the preparation of the Cry proteins the cultivation of both strains was realised as previously described until a 90% cell lyses was obtained. The culture was centrifuged at 5000 rpm, at 5 °C, for 15 minutes, and the concentrate obtained was washed with phosphate buffer (0.1 M NaH_2PO_4. H_2O + 0.1 M NaCl, pH 6.0). Spore, crystal and cell trace separation was realised by applying the bacterial suspension to a sacarose discontinuous gradient (67-79%), that was centrifuged at 9500 rpm, 5 °C for one hour. The bands deposited among different concentrations of sacarose were collected, washed with mili-Q water and observed by phase contrast microscopy. Next, proteins were dissolved in a pH 10 phosphate buffer (50 mM Na_2CO_3, 10 mM DTT, 5 mM EDTA, 0.1 mM PMSF), as described by Fiuza et al. (1996). The Protein concentration was determined by the Bradford method (Bradford, 1976), and the profile was evaluated in SDS-PAGE, at 10% (Laemmli, 1970).

2.3. Histopathology assays

In the treatments with the strains of *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73, 100 µL of suspensions (10^{10} cells.mL^{-1}) were applied on the Poitout and Bues diet surface (Poitout and Bues, 1970), arranged on acrylic mini-plates. In the tests, 30 caterpillars of the 1st instar of *S. frugiperda* were placed individually on the acrylic mini-plates containing the treatments. In the control samples, the test elements were substituted by distilled and sterilised water. Treatments were maintained in an acclimatised chamber (25 °C, 12-hour photophase and 70% Relative Humidity).

In the histological assessments, the *S. frugiperda* tissues were prepared with the paraplast© inclusion techniques (Brandtzaeg, 1982) and after application of the treatment, the caterpillars were collected in periods of 1, 3, 6, 9, 12 and 24 hours. After fixation in *Bouin Hollande* sublimate for 24 hours, the tissues were submitted to dehydration in ethanol solutions in an increasing order of graduation, followed by rapid xylol baths and impregnation with paraplast. Longitudinal histological sections were made with a 5 µm thickness. To remove the paraplast the slides containing the tissues were passed through xylol and ethanol baths in a decreasing order of graduation. The *S. frugiperda* tissues were stained with Heidenhain's Blue. The slides were assembled with *Etellan* and glass cover slips. The longitudinal sections of the digestive system of the *S. frugiperda* caterpillars, corresponding to the treatments with the two strains of *B. thuringiensis* and the control, were observed using the comparative histology system and optical microscopy.

2.4. Bioassays

Cry1Ab and Cry1Ac proteins were tested in 1st instar caterpillars of *S. frugiperda*. For each protein, the assays were made up of five protein concentrations (0.06 at 610 µg.mL^{-1} and 0.1 at 1050 µg.mL^{-1}, for Cry1Ab and Cry1Ac, respectively) and a control, representing 6 treatments of 30 insects and 3 repetitions, for a total of 540 insects evaluated by protein. The treatments were applied (100 µL) on the Poitout and Bues diet surface (Poitout and Bues, 1970), previously arranged on acrylic mini-plates, where the caterpillars were individualised. In the controls the proteins were substituted by distilled and sterilised water. The test slides were maintained in an acclimatized chamber at 25 °C, 70% Relative Humidity, with a 12 hour photophase. Mortality was evaluated until the seventh day after the application of the treatment, and then corrected using Abbott's formula (Abbott, 1925). The mean lethal concentrations (LC_{50}) of each protein (Cry1Ab and Cry1Ac) at the target species was determined by Probit's Analysis, with the Polo-PC LeOra Software program, 1987 (Haddad, 1998). Data was processed by Variance Analysis and the Tukey test ($p < 0.05$) for comparison of the averages.

