

## Current situation of pests targeted by Bt crops in Latin America

CA Blanco<sup>1,13</sup>, W Chiaravalle<sup>2,13</sup>, M Dalla-Rizza<sup>3,13</sup>,  
JR Farias<sup>4,13</sup>, MF García-Degano<sup>5,13</sup>, G Gastaminza<sup>5,13</sup>,  
D Mota-Sánchez<sup>6,13</sup>, MG Murúa<sup>5,13</sup>, C Omoto<sup>7,13</sup>,  
BK Pieralisi<sup>8,13</sup>, J Rodríguez<sup>9,13</sup>, JC Rodríguez-Maciel<sup>10,13</sup>,  
H Terán-Santofimio<sup>11,13</sup>, AP Terán-Vargas<sup>12,13</sup>, SJ Valencia<sup>9,13</sup>  
and E Willink<sup>5,13</sup>



Transgenic crops producing *Bacillus thuringiensis*- (Bt) insecticidal proteins (Bt crops) have provided useful pest management tools to growers for the past 20 years. Planting Bt crops has reduced the use of synthetic insecticides on cotton, maize and soybean fields in 11 countries throughout Latin America. One of the threats that could jeopardize the sustainability of Bt crops is the development of resistance by targeted pests. Governments of many countries require vigilance in measuring changes in Bt-susceptibility in order to proactively implement corrective measures before Bt-resistance is widespread, thus prolonging the usefulness of Bt crops. A pragmatic approach to obtain information on the effectiveness of Bt-crops is directly asking growers, crop consultants and academics about Bt-resistance problems in agricultural fields, first-hand information that not necessarily relies on susceptibility screens performed in laboratories. This type of information is presented in this report. Problematic pests of cotton and soybeans in five Latin American countries currently are effectively controlled by Bt crops. Growers that plant conventional (non-Bt) cotton or soybeans have to spray synthetic insecticides against multiple pests that otherwise are controlled by these Bt crops. A similar situation has been observed in six Latin American countries where Bt maize is planted. No synthetic insecticide applications are used to control corn pests because they are controlled by Bt maize, with the exception of *Spodoptera frugiperda*. While this insect in some countries is still effectively controlled by Bt maize, in others resistance has evolved and necessitates supplemental insecticide applications and/or the use of Bt maize cultivars that express multiple Bt proteins. Partial control of *S. frugiperda* in certain countries is due to its natural tolerance to the Bt bacterium. Of the 31 pests targeted and controlled by Bt crops in Latin America, only *S. frugiperda* has shown tolerance to certain Bt proteins in growers' fields, the most reliable indication of the status of Bt-susceptibility in most of the American continent.

### Addresses

<sup>1</sup> University of New Mexico, 544 Jurgensen Place, Landover, MD, USA  
<sup>2</sup> Ento Agro, Roberto Kock 4194, Montevideo, Uruguay  
<sup>3</sup> INIA, Ruta 48 Km 10, Canelones, Uruguay  
<sup>4</sup> Instituto Phytus, 2319 Duque de Caxias Street, Santa Maria, Rio Grande do Sul, Brazil

<sup>5</sup> EEAOC-CONICET-ITANOA, Sección Zoología Agrícola William Cross 3150, Las Talitas, 4101 Tucumán, Argentina

<sup>6</sup> Michigan State University, Department of Entomology, 1129 Farm Lane, East Lansing, MI, USA

<sup>7</sup> University of São Paulo, "Luiz de Queiroz" College of Agriculture (ESALQ), 11 Pádua Dias Av., Piracicaba, São Paulo, Brazil

<sup>8</sup> 260 Longswitch Road, Leland, MS, USA

<sup>9</sup> Centro Internacional de Agricultura Tropical, Km 17, Cali, Colombia

<sup>10</sup> Colegio de Postgraduados, Montecillo, Edo. Mex., Mexico

<sup>11</sup> Pioneer HiBreed, Carr. #3 km 154.9, Salinas, 00751 PR, USA

<sup>12</sup> Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Cuauhtémoc, Tamps, Mexico

Corresponding author: Blanco, CA ([carlos.blanco1206@gmail.com](mailto:carlos.blanco1206@gmail.com))

<sup>13</sup> The names of the authors were arranged alphabetically by last name.

**Current Opinion in Insect Science** 2016, **15**:131–138

This review comes from a themed issue on **Pests and resistance**

Edited by **Blair Siegfried** and **Juan Luis Jurat-Fuentes**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 10th May 2016

<http://dx.doi.org/10.1016/j.cois.2016.04.012>

2214-5745/© 2016 Elsevier Inc. All rights reserved.

## Introduction

Transgenic crops expressing *Bacillus thuringiensis*- (Bt) insecticidal genes (Bt crops) express Bt proteins similar to those produced by the Bt bacterium. The Bt proteins produced by these plants have a very narrow spectrum of activity [1], making them nearly pest-specific. Currently Bt cotton, Bt maize, and Bt soybeans are planted in ten Latin American countries, with activity against some lepidopteran and coleopteran pests [2], while other nations such as Cuba, Bolivia and Ecuador are close to the commercialization of Bt maize cultivars [3].

A potential positive effect on growers adopting genetically-engineered Bt crops can be evaluated by a significant reduction in the use of synthetic insecticides while at the same time providing excellent control of the most

problematic insect pests. For example, in a typical year prior to the availability of *Bt* cotton, a cotton farmer in Latin America needed to spray up to 12–25 times to obtain partial control of *Alabama argillacea*, *Heliothis virescens* (currently proposed as *Chloridea virescens*) or *Pectinophora gossypiella* [4,5]. Control of *H. virescens* necessitated multiple insecticide sprays prior to the use of *Bt* cotton, and now is no longer controlled with synthetic insecticides on *Bt* and non-*Bt* cotton in Mexico [6]. Planting *Bt* cotton across large areas may have been the most important force that decreased the overall population of *H. virescens*. Similarly, *P. gossypiella* is another example of a problematic pest nearly eradicated from a vast cotton-growing area of North America through the simultaneous use of multiple control tactics involving *Bt* cotton [7]. Large areas planted with *Bt* maize have also reduced the overall population of another serious pest in the United States, *Ostrinia nubilalis* [8]. The overall population of this pest has been reduced to levels such that growers using non-*Bt* maize have benefitted from not having to control *O. nubilalis* in their fields.

