

FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

WORKSHOP:

“Desafios da Pesquisa em Controle Biológico na Agricultura no Estado de São Paulo”

Bactérias entomopatogênicas – *Bacillus thuringiensis*



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Prof. Adjunto 1 FCAV/UNESP

TOPICOS

- 1 – Introdução: Caracterizar o *Bt* como agente de controle biológico**
- 2 – Modo de ação do *Bt* e sua relação com pesquisas básicas**
- 3 – Diversidade de toxinas Cry x diversidade de *Bt* bioinseticidas**
- 4 – Mercado dos *Bt* bioinseticidas: status, limitações e perspectivas**



Professor Shigetane Ishiwata
(1868-1941)

大日本醫學會報第百拾四號

論 論 説

● 創烈なる一種の軟化病(卒倒病)に就て(第一)

大日本醫學會技術委員農學士 石 普 雅

得たる實に就くべく、試に之れが爲めに焚れたる醫見の體操と桑葉に性味ある醫見に與ふるに同様の病状を呈して、死するよ同じく倒れたりしよりて之れが後段の後變を行ひ分離培養せしに一様の病の爲めに醫見の姿と被りたるものあるを聞く。此病原の何れよう來るやは未だ不明に屬す、然れども常に一因の醫見の此病に罹る見るに、傳染の皮の發大なるにもよるべしと最も抑も抑も亦給與したる桑葉に於けるか否かは能はざるなり、近頃東京の某處に試験着せるか否かは桑葉中にて野草の如き其の太郎氏、若狭氏は現今予更けるべから其の太郎氏は、當時の病院が醫見の如きの病の體操と検査より調査されたり。

Ishiwata, S., 1901. On a type of severe Xacherie (sotto disease). Dainihon Sanshi Kaiho 114, 1–5 [Original in Japonês].

Über die Schlaffsucht der Mehlmottenraupe (*Ephestia kühniella* Zell.) und ihren Erreger *Bacillus thuringiensis* n. sp.

Von

Ernst Berliner,
z. Zt. im Felde.

(Mit 7 Textabbildungen.)

1. Einleitung.

Im Jahre 1911 veröffentlichte ich in der „Zeitschrift für das gesamte Getreidewesen“ unter dem Titel: „Über die Schlaffsucht der Mehlmottenraupe“ eine vorläufige Mitteilung, in welcher ich eine Krankheit beschrieb, die unter günstigen Umständen unter den Raupen der als kosmopolitischen Schädling berüchtigten Mehlmotte (*Ephestia kühniella* Zell.) ausserordentliche Verheerungen anrichtete. Da ich durch meine Übersiedelung nach Halle gezwungen bin, die darauf bezüglichen Untersuchungen, welche in der „Versuchsanstalt für Getreideverarbeitung“ ausgeführt wurden, zum Abschluss zu bringen, seien die gewonnenen Ergebnisse hier niedergelegt. Den Herren Direktoren der Versuchsanstalt in Berlin, Prof. Dr. Buchwald und Dr. M. P. Neumann, möchte ich auch an dieser Stelle meinen aufrichtigen Dank für das dieser Arbeit entgegengebrachte Interesse aussprechen.

Die von mir in Anlehnung an die Benennung ähnlicher Raupenkrankheiten als Schlaffsucht bezeichnete seuchenhafte Erkrankung trat im Sommer 1909 in einer Sendung von Mehlmottenraupen auf, die ich zum Studium der Parasiten dieser Tiere aus einer thüringischen Mühle erhalten hatte, breitete sich bald in der Versuchsanstalt für Getreideverarbeitung aus und bot mir so mehrere Jahre hindurch bequeme Gelegenheit zu ihrem Studium.

2. Das Krankheitsbild.

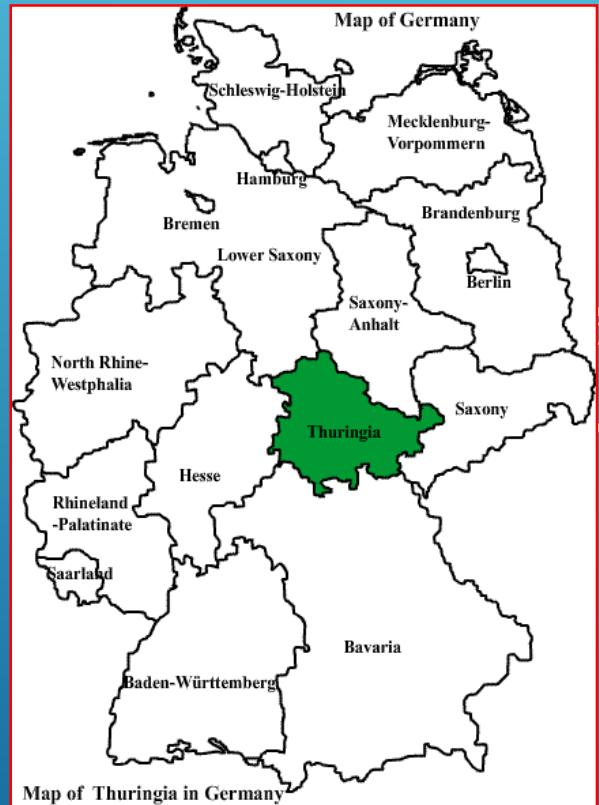
In ihren Anfangsstadien kann die Schlaffsucht mit Sicherheit nur durch die mikroskopische Untersuchung erkannt werden. Die befallenen Raupen unterscheiden sich äußerlich fast in nichts von den gesunden, nur dass sie meist ihren bisherigen Aufenthaltsort verlassen und gleich den vor der Verpuppung stehenden Larven sich auf die



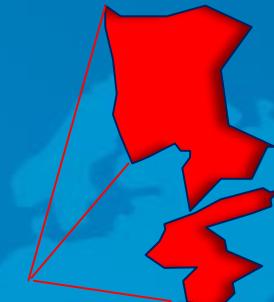
Berliner, E., 1915. Ueber die schlaffsucht der *Ephestia kuehniella* und *Bac. thuringiensis* n. sp. Z. Angew. Entomol. 2, 29–56.

Doença selvagem da traça-da-farinha (*Ephestia kuehniella* Zell.) e seu patógeno *Bacillus thuringiensis*

- Avaliação dos sintomas
- Morfologia e citologia
- Meios de cultura
- Bioensaios de suscetibilidade
- Experimentos de transmissão

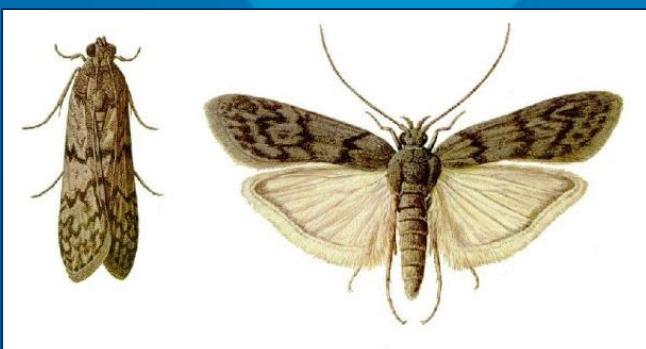


SPOREINE



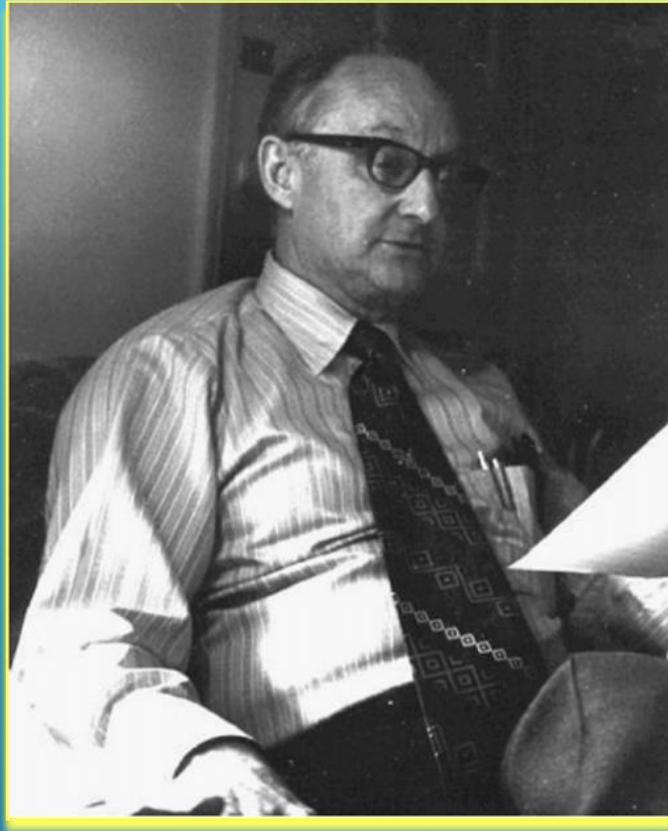
Hungria e Iugoslávia

Ephestia kuehniella



Ostrinia nubilalis





Angus, T., 1954. A bacterial toxin paralyzing silkworm larvae. Nature, 173, 545.

A Bacterial Toxin paralysing Silkworm Larvae

CERTAIN strains of *Bacillus cereus* are among the more violent known bacterial pathogens of the silkworm, *Bombyx mori* L.¹. Toumanoff and Vago^{2,3} isolated a strain of *B. cereus* (*B. cereus* var. *olesti*) which causes toxæmia, a septæmia and intermediate conditions in silkworm larvae. Another strain of *B. cereus* (*B. cereus* var. *sotoi*) was studied by Aoki and Chigasaki⁴, who suggested that its effect was due to a toxin. A culture of *B. sotoi* was kindly made available by Mr. Masataka Oono, of Tokyo, and the disease caused by it has been under investigation in this laboratory since early in 1953.

The work of Aoki and Chigasaki⁴ has been confirmed. Two conditions have been observed in larvae infected with *B. sotoi*: a profound paralysis 2 or 3 hr. after ingestion of old cultures and a septæmia without paralysis 12–24 hr. after injection of spores and cells into the body cavity. The rapid onset of paralysis, which took place without any discernible increase of the ingested bacteria, was suggestive of the presence of a pre-formed toxin; but no toxin could be demonstrated in culture media in which the bacteria had been grown.