3. Results and Discussion

In the histopathological analysis of the midgut of *S. frugiperda* caterpillars treated with the *B. thuringiensis thuringiensis* 407 (pH 408) strain, structural changes were observed six hours after application of the treatment (HAT), where there were cells in the intestinal lumen and elongation of the microvilosities, as compared to the control. After nine applications of the treatment (HAT) the action was intensified with vacuolisation of the cytoplasm, and the beginning of the degradation of the peritrophic membrane – this was entirely absent after 12 HAT. Treatment with *B. thuringiensis kurstaki* HD-73 strain was similar, except that rupture of the microvilosities (BBMV's) and vacuolisation of the cytoplasm began at 12 HAT.

Comparing the two treatments it can be seen that the *B. thuringiensis thuringiensis* 407 (pH 408) strain was more active, because the beginning of the degradation of microvilosity occurred at 9 HAT compared with 12 HAT for *B. thuringiensis kurstaki* HD-73 (Table 1).

Various other works report the cellular changes produced in the midgut of larvae intoxicated with the Cry proteins of *B. thuringiensis*, such as: an increase in the volume of the epithelium cells, rupture of microvilosities, vacuolisation of the cytoplasm, changes in the organelles of the cytoplasm and cell hypertrophy (Griego et al., 1980; Mathavan et al., 1989; Bravo et al., 1992). Similar changes were observed in intoxications with Cry1Ac and Cry1D in *S. frugiperda* (Aranda et al., 1996), thus confirming the data reported in this study for the Cry1Ac protein synthesised by the *B. thuringiensis kurstaki* HD-73 isolate.

Knaak and Fiuza (2005) tested the nuclear polyhedrosis virus of *Anticarsia gemmatalis* Hübner, 1818, (VPN*Ag*) and *B. thuringiensis kurstaki* HD-1 (Dipel®) in 2nd instar caterpillars of *A. gemmatalis* (Lepidoptera, Noctuidae), and observed that when both entomopathogens are utilised simultaneously they are more efficient, because they caused alterations in the intestinal cells after 6 HAT while when used separately they produced the alteration only after 12 HATs. This data is similar to that obtained in the present study because the treatment with *B. thuringiensis kurstaki*

Table 1. Histopathology of 1st instar *Spodoptera frugiperda* (Lepidoptera, Noctuidae) treated with *Bacillus thuringiensis thuringiensis* 407 (pH 408) (*Btt*) and *Bacillus thuringiensis kurstaki* HD-73 (*Btk*) isolates.

Treatments		Changes in peritrophic membrane	BBMVs changes	Cels./ intestinal light projection	Larger cell turgor	Celular hypertrophy	Cyoplasm vacuolization	Rupture of the peritrophic membrane	BBMVs rupture
Control		-	-	-	-	-	-	-	-
Btt	1HAT	-	-	-	-	-	-	-	-
	3HAT	-	-	-	-	-	-	-	-
	6HAT	+	+	+	-	-	-	-	-
	9HAT	+	+	+	+	+	+	+	+
	12HAT	+	+	+	+	+	+	+	+
	24HAT	+	+	+	+	+	+	+	+
Btk	1HAT	-	-	-	-	-	-	-	-
	3HAT	-	-	-	-	-	-	-	-
	6HAT	+	+	+	-	-	-	-	-
	9HAT	+	+	+	-	-	-	+	-
	12HAT	+	+	+	+	+	+	+	+
	24HAT	+	+	+	+	+	+	+	+

(+) Presence of changes in the midgut (-) absence of changes in the midgut, HAT = hours after treatment application, BBMV = microvillosities

HD-73 also demonstrated alterations in the microvilosities after 12 HATs.

Loeb et al. (2001) tested increasing concentrations of *B. thuringiensis* AA 1-9 and HD-73 toxins and found that they induced reductions related to the total number of cells in the midgut of *Heliothis virescens*. After two days of exposure at 800 $\rho g.\mu L^{-1}$ de AA 1-9 e 600 $\rho g.\mu L^{-1}$ de HD-73, the columnar and the globular cells showed a 20% decrease when compared with the control, thus leading to the conclusion that HD-73 toxin was more efficient than the AA 1-9 toxins. In this paper, *S. frugiperda* data show that the cytotoxic activity of the above-mentioned strain (*B. thuringiensis kurstaki* HD-73) is slower than that of *B. thuringiensis thuringiensis* 407 (pH 408).

