BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Integrated management of insect pests

Current and future developments

Emeritus Professor Marcos Kogan
Oregon State University, USA

Professor Elvis 'Short' Heinrichs
University of Nebraska-Lincoln, USA

E-CHAPTER FROM THIS BOOK

burleigh dodds
SCIENCE PUBLISHING

The role and use of genetically engineered insect-resistant crops in integrated pest management systems

Steven E. Naranjo and Richard L. Hellmich, USDA-ARS, USA; Jörg Romeis, Agroscope, Switzerland; Anthony M. Shelton, Cornell University, USA; and Ana M. Vélez, University of Nebraska-Lincoln, USA

1 Introduction

Integrated pest management (IPM) is the modern paradigm for mitigating the negative effects of pests through the strategic integration of multiple control tactics that accounts for environmental safety, risk reduction and favorable economic outcomes for growers and society at large. The components of IPM and how they interact can be conceptualized as a pyramid, the base of which is comprised of foundational tactics and knowledge that can largely help avoid pest problems in the first place (Fig. 1). When this foundation is insufficient for economic pest suppression, there is a need to resort to more prescriptive remedial tactics higher in the pyramid. Overall, an effective and sustainable approach to IPM will ultimately integrate multiple control tactics at all levels in the pyramid.

Among the key tactics in the IPM foundation for agricultural systems is host plant resistance (Bergman and Tingey, 1979; Quisenberry and Schotzko, 1994; Smith, 2005; Anderson et al., 2019). Farmers probably practiced

http://dx.doi.org/10.19103/AS.2019.0047.10
Published by Burleigh Dodds Science Publishing Limited, 2020.

Chapter taken from: Kogan, M. and Heinrichs, E. A. (ed.), *Integrated management of insect pests: Current and future developments*, Burleigh Dodds Science Publishing, Cambridge, UK, 2020, (ISBN: 978 1 78676 260 3; www.bdspublishing.com)

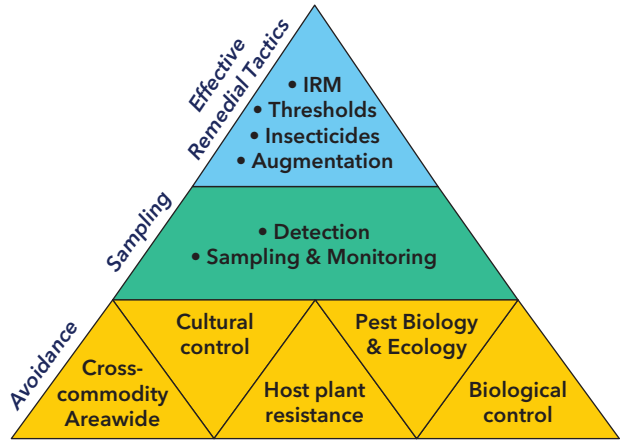

Figure 1 Conceptual model of IPM that emphasizes the importance of avoidance tactics to ameliorate pest problems. Source: modified from Naranjo (2001), with permission from Elsevier.

rudimentary forms of crop selection 3000 years ago, but it has only been in the last several centuries that cultivars with particular traits related to insect resistance were actively developed and cultivated (Smith, 2005). Three general categories of host plant resistance are recognized. In antixenosis, the behavior of the insect is affected such that it avoids the plant and moves on to other suitable plants. In contrast, antibiosis is manifested through direct effects on insect survival when feeding on the plant. Finally, the plant may express tolerance to the feeding activity of the insect such that it can withstand and/or recover from feeding injury without significant impacts on yield (Painter, 1951). In practice, it is likely that more than one of these characteristics may be involved in ultimately conferring pest resistance, and also likely that other IPM tactics will be needed to supplement pest suppression. Conventional breeding approaches have resulted in the development of effective host plant resistance against a number of pests in a variety of cropping systems (Smith, 2005). However, there has been relatively little success in developing crop resistance to lepidopteran and coleopteran insect pests, which are among the most significant pests globally.

Modern approaches such as marker-assisted selection and genetic engineering (GE) through the integration of targeted transgenes into the plant genome have accelerated the use of host plant resistance in modern agriculture. The first examples of successful GE were demonstrated over 30 years ago (Fischhoff et al., 1987; Vaeck et al., 1987). These groups inserted a chimeric and truncated gene from *Bacillus thuringiensis,* a common bacterium harnessed for pest control nearly 80 years ago (Sanahuja et al., 2011) and still a staple control tactic for organic agriculture, into tomato and tobacco plants.

Published by Burleigh Dodds Science Publishing Limited, 2020.

These genes were capable of producing functional Cry proteins, thereby enabling plants to be protected from certain caterpillar pests through antibiosis (Fischoff et al., 1987; Vaeck et al., 1987). Most Cry proteins have very narrow spectrums of activity against specific pest species and groups. This approach has since revolutionized the management of a number of key lepidopteran and coleopteran pest species in several crops including maize, cotton, soybeans, and most recently eggplant, cowpea and sugarcane, and has become an important tactic in the IPM toolbox. With a foundation of strong pest resistance through GE crops, other IPM tactics can be employed to achieve greater and more sustainable management for both the pests targeted by the resistance trait as well as other key and secondary pests in the system (Smith, 2005; Anderson et al., 2019; Romeis et al., 2019).

This chapter provides a broad overview of the application of host plant resistance through genetic engineering within an overall IPM context. We summarize the extent to which this technology is deployed in modern agriculture, examine regulatory and environmental risk assessment processes, show how GE crops are integrated into overall pest management systems and discuss resistance management and how it plays a vital role in ensuring the durability of the technology. We wrap up by summarizing how current GE technologies are being expanded to more pest targets and how new approaches such as RNAi and CRISPR offer even greater opportunities and challenges for IPM into the future.

2 Current genetically engineered (GE) crops

2.1 Bacillus thuringiensis *(Bt) crop adoption*

Since the first introduction of GE crops producing Cry proteins from *B. thuringiensis* in 1996, adoption rates have continued to grow with more crops and more countries using the technology every year (Table 1). The development and deployment of GE crops conferring high levels of antibiotic host plant resistance has been nothing short of a technological transformation in agriculture, and especially pest management (Shelton et al., 2002, 2008b). Australia, Mexico and the USA were the first adopting countries in 1996 and were collectively responsible for growing about 1.1 million ha of Bt maize, cotton and potato, and a total of 1.7 million ha of insect-resistant and herbicide-tolerant cultivars combined (James, 1997). Bt potato production in the USA, primarily for control of the Colorado potato beetle (*Leptinotarsa decemlineata*), ceased in 2000 due to perceived issues with consumer demand and the arrival of a new insecticide class (neonicitinoids) that would control beetle and other potato pests such as aphids (Smith, 2005; Grafius and Douches, 2008; Shelton, 2012). Argentina, Canada, China, Portugal, South

Table 1 Summary of global cultivation of currently approved insect-resistant genetically engineered (IRGE) crops conferring resistance to lepidopteran and coleopteran pests

	Bt Maize			Bt Cotton			Bt Soybean		
	Production (Ha × 1000)	% adoption[a] (first year)	IRGE proteins/dsRNA	Production (Ha × 1000)	% adoption (first year)	IRGE proteins	Production (Ha × 1000)	% adoption (first year)	IRGE proteins
North/Central America									
Canada	1780	86 (1997)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b], ECry3.1Ab[c], Cry34/35Ab[d], Cry3Bb, mCry3A, Vip3A						
Honduras	44	66 (2001)	Cry1Ab, Cry3Bb, Cry2Ab, Cry1A.105, Cry1F						
Mexico				210	96 (1996)	Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Vip3A			
USA	36790	80 (1996)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b], ECry3.1Ab[c], Cry34/35Ab[d], Cry3Bb, mCry3A, Vip3A, DvSnf7	4492	85 (1996)	Cry1Ac, Cry1Ab, Cry1F, Cry2Ab, Cry2Ae, Vip3A, mCry51Aa2[e]			
South America									
Argentina	5400	87 (1998)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b], ECry3.1Ab[c], Cry3Bb, mCry3A, Vip3A	320	100 (1998)	Cry1Ab, Cry1Ac, Cry2Ae	18100	17 (2015)	Cry1Ac
Brazil[f]	17550	85 (2008)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b], ECry3.1Ab[c], Cry34/35Ab[d], Cry3Bb, mCry3A, Vip3A	1175	59 (2005)	Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry2Ae, Vip3A	34742	58 (2013)	Cry1Ac

	Bt Maize			Bt Cotton			Bt Soybean		
	Production (Ha × 1000)	% adoption[a] (first year)	IRGE proteins/dsRNA	Production (Ha × 1000)	% adoption (first year)	IRGE proteins	Production (Ha × 1000)	% adoption (first year)	IRGE proteins
Colombia	375	20 (2007)	Cry1Ab, Cry1F, Vip3A	9	90 (2002)	Cry1Ac, Cry2Ab			
Paraguay	640	40 (2012)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b], Cry3Bb, Vip3A	11	100 (2011)	Cry1Ac, Cry2Ab	2790	34 (2013)	Cry1Ac
Uruguay	50	96 (2003)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b], Vip3A				1110	25 (2012)	Cry1Ac
Europe									
Portugal	116	6 (1999)	Cry1Ab						
Spain	345	36 (1998)	Cry1Ab						
Africa									
South Africa	2300	70 (1997)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b]	38	95 (1997)	Cry1Ac, Cry2Ab			
Sudan				120	98 (2012)	Cry1Ac			
Asia									
Bangladesh[g]									
China				3400	95 (1997)	Cry1A, Cry1Ac, Cry1Ab-Ac[h], CpTI			
India				12 400	93 (2002)	Cry1Ac, Cry1Ab-Ac[h], Cry1C, Cry2Ab			
Myanmar				249	92 (2006)	Cry1Ac			
Nigeria[i]				270	0 (2018)	Cry1Ac, Cry2Ab			

(*Continued*)

Table 1 (*Continued*)

	Bt Maize			Bt Cotton			Bt Soybean		
	Production (Ha × 1000)	% adoption[a] (first year)	IRGE proteins/dsRNA	Production (Ha × 1000)	% adoption (first year)	IRGE proteins	Production (Ha × 1000)	% adoption (first year)	IRGE proteins
Pakistan	1229	N/A(2018)	Cry1Ab, Cry1F, Cry2Ab, Cry1A.105[b]	2700	96 (2010)	Cry1Ac, Cry1Ab-Ac[h]			
Philippines	1378	44 (2003)	Cry1Ab, Cry1Fa2, Cry2Ab, Cry1A.105[b]						
Vietnam	1151	4 (2015)	Cry1Ab, Cry2Ab, Cry1A.105[b]						
Australasia									
Australia				530	96 (1996)	Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry2Ae, Vip3A			

[a] % adoption in 2017.
[b] Cry1A.105 chimeric with domains from Cry1Ab, Cry1Ac and Cry1F.
[c] eCry3.1Ab = chimeric Cry3A–Cry1Ab.
[d] Binary proteins.
[e] Under development for targeting *Lygus* spp. and thrips.
[f] Brazil approved the production of Bt sugarcane (Cry1Ab in 2017, Cry1Ac in 2018); the country produces 10.2 million ha; current Bt adoption rates are unknown.
[g] Bangladesh first approved the cultivation of Bt eggplant (Cry1Ac) in 2014; the country produces 50 000 ha; current Bt eggplant adoption rates are about 5%.
[h] Fusion protein.
[i] Nigeria approved Bt cowpea (Cry1Ab) for cultivation in 2018; the country produces 3.7 million ha; current Bt cowpea adoption rates are 0%.
Production and adoptions rates from www.cotton.org, www.fao.org, ISAAA (2017); IRGE data from www.isaaa.org/gmapprovaldatabase/.