Control of *Spodoptera frugiperda* (fall armyworm) in maize presents a different situation. This pest causes sporadic damage to cotton, where is satisfactorily controlled with *Bt* cultivars or with synthetic insecticides. Maize growers in Argentina, Brazil, Puerto Rico and Uruguay initially controlled this pest with *Bt* maize that expressed one *Bt* protein (Cry1Ab or Cry1F); now it is necessary to plant maize cultivars that produce two *Bt* proteins and/or spray synthetic insecticides on *Bt* maize to achieve satisfactory control [9,10\*\*,11]. In Mexico, where *Bt* maize has not yet been authorized for commercial planting, up to 12 synthetic insecticide applications target this pest alone (Mota-Sánchez, unpublished), while in Puerto Rico the number of synthetic insecticide sprays can reach 28 applications in a single growing season (Terán-Santofimio, unpublished). In some regions of Latin America, *Bt* maize that produces one or two toxins is sprayed 0–4 times with synthetic insecticides to achieve adequate control of *S. frugiperda*.

Due to the clear advantages for the environment and growers (e.g., less use and exposure to synthetic insecticides), and the ease of control of the majority of the problematic pests (e.g., reduced need to scout for pests while having an effective and consistent control), growers, consumers and the scientific community have expressed interest in preserving the benefits of planting *Bt* crops [12,13]. It is believed that on-time detection of incipient *Bt*-resistance in fields and implementing mitigation strategies to ameliorate its development will keep *Bt* crops effective for a long time. For nearly two decades, the goal of *Bt*-resistance monitoring programs worldwide has been to detect the areas where *Bt*-resistance is developing in a selected number of insect pests. In Latin America, screening of *Bt*-susceptibility has been done on an annual basis for some of the most problematic pests [14], and as

far as we know, with the exception of Brazil [15\*\*], there have been no *a priori* confirmed reports of *Bt*-resistance 'hotspots' in the field before actual crop damage was reported by growers. The problem of not accurately detecting *Bt*-resistance prior to field failures may be the case for *S. frugiperda* and *Diatraea saccharalis*. *Bt*-resistance monitoring efforts initially targeted other pests (e.g., *Helicoverpa zea*, *H. virescens*, *P. gossypiella*), and other problematic pests were not envisioned as potential candidates for *Bt*-resistance development until the first field reports attracted the attention of growers, regulators and the scientific community [16–18] (Figure 1).

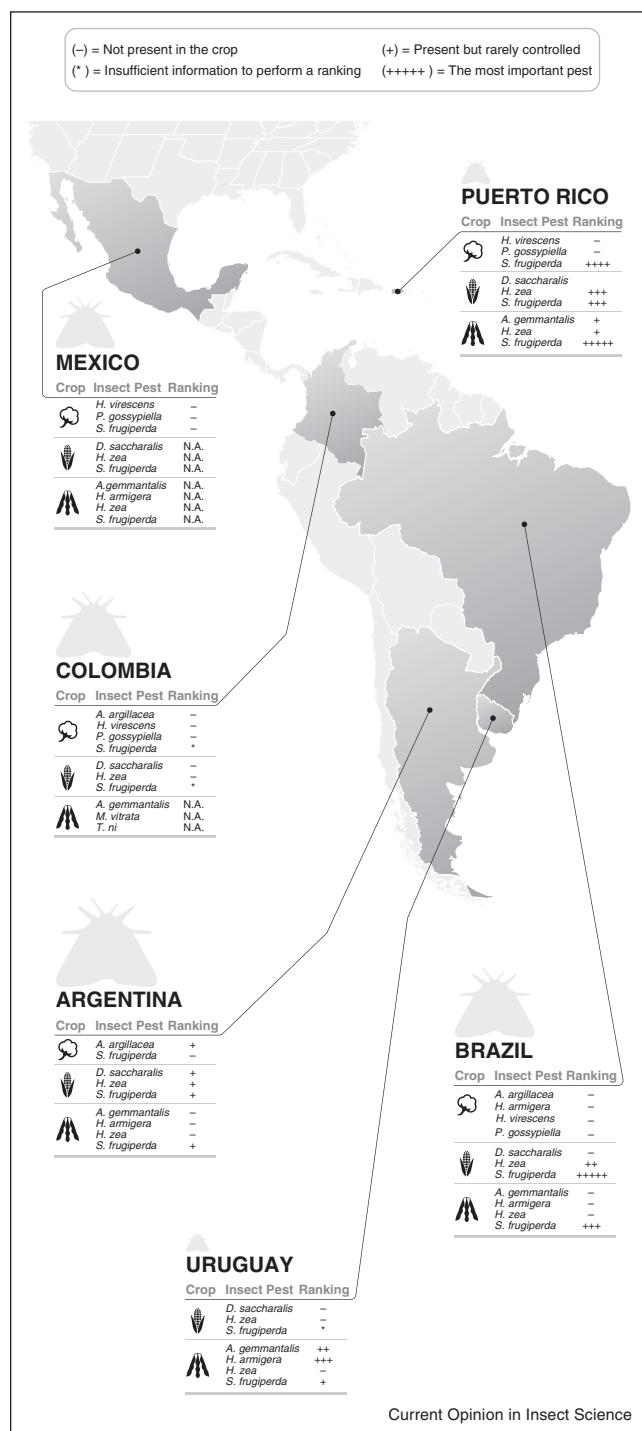
The constraints of early detection of resistance hotspots is likely the result of a number of factors, such as (1) the variability of the methodology performing pre and post-*Bt* crop deployment screening tests; (2) ecological and evolutionary factors that tend to eliminate resistant alleles from its populations primarily due to fitness costs [19,20\*\*]; but also see [21\*\*,22\*\*], (3) *Bt*-susceptibility screens are commonly performed under contract between industry and researchers, and their results seldom are included in scientific reports available to the public; and more importantly (4) the large areas in Latin America ( $\geq 130$  million hectares [23]) planted with cotton, maize and soybeans makes *Bt*-resistance monitoring efforts extremely challenging. The laborious and expensive screening for *Bt*-resistance in areas where random samples are taken has not generally yielded useful information before resistance has been observed in the fields. As far as we know, well-planned and carefully executed *Bt*-resistance monitoring programs [24–26] have failed to yield pertinent information to predict the development of resistance in the monitored areas (e.g., *Helicoverpa* spp.). Therefore, in this report we decided to focus our discussion using field-gathered information on the current effectiveness of *Bt* crops and relate those results when possible, to laboratory-generated *Bt*-susceptibility data.