It was found in our experiments that treatment of old cultures of *B. sotoi* with clarified silkworm gut juice yielded a toxic extract. As silkworm gut juice is normally alkaline (pH. 9.5–10.0), an old culture of *B. sotoi* was treated with dilute alkali and filtered through a *fritted* glass filter. The sterile filtrate caused paralysis of silkworm larvae. Hannay⁵ has recently observed alkali-soluble crystalline inclusions in sporulating cells of strains of *B. cereus* known to be pathogenic for insect larvae. Microscopic examination of *B. sotoi* revealed similar crystals. Treatment of a suspension of spores, crystals, and vegetative debris with alkali resulted in the dissolution of the crystals without affecting the viability of the spores.

An experiment was done to test whether there might be any foundation to the speculation made by Hannay⁵ that the crystals might be a toxic substance encouraging septæmia of the insect larvae. A suspension of crystals, spores and vegetative debris of *B. sotoi* was well washed by centrifugation, resuspended in water, and divided into two parts. One part was diluted and tested against silkworm larvae both by injecting a quantity of it into the body cavity and by feeding. The second part was treated with alkali until the crystals had dissolved, then the spores and debris were centrifuged out, well washed by centrifugation and resuspended in water. A

545
24 JULY 1954

THE EFFECT OF FEEDING AND INJECTION LARVAE OF *Bombyx mori* L. WITH FRACTION OF AN ALKALI-TREATED CULTURE OF *B. sotoi*

	Method of dosing larvae	
	By feeding	By injection
Original culture Spores and crystals 1.0 g. spores per liter of alkali-treated culture	Paralysis within 4 hr. Septæmia within 12 hr.	Septæmia within 12 hr. No paralysis
1. Spore fraction (1×10^6 spores per ml.)	No effect	Septæmia within 12 hr.
2. Supernatant	Paralysis within 4 hr. No septæmia	No effect
3. Supernatant diluted 10 times	Paralysis within 4 hr. No septæmia	No effect
4. Supernatant boiled at 70°C. for 30 min.	No effect	No effect

portion of the supernatant was dialysed against water and another portion heated at 70°C. for 30 min. All four fractions were tested by feeding and injection. The results are given in the accompanying table.

The results indicate that the septæmia following feeding is dependent upon the presence of a toxic substance which is heat-labile and non-dialysable. The toxin had a paralytic effect when fed but was non-toxic when injected. The crystal-free spores, on the other hand, were pathogenic when injected but non-invasive when fed.

By differential centrifugation of a suspension of spores and crystals, two fractions were obtained which differed significantly in the ratio of spores to crystals. It was found by feeding various dilutions of the two fractions that toxicity varied as the crystal content and was independent of the number of spores present.

The results presented above indicate that the toxic component in old cultures of *Bacillus sotoi* responsible for paralysis in silkworm larvae is associated with the crystalline inclusions described by Hannay. The investigation of its nature and mode of action is being continued.

T. A. ANGUS

Laboratory of Insect Pathology,
Sault Ste. Marie,
Ontario,
Nov. 27.

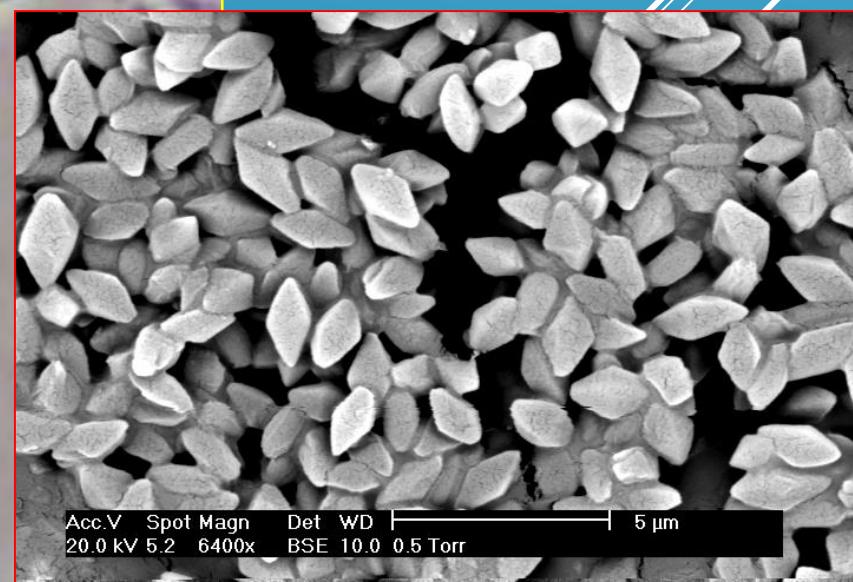
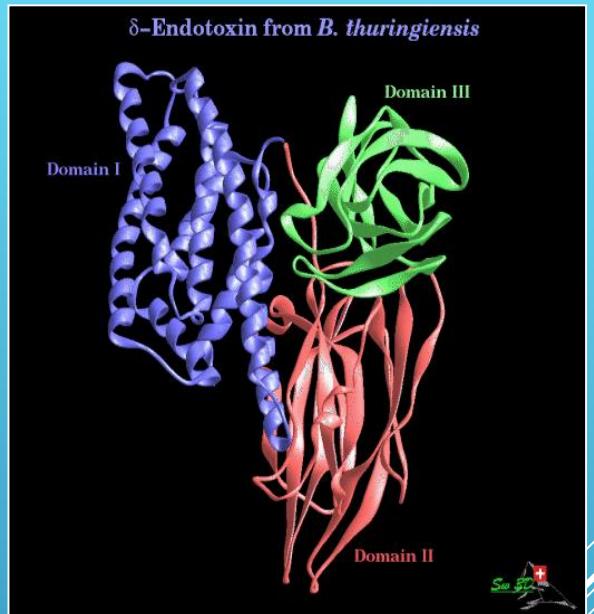
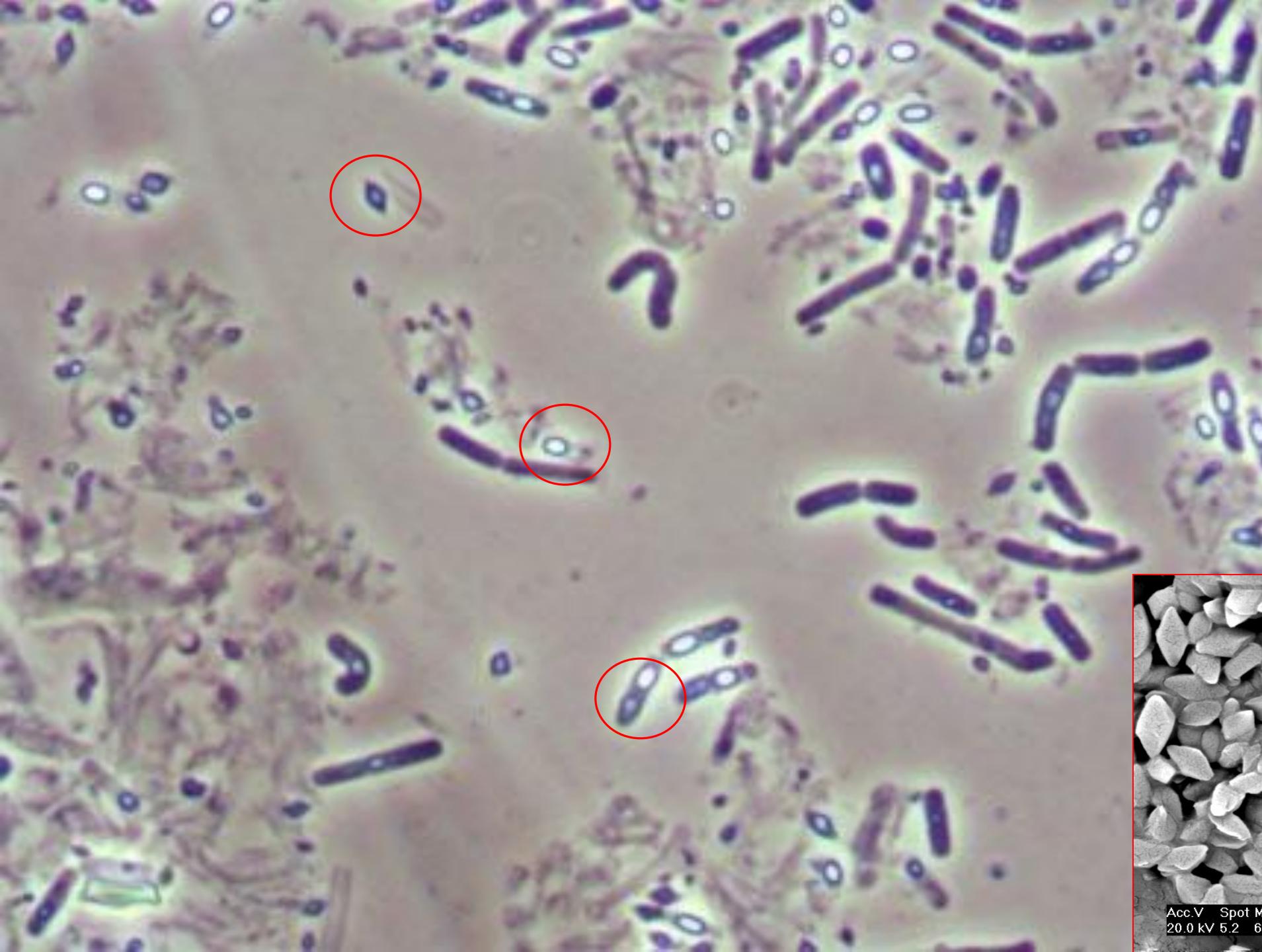
¹ STERKSEN, T. A., "Principles of Insect Pathology" (Oxford-Blk. N.Y., 1946).

² TOUMANOFF, C., and VAGO, C., Ann. Inst. Pasteur, **84**, 316 (1954).

³ TOUMANOFF, C., and VAGO, C., Ann. Inst. Pasteur, **82**, 512 (1952).

⁴ AOKI, K., and CHIGASAKI, Y., Mts. Med. Polycl. Inst. Univ. Tokyo, **14**, 58 (1951).

⁵ HANNAY, C. L., Nature, **172**, 300 (1954).