Africa and Spain began production of GE crops in the latter half of the 1990s and many others joined the ranks in the early to mid-2000s. France (1998), Germany (2000), Czech Republic (2005), Slovakia (2006), Poland (2007) and Romania (2007) grew Bt maize for several years before ceasing production. France and Germany banned cultivation of Bt maize in 2009 (https://www.nature.com/news/2009/090414/full/news.2009.364.html), and Poland banned production in 2013 (https://phys.org/news/2013-01-poland-cultivation-gm-maize-potatoes.html). Czech Republic, Slovakia and Romania ceased production in 2017 due primarily to market issues but have not banned production and remain interested in using biotechnology to enhance competitiveness for their growers (ISAAA, 2017). Bt cotton was grown in Burkina Faso starting in 2008, primarily for control of old-world bollworm (*Helicoverpa armigera*), but production was ceased in 2016 due to several factors including issues with the lint quality of available cultivars (Sanou et al., 2018). Bangladesh began growing Bt eggplant in 2014 and Vietnam commenced Bt maize production in 2015. Bangladesh is perhaps most notable for being the first developing nation to commercially grow a GE vegetable, in this case for control of the notorious eggplant fruit and shoot borer (*Leucinodes orbonalis*). Seed was distributed to 20 farmers in 2014 and by 2018 this increased to 27,012 farmers or about 5% of the crop countrywide (Prodhan et al., 2018; Shelton et al., 2018). The most recent crops approved for cultivation are Bt cowpea in Nigeria to help protect the crop from damage by the bean pod borer, *Maruca vitrata* (IITA, 2019) and Bt sugarcane in Brazil for control of stem borers (ISAAA, 2017).

Levels of adoption of insect-resistant and herbicide-tolerant GE crops skyrocketed by the end of 2017, with 22 countries now producing 190 million ha overall with over 101 million ha of Bt crops (Table 1; ISAAA, 2017). For Bt crops alone this represents over a 9000% change in production since 1996. Up until about 2011, the majority of GE crops were being grown in developed countries. However, since then, more GE crops are being cultivated in developing nations and in 2017 about 53% of all GE crops were being grown in developing countries (ISAAA, 2017). To further emphasize this pattern, 14 of the 18 'mega-countries,' or those producing more than 50 000 ha of GE crops, are developing countries in Africa, Asia and Latin America.

2.2 Current Bt proteins in GE crops

Cry proteins are δ-endotoxins produced by bacteria during sporulation and have very specific interactions within the gut of susceptible insects after ingestion, ultimately leading to the formation of holes in the gut wall and death by sepsis (Bravo et al., 2007). *B. thuringiensis* is also the source of vegetative insecticidal proteins (VIPs), which as the name implies are produced vegetatively by the bacteria (Estruch et al., 1996). These VIPs have become more common in

pyramids along with other Cry proteins to enhance efficacy and help mitigate resistance development (see Section 5.2). While there are hundreds of known Cry proteins (Glare and O'Callaghan, 2000), relatively few (Cry1, 2 and 3, and their modifications) have been harnessed for use in Bt crops. The current Bt crops available primarily target key lepidopteran pests and, in the USA and some parts of Latin America, also coleopteran pests, specifically, species of corn rootworm (*Diabrotica* spp.). The Bt Cry proteins have a very narrow spectrum of activity, with Cry1 and Cry2 type proteins targeting caterpillars and certain Cry3 proteins targeting coleopterans within the family Chrysomelidae. While VIP proteins have a very different mode of action to Cry proteins (Lee et al., 2003), the spectrum of activity of the currently used protein is again very narrow, affecting only Lepidoptera (Raybould and Vlachos, 2011; Whitehouse et al., 2014).

In the early years of Bt-crop production, single Cry proteins were common (e.g. Cry1Ac in cotton and Cry1Ab in maize), but pyramids of two or more proteins targeting a group of pests (e.g. Lepidoptera) are now dominant and these are almost always stacked with one or more genes providing tolerance to various herbicides. These pyramids of several proteins provide more effective target pest control and also contribute to the management of resistance evolution (see Section 5.2). In the case of maize, pyramids and stacks (especially in the USA) contain multiple Cry proteins with efficacy against both lepidopteran and coleopteran pests. The most extreme examples come from SmartStax® maize in which the plant produces six different proteins for control of lepidopteran and coleopteran pests along with two herbicide-tolerance genes (Head et al., 2017). At the other extreme, only single gene maize events (Cry1Ab) are produced in Spain and Portugal, and Bt soybeans produced in several South American countries are currently single gene events (Cry1Ac) (Table 1). Although not detailed in Table 1, there are dozens of different varieties for Bt maize and cotton, often containing insect resistance and herbicide tolerance, marketed by a number of seed companies in adopting countries.

2.3 Pest targets of Bt crops

As noted previously, the host plant resistance embodied in GE crops producing proteins from *B. thuringiensis* has fairly narrow spectrums of activity (see Section 3.4). Cry1, Cry2 and Vip3A proteins have specific activity against lepidopteran species, although not all species within the order are equally susceptible (see summaries in Hellmich et al., 2008; Naranjo et al., 2008). Primary targets vary from country to country and even regions within countries (Table 2). For example, pink bollworm (*Pectinophora gossypiella*) is the primary target of Bt cotton in the southwestern USA, but cotton bollworm (*Helicoverpa zea*) is a primary target in other areas of the USA Cotton Belt. Overall, a broad

Published by Burleigh Dodds Science Publishing Limited, 2020.

Table 2 Summary of target pests of Bt crops in adopting countries

Species	Family	Common name	Bt Maize	Bt Cotton	Bt Soybean	Bt Eggplant	Bt Cowpea	Bt Sugarcane
Lepidoptera								
Agrotis spp.	Noctuidae	Cutworm	1, 5, 8, 13, 15, 16, 17, 18, 21, 22	2				
Alabama argillacea	Noctuidae	Cotton leafworm		1, 4, 7, 14				
Amsacta moorei	Arctiidae	Red hairy caterpillar		9				
Anomis flava	Noctuidae	Cotton looper		2, 6, 9				
Anticarsia gemmatalis	Noctuidae	Velvetbean caterpillar			1, 4, 14, 20			
Bucculatrix gossypii	Tineidae	Cotton leaf perforator		2				
Bucculatrix thurberiella	Tineidae	Cotton leaf perforator		7, 10, 21				
Busseola fusca	Noctuidae	African maize stalk borer	17					
Chilo partellus	Crambidae	Spotted stalk borer	15, 17, 22					
Crocidosema plebejana	Tortricidae	Cotton tip worm		2				
Diatraea grandiosella	Crambidae	Southwestern corn borer	8, 21					
Diatraea saccharalis	Crambidae	Sugarcane borer	1, 4, 7, 8, 14, 20, 21	1				4
Diparopis castanea	Noctuidae	Red bollworm		17				
Earias biplaga	Noctuidae	Spiny bollworm		17				

(*Continued*)

Table 2 (*Continued*)

Species	Family	Common name	Bt Maize	Bt Cotton	Bt Soybean	Bt Eggplant	Bt Cowpea	Bt Sugarcane
Earias huegeliana	Noctuidae	Rough bollworm		2				
Earias insulana	Noctuidae	Spiny bollworm		9, 11, 13, 17				
Earias spp.	Noctuidae	Spiny bollworm		6				
Earias vittella	Noctuidae	Spotted bollworm		9, 11, 13				
Estigmene acrea	Arctiidae	Saltmarsh caterpillar		7, 21				
Helicoverpa armigera	Noctuidae	Old-world bollworm	13, 15, 16, 18, 22	2, 4, 6, 9, 11, 12, 13, 14, 17, 19	1, 4, 14, 20			
Helicoverpa gelotopoeon	Noctuidae	Bollworm		1				
Helicoverpa punctigera	Noctuidae	Native budworm		2				
Helicoverpa zea	Noctuidae	Corn earworm, Cotton bollworm	1, 4, 5, 7, 8, 14, 20, 21	4, 7, 10, 14, 21	1, 4, 14, 20			
Heliothis virescens	Noctuidae	Tobacco budworm		1, 4, 7, 10, 14, 21				
Leucinodes orbonalis	Crambidae	Eggplant fruit/shoot borer				3		
Maruca vitrata	Crambidae	Bean pod borer					12	
Mythimna separata	Noctuidae	Oriental armyworm	13					
Ostrinia furnacalis	Crambidae	Asian corn borer	15, 17, 22	6				
Ostrinia nubilalis	Crambidae	European corn borer	5, 16, 18, 21	21				
Pectinophora gossypiella	Gelechiidae	Pink bollworm		1, 2, 4, 6, 7, 9, 10, 11, 13, 14, 17, 21				

Species	Family	Common name	Bt Maize	Bt Cotton	Bt Soybean	Bt Eggplant	Bt Cowpea	Bt Sugarcane
Pectinophora scutigera	Gelechiidae	Pink spotted bollworm		2				
Pseudaletia unipuncta	Noctuidae	Armyworm	1, 5, 16, 18, 21					
Pseudoplusia includens	Noctuidae	Soybean looper		4, 7, 21				
Sacadotes pyralis	Noctuidae	S.A. bollworm		7				
Sesamia nonagrioides	Noctuidae	Mediterranean corn borer	16, 17, 18					
Spodoptera exigua	Noctuidae	Beet armyworm	8	10				
Spodoptera frugiperda	Noctuidae	Armyworm	1, 4, 5, 7, 8, 14, 20, 21	1, 2, 4, 6, 7, 9, 10, 11, 12, 13, 14, 17, 19, 21	1, 4, 14, 20			
Striacosta albicosta	Noctuidae	Western bean cutworm	5, 21					
Sylepta derogata	Pyralidae	Cotton leaf roller		9, 11, 13, 17				
Trichoplusia ni	Noctuidae	Cabbage looper		7, 10, 21				
Coleoptera								
Diabrotica spp.	Chrysomelidae	Corn rootworms	1, 4, 5, 8, 21					