## Methodology

The information in this report represents the compiled current opinion of researchers, regulators and crop advisors of six Latin American countries on the effectiveness of *Bt* crops against key insect pests. Due to (1) the variability in the susceptibility of insect pests from different regions to *B. thuringiensis* proteins, (2) the discrepancies in methodology among laboratories, and (3) the lack of published reports of routine *Bt*-susceptibility screening from different Latin American countries, this report presents a consensus on the current effectiveness of field-planted *Bt* crops to control specific pests. This is a pragmatic approach of what can be found among scarce published reports of laboratory screenings.

A questionnaire similar to what appears in Tables 1–3 was distributed among researchers and crop advisors directly involved with *Bt* crops in the six countries appearing in

Figure 1



Status of insect pests in Bt crops (cotton, maize, soybean) in six Latin American countries.

**Table 2.** The severity of the pest was ranked as “0” when the pest did not occur in the crop; and between “1” (seldom occurrence in the crop, usually not sprayed with insecticides) and “5” (the most problematic pest triggering

high use of insecticide and/or the adoption of Bt cultivars). These data were gathered for Bt and non-Bt cotton, maize and soybeans in 2015.

Cotton and maize that express *B. thuringiensis* proteins have been commercially planted in some Latin American countries since 1996. Bt soybeans are a recent commercial introduction in Brazil and Argentina. The limited/partial information on the adoption of Bt crops in Latin America is available using the Internet links that appear below.<sup>14</sup> They provide an indication of the Bt-resistance selection pressure over time in some of these countries. Table 4 presents the events approved in each country, but it does not necessarily reflect the current commercial availability of the cultivars.

## Results and discussion

Twenty-seven important lepidopteran and four coleopteran pests may be controlled by Bt crops in six Latin American countries. Their current impact on conventional (non-Bt) and Bt crops varies greatly (Tables 1–3). The great majority (~80%) of lepidopteran and coleopteran pests according to pest control professionals, regulators and academics, are better controlled with Bt crops than with the use of synthetic insecticides on conventional (non-Bt) crops. However, their control depends on the type of Bt crop, the number and type(s) of Bt protein(s) produced, as well as the type of crop rotation and the geography where the pest occurs.

For example, *Agrotis ipsilon* is not difficult to control on Bt and conventional maize in Argentina and Brazil, is not a serious pest of Bt maize in Colombia and Uruguay (Table 2), and it does not have a pest status in Bt cotton in Brazil nor in the rest of the countries (Table 1). In contrast, *Spodoptera cosmioides* and *S. eridania* seem to be more problematic on Bt and conventional soybeans in Brazil, but this is not true for Uruguay, where they are classified as of no importance on this crop (Table 3). In Argentina, the most damage to soybean crops is produced by *Anticarsia gemmatalis*, *Rachiplusia nu*, *Chrysodeixis includens* and *S. cosmioides*. With Bt soybean, which has recently been introduced in Argentina, the only pest mentioned above that is not controlled by this technology is *S. cosmioides*. As a consequence, it is necessary to continue traditional lepidopteran management techniques involving seed treatments and the use of insecticides on soybean in Argentina and Brazil (Murúa, unpublished; Farias, unpublished; [27]).

<sup>14</sup> Argentina: Listado de eventos aprobados, <http://www.argenbio.org/index.php?action=novedades&note=712>. Mexico: Sistema Nacional de Información sobre Bioseguridad: <http://www.conacyt.mx/cibiosem/index.php/sistema-nacional-de-informacion/estadisticas>. Situación nacional, Uruguay: <http://www.cus.org.uy/biotecnologia/situacion-nacional>.

**Table 1****Insect pests affecting cotton cultivation in five Latin American countries.**

	Argentina		Brazil		Colombia		Mexico		Puerto Rico	
	CO	Bt	CO	Bt	CO	Bt	CO	Bt	CO	Bt
<i>Agrotis ipsilon</i>			2	0						
<i>Alabama argillacea</i>	5	1	2	0	4	0				
<i>Chrysodeixis includens</i>	4	2	4	0						
<i>Diabrotica barberi</i>									1	1
<i>Diabrotica virgifera</i>									1	1
<i>Helicoverpa armigera</i>			5	1						
<i>Helicoverpa zea</i>							5	0	5	0
<i>Heliothis virescens</i>			3	0	4	0	2	0	2	0
<i>Pectinophora gossypiella</i>			2	0	4		1	0	3	0
<i>Sacades pyralis</i>					4	0				
<i>Spodoptera exigua</i>							4	0	4	0
<i>Spodoptera frugiperda</i>	4	0	4	1	5	*	4	0	4	4
<i>Trichoplusia ni</i>							3	0	4	0

CO, conventional cotton; Bt, Bt cotton; 0, not present in the crop; 1, present but rarely controlled; 5, the most important pest.

\* Insufficient information to perform a ranking.

