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Current situation of pests targeted by Bt crops in Latin America

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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.

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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 of 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).

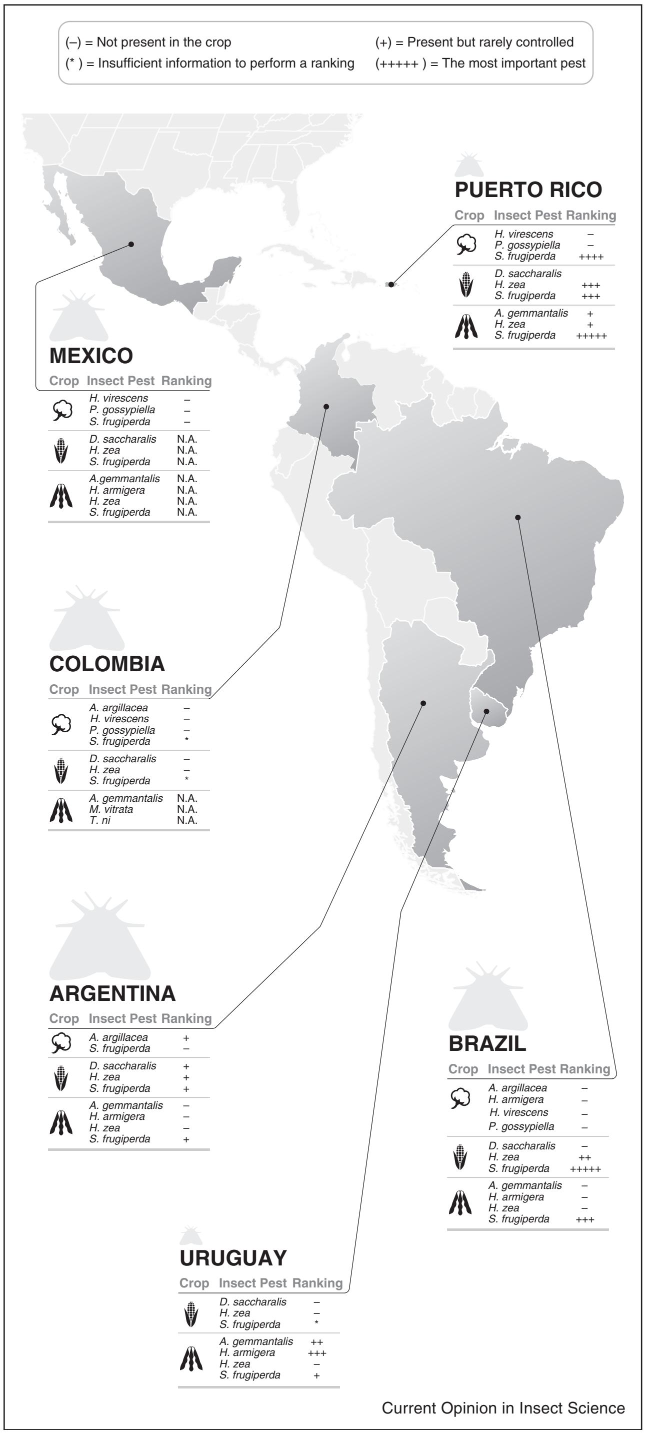
The constrains of early detection of resistance hotspots is likely the result of a number of factors, such as (1) the variability of the methodology performing pre and post-*Bt* crop deployment screening tests; (2) ecological and evolutionary factors that tend to eliminate resistant alleles from its 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 (≥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.[14] 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]).

[14] 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/cibiogem/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
Agrotis ipsilon			2	0						
Alabama argillacea	5	1	2	0	4	0				
Chrysodeixis includens	4	2	4	0						
Diabrotica barberi									1	1
Diabrotica virgifera									1	1
Helicoverpa armigera			5	1						
Helicoverpa zea							5	0	5	0
Heliothis virescens			3	0	4	0	2	0	2	0
Pectinophora gossypiella			2	0	4	*	1	0	3	0
Sacadodes pyralis					4	0				
Spodoptera exigua							4	0	4	0
Spodoptera frugiperda	4	0	4	1	5	*	4	0	4	4
Trichoplusia ni							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
Agrotis ipsilon	3	3	3	3	2	1	4				3	0
Chrysodeixis includens											1	0
Diabrotica barberi							2		1	1		
Diabrotica virgifera	2	0					2		1	1	1	0
Diatraea saccharalis	5	1	3	0	4	0	2				2	0
Helicoverpa armigera			2	0							2	0
Helicoverpa gelotopoeon											2	0
Helicoverpa zea	3	1	3	2	4	0	4		5	3	4	0
Peridroma saucia											3	0
Spodoptera exigua							3					
Spodoptera frugiperda	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*	CO	Bt*	CO	Bt	CO	Bt
Achyra bifidalis	4	1										
Agrotis ipsilon	3	1	3	3			3				3	1
Anticarsia gemmatalis	5	0	5	0	3		2		1	1	5	2
Cerotoma tingomariana					1							
Chrysodeixis includens	3	0	5	0			5		5	3	4	1
Colias lesbia	4	0										
Crocidosema aporema	3	0	2	0					1	0	3	1
Diabrotica barberi									1	0		
Diabrotica virgifera									1	0	1	0
Diatraea saccharalis											1	0
Elasmopalpus lignosellus	3	0	4	0								
Helicoverpa armigera	1	0	5	0							5	3
Helicoverpa gelotopoeon	4	0									3	1
Helicoverpa zea	3	0	1	0			3		4	1	1	0
Heliothis virescens	4	0	4	0							1	0
Maruca vitrata					2							
Rachiplusia nu	2	0	2	0								
Rhyssomathus subtilis	5	5										
Spilosoma virginica	4	0										
Spodoptera cosmidoides	3	1	3	3							2	1
Spodoptera eridania	3	1	3	3							2	1
Spodoptera exigua							3				1	0
Spodoptera frugiperda	3	1	3	3			3		3	5	1	1
Trichoplusia ni			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 agroecologial 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.

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- • of special interest
- •• of outstanding interest

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