1 = Argentina, 2 = Australia, 3 = Bangladesh, 4 = Brazil, 5 = Canada, 6 = China, 7 = Colombia, 8 = Honduras, 9 = India, 10 = Mexico, 11 = Myanmar, 12 = Nigeria, 13 = Pakistan, 14 = Paraguay, 15 = Philippines, 16 = Portugal, 17 = South Africa, 18 = Spain, 19 = Sudan, 20 = Uruguay, 21 = USA, 22 = Vietnam.
Compiled from various sources including Hellmich et al. (2008); Naranjo et al. (2008); Blanco et al. (2016); Rocha-Munive et al. (2018), personal communications.

range of target pests can be found in adopting countries (Table 2). The most significant targets in Bt maize include *Agrotis* spp. cutworms, stalk borers like *Diatraea* spp., *Ostrinia* spp., *Busseola fusca* and *Sesamia nonagrioides*, and ear and foliar pests such as the corn earworm (*H. zea*) and fall armyworm (*Spodoptera frugiperda*). This latter insect has recently invaded Africa and Asia (e.g., Goergen et al., 2016; Ganiger et al., 2018; Nagoshi et al., 2018). The primary targets of Bt cotton include the cotton leafworm (*Alabama argillacea*) in South America, old-world bollworm (*H. armigera*) throughout Asia, Australasia and South America, boll and budworm (*H. zea* and *Heliothis virescens*) in the Americas, and *P. gossypiella* and *S. frugiperda*, both of which have wide global distributions. The distribution of *H. armigera* is growing with the relatively recent invasion of South America (Tay et al., 2013). At present, Bt soybean production is limited to several countries in South America, where the primary targets are the velvetbean caterpillar (*Anticarsia gemmatalis*), *H. armigera*, *H. zea* and *S. frugiperda* (Table 2). *Diabrotica* spp. corn rootworms represent the sole coleopteran targets of Bt maize that are cultivated only in the Americas. As noted, the degree of efficacy of Bt crops on these target pests is variable. Due to this factor and the presence of other key arthropod pests not susceptible to Bt proteins, this type of host plant resistance typically represents only one tactic in an overall IPM strategy (see Section 4).

3 Environmental aspects

GE crops, like other agricultural technologies, can cause impacts on the environment. In general, those impacts can be subdivided into direct and indirect effects (Sanvido et al., 2007). Direct effects are caused by the genotype or phenotype of the GE crop and include a change in persistence and invasiveness of the crop (weediness), hybridization with sexually compatible plants (gene flow) and effects on biodiversity (non-target organisms and ecosystem functions). Indirect effects on the environment can be caused when the target pest develops resistance against the introduced trait (see Section 5) and thus alternative control measures need to be undertaken such as changes in GE crop management (e.g. changes in pesticide application patterns; see Section 4).

3.1 Regulatory aspects

The potential of GE crops to cause unacceptable harm to the environment is assessed prior to their cultivation during an Environmental Risk Assessment (ERA). While GE plants are regulated products in most jurisdictions (Craig et al., 2008), the triggers for regulation differ. In the case of GE plants with insecticidal traits, three principles can be identified (Schiemann et al., 2019). In many

jurisdictions, including the European Union (EU), Brazil, Argentina and India, regulation is triggered by the technology applied to introduce a certain trait (process-based regulation). In contrast to this, in Canada, it is the novelty of the trait that is the trigger (product-based regulation). The situation in the USA is somewhere in between as the legislative focus is placed on the characteristics of the product. However, the trigger for regulatory oversight is that the GE plant could pose a plant risk (i.e., when the plant is transformed using *Agrobacterium tumefaciens*, which is regarded as a plant pathogen). Internationally, the Cartagena Protocol on Biosafety (CBD) facilitates the establishment of national biosafety regulatory systems. The aim of the CBD is '…ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.' It thus '…creates an enabling environment for the environmentally sound application of biotechnology.' This protocol was adopted in January 2000 at the Convention on Biological Diversity and entered into force in September 2003 (Devos et al., 2012). However, major developers and adopters of GE plants such as the USA, Argentina and Canada have not ratified the protocol (https://bch.cbd.int/protocol/parties/). Furthermore, for those countries that have signed the agreement, there have been difficulties in developing a consensus document to guide risk assessment of GE crops under the CBD. Some countries may also consider that participating in the agreement will limit them from being able to utilize GE crops for the benefit of their countries (Hokanson, 2019).

3.2 Gene flow

Another concern with the use of GE plants is that the gene could be transferred to wild and weedy relatives of the GE crop, which could then gain a fitness advantage with potential adverse ecological consequences (Poppy and Wilkinson, 2005). What is important to note is that from an environmental point of view, it is not the gene flow itself that is a concern, but the potential for any ecological consequences.

The process of gene flow and introgression is complex and requires multiple steps (Stewart et al., 2003). For gene flow to occur, the GE crop and its wild relatives must grow in the same region within the pollen dispersal distance and they must flower at the same time. Successful fertilization must then lead to the development of an embryo and a seed. Subsequently, the hybrid seed must establish, flower and further hybridize with the wild type plants. However, a key element for potential ecological effects is whether the hybrid plant might increase in competitiveness due to the novel trait (i.e. insect resistance) that

it has obtained (Hails and Morley, 2005). So, two factors are important. First, the transgenic trait needs to provide a selective advantage to the wild plant. Second, the trait must be able to establish in the natural population (Sanvido et al., 2007). In the case of insect-resistance traits this depends on whether the fitness of the wild plant is affected by herbivores, and to what extent, and whether the novel trait comes with any fitness costs, which become important in the absence of herbivores.

One example comes from Bt sunflower (*Helianthus annuus*) producing Cry1Ac, developed to protect the plant from damage by a complex of lepidopteran pests in the USA (Snow et al., 2003). Field experiments using male-sterile hybrids with wild *H. annuus* demonstrated a significant fitness benefit to the Bt plants suggesting that the release of Bt-transgenic sunflower would likely result in a spread of the transgene to wild and weedy sunflower populations. This crop was never commercialized. A more pertinent example has been reported from Europe (Devos et al., 2018). The only GE crop cultivated there is Bt maize MON810 that produced Cry1Ab. Initially, gene flow has not been an issue because no wild relatives of maize were grown in Europe. This situation changed when Teosinte, the progenitor of maize, was observed as a weed. Thus, concerns were raised that gene flow to Teosinte might occur and that Teosinte x maize hybrids could exaggerate weed problems. Recently, risk assessment experts have addressed this question by identifying plausible pathways through which harm to the environment could occur. This enabled the identification of events that have to occur and helped derive risk hypotheses about the likelihood and severity of these events. Using relevant and available information, it was demonstrated that there is negligible risk that gene flow to Teosinte would cause harmful effects to the environment that are greater than those caused by conventional maize (Devos et al., 2018).

Concerns about gene flow into areas of crop origin have been raised because these areas often have the greatest diversity of wild relatives. Consequently, the Brazilian authority has established exclusion zones for transgenic cotton to minimize gene flow to other *Gossypium* species (Fitzpatrick et al., 2009). Similar restrictions apply to cultivation of Bt cotton in certain areas of Hawaii and Florida in the USA (McHughen and Smyth, 2008).

3.3 Invasiveness and persistence of GE crops

Increased invasiveness and persistence of the GE crop could pose a threat to agricultural production and biodiversity in natural habitats if they spread and reduce the abundance of other (valued) species. Given that the field crops grown today are highly domesticated, it is highly unlikely that the addition of a single trait would turn them into a weed because numerous factors have to be fulfilled for a plant to be weedy (Keese et al., 2014). Nevertheless, some studies

have been performed to experimentally assess the invasiveness/persistence of GE crops with insecticidal traits. In a long-term study, Crawley et al. (2001) monitored the performance of GE potatoes producing either a Bt Cry protein or pea lectin in 12 different habitats over a period of 10 years. In no case did the data suggest that GE potatoes were more invasive or persistent than their conventional counterpart. A study in Northern Australia demonstrated no increased fitness of weeds in Bt cotton (Eastick and Hearnden, 2006). In a more recent study, Raybould et al. (2012) studied the performance of several Bt maize events in non-agricultural habitats in Texas, USA. Overall, the GE maize was found to behave similar to non-transgenic maize and was not able to establish self-sustaining feral populations. In summary, there is no evidence available to date that insect-resistant GE crops are more invasive of natural habitats than are conventional crops (Sanvido et al., 2007).

3.4 Non-target risk assessment

One of the major concerns with the use of GE plants with insecticidal traits is their potential to harm non-target species and biodiversity in general. This is considered a potential harm because biodiversity provides numerous important ecosystem services (MEA, 2005). Arthropods, in particular, can provide cultural services (e.g. species that are valued because they are rare or endangered), supporting services (e.g. nutrient cycling supported by detritivores, pollination) and regulating services (e.g. biological control). From an IPM perspective, of course, this latter service is important.

Consequently, the non-target risk assessment is a fundamental component of the ERA of GE plants with insecticidal traits. A typical risk hypothesis that is addressed is that the insecticidal compound does not harm non-target species at the concentration at which it is present in the field (Garcia-Alonso et al., 2006; Romeis et al., 2008). This hypothesis is tested within a tiered framework that progresses from laboratory studies under worst-case exposure conditions to semi-field and field studies if required. Higher tier studies are indicated when harm cannot be excluded based on the laboratory studies or when unacceptable uncertainty exists. Analyzing the published evidence available at that time, Duan et al. (2010) suggest that such early-tier laboratory studies can conservatively predict non-target effects expected in the field. It is important to note that testing the risk hypothesis does not automatically require studies. It might be possible to address the hypothesis using existing data that have been collected previously, for example, as part of an earlier risk assessment (Romeis et al., 2009; Raybould and Quemada, 2010; Ba et al., 2018). The same applies to higher tier studies (Garcia-Alonso et al., 2014; Corrales Madrid et al., 2018).

In cases where studies need to be conducted, care has to be taken regarding which species should be used for laboratory testing. The criteria on

how to select the most appropriate test species has been described in detail elsewhere (Carstens et al., 2014; Romeis et al., 2013a; Wach et al., 2016). In short, the species should first represent valued taxa or functional groups that are most likely to be exposed to the insecticidal factor in the field (relevance criteria). Second, species should be selected that are most likely to be sensitive to the insecticidal factor (sensitivity criteria). Sensitivity can be anticipated based on what is already known about the spectrum of activity and the mode of action of the compound. Third, the species should be amenable and available to testing (availability criteria). This means that a sufficient number of good quality insects of the most appropriate life stage are available for testing. Furthermore, test protocols that lead to robust results are required.