Rausel et al. (2000a) observed the vacuolisation of the cytoplasm and rupture of the microvilosities in *Lymantria monacha* Linnaeus, 1758 caterpillars exposed to Cry1A toxins, and for the Cry1Ac protein, as well as the effects mentioned, found in vitro tests disorganisation of the midgut and hypertrophy of the epithelium cells, which were loosened and ejected into the intestinal lumen. Furthermore, Baines et al. (1997), when testing Cry1A toxins of *B. thuringiensis* in Lepidoptera, stated that the collapse provoked in the electro-chemical gradient in the midgut epithelium causes the death of the insect, thus confirming the insecticidal activity of this class of Cry proteins against the lepidopteras.

During in vitro tests by Peyronnet et al. (1997) they used the Cry1Aa protein in *Lymantria dispar* Linnaeus, 1758, cells to cause a quick and irreversible depolarisation of the membrane, but the same effect was not observed with the Cry1Ab and Cry1Ac proteins, so that these two must be considered inactive against the Lepidoptera species. On the other hand, Lambert et al. (1996) have shown that the same Cry protein can present different effects for two species of the same insect order - for example, Cry1Ac was very toxic to *H. virescens*, but non-toxic to *Heliothis armigera* Hübner, 1808. Also, Aranda et al. (1996) suggest that the explanation for the different interactions between Cry1Ab and Cry1Ac is that the toxins interact with different receptors in *S. frugiperda*.

As for the effectiveness of the *B. thuringiensis* strain in the *S. frugiperda* midgut, the manner in which way the proteins bind to the receptor is relevant (Fiuza, 2004), because, according to Ferre et al. (1991), Lee et al. (1995a) and MacIntosh et al. (1992), it is the key factor of the specificity, toxicity and resistance of the insects to Cry proteins. Considering the two proteins used in this study, one should mention that an amino-peptidase N of 120kDA, located in the membrane of the intestinal epithelium of the Lepidoptera for Cry1Ac (Knight et al., 1994) and cadherin correspond to the receptor for Cry1Ab (Vadlamudi et al., 1993). These epithelium receptors of the midgut of Lepidoptera larvae can be shared by different Cry proteins (Ballester et al., 1999; Martinez-Ramirez et al., 1999), and alterations can make the insects resistant to these toxins (Lee et al., 1995; Schnepf et al., 1998). Estela et al. (2004) have shown that Cry1Aa, Cry1Ab and Cry1Ac compete for receptor sites on the membrane of the midgut of *H. armigera*, and that Cry1Ac and Cry1Ab use different epitopes to bind to the membrane. Competition experiments have shown that CrylAc and CrylBa share a binding site on the *C. suppressalis* membrane, and that this location is also used as a binding site for CrylAa

Table 2. Mean Lethal Concentration of Cry1Ab and Cry1Ac proteins of *Bacillus thuringiensis* to 1st instar *Spodoptera frugiperda* (Lepidoptera, Noctuidae).

Treatments	Proteins (μg.cm^{-2})		
	LC_{50}	CI (IL-UL)*	χ^2
Cry1Ab protein	9.29	4.34-28.46	1.59
Cry1Ac protein	1.79	0.96-3.76	1.08

*CI = Confidence Interval, estimated at 95% probability through Probit's Analysis; IL = Inferior Limit; UL = Upper Limit.

(Fiuza et al., 1996). Cry1Ab and Cry1Ac identify the same binding site on *Ostrinia nubilalis* Hübner, 1796 epithelium effectively competing for the same location (Denolf et al., 1993) and are closely related to each other sharing 84% of the amino acids (Lee et al., 1995a). This data can also justify the way that *B. thuringiensis* acts in the S. *frugiperda* midgut.