**Table 2****Insect pests affecting maize cultivation in six Latin American countries.**

	Argentina		Brazil		Colombia		Mexico		Puerto Rico		Uruguay	
	CO	Bt	CO	Bt	CO	Bt	CO	Bt*	CO	Bt	CO	Bt
<i>Agrotis ipsilon</i>	3	3	3	3	2	1	4				3	0
<i>Chrysodeixis includens</i>											1	0
<i>Diabrotica barberi</i>							2		1	1		
<i>Diabrotica virgifera</i>	2	0					2		1	1	1	0
<i>Diatraea saccharalis</i>	5	1	3	0	4	0	2				2	0
<i>Helicoverpa armigera</i>			2	0							2	0
<i>Helicoverpa gelotopoeon</i>											2	0
<i>Helicoverpa zea</i>	3	1	3	2	4	0	4		5	3	4	0
<i>Peridroma saucia</i>											3	0
<i>Spodoptera exigua</i>							3					
<i>Spodoptera frugiperda</i>	5	1	5	5	5	*	5		5	3	5	**

CO, conventional maize; Bt, Bt maize; 0, not present in the crop; 1, present but rarely controlled; 5, the most important pest.

\* Insufficient information to perform a ranking.

\*\* *Spodoptera frugiperda* continues to be a serious problem on Bt maize, more so in hybrids expressing only one protein.

*Spodoptera frugiperda* has not been difficult to control on cotton and soybeans, but has been the number one problem of maize production in Latin America for many years (Tables 1–3). The importance of *S. frugiperda* on cotton depends on the country and the type of Bt cotton cultivar that it feeds on. Bt cotton that only produces Cry1Ac tends to have more damage than cultivars expressing two Bt proteins (Tables 1 and 4). The same can be said about this pest in Bt soybeans, where it has been satisfactorily controlled in Argentina, Puerto Rico and Uruguay, but not so well in Brazil (Table 3). *Spodoptera frugiperda* is still a serious problem in conventional and Bt maize in Argentina, Brazil, Colombia, Mexico, Puerto Rico and Uruguay, but its damage to Bt maize is restricted to cultivars that express only one Bt protein (Cry1Ab, or Cry1F) and that have been in commercial production for a number of years (Tables 2 and 4). Due to the intense selection pressure for the past 3–5 years, this insect has

developed tolerance to Cry1Ab, Cry1Ac, and Cry1F [10,28,29,30,31,32\*\*] under field conditions; however, this may be also due to natural tolerance to Cry1Fa, Cry1Ab and Cry1Ac among certain *S. frugiperda* populations [33], and cases where it has been subjected to selection pressure with Bt crops [28,29] and synthetic insecticides. Similar to the current situation in Bt maize fields, Bt cotton that expresses two Bt toxins provides better control of the fall armyworm (Table 1). Larvae of *S. frugiperda* are still responsible for sporadic damage on soybeans, occasional heavy damage to cotton, and yield reductions of up to 100% in maize when they are not properly controlled. On non-Bt cotton, this insect is usually controlled satisfactorily with one or two insecticide applications, rarely requires control in soybeans, but on occasion may result in 12 (Mota-Sánchez, unpublished [9,34]) to 28 (Terán-Santofimio, unpublished) insecticide applications over non-Bt maize to minimize

**Table 3**

Insect pests affecting soybean cultivation in six Latin American countries.

	Argentina		Brazil		Colombia		Mexico		Puerto Rico		Uruguay	
	CO	Bt	CO	Bt	CO	Bt <sup>†</sup>	CO	Bt <sup>†</sup>	CO	Bt	CO	Bt
<i>Achyra bifidalis</i>	4	1									3	1
<i>Agrotis ipsilon</i>	3	1	3	3			3				5	2
<i>Anticarsia gemmatalis</i>	5	0	5	0	3	1	2		1	1		
<i>Cerotoma tingomariana</i>												
<i>Chrysodeixis includens</i>	3	0	5	0			5		5	3	4	1
<i>Colias lesbia</i>	4	0							1	0		
<i>Crocidozema aporema</i>	3	0	2	0					1	0	3	1
<i>Diabrotica barberi</i>									1	0		
<i>Diabrotica virgifera</i>									1	0	1	0
<i>Diatraea saccharalis</i>											1	0
<i>Elasmopalpus lignosellus</i>	3	0	4	0								
<i>Helicoverpa armigera</i>	1	0	5	0							5	3
<i>Helicoverpa gelotopoeon</i>	4	0									3	1
<i>Helicoverpa zea</i>	3	0	1	0			3		4	1	1	0
<i>Heliothis virescens</i>	4	0	4	0							1	0
<i>Maruca vitrata</i>					2							
<i>Rachiplusia nu</i>	2	0	2	0								
<i>Rhysomathus subtilis</i>	5	5										
<i>Spilosoma virginica</i>	4	0										
<i>Spodoptera cosmidoides</i>	3	1	3	3							2	1
<i>Spodoptera eridania</i>	3	1	3	3							2	1
<i>Spodoptera exigua</i>						3					1	0
<i>Spodoptera frugiperda</i>	3	1	3	3			3		3	5	1	1
<i>Trichoplusia ni</i>			1	1	1		4		1	3		

CO, conventional soybean; Bt, Bt soybean; 0, not present in the crop; 1, present but rarely controlled; 5, the most important pest.

\* Cultivation of Bt soy is not approved, therefore there is no field-generated information to perform a ranking.

its damage. The control of *S. frugiperda* with synthetic insecticides may require up to 500 g of insecticidal active ingredient in every application [35]; therefore, alternative measures such as effective Bt proteins are necessary to achieve satisfactory control and reduce chemical contamination from multiple applications, an economic burden to maize growers.

To better understand the complicated control of *S. frugiperda*, it needs to be considered that this insect responds significantly different to Bt proteins within and between Bt toxin families [32,36], and variability among *S. frugiperda* populations [33]. These two factors make it difficult to select the most efficacious proteins for Bt crops. In countries such as Brazil, Argentina and Mexico where 15.5, 3.0, and 7.0 million hectares are planted with maize, respectively [23], each with very different agroecological conditions, it is necessary to develop a better understanding of the performance of maize cultivars and the response to Bt proteins in local pest populations [37].