We have a good understanding about the flow of plant-produced insecticidal proteins within the environment in general and within arthropod-food webs in particular (Romeis et al., 2019). In general, Bt proteins are diluted when moving along the food chain due to excretion and digestion at each trophic level. Consequently, exposure is highest for natural enemy species that directly consume plant materials that contain high amounts of the insecticidal compounds such as pollen from some Bt maize events (Romeis et al., 2019). This, together with our knowledge about the beneficial arthropod species that are present in field crops allows the identification of the most relevant test species. Databases comprising this knowledge have been established and it has been demonstrated how the information can be used for test species selection in the case of Bt maize in Europe (Romeis et al., 2014), Bt rice in China (Li et al., 2017), Bt cowpea in West Africa (Ba et al., 2018), Bt sweet potato in Uganda (Rukarwa et al., 2014) and Bt pine trees in New Zealand (Todd et al., 2008).

Once test species have been identified, care has to be taken to design laboratory studies that have a low risk of deriving erroneous results. In particular, false negatives need to be avoided because they could lead to the cultivation of GE plants that cause harm to non-target species. However, false positives also can cause damage as they may result in the ban of safe products (e.g. Romeis et al., 2013b). This puts a high demand on the design and execution of such laboratory tests. An international working group has developed and proposed general recommendations on how to conduct these studies (Romeis et al., 2011).

To avoid false negative results, the following criteria are necessary: (1) the non-target test species has ingested high amounts of the bioactive test substance and (2) the study design can detect adverse effects. In the case of false positives, care has to be taken that the adverse results obtained were caused by the stressor of concern and not artifacts due to poor study design. This appears to be most problematic in cases where GE plant material was used as the test substance because it has to be ensured that the nearest non-GE plant is used for comparison. This is important to link any effect back to the

Published by Burleigh Dodds Science Publishing Limited, 2020.

stressor of concern. Furthermore, many erroneous results were reported from tri-trophic studies where a natural enemy was not harmed by the insecticidal protein per se but by the quality of the herbivore used as host or prey. In these cases, host of prey insects were adversely affected by the insecticidal compound. There are many examples of such false positive outcomes in prey/host-quality mediated effects available in the literature (see Naranjo, 2009; Romeis et al., 2013b, 2019 for a detailed discussion).

Overall, studies conducted on the currently deployed Cry proteins from Bt have shown that the proteins have a very narrow spectrum of activity and pose negligible risk to non-target species outside of the taxonomic order of the target pest(s). The available literature has been summarized in meta-analyses (Wolfenbarger et al., 2008; Naranjo, 2009; Duan et al., 2010; Comas et al., 2014) and reviews (Romeis et al., 2019).

3.5 Change in insecticide use patterns

GE plants with insecticidal traits aim to improve pest control and thus replace synthetic insecticides. Consequently, the adoption of Bt-transgenic varieties leads to changes in agricultural practice and typically to a decline in the use of insecticides with positive consequences for the environment and non-target species in particular (Lu et al., 2012; Klümper and Qaim, 2014; Ellsworth et al., 2018; Brookes and Barfoot, 2018). Unfortunately, the decline in insecticide use in GE crops has been countered somewhat in cotton and maize, but not eggplant, by the steady increase in the use of systemic insecticides applied as seed treatments (Douglas and Tooker, 2015; Papiernik et al., 2018) even though there is no direct link to their use in connection to GE plant varieties (also see Section 4.5).

There is a sound body of literature showing that in systems where insecticidal GE varieties replace insecticides, non-target organisms and biological control, in particular, benefits (Romeis et al., 2006; Wolfenbarger et al., 2008; Naranjo, 2009; Lu et al., 2012).

4 Integration into IPM

Currently available GE insect-resistant crops (Bt crops) represent powerful forms of host plant resistance that provide the selectivity and persistence in pest control that has long been sought in IPM programs. The proteins currently produced in Bt crops target several key lepidopteran and coleopteran pests (see Table 2), including some that are often cryptic in nature and difficult to effectively control by traditional foliar or soil insecticides. Examples include *O. nubilalis* larvae that bore into maize stalks, *P. gossypiella* larvae that feed within seeds of cotton bolls and *Diabrotica* spp. that feed on maize roots. As

a foundational element of an overall IPM strategy (see Fig. 1), Bt crops have enabled cascading benefits within the agroecosystem through positive interactions with other IPM tactics that would not have been possible with the alternative use of conventional control systems such as broad-spectrum insecticides.

4.1 The upsides and downsides of Bt selectivity

As discussed in Section 3.4, the selectivity of Bt proteins has helped support the conservation of arthropod natural enemies and thus the biological control that functions to regulate pest populations (also see Romeis et al., 2019). From a broader ecological perspective, the lack of harm by the currently approved Bt crops also helps preserve arthropods and other non-target biota that collectively provide critical functions such as pollination and nutrient recycling in the soil.

However, from another vantage point, the selectivity of Bt crops can be considered a limitation compared to broader-spectrum insecticides. For example, problems can occur if there is a complex of lepidopteran pests that need to be controlled and the particular protein chosen to control one key pest does not control others. This situation has occurred with the western bean cutworm (*Striacosta albicosta*), historically found in the western USA Corn Belt as a common pest of dry beans and only a sporadic pest of maize (Hagen, 1962; Michel et al., 2010; Hutchison et al., 2011; Paula-Moraes et al., 2013). Starting in 2000, economic damage from this pest was found on maize in Iowa and Minnesota (O'Rourke and Hutchison, 2000) and it has continued to expand its range eastward to become a key pest in many states (Dorhout and Rice, 2004; DiFonzo and Hammond, 2008; Tooker and Fleisher, 2010). Unfortunately, many of the Bt maize varieties commercialized to control other Lepidoptera, especially *O. nubilalis*, are not effective against *S. albicosta* (https://lubbock.tamu.edu/files/2018/11/BtTraitTableNov2018.pdf) (see Section 5 for resistance issues in this pest). Therefore, growers need to be cognizant of their pest complex and select the maize varieties that have the relevant traits.

The field crops for which we have Bt varieties are affected by a range of pest species outside of Lepidoptera and one family within Coleoptera. Thus, besides selecting the trait that will best control the Bt-targeted pest complex, it is also important to consider how using a Bt trait will affect other non-target members of the pest complex. Specifically, what will happen to other arthropod pests when Bt crops replace largely broad-spectrum insecticides? For example, in China, widespread adoption of Bt cotton, and the associated decreased use of broad-spectrum insecticides, was correlated with an increased abundance of mirid bugs not only in Bt cotton fields, but also in other affected crops in the region (Lu et al., 2010). Similar scenarios have played out with the green

mirid in Australia, with mirids and stink bugs in the southern USA, plant bugs in South Africa, and mealy bugs, thrips and leafhoppers in India. The alternate use of broader-spectrum insecticides for control of these non-target pests in Bt crops has then led to other pest outbreaks precipitated by disruption of natural enemy communities (see Naranjo et al., 2008 for summary of non-target pest issues).

However, there are other instances in which the use of Bt crops has provided enhanced control of non-target pests. For example, the introduction of Cry1Ac-containing Bt cotton varieties in 1996 helped to usher in a new era of selective pest control in Arizona (Naranjo and Ellsworth, 2009; Anderson et al., 2019). Bt cotton provided excellent control of the pink bollworm without the use of traditional foliar insecticides that were often applied early to mid-season. This coupled with the availability of selective insect growth regulators and selective feeding inhibitors for *Bemisia tabaci* and *Lygus hesperus*, respectively, two other keys pests in the system enabled biological control of key and secondary pests by a suite of native arthropod natural enemies. The resulting program manages all cotton pests in an integrated manner with biological control and the limited use of selective insecticides. Use of broad-spectrum insecticides is essentially eliminated. Overall insecticide use has been reduced over 80% with cotton growers saving over $500 million in yield loss and control costs since 1996 (Naranjo and Ellsworth, 2009; Ellsworth et al., 2018).

4.2 Do Bt crops fit the IPM paradigm?

There is a debate about whether the prophylactic practice of planting a Bt crop is compatible with the fundamental concept of IPM whereby a control tactic should only be implemented after sampling to determine whether an economic injury level has been reached (Stern et al., 1959). To explore this question in more detail, it is important to consider that the typical densities of a pest population, the amplitude of its fluctuations and the pest's destructiveness all influence the value of sampling a population to determine if a treatment is necessary (Onstad, 1987). It has been argued that in cases where there is a high likelihood of the pest causing economic damage, sampling and economic thresholds may not be economically worthwhile (Poston et al., 1983; Nyrop et al., 1986, 1989; Onstad, 1987). In such situations, the prophylactic use of Bt plants appears justified for controlling the pest. This is the nature of the deployment of host plant resistance, whether produced by GE or traditional plant breeding methods, in IPM programs. One cannot decide later that certain varieties should have been planted – they have to be used proactively. However, considerations also should be made about whether such prophylactic use will have other consequences such as increased likelihood of promoting the evolution of resistance in the pest to the proteins produced in the plant.

Crowder et al. (2006) used a simulation model to investigate whether economic thresholds can be used to improve IPM and insect resistance management (IRM) when Bt maize is used to manage *Diabrotica* spp., serious and perennial pests throughout much of the USA Corn Belt. Their model evaluated whether the use of sampling and economic thresholds could make IPM more efficient and IRM more effective in helping farmers decide whether to plant Bt maize. They explored the use of economic thresholds that determine the planting of Bt maize with and without the use of spatial refuges. Their findings suggested the use of economic thresholds only slightly slowed the evolution of resistance to Bt maize and the use of sampling and economic thresholds generated similar returns compared with strategies of planting Bt maize every season. Because Bt crops are extremely effective, farmers are often inclined to plant transgenic crops every season rather than implementing costly and time-consuming sampling protocols. Still, the deployment of host plant resistance can be viewed prescriptively. A Bt maize Economic Tool (BET) was developed at Penn State University that uses maize and *O. nubilalis* development models based on degree days to predict when Bt maize deployment is most economical (https://ento.psu.edu/extension/field-crops). This approach merges transgenic technology with more traditional insect IPM tools based on need (Hellmich et al., 2005).