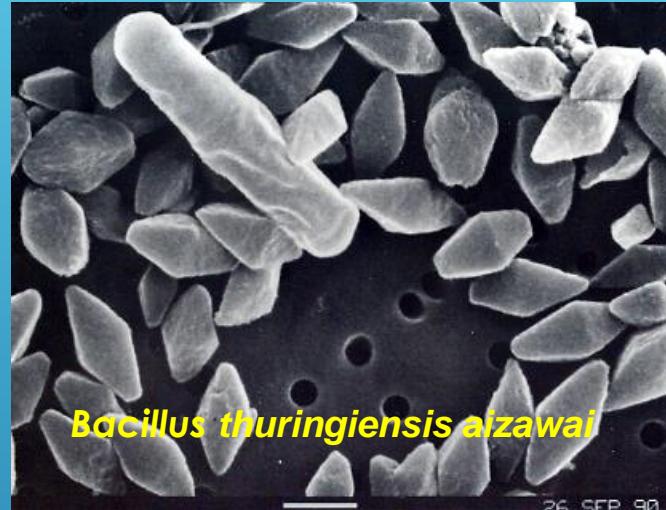
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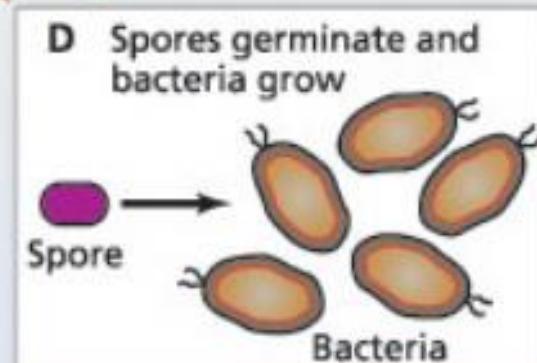
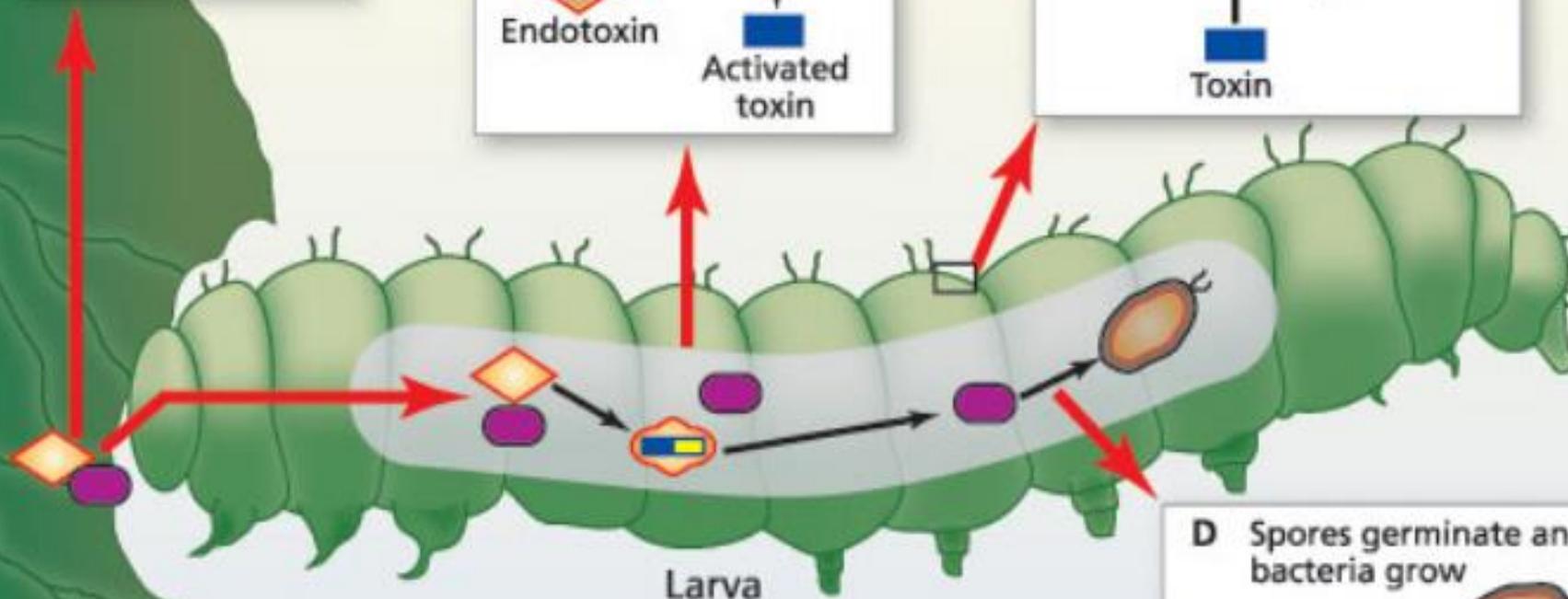
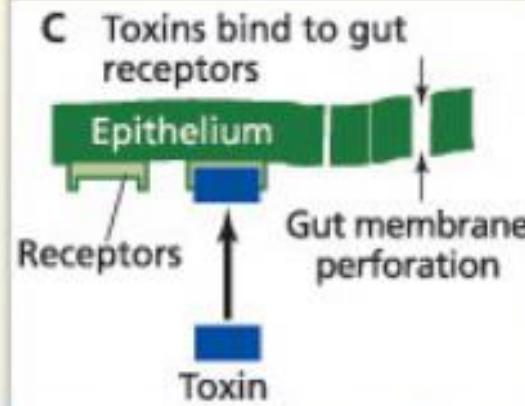
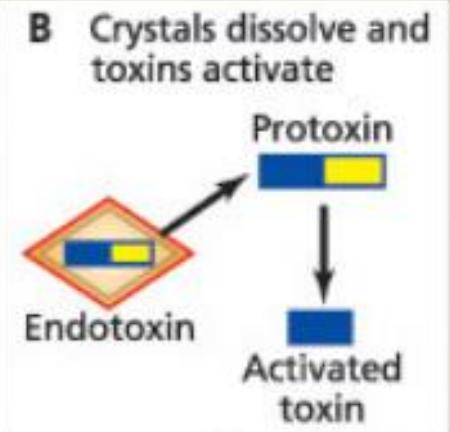
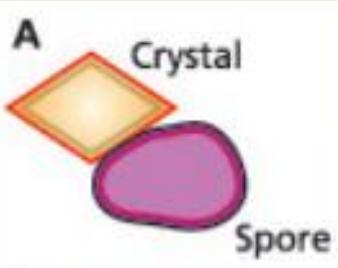
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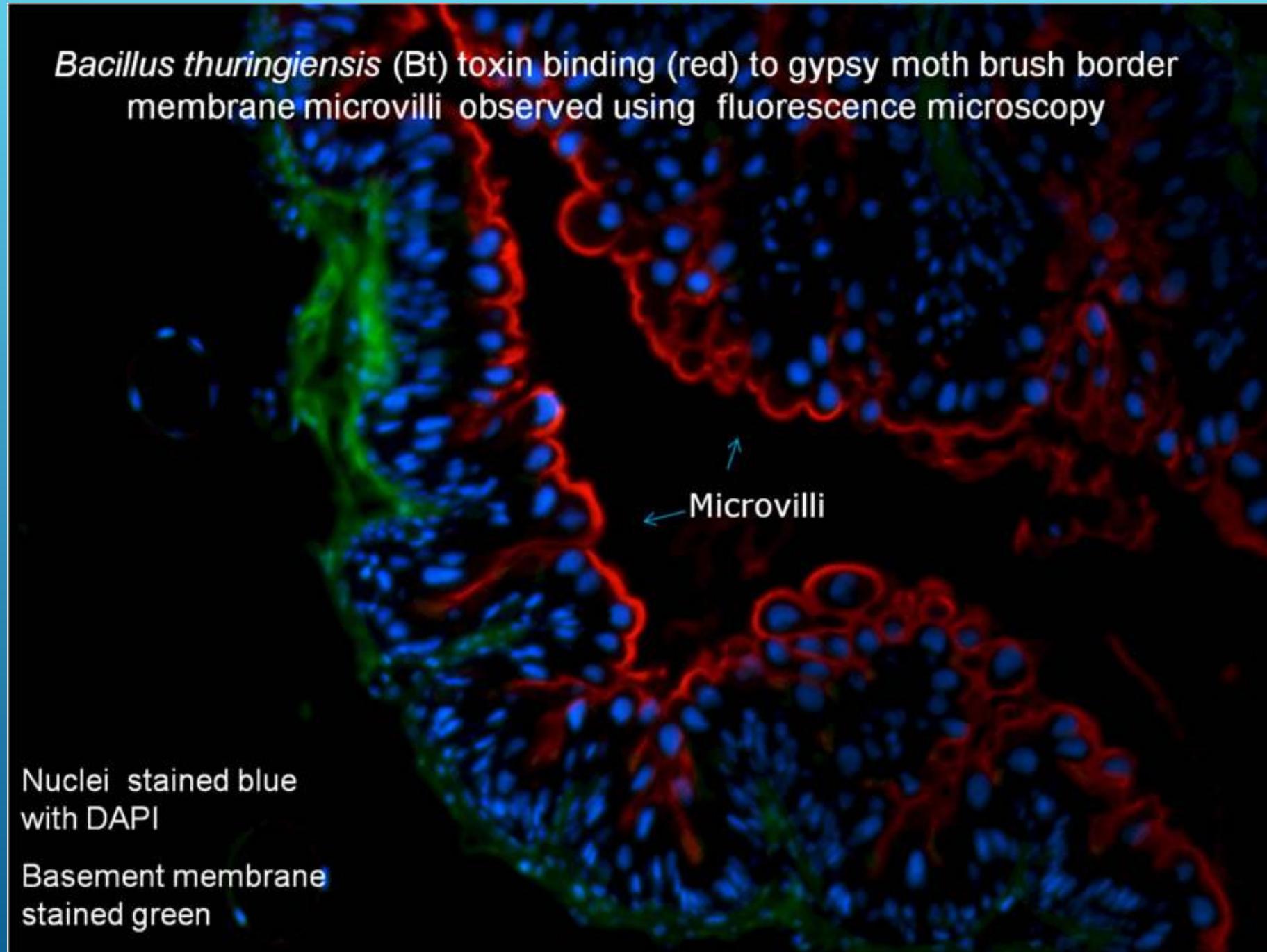
- Diversidade de formas
- Diversidade de toxinas
- Diversidade de numero

B. thuringiensis Life Cycle



- 1) INGESTAO
- 2) DISSOLUCAO CRISTAL
- 3) ATIVACAO
- 4) LIGACAO
- 5) FORMACAO DO PORO
- 6) SEPTICEMIA
- 7) MORTE DO INSETO

Bacillus thuringiensis (Bt) toxin binding (red) to gypsy moth brush border membrane microvilli observed using fluorescence microscopy