Another pest challenge for the near future of Bt crops is *Helicoverpa armigera*, introduced in South America around 2013 with an apparent northward expansion [38]. Recently, this pest has been detected in Brazil [39], Paraguay [40], Argentina [41], Bolivia, Uruguay [38], and Puerto Rico [42]. *Helicoverpa armigera* has been very problematic in Asia, Australia and Europe where it attacks multiple

crops and has developed resistance to both synthetic insecticides and *B. thuringiensis* proteins [43–47]. A similar multiple-resistance situation has also been found in *S. frugiperda* [48,49,50\*].

To further illustrate the complexity of the challenge, in Argentina the Heliothinae complex is formed by *Helicoverpa gelotopoeon*, *H. zea*, *H. armigera*, and *Heliothis virescens*. The differentiation of the Heliothinae complex is very difficult, and only the adults can be distinguished by the wing pattern design and male genitalia using traditional taxonomic methods or molecular techniques [51–57]. A further complication is that *H. armigera* and the native *H. zea* produce the same pheromone compounds, although in different concentrations [51]. Thus, males of both species are attracted to sex pheromone lures in the field. Yet another complication is the fact that *H. armigera* and *H. zea* have been shown to hybridize under laboratory conditions and could well be doing the same in the field [58,59]. Nonetheless, it is still unclear whether *H. armigera* can hybridize with other endemic Heliothinae species such as *H. gelotopoeon*. Studies made in Argentina have shown that regardless of province, county, crop, and year, the predominant species is *H. gelotopoeon*, especially in chickpeas (*Cicer arietinum*) and soybeans. However, *H. armigera* has been recorded in a much larger geographical area that now includes 8 provinces and 20 counties of Argentina (Murúa, unpublished).

**Table 4*****Bacillus thuringiensis* proteins expressed by Bt-crops registered for commercial cultivation in Latin American countries in 2015.**

	Bt cotton	Bt maize	Bt soy
Argentina	Cry1Ac	Cry1Ab, Cry1F, Cry1A.105, Cry2Ab, Cry3Bb, Cry3A, Vip3Aa20	Cry1Ac
Brazil	Cry1F, Cry1Ac, Cry1Ab, Cry2Ae, Cry2Ab2	Cry1Ab, Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, Cry35Ab1, VIP3Aa20	Cry1Ac
Chile		Cry1Ab	
Colombia	Cry1Ac, Cry2Ab2, Cry2Ae	Cry1Ab, Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1 y VIP3Aa20	Cry1Ac*
Costa Rica	Cry1Ab, Cry1Ac, Cry1F, Cry2Ab2, VIP3A(a)		
Honduras		Cry1Ab, Cry1A.105, Cry1Fa2, Cry2Ab2, Cry3Bb1	
Mexico	Cry1Ab, Cry1Ac, Cry2Ab2, Cry2Ae		
Panama		Cry1Fa2	
Paraguay	Cry1Ac	Cry1Ab, Cry1A.105, Cry1Fa2, Cry2Ab2, Cry3Bb1, VIP3Aa20	Cry1Ac
Puerto Rico	See below	Cry1Ab, Cry1Ac, Cry1A.105, Cry1Fa2, Cry2Ab2, Cry3Bb1, Cry34Ab1, Cry35Ab1, eCry3.1Ab, mCry3A, VIP3Aa20	See below
Uruguay		Cry1Ab, Cry1A.105, Cry1F, Cry2Ab2, Cry3A, Cry3Bb1, VIP3Aa20	Cry1Ac

\* As of December 2015, Bt soy is approved for cultivation but it has not been planted in the field.

Bt cottons expressing Cry1Ab, Cry1Ac, Cry1F, Cry2Ab2, Cry2Ae, and/or VIP3A(a) are prohibited for commercial cultivation in Puerto Rico. They can be planted for research purposes under restrictive conditions. Bt soybeans expressing Cry1Ac, Cry1A.105, Cry1F and/or Cry2Ab2 are registered for commercial cultivation and currently are planted for seed increases exclusively.

The complexity of factors involved in the deployment of new *Bt* crops, especially into countries that have not yet commercialized them, poses a significant challenge. In order to provide effective and a sustainable pest control tool for growers, mitigation of *Bt*-resistance will necessitate the use of multiple tactics, such as crop rotation, pyramided *Bt* toxins expressed by different crops, and a better understanding of pest biology and ecology. Finding a satisfactory control for *S. frugiperda* may provide practical answers for the other 30 pests associated with *Bt* crops in the region and for the preservation of this useful technology.

## Acknowledgements

We wish to recognize the invaluable assistance of Alan Reynolds, Alberto Rodríguez, Alejandra Bravo, Blair Siegfried, Daniel Gutiérrez, Jodi Scheffler, Juan Luis Jurat-Fuentes, Khamkeo D. Vongpaseuth, Pedro Macías-Canales, and Subray Hegde to this study.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Bravo A, Gil SS, Soberón M: **Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control.** *Toxicon* 2007, **49**:423-435.
2. ISAAA: *International Service for the Acquisition of Agri-Biotech Applications.* GM approval database; 2015.. <http://www.isaaa.org/gmapprovaldatabase/default.asp> [Accessed on 08.09.15]..
3. James C: *Global status of commercialized biotech/GM crops: 2011.* 2014.. ISAAA briefs 43. <http://www.isaaa.org/resources/publications/briefs/43/download/isaaa-brief-43-2011.pdf> [10.12.15].
4. García R, Baraona M, Duval G, Fernández L, Tudela F: *Deterioro ambiental y pobreza en la abundancia productiva (el caso de la Comarca Lagunera).* México: CINVESTAV; 1988.. monografía #9.
5. Bellotti AC, Melo EL, Arias B, Herrera CJ, Hernández MP, Holguín CM, Guerrero JM, Trujillo H: **Biological control in the neotropics: a selective review with emphasis on cassava.** *Second International Symposium on Biological Control of Arthropods.* 2005. [https://www.researchgate.net/profile/Claudia\\_Holguin3/publication/242282705\\_BILOGICAL\\_CONTROL\\_IN\\_THE\\_NEOTROPICS\\_A\\_SELECTIVE REVIEW\\_WITH\\_EMPHASIS\\_ON\\_CASSAVA/links/548082190cf20f081e7257da.pdf](https://www.researchgate.net/profile/Claudia_Holguin3/publication/242282705_BILOGICAL_CONTROL_IN_THE_NEOTROPICS_A_SELECTIVE REVIEW_WITH_EMPHASIS_ON_CASSAVA/links/548082190cf20f081e7257da.pdf) [10.12.15].
6. Blanco CA: ***Heliothis virescens* and Bt cotton in the United States.** *GM Crops Food: Biotechnol Agric Food Chain* 2012, **3**:201-212.
7. APHIS U.S.: *Department of Agriculture, Animal and Plant Health Inspection Service.* 2014 pink bollworm eradication update. 2015.. [https://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/cotton\\_pests/downloads/pbw-map.pdf](https://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/downloads/pbw-map.pdf) [10.12.15].
8. Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamsen M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt TE, Wright RJ, Pecinovsky K, Rabaei TL, Flood BR, Rauh ES: **Areawide suppression of European corn borer with Bt maize reaps saving to non-Bt maize growers.** *Science* 2015, **330**:222-225.