4.3 Landscape effects of Bt plants

Any pest management tactic, whether the release of a biological control agent or a new insecticide, will have an impact on a larger scale than a single farm because of insect movement. Such landscape effects have been observed with Bt crops, especially those that have been widely adopted in a region, such as maize and cotton. In the Midwest USA where Bt maize has been widely adopted, there has been regional suppression of *O. nubilalis* that has benefited Bt maize growers as well those who did not grow Bt maize, a phenomenon referred to as a 'halo effect' (Hutchison et al., 2010). In a recent study in the Mid-Atlantic USA, an analysis of data collected from 1976 to 2016 concluded that vegetable growers have benefited from widespread adoption of Bt maize on a regional basis due to pest suppression of *O. nubilalis* and *H. zea*, pests that attack numerous vegetables in the region (Dively et al., 2018). Here, vegetable growers benefited from decreased crop damage and a reduced need for insecticide applications.

Regional suppressive effects of pests by Bt cotton also have been observed. The adoption of Bt cotton in Arizona dramatically reduced populations of *P. gossypiella* (Carrière et al., 2003) and was a major component of a multi-national program that successfully eradicated this pest in late 2018 across the entire USA and northern Mexico (Western Farm Press 2018). In China, the use

of Bt cotton not only suppressed populations of *P. gossypiella* in cotton (Wan et al., 2012), but also *H. armigera*, a pest with a much wider host range. Reports indicate regional suppression of *H. armigera* in China also has provided benefits to farmers of other crops, including high value vegetables (Wu et al., 2008).

Landscape effects have not only been seen with pest suppression, but also with increases in natural enemies and the biological control services they provide. For example, with the adoption of Bt cotton in northern China, and its resulting reduction in broad-spectrum insecticides, long-term data suggest increases in general predator abundance throughout the landscape, with biological control benefits realized in cotton and other crops such as maize, soybeans and peanuts in the region (Lu et al., 2012).

4.4 Integrating other management tactics with Bt crops

As discussed, biological control integrates well with Bt crops as part of an overall IPM program. This positive interaction of Bt crops and biological control can be enabled even further with other tactics such as selective insecticides for other key and secondary pests, as demonstrated by the pest management program for cotton in Arizona (Naranjo and Ellsworth, 2009, 2010). Other traditional and effective strategies, including cultural practices, also need to be part of an overall IPM program. However, some of the traditional cultural practice options like crop rotation may no longer be as effective for certain pests in crops where the Bt trait is available.

For example, the cultural practice of crop rotation has been an effective strategy to manage *Diabrotica* spp. on maize when the insects had one generation per year and the females laid their eggs in maize fields where they would overwinter. However, *Diabrotica barberi* was the first insect documented to subvert annual crop rotation by evolving extended diapause enabling adults to emerge two or three years later (Krysan et al., 1986; Levine et al., 1992). In contrast, some strains of *Diabrotica virgifera virgifera* have evolved to deposit eggs outside maize fields, often in soybean fields (Levine et al., 2002). Because of the common practice of rotating the non-host soybeans with maize, the larvae that hatch from eggs laid in soybean field may find themselves in their preferred maize host when they hatch next season. The evolution of these strains in some areas makes crop rotation a less effective strategy for combining with Bt maize into an overall IPM and IRM strategy.

Although we are not aware of any current management systems that use trap cropping in combination with Bt plants, research has shown possible areas where this strategy may be useful (Shelton and Badenes-Perez, 2006). Using Bt potatoes, Hoy et al. (2000) showed that when they were planted early in the season along borders, they attracted immigrating Colorado potato beetle and prevented colonization of the interior of the field planted to non-Bt potatoes.

Another example used the concept of 'dead-end' trap cropping, in which the trap crop is more attractive for oviposition than the cash crop but on which the pest insect cannot survive (Shelton and Nault, 2004). Dead-end trap crops thus serve as a sink for pests, preventing their movement from the trap crop to the main crop. Greenhouse studies with the diamondback moth (*Plutella xylostella*) demonstrated that Bt Indian mustard and Bt collards significantly reduced the insect population and decreased damage to the cash crop, cabbage (Shelton et al., 2008a). Another example is that of Bt rice with resistance to stemborers such as *Chilo suppressalis*. Under laboratory, greenhouse and field conditions, females were found to prefer undamaged Bt rice plants for oviposition when given a choice with caterpillar-damaged non-Bt rice (Jiao et al., 2018). Consequently, Bt rice could act as a dead-end trap crop protecting non-Bt rice in the vicinity. Interestingly, the opposite was observed for a non-Lepidoptera pest, the planthopper *Nilaparvata lugens*. When given a choice, planthoppers showed a strong preference for caterpillar-damaged plants (Wang et al., 2018). Thus, this non-target pest appears to be attracted to non-Bt refuge plants grown near Bt rice.

4.5 Prophylactic seed treatments

Though not specific to Bt crops, the use of neonicotinoid-treated seed has become a standard practice in field crop production in the USA and has the potential to partially reverse many of the positive gains in reduced foliar and soil insecticides use with adoption of Bt crops (Douglas and Tooker, 2015; Papiernik et al., 2018; Sappington et al., 2018). Like Bt crops, the decision to use these treated seeds is made at the beginning of the season and not prescriptively as would be dictated within an IPM perspective. Use of seed treatments varies somewhat by region. The practice is common in mid-southern and southeastern cotton production areas for the control of thrips during seedling establishment (Allen et al., 2018; North et al., 2018; Toews et al., 2010) and some research suggests it is an economically viable approach (North et al., 2018). In other cotton production areas like Arizona, the practice is relatively rare (P. Ellsworth, personal communication). Here, thrips are not a major pest because plants in this environment can compensate for minor damage. In addition, thrips species such as *Frankliniella occidentalis* are considered important natural enemies of some cotton pests including mites and whiteflies (Gonzalez et al., 1982; Trichilo and Leigh, 1986; Naranjo, personal observation). In maize, treated seed is seen as a guard against sporadic early-season pests that are not easy to control by other means (Gray, 2011; Sappington et al., 2018). The effects of treated seed on arthropod natural enemies is not well understood in cotton (Saeed et al., 2016), but some evidence suggests treated maize seeds may disrupt some early-season natural enemy populations (Disque et al., 2019).

Published by Burleigh Dodds Science Publishing Limited, 2020.

4.6 The economics of Bt crops

The global impact of Bt crops for growers has been immense. Brookes and Barfoot (2017) estimated a net benefit of $3.4 billion in 2015 to growers of Bt maize targeting lepidopteran pests. This figure is $33.4 billion if benefits are summed since 1996 when Bt crops first became commercially produced. These income gains are mostly a result of protected yields but reductions in insecticide use also play a large role. For Bt maize targeting *Diabrotica* spp. the overall gains have been smaller because production did not start until 2003 and there are fewer countries benefiting from this technology. In 2015 the estimated net benefits in the USA for Bt maize targeting *Diabrotica* spp. was $1.1 billion and in Canada $53.1 million. Globally, since 2003, the net benefit has been $12.6 billion for Bt maize targeting *Diabrotica* spp. Benefits for Bt cotton have been even greater, with global net benefits of $50.3 billion since 1996. Again, these benefits arise from a combination of yield increases and reduced insecticide costs (Brookes and Barfoot, 2017). Meta-analyses suggest a 68.8% increase in farm profits with the use of Bt maize and cotton between 1995 and 2014, compared with growers not adopting the technology (Klümper and Qaim, 2014).

The other Bt crops currently grown are Bt soybean in South America and Bt eggplant in Bangladesh. We are not aware of national economic analyses for Bt soybeans. In Bangladesh, Bt eggplant was first grown by 20 farmers in 2014 and by >27 000 farmers in 2018 (Shelton et al., 2018). An ex-ante study in Bangladesh estimated that adoption of Bt eggplant would reduce insecticide use by 80% and increase the gross profit margins by nearly 45% (Islam and Norton, 2007). In a study conducted in 35 districts during the 2016–2017 cropping season using 505 Bt eggplant farmers and 350 non-Bt eggplant farmers, net returns per hectare were $2151/ha for Bt eggplant as compared to $357/ha for non-Bt eggplant, a six fold difference (Rashid et al., 2018). In a more intensive study in only one district with one of the four available Bt lines, the economic benefit was $368/ha for Bt eggplant growers (Ahmed et al., 2019), a princely sum considering the annual household income per capita was $602 in 2016 (https://www.ceicdata.com/en/indicator/bangladesh/annual-household-income-per-capita).

5 Resistance management

The high efficacy of insect control combined with season-long production of insecticidal proteins in Bt crops exerts considerable selection pressure for insect resistance. Such a challenge is not unique to Bt crops but occurs with other forms of host plant resistance developed without genetic engineering (Smith, 2005). Scientists and regulators were aware of this challenge before Bt

maize and cotton became commercially available and a number of Scientific Advisory Panels were held to advise the USA Environmental Protection Agency (USA-EPA) so that a proactive insect resistance management (IRM) strategy could be developed and implemented (Glaser and Matten, 2003). Models and some laboratory and field experiments were used to develop an IRM strategy based on refuges (Gould, 1998; Roush, 1998; Caprio, 1998; Shelton et al., 2000; Tang et al., 2001; Onstad, 2013) and more than 20 years after the initial commercialization of GE maize and cotton, predictions from the models were mostly correct (Tabashnik et al., 2013). The GE crop and targeted-insect combinations that satisfied the most stringent assumptions of the models have been successful, while those GE crop/insect combinations that have not met these IRM assumptions have had resistance challenges. This has been especially evident in the case of Bt products to control *Diabrotica* spp. (see below).

Evidence started to emerge in 2004 that *H. zea* was starting to develop resistance to Cry1Ac cotton (Ali and Luttrell, 2007; Tabashnik et al., 2013). This was followed by cases of *S. frugiperda* resistance to Cry1F maize in Puerto Rico (Storer et al., 2010) and African stem borer, *B. fusca*, resistance to Cry1Ab maize in South Africa (Van Rensburg, 2007). Understanding why some insect Bt-crop combinations were problematic and others were not requires a closer look at factors that influence resistance evolution including the dose of the protein, the number of proteins expressed and the use of a refuge IRM strategy.

The refuge strategy is based on the concept that rare resistant insects (homozygous, rr) that develop on Bt plants, instead of mating with each other, mate with individuals among the overwhelming number of susceptible moths (SS) from the refuge (Fig. 2a). These matings produce offspring that are heterozygous for resistance (Sr) (Georghiou and Taylor, 1977; Gould, 1998; Tabashnik and Croft, 1982). Controlling these heterozygous insects is another key part of the strategy. When toxin expression is at a high dose, most if not all the heterozygous insects are killed when resistance is functionally recessive. The reason high dose is such an important part of this strategy is that during the early stages of resistance evolution, most of the resistance genes in a population occur in heterozygous insects. Under non-high-dose conditions, some heterozygous insects escape control, which compromises the IRM refuge strategy (Gould, 1998; Tabashnik et al., 2013). If inheritance of resistance is recessive, this process essentially dilutes resistance genes and maintains a population of susceptible insects. The refuge strategy should be effective as long as plants express a high dose of the toxin, genes conferring resistance are rare, and there are many insects from the refuge available to mate randomly with resistant insects (Gould, 1998; Bates et al., 2005; Tabashnik, 2008). Other factors that influence the success of the refuge strategy are fitness costs and incomplete resistance (Gassmann et al., 2009; Carrière et al., 2010; Tabashnik et al., 2013). Recent models suggest

Published by Burleigh Dodds Science Publishing Limited, 2020.