Aronson et al. (1999), examining the steps required to insert toxins into the membrane, and the possible formation of ionic channels, observed that the oligomerisation chain of the Cry1Ac toxin on the membrane of the *H. virescens* midgut was longer than that of *Manduca sexta* Linnaeus, 1763, and the binding would correspond directly to the toxicity. Furthermore, with reference to the action mode, Zhuang et al. (2002) report that the integrity of lipid pouches is related to the Cry1Ab activity and the consequent formation of the pores that lead to the death of the insect. In the *B. thuringiensis* action sequence, fixing the toxin onto the membrane is a necessary step for formation of the pore (Schnepf et al., 1998; Aronson and Shai, 2001).

In this research, data on the toxicity of Cry1Ab and Cry1Ac proteins, synthesised by *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73, respectively, showed a Mean Lethal Concentration (LC_{50}) of 9.29 and 1.79 μg.cm^{-2} to 1st instar of *S. frugiperda* caterpillars.

The data on CL_{50} shown in Table 2, and the respective confidence intervals, are regarded as dependable because, according to χ^2, the data obtained agrees with Probit's model, and the estimated value of χ^2 is smaller than the tabulated value (8.89), and is therefore, not significant.

Praça et al. (2004), selecting *B. thuringiensis* strains that are effective against 2nd - instar *S. frugiperda*, compared new strains to *B. thuringiensis kurstaki* HD-1 (LC_{50} de 0.285 μg.cm^{-2}), and obtained a LC_{50} of 0.09 and 0.52 μg. cm^{-2}, for S234 and S997 respectively, which showed the new S234 strain as the most toxic to *S. frugiperda*. In studies with Cry proteins, Aranda et al. (1996) determined that the LC_{50} for *S. frugiperda* of Cry1Ab and Cry1Ac proteins was higher than 2 μg.cm^{-2}, and therefore differ somewhat from the results of this study, wherein the Cry1Ac protein (1.79 μg.cm^{-2}) was significantly more toxic to the target species when compared to the Cry1Ab protein (9.29 μg. cm^{-2}). Rausell et al. (2000) have reported that when they analysed the larval development of *Thaumetapoea pityocampa* Denis & Schiffermuller, 1775 and *L. monacha*, the Cry1Ab and Cry1Ac toxicity decreased as the insect's age increased, although the loss of activity was more marked for Cry1Ab.

Meng et al. (2003) calculated the LC_{50} of Cry1Ac and Cry1Ab proteins from commercial products of *B. thuringiensis* for newly-born larvae of *Chilo suppressalis* (Walker) (Lepidoptera, Pyralidae), which were from 15 to 157 mg (AI)/L and 2 to 34 mg (AI)/L, respectively. On the other hand, however, Fiuza et al. (1996) when testing *B. thuringiensis* Cry proteins in 2nd instar *C. suppressalis* caterpillars, observed that the LC_{50} for Cry1Ac was 2.24 μg.cm^{-2}.

The present study concludes that the Cry1Ac protein was more toxic to the target insect than Cry1Ab. Waquil et al. (2002a, b) verified that corn cultures expressing the Cry1Ab and Cry1 proteins were moderately resistant to *S. frugiperda* reducing both the survival and the development of the larvae. In laboratory and field studies with eight corn hybrids evaluated for resistance to *S. frugiperda* and *Diatraea grandiosella* Dyar, 1911, Williams et al. (1997) concluded that the expression of the Cry1Ab toxin produced characteristics of high resistance to the *S. frugiperda* and practically conferred immunity of the *D. grandiosella*. In tests utilising cotton expressing the Cry1Ac protein, Sivasupramaniam et al. (2008) found mortality rates between 20-69% for caterpillars of the 2nd instar of *S. frugiperda*.

These results confirm that strains and proteins synthesised by *B. thuringiensis thuringiensis* 407 (pH 408) and *B. thuringiensis kurstaki* HD-73 are efficient in controlling *S. frugiperda*, and that Cry1Ac protein was the most effective. Furthermore, they can be used to make new biopesticides or the genes utilised to genetically transform the *Oryza sativa* L. in order to resist the *S. frugiperda* armyworm.

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