9. Murúa MG, Juárez ML, Prieto S, Gastaminza G, Willink E: **Distribución temporal y espacial de poblaciones de *Spodoptera frugiperda* (Smith) (Lep.: Noctuidae) en diferentes hospederos en provincias del norte de Argentina.** *Rev Ind Agric Tucumán (RIAT)* 2009, **86**:25-36.

10. Farias JR, Andow DA, Horikoshi RJ, Sorgatto RJ, Fresia P, Dos Santos A, Omoto C: **Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil.** *Crop Prot* 2014, **64**:150-158.

Extremely high levels of resistance were detected in fall armyworm populations in Brazil. The bioassay methodology in this report exemplifies the most accurate procedure to detect rare alleles and estimate levels of resistance.

11. Balbi EI, Flores Fm: **Evaluación del daño causado por el “Cogollero de maíz” (*Spodoptera frugiperda*) y presencia de la “Isoca de la espiga” (*Helicoverpa zea*) en diferentes híbridos de maíz transgénico..** 2015:. Available: <http://inta.gob.ar/documentos/evaluacion-del-dano-causado-por-el-201ccogollero-de-maiz201d-spodoptera-frugiperda-y-presencia-de-la-201cisoca-de-la-espiga201d-helicoverpa-zea-en-diferentes-hibridos-de-maiz-transgenico> [10.12.15].

12. Andow DA, Hutchison WD: **Bt-corn resistance management.** In *Now or Never: serious new plans to save a natural pest control.* Edited by Mellon M, Rissler J. 1998.

13. Gould F, Tabashnik B: **Bt-cotton resistance management.** In *Now or Never: serious new plans to save a natural pest control.* Edited by Mellon M, Rissler J. 1998.

14. ICA: *Instituto Colombiano Agropecuario: Resolución 682 de 2009.* 2015:. <http://www.ica.gov.co/Normatividad/Normas-Ica/Resoluciones/2009/2009R682.aspx> [10.12.15].

15. Farias JR, Horikoshi RJ, Santos AC, Omoto C: **Geographical and temporal variability in susceptibility to Cry1F toxin from *Bacillus thuringiensis* in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Brazil.** *J Econ Entomol* 2014, **107**:2182-2189.

A three-year field study documenting the trend of higher resistance of the fall armyworm to the Cry1F protein of *B. thuringiensis* expressed in maize in different regions of Brazil.

16. Matten SR, Reynolds AH: **Current resistance management requirements for Bt cotton in the United States.** *J New Seeds* 2003, **5**:137-178.

17. ASA: *Asociación de Semilleros Argentinos: Daños no esperados de *Diatraea saccharalis* en maíces Bt en San Luis.* 2014:. Available: <http://www.asabiotecnologia.com.ar/mostrarnoticia/7/140> [10.12.15].

18. Grimi DA, Ocampo F, Martinelli S, Head GP: **Detection and characterization of *Diatraea saccharalis* resistant to Cry1A.105 protein in a population of Northeast of San Luis province in Argentina.** P 9-3 in *IX Congreso Argentino de Entomología; Posadas, Misiones, Mayo, Argentina: 2015.*

19. Gassmann AJ, Carrière Y, Tabashnik BE: **Fitness costs of insect resistance to *Bacillus thuringiensis*.** *Annu Rev Entomol* 2009, **54**:147-163.

20. Renato J, Horikoshi RJ, Bernardi O, Bernardi D, Okuma DM, Farias JR, Miraldo LL, Amaral FSA, Omoto C: **Near-isogenic Cry1F-resistant strain of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to investigate fitness cost associated with resistance in Brazil.** *J Econ Entomol* 2016, **109**:854-859.

Fitness costs in resistant insects is believed to influence the rate of resistance evolution. This study conducted with a fall armyworm strain isolated from Bt-maize in Brazil and fed artificial diet, demonstrated the high fitness costs of Cry1F-resistant compared with Bt-susceptible *S. frugiperda*.

21. Jakka SRK, Knight VR, Jurat-Fuentes JL: **Fitness costs associated with field-evolved resistance to Bt maize in *Spodoptera frugiperda* (Lepidoptera: Noctuidae).** *J Econ Entomol* 2014, **107**:342-351.

This report demonstrated that the lack of fitness costs is relevant to stability of field-evolved resistance to Bt maize in a fall armyworm Bt-resistant strain isolated from maize, helping to explain the stability of Bt-resistance in *S. frugiperda* populations in Puerto Rico.

22. Vélez AM, Spencer TA, Alves AP, Crespo ALB, Siegfried BD: **•• Fitness costs of Cry1F resistance in fall armyworm, *Spodoptera frugiperda*.** *J Appl Entomol* 2013 <http://dx.doi.org/10.1111/jen.12092>.