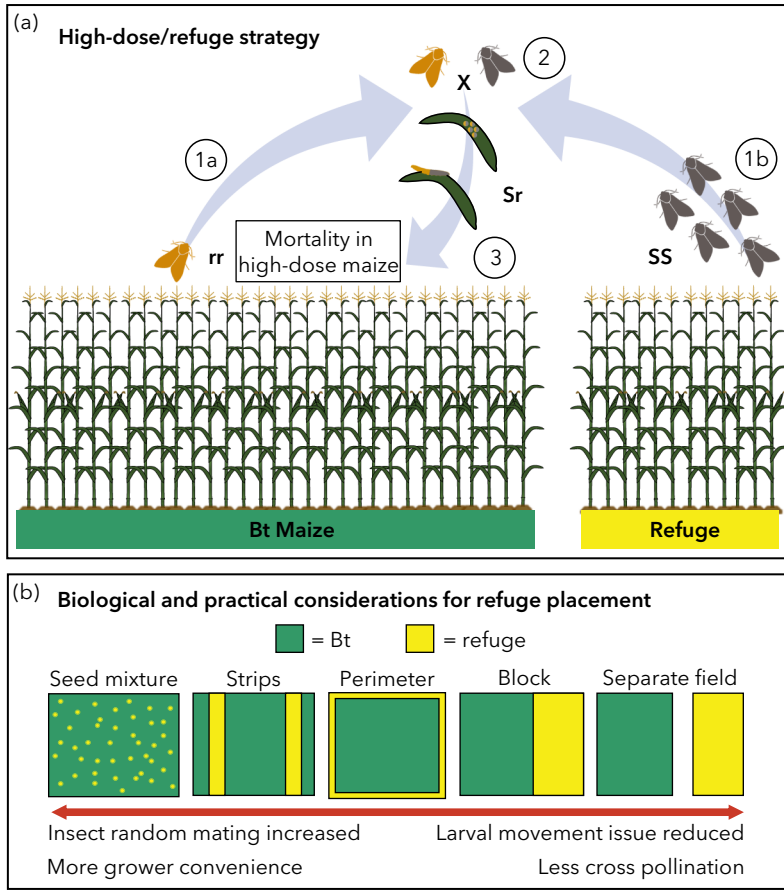

Figure 2 (a) High-dose refuge strategy used to delay the evolution of resistance in Bt crops. (1a) Few homozygous resistant insects emerge from Bt maize; (1b) multiple homozygous susceptible insects emerge from the non-Bt maize refuge; (2) homozygous susceptible insects mate with homozygous resistant insects; (3) functionally recessive resistance generates heterozygous offspring that die with the high-dose expressed in Bt maize. Source: adapted from Vélez et al. (2016b). (b) Biological and practical considerations for refuge placement. Refuge configurations for GE crop with a continuum of biological and practical considerations for refuge placement. Source: adapted from Siegfried and Hellmich (2012).

that pest population dynamics (e.g. density dependence, growth rate) also influence the evolution of resistance (Martinez et al., 2018). Because of the importance of high dose and refuge, this strategy commonly is called the 'high-dose/refuge (HDR) strategy'. The three cases of insect resistance mentioned above violated, at a minimum, the high-dose component of the HDR strategy. Since 2006, several other cases of insect resistance to Bt crops have emerged.

5.1 Implementing the refuge strategy

Scientists were concerned that some growers would reject the refuge concept because non-Bt crops with insect injury might have lower yields, and controlling pests by setting aside crops to produce pests is somewhat counterintuitive. Indeed, refuge compliance is an issue in many parts of the world (Bourguet et al., 2005; Kruger et al., 2009, 2012; Tabashnik et al., 2013; Reisig, 2017). Persistent educational outreach programs from academics and industry made convincing arguments for the HDR strategy (Ostlie et al., 1997; Hurley et al., 2001). Now, non-Bt refuge plants that produce Bt-susceptible insects are commonly used where GE crops are grown and are mandated in some countries (Head and Greenplate, 2012). However, monitoring for adherence to planting refuges remains a challenge, especially in developing countries (Kranthi et al., 2017). Practical implementation of refuge sometimes is challenging because it requires growers to consider both size and placement of the refuge. Theoretically, refuges should produce susceptible moths (SS) that outnumber Sr and rr insects by a ratio of at least 500:1 (USA-EPA, 1998), which generally translates to 5–20% or more refuge area. Furthermore, refuges should be placed close enough to Bt crops to ensure random mating of resistant and susceptible insects. Many GE crops have multiple pests, so refuge placement recommendations are influenced by the biology of all the pests and defaults to the most conservative recommendation. For example, refuge placement recommendations in the USA Corn Belt for Bt maize that targets *O. nubilalis* is one-half mile (~800 m) because moths commonly fly 800 m or more before mating (Showers et al., 2001; Matten et al., 2004). This recommendation was altered slightly in 2003 after the introduction of Bt maize for *Diabrotica* spp. The 20% or greater refuge recommendation remained the same; however, the placement recommendation was changed such that the refuge is adjacent to the Bt maize field. *Diabrotica* spp. beetles are more likely to mate within the field compared with *O. nubilalis*; thus the refuge for rootworms had to be closer to the Bt maize field to increase the chances that resistance beetles would mate with susceptible beetles. Thus, refuge recommendations are made on a case-by-case basis depending on the biology of the targeted pests for the GE crop and the landscape in which it occurs (e.g. whether there are other Bt crops). In general, to achieve the basic size and proximity requirements, refuge schemes fall into three general categories: structured, mixed-seed and natural. However, in the future, there is the possibility of creating 'within-plant refuges'. In this strategy, the Bt protein would only be expressed in plant tissue needing protection (e.g. cotton boll, maize roots) or be expressed only at a particular time to prevent damage to the marketable part of the plant (Bates et al., 2005).

Presently, growers that choose structured refuges plant a specific proportion of their crop into a non-Bt variety, either within (strips or blocks),

adjacent (edges or headlands) or within a designated distance (separate fields) from the Bt crop (Fig. 2b; Siegfried and Hellmich, 2012). Non-Bt crops used for refuge should be selected based on equivalent maturity to Bt crops, planted in similar fields within the same planting window and managed with similar fertilization, weed and pest management and irrigation practices. Otherwise, insects could emerge from Bt and refuge hybrids at different times, leading to assortative (non-random) mating between resistance and susceptible individuals, and thus, weaken IRM. Additionally, if growers use insecticides on refuge crops to control a targeted pest, the percentages of refuge may increase to compensate for killed susceptible insects.

The second type of refuge involves blends of Bt and non-Bt seeds. This approach is known as 'refuge in the bag' (RIB) and is convenient for growers because it guarantees refuge compliance and avoids size and placement concerns (Fig. 2b; Onstad et al., 2018). Most of the seed blends involve pyramided crops where refuge size requirements are lower. Besides mating behavior, another important biological consideration is plant-to-plant movement of larvae. Such movement is primarily a concern with seed-mixture and narrow-strip refuges as larval movement among Bt and non-Bt plants could violate the high-dose component of HDR (Mallet and Porter, 1992; Davis and Onstad, 2000; Wangila et al., 2013). This could occur if a young larva tastes a Bt plant, becomes sick and moves to a non-Bt plant and survives; or the reverse, an older larva moves from non-Bt to Bt plant. In these scenarios, if larvae with one copy of a resistance gene (heterozygote, Sr) have greater fitness than susceptible insects, then the high-dose component of the HDR strategy is compromised (Mallet and Porter, 1992; Gassmann et al., 2009). Tests comparing spatially separate refuges to mixed Bt and non-Bt plants revealed the benefit of a separate refuge for an insect whose larvae could move between plants (Tang et al., 2001). Similarly, recent research suggests that reproductive tissues of Bt plants (e.g. maize ears) can produce a mixture of Bt and non-Bt tissues depending on whether the pollen is from a Bt or non-Bt plant (Chilcutt and Tabashnik, 2004; Caprio et al., 2016). Seed blends are frequently used in the USA Corn Belt where the primary pest is *D. v. virgifera*, which has limited movement between plants, but are not recommended in the southern USA or the tropics, where primary lepidopteran pests are often from the Noctuid family, including *S. frugiperda* and *Helicoverpa* spp.

In some cases, occurrence of target insects in other non-Bt crops or in semi-natural or natural plants is sufficient and no refuge is required (Gustafson et al., 2006; Head et al., 2010). This is the case for control of heliothines in pyramided Bt cotton from West Texas to the East Coast (USA-EPA, 2007; Head and Greenplate, 2012). The natural refuge is effective for prolonging resistance to *H. virescens*, but not *H. zea* (Tabashnik and Carrière, 2017). In northern China,

structured refuges are not mandated because non-cotton host plants provide an estimated 56% refuge for *H. armigera* (Jin et al., 2015a).

The best placement strategy for the refuge varies depending on the crop landscape and biology of each targeted pest species (Fig. 2b). Seed mixtures are the best strategy for maximizing random mating of adults but the riskiest strategy when larval movement or Bt pollen contamination are important factors. Another consideration is that seed mixtures may lead to farmers wondering if any damage they see is due to the failure of the Bt plant rather than being confined to refuge plants. This concern, along with the inter-plant movement of *L. orbonalis*, led to the recommendation of using a separate refuge as part of the IRM strategy for Bt eggplant. For Bt maize and Bt cotton, experience suggests that refuge placement for Lepidoptera is probably best optimized with separate blocks or fields, but in the case of Coleoptera, Bt maize within field strips or even seed mixtures may be optimal. From a grower perspective, however, refuge placement that is most convenient may be the most important factor (Fig. 2b).

5.2 Pyramids

Initially, all Bt crops produced one Bt toxin. However, the introduction of pyramided crops producing two or more traits targeting the same pest has dramatically changed options for managing insect pest resistance (Tabashnik, 1989; Caprio, 1998; Roush, 1998; Zhao et al., 2003). Two or more traits results in 'redundant killing' and reduces chances that insects will evolve resistance (Fig. 3), especially when each of the traits satisfies the high-dose criteria and has no or little cross-resistance (Carrière et al., 2015). Pyramided crops have two advantages over single-trait crops (Bates et al., 2005). First, two genes provide a wider spectrum of control, which is especially useful for systems that have multiple pests. Second, crops with pyramided traits require smaller refuges. For example, models suggest two traits deployed in a pyramid would require about 5% refuge compared with 30–40% refuge for traits deployed sequentially to delay resistance for about the same number of generations (Roush, 1997). A smaller requirement for refuge, in the 5% range, makes blended seed options more practical and perhaps more acceptable to growers.