Interactions of *Bacillus thuringiensis* Crystal Proteins with the Midgut Epithelial Cells of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Role of Alkaline Phosphatase from *Manduca sexta* in the Mechanism of Action of *Bacillus thuringiensis* Cry1Ab Toxin*

Received for publication, November 14, 2009, and in revised form, January 14, 2010. Published, JBC Papers in Press, February 22, 2010, DOI 10.1074/jbc.M109.085266

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Genetic Variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) Populations from Latin America Is Associated with Variations in Susceptibility to *Bacillus thuringiensis* Cry Toxins[▽]

Rose Monnerat,¹ Erica Martins,¹ Paulo Queiroz,¹ Sergio Ordúz,² Gabriela Jaramillo,² Graciela Benintende,³ Jorge Cozzi,³ M. Dolores Real,⁴ Amparo Martinez-Ramirez,⁴ Carolina Rausell,⁴ Jairo Cerón,⁵ Jorge E. Ibarra,⁶ M. Cristina Del Rincon-Castro,⁶ Ana M. Espinoza,⁷ Luis Meza-Basso,⁸ Lizbeth Cabrera,⁹ Jorge Sánchez,⁹ Mario Soberon,⁹ and Alejandra Bravo^{9*}

Variation in Susceptibility to *Bacillus thuringiensis* Toxins among Unselected Strains of *Plutella xylostella*

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PROJETOS DESENVOLVIDOS PELO LCMAP COM *Bacillus thuringiensis*

Projeto 1: *Plutella xylostella*: variabilidade populacional e suscetibilidade a táticas de controle.
(FAPESP 2010/12438-2)

Projeto 2: Investigação de fatores envolvidos na resistência ao *Bacillus thuringiensis* Berliner
em populações nativas de *Plutella xylostella* (L.,1758) (Lepidoptera: Plutellidae) (FAPESP 2015/05891-6)

Projeto 3: Interação da toxina Cry1Ac de *Bacillus thuringiensis* às microvilosidades apicais das células
colunares (BBMVs) do intestino médio de diferentes instares larvais de *Helicoverpa armigera*
(Lepidoptera:Noctuidae) (FAPESP 2015/24330-5)

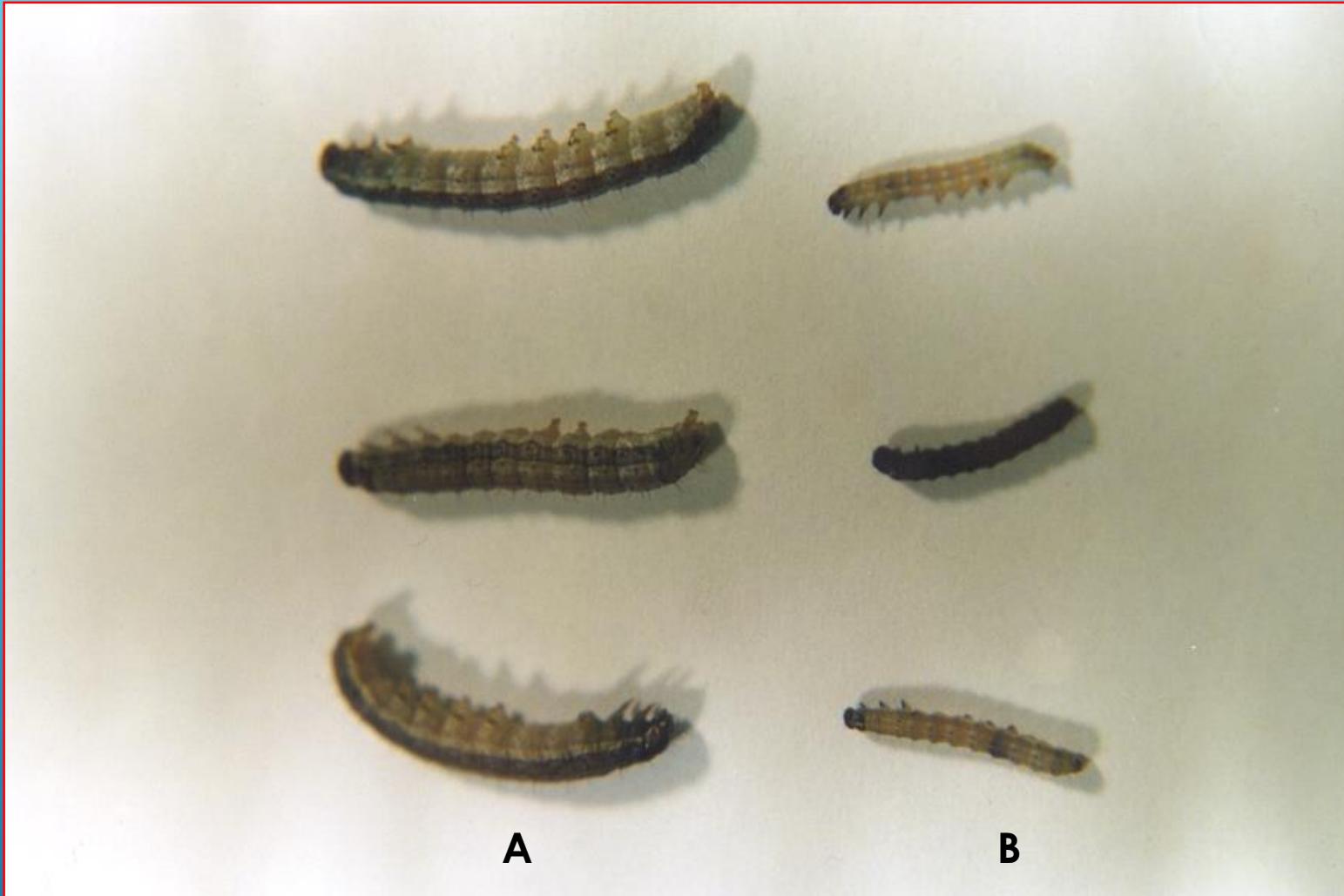


0 que avaliar ?

Bacillus thuringiensis
X
Inseto praga

Bioensaios de patogenicidade

Bioensaios de virulência (Estimativa da CL₅₀)



Lagartas de *Spodoptera frugiperda* da testemunha (A) e sobreviventes (B) ao *Bt*



Uptake and Transfer of a *Bt* Toxin by a Lepidoptera to Its Eggs and Effects on Its Offspring

Débora Pires Paula^{1*}, David A. Andow², Renata Velozo Timbó³, Edison R. Sujii¹, Carmen S. S. Pires¹, Eliana M. G. Fontes¹

1 Department of Biological Control, Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil, **2** Department of Entomology, University of Minnesota, St. Paul, Minnesota, United States of America, **3** Department of Molecular Biology, University of Brasília, Brasília, DF, Brazil

Abstract

Research on non-target effects of transgenic crop plants has focused primarily on bitrophic, tritrophic and indirect effects of entomotoxins from *Bacillus thuringiensis*, but little work has considered intergenerational transfer of Cry proteins. This work reports a lepidopteran (*Chlosyne lacinia*) taking up a *Bt* entomotoxin when exposed to sublethal or low concentrations, transferring the entomotoxin to eggs, and having adverse effects on the first filial generation (F1) offspring. Two bioassays were conducted using a sublethal concentration of toxin (100.0 ng/μl Cry1Ac) for adults and a concentration equal to the LC10 (2.0 ng/μl Cry1Ac) for larvae. Cry1Ac is the most common entomotoxin expressed in *Bt* cotton in Brazil. In the adult diet bioassay there was no adverse effect on the parental generation (P0) adults, but the F1 larvae had higher mortality and longer development time compared to F1 larvae of parents that did not ingest Cry1Ac. For the 3rd instar larvae, there was no measurable effect on the P0 larvae, pupae and adults, but the F1 larvae had higher mortality and longer development time. Using chemiluminescent Western Blot, Cry1Ac was detected in F1 eggs laid by P0 butterflies from both bioassays. Our study indicates that, at least for this species and these experimental conditions, a ~65 kDa insecticidal protein can be taken up and transferred to descendants where it can increase mortality and development time.

BIOLOGICAL AND MICROBIAL CONTROL

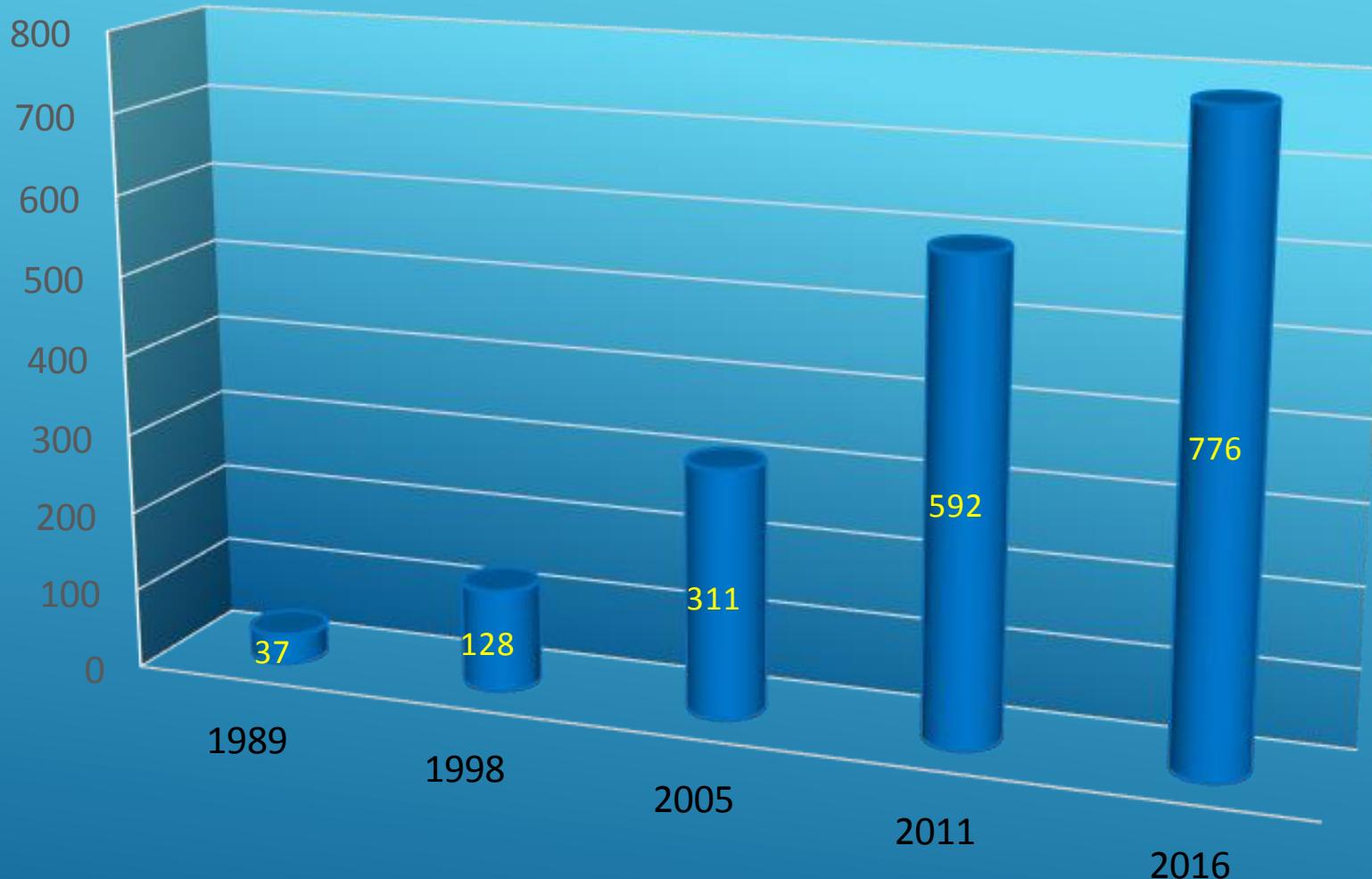
Bacillus thuringiensis Insecticidal Crystal Proteins Affect Lifespan and Reproductive Performance of *Helicoverpa armigera* and *Spodoptera exigua* Adults