Another fitness study conducted with a different fall armyworm Bt-resistant strain from Puerto Rico, as the one of reference 21, also shows lack of fitness costs in the Bt-resistant fall armyworm, suggesting that lacking fitness costs may have accelerated the evolution of resistance in that country.

23. USDA-FAS: *United States Department of Agriculture, Foreign Agricultural Service: World agricultural production. Circular series WAP 10-15, October 2015.* 2015:. <http://apps.fas.usda.gov/psdonline/circulars/production.pdf> [10.12.15].

24. Downes S, Mahon R, Olsen K: **Monitoring and adaptive resistance management in Australia for Bt-cotton: current status and future challenges.** *J Invert Pathol* 2007, **95**:208-213.

25. Gujar GT, Kalia V, Kumari A, Singh BP, Mittal A, Nair R, Mohan M: ***Helicoverpa armigera* baseline susceptibility to *Bacillus thuringiensis* Cry toxins and resistance management for Bt cotton in India.** *J Invert Pathol* 2007, **95**:214-219.

26. Wu K: **Monitoring and management strategy for *Helicoverpa armigera* resistance to Bt cotton in China.** *J Invert Pathol* 2007, **95**:220-223.

27. Casmuz A, Cazado L, Fadda LA, Tuzza MF, Fadda C, Jadur A, Merlini A, Orce JD, Vera MA, María MN, Fernández RA, Gastaminza G, Lane Wilde B: **Momento de aplicación de los insecticidas y su relación con el control ejercido sobre el complejo de orugas defoliadoras de la soja.** In *El cultivo de la soja en el norteño argentino. Campaña 2014/2015.* Edited by Devani MR, Ledesma F, Sánchez JR. Las Talitas, Tuc, Argentina: Publicación Especial EEAOC n° 51. EEAOC; 2015. ISSN: 0328-7300, <http://www.eeaoc.org.ar/publicaciones/categoría/16/539/62-Zoología-621Defoliadoras.html> [10.12.15].

28. Blanco CA, Portilla M, Jurat-Fuentes JL, Sánchez JF, Viteri D, Vega-Aquino P, Terán-Vargas AP, Azuara-Domínguez A, López JD Jr, Arias R, Zhu Y-C, Lugo-Barrera D, Jackson R: **Susceptibility of isofamilies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1Ac and Cry1Fa proteins of *Bacillus thuringiensis*.** *Southwest Entomol* 2010, **35**:409-415.

29. Storer NP, Babcock JM, Schlenz M, Meade T, Thompson GD, Bing JW, Huckaba RM: **Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico.** *J Econ Entomol* 2010, **103**:1031-1040.

30. Storer NP, Kubiszak ME, King JE, Thompson GD, Santos AC: **Status of resistance to Bt maize in *Spodoptera frugiperda*: lessons from Puerto Rico.** *J Invert Pathol* 2012, **110**:294-300.

31. Binning RR, Coats J, Kong X, Hellmich RL: **Susceptibility and aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt maize and considerations for insect resistance management.** *J Econ Entomol* 2014, **107**:368-374.

32. Jakka SRK, Gong L, Hasler J, Banerjee R, Sheets JJ, Narva K, Blanco CA, Jurat-Fuentes JL: **Field-evolved mode 1 resistance of the fall armyworm to *Bacillus thuringiensis* corn associated with reduced Cry1Fa toxin binding and midgut alkaline phosphatase expression.** *Appl Environ Microbiol* 2016, **82**:1023-1034 This is the most up to date study on *B. thuringiensis*-resistance mechanisms in *S. frugiperda*. A thorough mechanistic description of field-evolved resistance to the Bt protein Cry1Fa, in the fall armyworm described in reference 21, supports an association between resistance and reduced Cry1Fa toxin binding and levels of a putative Cry1Fa toxin receptor, and alkaline phosphatase in the midgut of *S. frugiperda* larvae..

33. Monnerat R, Martins E, Queiroz P, Ordúz S, Jaramillo G, Benintende G, Cozzi J, Real MD, Martínez-Ramírez A, Rausell C, Cerón J, Ibarra JE, Del Rincón-Castro MC, Espinoza AM, Meza-Basso L, Cabrera L, Sánchez J, Soberón M, Bravo A: **Genetic variability of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) populations from Latin America is associated with variations in susceptibility to *Bacillus thuringiensis* Cry toxins.** *Appl Environ Microbiol* 2006, **72**:7029-7035.

34. Willink E, Osores VM, Costilla MA: **Daños, pérdidas y niveles de daño económico por *Spodoptera frugiperda* (Lepidoptera: Noctuidae) en maíz.** *Rev Ind Agric Tucumán* 1993, **70**:49-52.

35. Blanco CA, Pellegaud JG, Nava-Camberos U, Lugo-Barrera D, Coello J, Terán-Vargas AP, Vargas-Campilis J: **Maize pests in Mexico and challenges for the adoption of integrated pest management programs.** *J Integ Pest Mgmt* 2014, **5** <http://dx.doi.org/10.1603/IPM14006>.

36. Santos KB, Neves P, Meneguim AM, dos Santos RB, dos Santos WJ, Villas Boas G, Dumas V, Martins E, Botelho Praça L, Queiroz P, Berry C, Monnerat R: **Selection and characterization of the *Bacillus thuringiensis* strains toxic to *Spodoptera eridania* (Cramer), *Spodoptera cosmioides* (Walker) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae).** *Biol Control* 2009, **50**:157-163.

37. Valicente FH, Barreto MR: ***Bacillus thuringiensis* survey in Brazil: geographical distribution and insecticidal activity against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae).** *Neotrop Entomol* 2003, **32**:639-644.

38. Kriticos DJ, Ota N, Hutchison WD, Beddow J, Walsh T, Tay WT, Borchert DM, Paula-More SV, Czepak C, Zalucki MP: **The potential distribution of invading *Helicoverpa armigera* in North America: is it just a matter of time?** *PLoS ONE* 2015, **10**:e0119618.