Model predictions related to pyramids were evaluated in greenhouse tests with *P. xylostella* and transgenic broccoli. These tests showed broccoli expressing two genes in a pyramid (*cy1Ac* and *cry1C*) delayed resistance longer than when Cry1Ac plants and Cry1C plants were used in a mosaic (i.e. simultaneously in the same area) or when Cry1Ac plants and Cry1C plants were deployed sequentially (Zhao et al., 2003). Follow-up tests using the same system showed concurrent use of pyramids Bt plants and plants expressing either trait singly results in faster resistance development than using the pyramid plants

Published by Burleigh Dodds Science Publishing Limited, 2020.

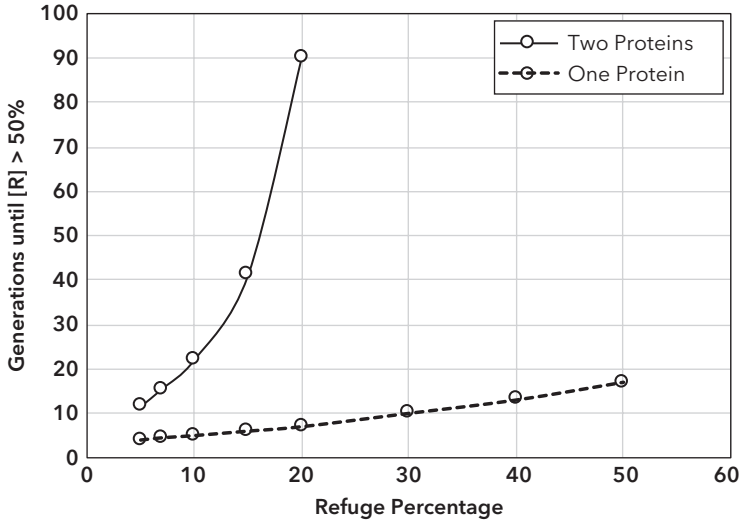

Figure 3 Refuge size effect on the rate of Bt resistance evolution to crop with one Bt protein versus crop with two Bt proteins. Source: adapted from Roush (1998).

alone (Zhao et al., 2005). These studies have practical implications. Often two or more companies develop separate traits that are deployed singly instead of pyramided (Roush, 1997). Sometimes by the time the traits are put into pyramided crops, insect resistance has already compromised one or more of the traits (Carrière et al., 2019).

5.3 Evaluating Bt-crop successes and failures

After nearly two decades of experience, the most successful Bt crops are those that have met the high-dose or near high-dose criteria for targeted pest insects. Notably, *O. nubilalis*, after nearly two decades is still controlled by Cry1Ab, Cry1A.105, Cry2Ab and Cry1F producing maize in the USA (Siegfried et al., 2007). Other successful Bt-crop/insect combinations that have remained effective for 15 years or more include: Cry1Ac cotton/*H. armigera* and *H. punctigera* in Australia (Mahon et al., 2007; Downes, 2016); Cry1Ab maize/*O. nubilalis* and *Sesamia nonagrioides* in Spain (Castañera et al., 2016; EFSA, 2017; Farinós et al., 2018) and Cry1Ac cotton/*P. gossypiella* in China (Wan et al., 2017) and the USA (Tabashnik et al., 2010). On the other hand, Bt-crop/insect combinations that have had resistance challenges, besides those mentioned above, include Cry2Ab cotton/*H. zea* in the USA (Ali and Luttrell, 2007); Cry1Ac and Cry2Ab cotton/*P. gossypiella* in India (Dhurua and Gujar, 2011; Kranthi, 2015; Mohan et al., 2016); Cry3Bb, mCry3A, eCry3.1Ab and Cry34/35Ab maize/*D. v. virgifera* and *D. barberi* in the USA (Gassmann et al.,

2011, 2014, 2016; Jakka et al., 2016; Zukoff et al., 2016; Calles Torrez et al., 2017); Cry1F, Cry1Ab maize/*S. frugiperda* in Brazil (Farias et al., 2014, 2016; Omoto et al., 2016); Cry1A.105 maize/*D. saccharalis* in Argentina (Grimi et al., 2015); Cry1Ab, Cry1A.105 maize/*H. zea* in the USA (Dively et al., 2016) and recently Cry1F maize/*S. albicosta* in the USA and Canada (Ostrem et al., 2016; Smith et al., 2017). Western bean cutworm, *S. albicosta*, is notable because of its recent eastern range expansion from the western Great Plains across the Corn Belt (see Section 4.1). Control of this lepidopteran has many scientists worried because of its resistance to Cry1F maize (DiFonzo et al., 2016) and its control is limited to a single trait – Vip3A.

Most of the occurrences of insect resistance to GE crops mentioned above are due to violation of high-dose criteria, which is particularly problematic for *Diabrotica* spp. and Lepidoptera from the family Noctuidae (see Tabashnik and Carrière, 2017 review for current lists of resistant and susceptible species).

6 Future GE crops

Given the success of the Bt-transgenic crops and developments of molecular tools to engineer crop varieties, we expect to see more improved varieties that are resistant to additional key insect pests through various means and modes of action. The use of emerging molecular tools will not only increase the ability to transform new crops but also control pests that are not susceptible to currently available Bt proteins.

6.1 New Bt events/crops and other traits

New Bt crops include the production of multiple lepidopteran-active Cry proteins from Bt in rice, which has been explored over the past 20 years. Two Bt rice lines have been approved in China, but these lines are not yet commercialized due to political and societal issues (Li et al., 2016). Also, in China, *Populus nigra* trees producing Cry1Ac were developed in 1993 and have been field tested since 1994. *Bt* poplars were first commercialized in 2001 and occupied 490 ha in China up through 2014 but are no longer planted (Hu et al., 2017). Production of the Cry2Aa protein in pigeon pea has demonstrated successful control of the gram pod borer, *H. armigera* (Singh et al., 2018), but is not yet commercialized. Bt genes have been inserted into several Brassica species for control of Lepidoptera, primarily *P. xylostella*. These include Cry1Ac and Cry1C in broccoli (Bhattacharya et al., 2002; Cao et al., 2002), Cry1C in cauliflower (Cao et al., 2003) and Cry1Ac and Cry1C in collards (Cao et al., 2005) and all have provided excellent control. A private–public partnership to commercialize Bt crucifers in Asia and Africa (Russel et al., 2008) showed great potential but ended without getting a product to market.

Published by Burleigh Dodds Science Publishing Limited, 2020.

Current Bt crops deploy proteins to control lepidopteran and coleopteran pests. Early transgenic plants producing the β-pore-forming protein Cry51Aa2 did not exhibit effective protection from *Lygus* spp. (mirid) damage (Baum et al., 2012). However, through optimization strategies, the insecticidal activity of Cry51Aa2 toward *Lygus* spp. was increased. The variant protein (Cry5Aa2.834_16) was then produced in transgenic cotton and demonstrated a reduction of *Lygus* spp. populations and a transgenic event (MON88702) has been selected for further development (Gowda et al., 2016). Additional studies also found this event to be effective in the control of thrips such as *Frankliniella* spp. (Bachmann et al., 2017; Graham and Stewart, 2018), but it may also cause effects on non-target beneficial Hemiptera such as *Orius* spp. (Bachmann et al., 2017). Studies are currently ongoing to evaluate whether this poses any unacceptable risk.

Lastly, *Pseudomonas chlororaphis*, a gram-negative bacterium, was found through a screening of soil-isolated microbial strains and in 2016, the protein IPD072Aa was identified and demonstrated to be highly toxic to *Diabrotica* spp. larvae. Transformed maize plants producing the IPD072Aa protein protected plants against *D. v. virgifera* injury under field conditions (Schellenberger et al., 2016). The insecticidal mechanism of IPD072Aa is under investigation, but it seems that this protein has functional similarities to Bt proteins. Effects of IPD072Aa on non-target insects were only observed within Coleoptera at high concentrations and caused reduced growth and developmental delays. These data will help guide risk assessment for IPD072Aa maize (Boeckman et al., 2019). *P. chlororaphis* protein IPD072Aa is the first case of successful identification, plant transformation and plant protection under field conditions of a protein other than Bt, suggesting the possibility of finding new sources of insecticidal proteins from soil bacteria (Schellenberger et al., 2016).

6.2 RNAi-based crops

RNA interference (RNAi) is a conserved immune response in eukaryotes that refers to the process by which double-stranded RNA (dsRNA) directs sequence-specific gene repression (Fire et al., 1998). RNAi was initially used as a tool to study gene function, but when it was shown that oral delivery of dsRNA elicited an RNAi response, scientists began exploring this technique as a potential pest management strategy. RNAi-based crops exploit this mechanism by expressing transgene-encoded dsRNA that targets vital function genes (Baum et al., 2007; Bolognesi et al., 2012; Fishilevich et al., 2019; Hu et al., 2016; Mao et al., 2007; Ramaseshadri et al., 2013), and more recently genes targeting reproduction (Niu et al., 2017) in herbivorous pests. The consumption of a plant expressing a gene-specific dsRNA can repress the expression of the target gene, thereby preventing gene function and causing insect mortality.

Two studies first demonstrated effective gene knockdown of transgene-encoded dsRNA for targeting insect pests – *H. armigera* in cotton (Mao et al., 2007) and *D. v. virgifera* (Baum et al., 2007) in maize – and providing plant protection. Further research has demonstrated the great potential of RNAi for crop protection (Price and Gatehouse, 2008; Zhang et al., 2017). However, the response to orally delivered dsRNA (dietary RNAi) is highly variable between arthropods and has limited the ability for broad use of RNAi in pest control. To date, dietary dsRNA has been described as highly effective in species of the order Coleoptera and highly variable on insect orders such as Diptera, Lepidoptera, Hemiptera and Orthoptera (Baum and Roberts, 2014; Christiaens and Smagghe, 2014; Niu et al., 2018; Zhang et al., 2017). Insensitivity to dietary RNAi appears linked to limited cellular uptake of dsRNA (Shukla et al., 2016; Yoon et al., 2017), extra-oral digestion (Allen and Walker, 2012; Zhu et al., 2016), stability of dsRNA in the gut or hemolymph (Allen and Walker, 2012; Garbutt et al., 2013; Liu et al., 2013; Luo et al., 2013; Christiaens et al., 2014; Wynant et al., 2014) and viral interactions (Christiaens and Smagghe, 2014). The sensitivity to dietary RNAi not only varies between orders but also between species (Terenius et al., 2011), populations (Chu et al., 2014) and life stages (Pereira et al., 2016). Understanding the factors that affect the response to dietary dsRNA in different insects might allow the development of strategies to overcome these limitations and provide broader RNAi-based crops for other insect orders.