YING ZHANG,¹ YAN MA,² PIN-JUN WAN,¹ LI-LI MU,¹ AND GUO-QING LI^{1,3}

J. Econ. Entomol. 106(2): 614–621 (2013); DOI: <http://dx.doi.org/10.1603/E12413>

Interação entre *Bacillus thuringiensis* e a fase jovem e adulta de *Helicoverpa armigera* (Lepidoptera: Noctuidae). Bolsa de Produtividade. Processo CNPq 312618/2014-0.

DIVERSIDADE DE TOXINAS Cry



Cry1Aa1 – Cry74Aa

Dr Neil Crickmore



Senior Lecturer in Molecular Genetics
Department of Biochemistry
School of Life Sciences
University of Sussex, Falmer, Brighton
BN1 9QG UK

Numero de toxinas Cry de *Bacillus thuringiensis* caracterizadas ao logo do tempo



Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/yjipa



Minireview

Insecticidal activity of *Bacillus thuringiensis* crystal proteins

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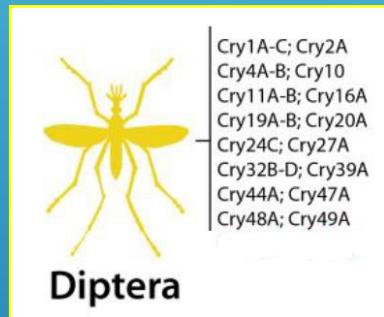
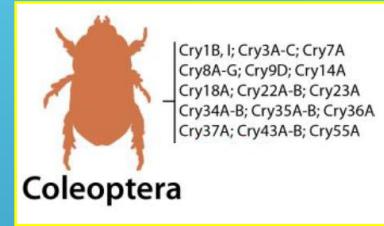
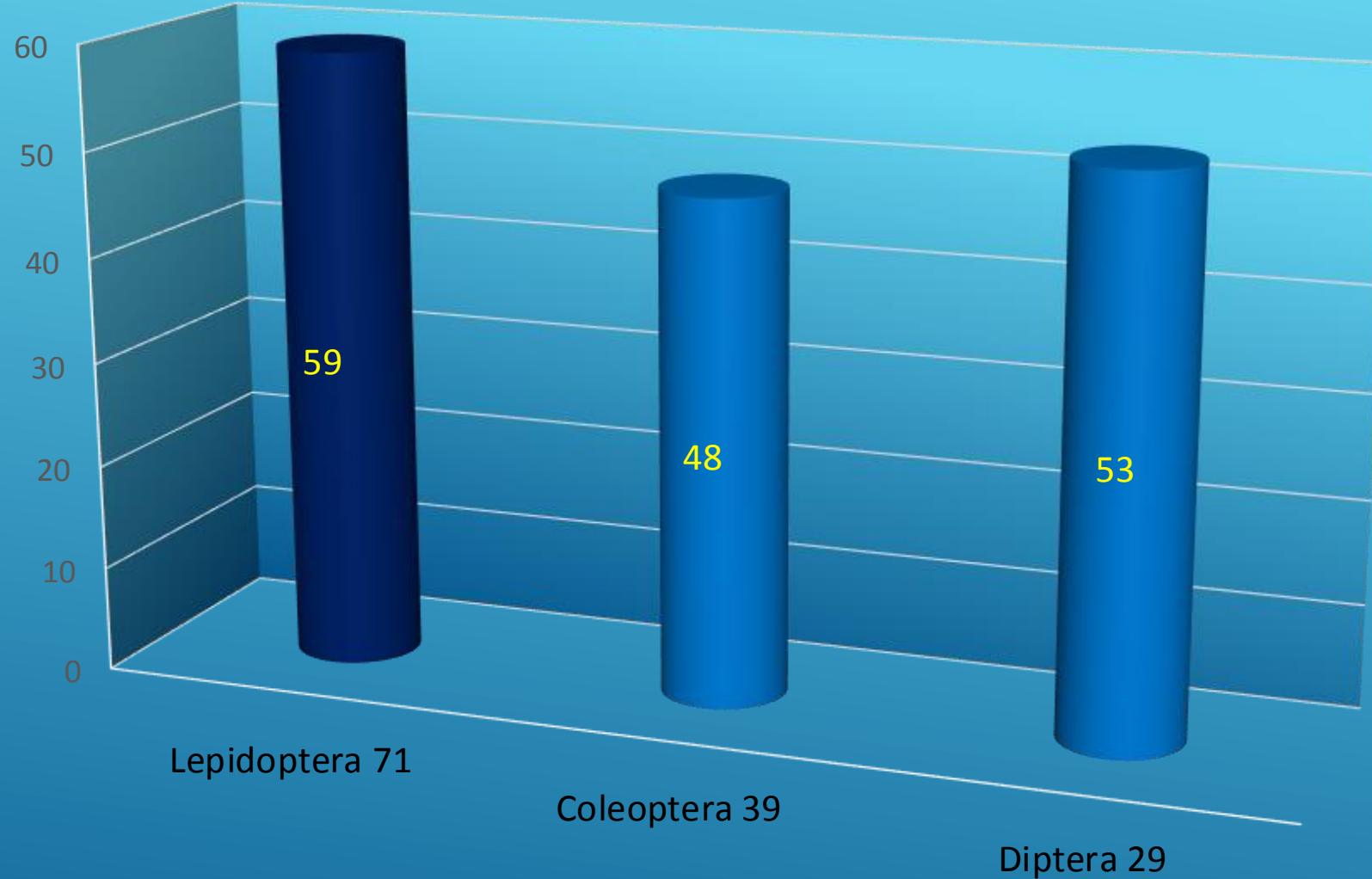
Specificity

Toxicity

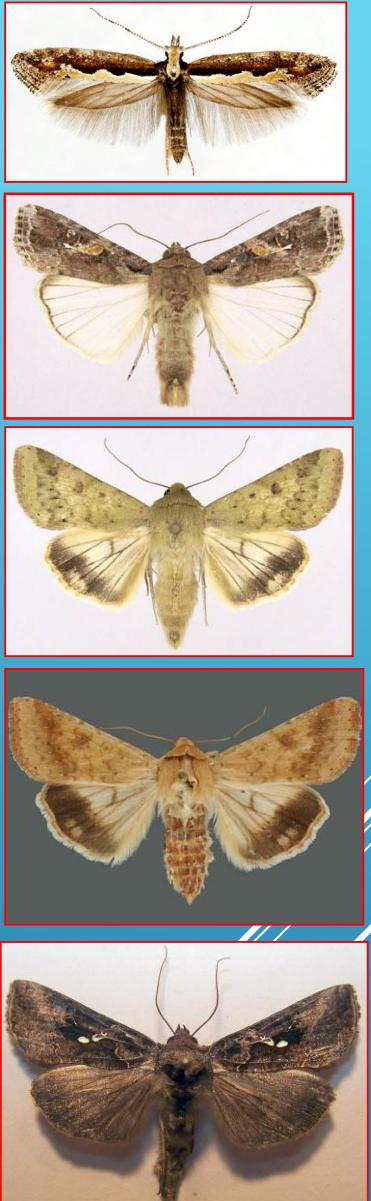
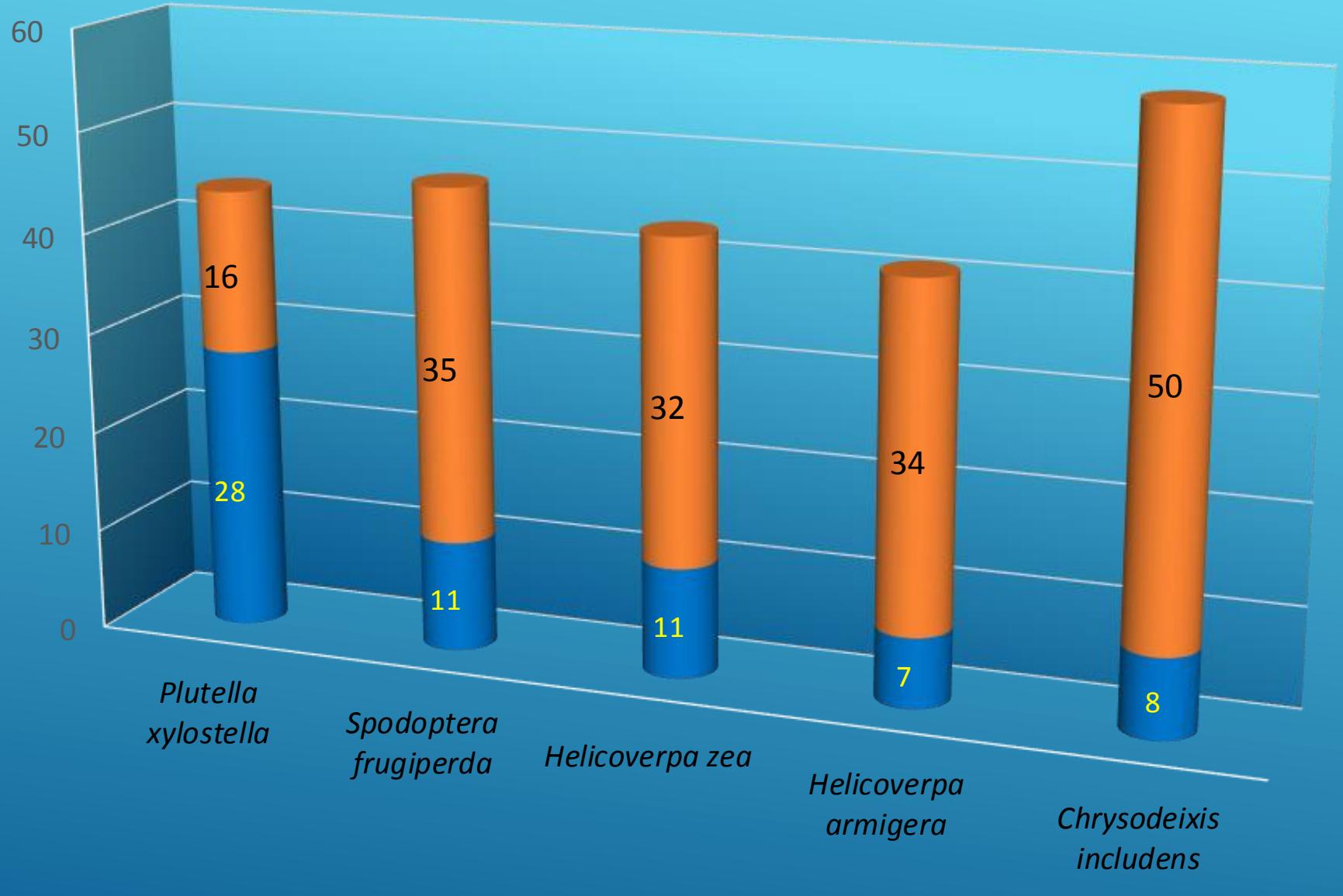
ABSTRACT