39. Czepak C, Cordeiro Albernaz K, Vivan LM, Guimarães HO, Carvalhais T: **Primeiro registro de ocorrência de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) no Brasil.** *Pesqui Agropecu Trap* 2013, **43**:110-113.

40. Senave: **Alerta tras ingreso de peligrosa plaga agrícola.** 2013.: <http://www.abc.com.py/edicion-impresa/economia/senave-en-alerta-tras-ingreso-de-peligrosa-plaga-agrícola-629240.html> [10.12.15].

41. Murúa MG, Scalora FS, Navarro FR, Cazado LE, Casmuz AS, Villagrán ME, Lobos E, Gastaminza G: **2014. First record of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Argentina.** *Fla Entomol* 2014, **97**:854-856.

42. APHIS: **U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Old World Bollworm.** 2015: [https://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planhealth/sa Domestic pests\\_and\\_diseases/sa\\_pests\\_and\\_diseases/sa\\_insects/sa\\_owb/lut/p/a/04\\_Sj9CPykssy0xPLMnMz0vMAfGjzOK9\\_D2MDJ0Mj0zd3V2dDDz93HwCzL29jAx8TfULsh0VAY\\_1WKEI/](https://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planhealth/sa Domestic pests_and_diseases/sa_pests_and_diseases/sa_insects/sa_owb/lut/p/a/04_Sj9CPykssy0xPLMnMz0vMAfGjzOK9_D2MDJ0Mj0zd3V2dDDz93HwCzL29jAx8TfULsh0VAY_1WKEI/) [10.12.15].

43. Armes NJ, Jadhav DR, DeSouza KR: **A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent.** *B Entomol Res* 1996, **86**:499-514.

44. Torres-Vila LM, Rodríguez-Molina MC, Lacasa-Plasencia A, Bielza-Lino P: **Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case.** *Crop Prot* 2002, **21**:1003-1013.

45. Li GP, Wu KM, Gould F, Wang JK, Miao J, Gao XW, Guo YY: **Increasing tolerance to Cry1Ac cotton from cotton bollworm, *Helicoverpa armigera*, was confirmed in Bt cotton farming area of China.** *Ecol Entomol* 2007, **32**:366-375.

46. Gao Y, Wu K, Gould F, Shen Z: **Cry2Ab tolerance response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) populations from Cry1Ac cotton planting region.** *J Econ Entomol* 2009, **102**:1217-1223.

47. Bird LJ, Downes SJ: **Toxicity and cross-resistance of insecticides to Cry2Ab-resistant and Cry2Ab-susceptible *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae).** *J Econ Entomol* 2014, **107**:1923-1930.

48. Ríos-Díez JD, Saldamando-Benjumea CI: **Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from Central Colombia to two insecticides, methomyl and lambda-cyhalothrin: a study of the genetic basis of resistance.** *J Econ Entomol* 2011, **104**:1698-1705.

49. Carvalho RA, Omoto C, Field LM, Williamson MS, Bass C: **Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*.** *PLoS ONE* 2013, **8**:e62268.

50. Zhu Y-C, Blanco CA, Portilla M, Adamczyk J, Luttrell R, Huang F: **• Evidence of multiple/cross resistance to Bt and organophosphate insecticides in Puerto Rico population of the fall armyworm, *Spodoptera frugiperda*.** *Pestic Biochem Phys* 2015, **122**:15-21.

Bioassays conducted with a fall armyworm strain described in reference 21 indicate that this insect strain originally collected in Puerto Rico is also highly resistant to acephate. The intense insecticide selection pressure on the *S. frugiperda* population in Puerto Rico has forced field managers to apply synthetic insecticides up to 28 times over a single maize crop season.

51. Pogue MG: **A new synonym of *Helicoverpa zea* (Boddie) and differentiation of adult males of *H. zea* and *H. armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae).** *Ann Entomol Soc Am* 2004, **97**:1222-1226.

52. Navarro FR, Saini ED, Leiva PD: **Clave pictórica de polillas de interés agrícola, agrupadas por relación de semejanza.** Pergamino, Buenos Aires, Argentina: INTA, EEA PERGAMINO; 2009.

53. Behere GT, Tay WT, Russel DA, Batterham P: **Molecular markers to discriminate among four pest species of *Helicoverpa* (Lepidoptera: Noctuidae).** *Bu Entomol Res* 2008, **98**:599-603.

54. Specht A, Sosa-Gómez DR, Paula-Moraes SV, Yano SAC: **Identificação morfológica e molecular de *Helicoverpa armigera* (Lepidoptera: Noctuidae) e ampliação de seu registro de ocorrência no Brasil.** *Pesq Agropec Bras* 2013, **48**:689-692.

55. Tay WT, Soria MF, Walsh T, Thomazoni D, Silvie P, Behere GT: **A brave New World for an Old World Pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil.** *PLoS ONE* 2013, **8**:e80134.

56. Leite NA, Alves-Pereira A, Corrêa AS, Zucchi MI, Omoto C: **Demographics and genetic variability of the new world bollworm (*Helicoverpa zea*) and the old world bollworm (*Helicoverpa armigera*) in Brazil.** *PLoS ONE* 2014, **9**:e113286.

57. Arneodo JD, Balbi EI, Flores FM, Sciocco-Cap A: **Molecular Identification of *Helicoverpa armigera* (Lepidoptera: Noctuidae: Heliothinae) in Argentina and development of a novel PCR-RFLP method for its rapid differentiation from *H. zea* and *H. gelotopoeon*.** *J Econ Entomol* 2015, **108**:2505-2510.

58. Hardwick DF: **The corn earworm complex.** *Mem Entomol Soc Can* 1965, **97**:5-247.

59. Laster ML, Hardee DD: **Intermating compatibility between North American *Helicoverpa zea* and *Heliothis armigera* (Lepidoptera: Noctuidae) from Russia.** *J Econ Entomol* 1995, **88**:77-80.