Published data on insecticidal activity of crystal proteins from *Bacillus thuringiensis* are incorporated into the Bt toxin specificity relational database. To date, 125 of the 174 holotype known toxins have been tested in ~1700 bioassays against 163 test species; 49 toxins have not been tested at all; 59 were tested against 71 Lepidoptera species in 1182 bioassays; 53 toxins were tested against 23 Diptera species in 233 bioassays; and 47 were tested against 39 Coleoptera species in 190 bioassays. Activity spectra of the tested toxins were summarized for each order. Comparisons of LC₅₀ values are confounded by high variability of the estimates, mostly due to within-species variation in susceptibility, and errors associated with estimation of toxin protein content. Limited analyses suggest that crystal protein toxicity is not affected by quaternary toxin rank or host used for gene expression, but that pre-ingestion treatment by solubilization or enzymatic processing has a large effect. There is an increasing number of toxin families with cross-order activity, as 15 of the 87 families (secondary rank) that are pesticidal are active against more than one order. Cross-order activity does not threaten environmental safety of *B. thuringiensis*-based pest control because toxins tend to be much less toxic to taxa outside the family's primary specificity range.

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Numero de toxinas Cry de *Bacillus thuringiensis* testadas contra insetos
(Van Frankenhuyzen, 2009 – JIP)



Numero de toxinas Cry de *Bacillus thuringiensis* ativas e com atividade desconhecida para algumas pragas que ocorrem no Brasil

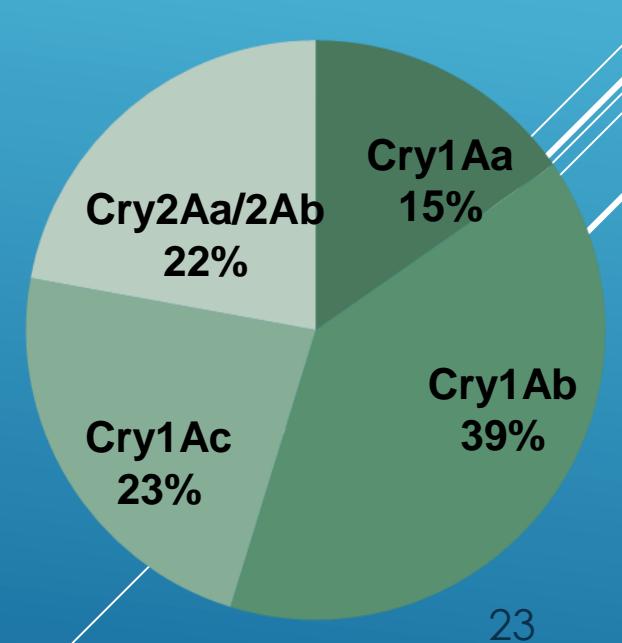
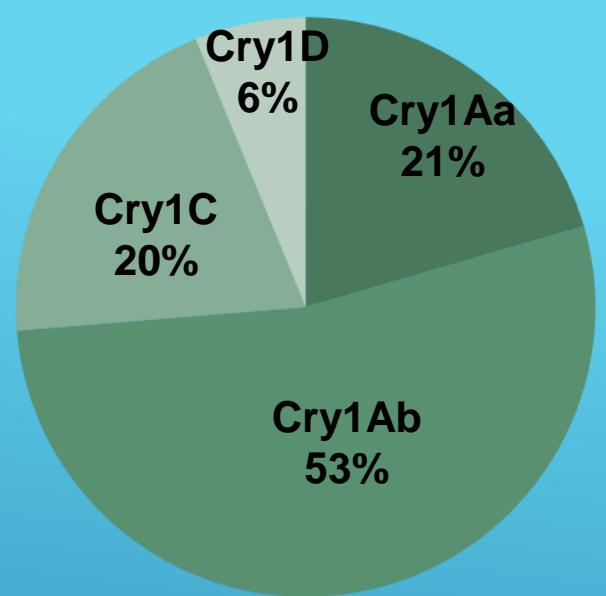
Cepas de *Bt* utilizadas na formulação de bioinseticidas

Cepa	Toxinas
<i>Bacillus thuringiensis kurstaki</i> HD-1	Cry1Aa, Cry1Ab, Cry1Ac, Cry2A e Cry2B
<i>Bacillus thuringiensis kurstaki</i> HD-73	Cry1Ac
<i>Bacillus thuringiensis aizawai</i> HD-112	Cry1Aa, Cry1Ab, Cry1C, Cry1D, Cry 1G e Cry2
<i>Bacillus thuringiensis aizawai</i> HD-113	Cry1Aa, Cry1Ab, Cry1C, e Cry1D
<i>Bacillus thuringiensis thuringiensis</i> HD-2	Cry1A e Cry1B
<i>Bacillus thuringiensis tenebrionis</i>	Cry3A

Bacillus thuringiensis aizawai HD-113



Bacillus thuringiensis kurstaki HD-1



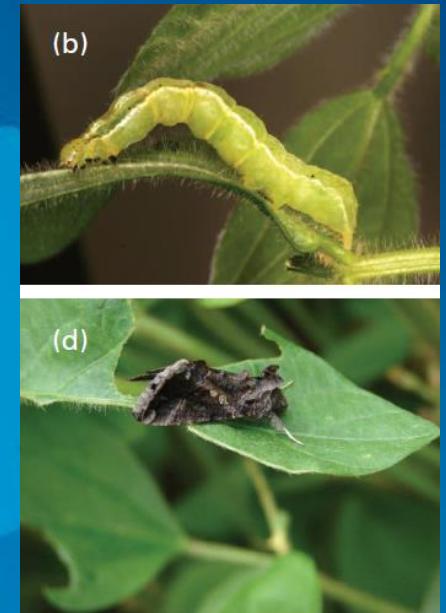
MERCADO BRASILEIRO DE BIOINSETICIDAS A PARTIR DE 2010

Able
Agree
Bac Control (EC e WP)
Bactur WP
Dipel (WP – WG)
Thuricide
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Xentari

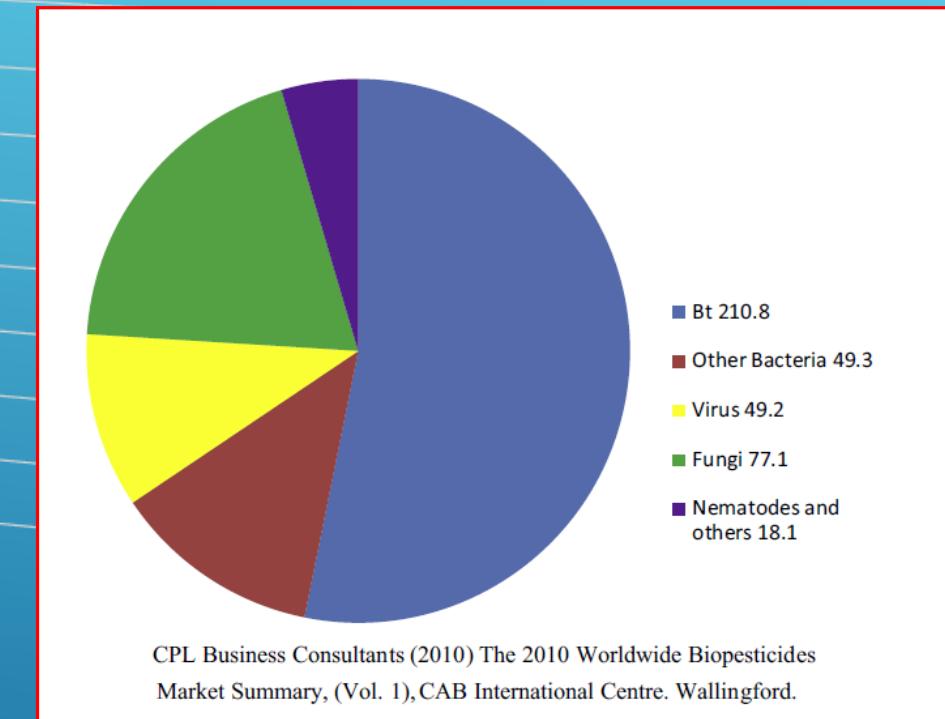
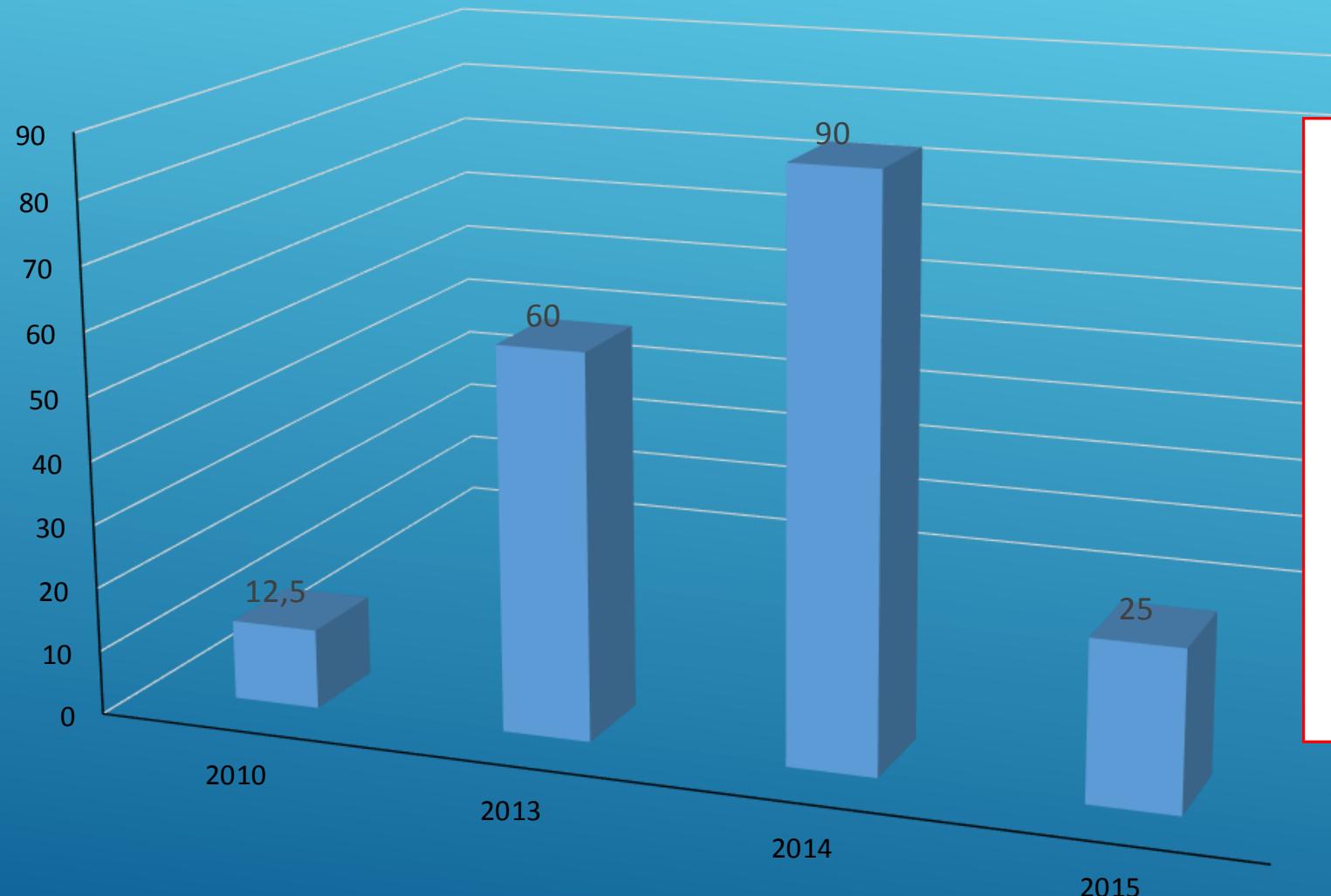


Chrysodeixis includens
10 milhões de hectares

Helicoverpa armigera

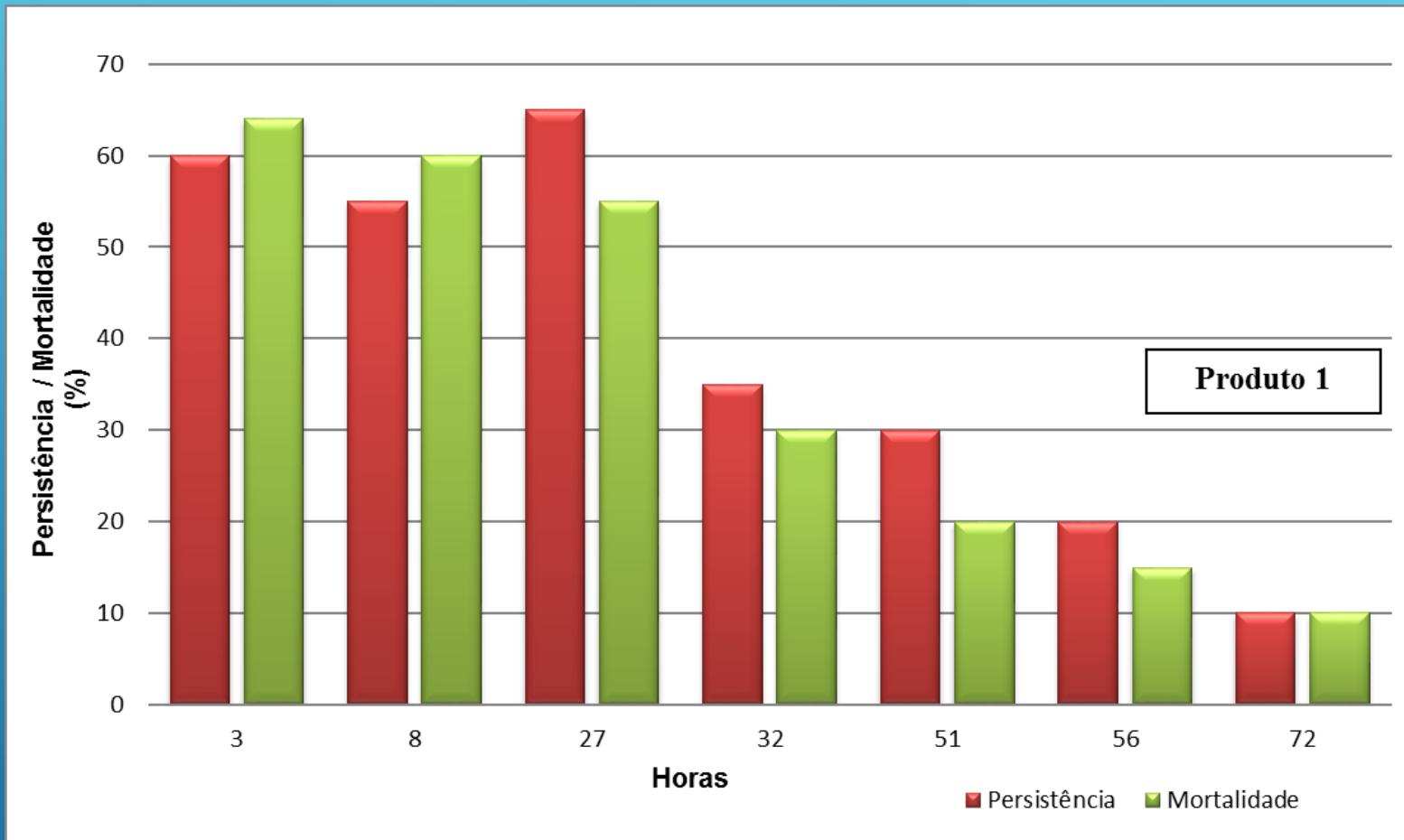


ESTIMATIVA DO MERCADO BRASILEIRO DE BIOINSETICIDAS A PARTIR DE 2010 (milhoes de dolares)

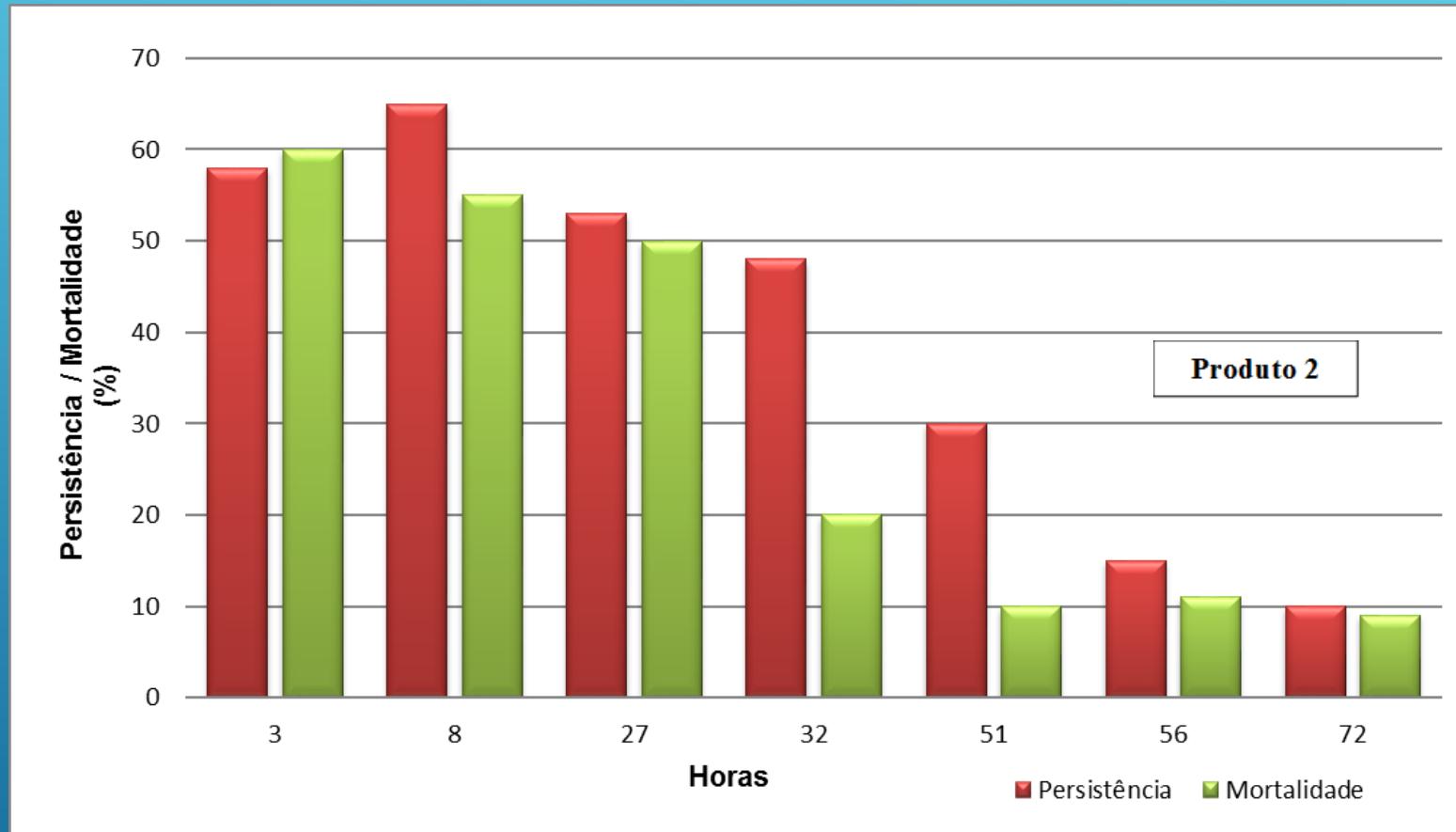


Razões para a redução do mercado de *Bt* bioinseticidas

- Soja *Bt* Cry1Ac
- Condições climáticas (excesso de chuva)
- *Bt* “caseiro” (1000 L de água, 2 L de Dipel, 10 L de creme de milho e 8 kg de açúcar)
- Mistura de produtos
- Redução da ocorrência de *H. armigera*
- Importação benzoato
- Baixa persistência



Persistência de bioinseticidas a base de *Bt* x mortalidade *Helicoverpa armigera* na cultura do milho



Persistência de bioinseticidas a base de *Bt* x mortalidade *Helicoverpa armigera* na cultura do milho

Desenvolvimento de microcápsulas de defensivos agrícolas orgânicos por spray dryer



Ecdytolopha aurantiana



Diatraea saccharalis

SAFRA 2011 - 2012				
CULTURA	ALGODÃO		CULTURA	ALGODÃO
PRODUTO	DOSE		PRODUTO	DOSE
CELEIRO	0,8		CERCOBIM	1
DIPEL	0,5		SUMILEX	0,8
KARATE	0,12		KRAFT	0,25
SAURUS	0,2		SAURUS	0,2
KRAFT	0,25		XENTARI	0,5
AUREO	0,2		AUREO	0,2
BOROMAG	1			

SAFRA 2011 - 2012				
CULTURA	ALGODÃO		CULTURA	ALGODÃO
PRODUTO	DOSE		PRODUTO	DOSE
LARVIM		0,4	POLO	0,6
DIPEL		0,5	MERTIM	0,5
SAURUS		0,2	BENDAZOL	1
KARATE		0,12	DIPEL	0,5
CERCOBIM		1	KARATE	0,12

Mistura de bioinseticidas a base de *Bt* com inseticidas em algodão. LEM (Bahia)

Perspectivas para o mercado de *Bt* bioinseticidas

- Alto custo dos produtos químicos clássicos de amplo espectro
- Alto impacto dos inseticidas convencionais sobre o meio ambiente

Avaliação integrada dos impactos dos agrotóxicos na saúde e ambiente em Lucas do Rio Verde – MT, com ênfase na contaminação do leite materno.



Dr. Wanderlei Pignati – UFMT / ISC

Audiência Pública na Câmara dos Deputados, anexo II, plenário 8
Brasília, 03/07/2012; Comissão do meio ambiente e desenvolvimento sustentável

Data das imagens: 7/5/2007

Image © 2012 DigitalGlobe

Google earth

Altitude do ponto de visão 9.49 km

Ciência Rural, Santa Maria, v.42, n.10, p.1715-1721, out, 2012

ISSN 0103-8478

Risco de contaminação das águas de superfície e subterrâneas por agrotóxicos recomendados para a cultura do arroz irrigado

Risk assessment of surface and groundwater contamination by the rice pesticides

Luiz Fernando Dias Martini^I Sergiane Souza Caldas^{II} Catia Marian Bolzan^{II}
Angela Da Cas Bundt^I Ednei Gilberto Prime^{II} Luis Antonio de Avila^{I*}

O controle biológico de pragas também é uma questão de saúde pública

Perspectivas para o mercado de *Bt* bioinseticidas

- Alto custo dos produtos químicos clássicos de amplo espectro
- Alto impacto dos inseticidas convencionais sobre o meio ambiente
- Grande diversidade de toxinas inexploradas

Potencial de uso de toxinas Cry de *Bt* para Lepidoptera e Coleoptera

Bioinseticidas



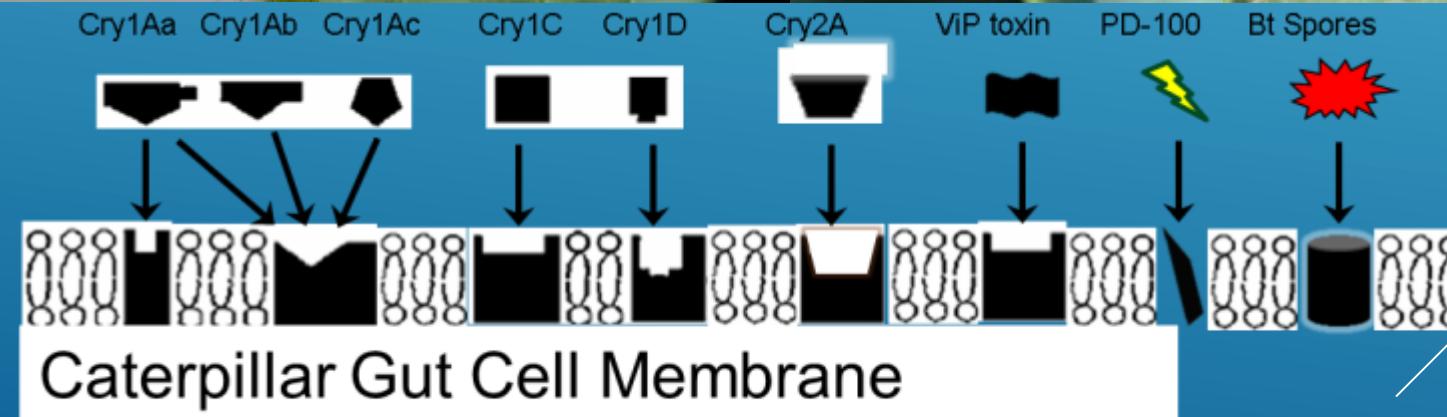
Lepidoptera	Coleoptera
Cry1 (A – G)	Cry1 (B e I)
Cry 2A	Cry 3 (A,B e C)
Cry 1H	Cry7A
Cry1J	Cry 8 (A e G)
Cry1 K	Cry 9D
Cry7B	Cry 14 A
Cry8D	Cry18A
Cry9 (A,B,C e E)	Cry 22 (A e B)
Cry15A	Cry 23 A
Cry22A	Cry 34 (A e B)
Cry32A	Cry 35 (A e B)
Cry51A	Cry 36 A
	Cry 37 A
	Cry 43 (A e B)
	Cry 55 A

Perspectivas para o mercado de *Bt* bioinseticidas

- Alto custo dos produtos químicos clássicos de amplo espectro
- Alto impacto dos inseticidas convencionais sobre o meio ambiente
- Grande diversidade de toxinas inexploradas
- Perspectiva de melhoria das formulações
- Durabilidade da tecnologia plantas *Bt*
- Mercado internacional extremamente ativo



6 Cry Toxins – Broadest spectrum lepidopteran control



(Adapted from Ferré & van Rie, 2002)

Perspectivas para o mercado de *Bt* bioinseticidas

- Alto custo dos produtos químicos clássicos de amplo espectro
- Alto impacto dos inseticidas convencionais sobre o meio ambiente
- Grande diversidade de toxinas inexploradas
- Perspectiva de melhoria das formulações
- Durabilidade da tecnologia plantas *Bt*
- Mercado internacional extremamente ativo
- O *Bt* é um agente de controle biológico “multidisciplinar”

Atualização

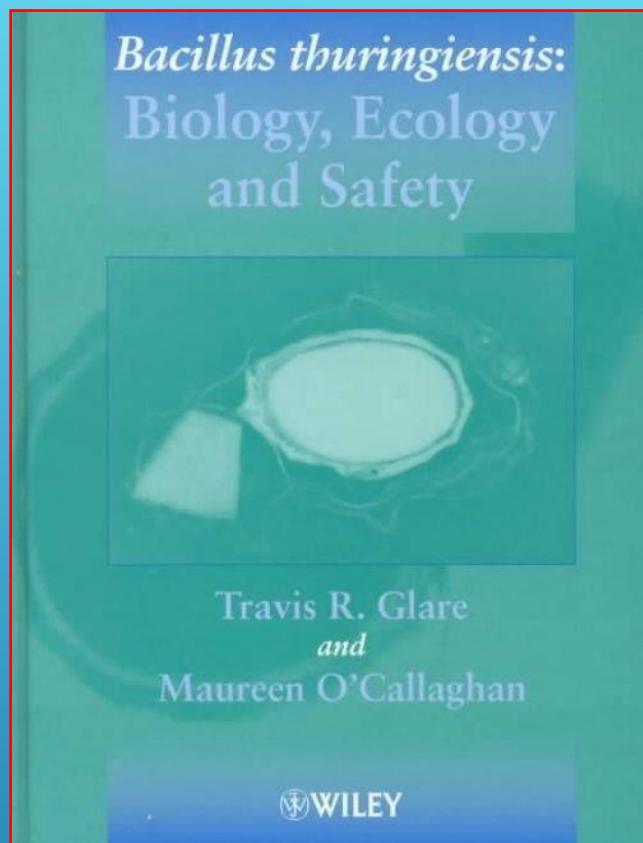
Current Comments

Potencial de *Bacillus thuringiensis israelensis* Berliner no controle de *Aedes aegypti*

Potential of *Bacillus thuringiensis israelensis* Berliner for controlling *Aedes aegypti*

Ricardo Antonio Polanczyk, Marcelo de Oliveira Garcia e Sérgio Batista Alves

Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Escola Superior de Agricultura "Luiz de Queiroz" da Universidade de São Paulo. São Paulo, SP, Brasil



Bt aizawai, *Bt* amagiensis, *Bt* canadensis, *Bt* darmstadiensis, *Bt* entomocidus, *Bt* fukuakensis, *Bt* galleriae, *Bt* jagathesan, *Bt* kenyae, *Bt* kurstaki, *Bt* kyushuensis, *Bt* malaysiensis, *Bt* medellin, *Bt* morrisoni, *Bt* pakistani, *Bt* thompsoni, *Bt* thuringiensis, *Bt* tochigiensis, *Bt* tolworthi.