Even though RNAi has been challenging for lepidopterans, there are few cases where plant transformation to express transgene-encoded dsRNA has been successful (Han et al., 2017; Mao et al., 2007; Ni et al., 2017). Ni et al. (2017) transformed cotton to express Bt proteins and RNAi against *H. armigera*. They not only demonstrated the effectiveness of a dsRNA targeting an enzyme involved with juvenile hormone synthesis, but also demonstrated that combining a Bt toxin and RNAi delayed the evolution of resistance to Bt. One strategy that has allowed improvement in RNAi efficiency in Coleoptera and target recalcitrant lepidopterans is the expression of dsRNA in plastids (Bally et al., 2016; Burke et al., 2019; Jin et al., 2015b; Zhang et al., 2015).

Presently, the most successful case of RNAi-based crops is with *D. v. virgifera*. This success is in part due to the susceptibility of this insect to dietary RNAi and a biotechnology company's investment for the management of this insect. In 2017, the USA-EPA approved the first trait expressing the *DvSnf7* (involved with cellular transport) dsRNA transgene for production and consumption in the USA. This product will be marketed under the trade name of SmartStax Pro™ and will provide farmers with the first novel mode of action for control of *Diabrotic*a spp. since the release of the Cry34/35Ab1 protein in 2005 (USA-EPA, 2017). *DvSnf7* dsRNA will be stacked with Cry3Bb1 and Cry34/35Ab1 to include three different modes of action to aid in delaying the evolution of resistance (Head et al., 2017).

Published by Burleigh Dodds Science Publishing Limited, 2020.

RNAi-based crops have a great potential given that they can silence an essential gene in a sequence-specific manner, thus creating a very specific means of control. While the specificity of current Bt proteins ranges from order to families, dsRNA can be as specific as subfamilies and with careful design, even species (Whyard et al., 2009). As shown with *Diabrotica* spp., the biggest hurdle for developing RNAi crops may be the fact that resistance affects the dsRNA uptake mechanism making resistant insects immune to any novel dsRNA construct (see below).

Risks associated with Bt plants are related to newly expressed proteins that could generate toxicity. This is not the case for RNAi given that this strategy aims to reduce the amount of endogenous protein. However, a reduction of the target gene could have biological implications in non-target organisms (Casacuberta et al., 2015). The ecological risk assessment for plants expressing transgene-encoded dsRNA has used the framework developed for Bt proteins (Roberts et al., 2015). However, differences in the characteristics of these traits should be considered. Even though different dsRNAs use the same machinery, the targeted genes have different biological functions and some genes are more likely to be conserved. Consequently, each dsRNA should be evaluated and considered as a different trait. Bioinformatic analyses also have been suggested as a potential tool to identify species that are likely to be affected by a particular dsRNA compound and that should be selected for non-target testing, and/or reduce the number of non-target species required to be tested (Casacuberta et al., 2015). However, a few studies suggest the possibility of off-target gene silencing (Jarosch and Moritz, 2012; Kulkarni et al., 2006; Ma et al., 2006; Senthil-Kumar and Mysore, 2011). More research is needed to determine the connection between potential nucleotide matches and off-target gene knockdown with biological activity in different insect orders. This approach also will require advances in bioinformatics and more insect genomes before we can rely on the use of bioinformatics to include/exclude testing of non-target species (Casacuberta et al., 2015).

The initial characterization of the insecticidal activity of dsRNA targeting *D. v. virgifera DvSnf7* showed that the spectrum of activity was narrow and activity was only observed in the subfamily Galerucinae within the Chrysomelidae family. Non-target studies included predators, parasitoids, pollinators and soil invertebrates, and aquatic and terrestrial vertebrate species. Bioassays evaluated survival, growth, development and reproduction to assess adverse effects. No effect was observed at or above the maximum expected concentration (>10-fold), suggesting that direct and indirect exposure to *DvSnf7* was safe at the expected field exposure (Bachman et al., 2013, 2016; Tan et al., 2016). No non-target effects were observed in additional field level evaluations of maize expressing Cry3Bb1 and *DvSnf7* (Ahmad et al., 2016).

The effect of *vATPase-A* dsRNA targeting *Diabrotica* spp. also has been tested on predators, pollinators and soil decomposers. No effects or negligible

effects were reported for the honeybee, the monarch butterfly or the soil micro-arthropod, *Sinella curviseta* (Pan et al., 2016, 2017; Vélez et al., 2016a). However, feeding bioassays with dsRNA at concentrations several orders of magnitude higher than expected in the field showed that *vATPase-A* dsRNA generated prolonged developmental time in the coccinellids *Adalia bipunctata* and reduced survival rate in *Coccinella septempunctata*. This study suggests a connection between the number of 21-nucleotide-long matches and biological activity; *C. septempunctata* had 34 matches, while *A. bipunctata* had 6 (Haller et al., 2019).

The stability of dsRNA in the environment also has been evaluated and it was found that *DvSnf7* was not detectable after 48 hours in three types of agricultural soils and thus is unlikely to accumulate in the environment (Dubelman et al., 2014). Further studies demonstrated that *DvSnf7* degraded after seven days in aerobic water-sediment systems, again suggesting that dsRNA accumulation is unlikely (Fischer et al., 2017). However, Parker et al. (2019) reported that dsRNA could be adsorbed by soil particles and taken up by soil microorganisms suggesting more studies are needed to better understand the fate of dsRNA in the environment.

6.3 Resistance management in RNAi crops

In order to better understand the potential mechanism of resistance to RNAi in *D. v. virgifera*, researchers developed a resistant colony. Characterization of this strain indicated that dsRNA *DvSnf7* dsRNA had impaired luminal uptake and cross-resistance with other dsRNAs tested (Khajuria et al., 2018). In line with this, Vélez et al. (2016c) also reported that the knockdown of the RNAi machinery genes decreased mortality by *vATPase-A* dsRNA in *D. v. virgifera*. These studies suggest that once resistance evolves in the field, insects will be resistant to any dsRNA construct.

DvSnf7 dsRNA is not planned to be deployed as a single-trait product to delay the evolution of resistance. As previously indicated, SmartStax Pro™ stacks *DvSnf7* dsRNA and two Bt proteins with different modes of action, Cry3Bb1 and Cry34/35 (Head et al., 2017). Even with three modes of action, concerns remain given that several areas in the USA Corn Belt have reported resistance to Cry3Bb1 and Cry34/35Ab1 (see Section 5.3).

6.4 CRISPR-based crops

CRISPR-based genome editing has revolutionized the way genomes can be manipulated (Sternberg and Doudna, 2015). For example, CRISPR-Cas9 has already successfully been used for enhancing biotic and abiotic stress tolerance in crops. Biotic stressors have included viral, bacterial and fungal

disease resistance (Jaganathan et al., 2018). Only one study has so far demonstrated the use of CRISPR-based crops for the management of insect pests. Rice was transformed to generate mutants with an inactivated *CYP71A1* gene that eliminates the production of serotonin, allowing for higher salicylic acid levels, making the plant resistant to the brown planthopper, *N. lugens* (Lu et al., 2018). More examples of CRISPR-Cas9 transformed plants for insect pest management are expected to be developed in the next decade focusing on enhancing plant tolerance to insect damage. One advantage of CRISPR-based crops is the likelihood of targeting insect pests that have not been susceptible to Bt and RNAi technologies.

One challenge with crop varieties that use CRISPR-Cas9 technology is the uncertainty and inconsistency of their regulation in different jurisdictions (Duensing et al., 2018; Schiemann et al., 2019). In the EU, genome-edited plants are considered GE and regulated accordingly while in Argentina they are excluded from GE regulation and other countries are developing their own regulations (Whelan and Lema, 2015). After public notification of a ruling on plants produced through innovative breeding techniques, the USA Secretary of Agriculture issued a statement clarifying that genome editing is excluded from the department's oversight (USDA, 2018).

7 Conclusion

The application of genetic engineering has significantly accelerated the deployment of host plant resistance as a foundational element of IPM in several cropping systems and has the potential to expand to many more pests and crops with continued advances in Bt, RNAi, CRISPR and other technologies. Current GE crops represent virulent forms of antibiotic host plant resistance for multiple pest species. As of 2018, Bt crops have been adopted on >100 million ha in 22 countries, and one RNAi-based crop was recently approved in the USA.

Extensive global data have demonstrated the selectivity and associated environmental safety of current GE crops. Available data also show that Bt crops in particular have had immense economic impacts due to improved crop yields and reductions in insecticide use. This latter factor has in turn facilitated the broader use of biological control by natural enemies important in control of both Bt target and non-target pests, thus providing for further positive cascading impacts. The broad regional impacts of GE crops adoption have also been demonstrated in several countries, enabling non-adopters of the GE crop or even producers of other crops to benefit from wide-scale pest control, especially when the target pest is polyphagous. The integration of GE crops with other pest management tactics is possible, suggesting even further gains in overall IPM.

GE crops that produce insecticidal proteins continuously during the cropping season place incredible selection pressure on target pests, and it is thus not surprising that predictions of the evolution of resistance to multiple Cry proteins have become a reality in several systems globally. In large measure, the deployment of resistance management through the use of effective refuge strategies and pyramided cultivars producing several Bt proteins have helped to preserve the efficacy of Bt crops in most regions. Nonetheless, practical resistance has developed to a number of Cry proteins in a variety of pests, further emphasizing the need to recognize that GE crops represent only a single component in what needs to be a more inclusive IPM strategy.

Many additional GE crops using Bt technology have been developed and tested but have yet to be approved for commercial production for a variety of reasons. Also, there are still many additional Bt-based proteins (Cry and VIP) and proteins from other bacterium yet to be exploited. RNAi and CRISPR technologies offer avenues to produce additional crops with highly selective spectra of activity that may extend to pest species beyond Lepidoptera and Coleoptera. Combining these approaches with Bt technology may both prolong the durability of GE crops overall through more effective resistance management and increase the number of target species that can be managed.

Host plant resistance, whether through traditional breeding approaches or through the use of genetic engineering, enables producers to avoid pest problems and will remain a critical component of IPM for the future. Continued advances in our understanding of the biology, ecology and genetics of crops and insect pests will further facilitate the development of host plant resistance-based strategies for economically viable and environmentally safe agricultural production benefiting all of society.

8 Where to look for further information

There is an extensive literature on GE crops within many contexts. Within those contexts relevant to the subject of this chapter, the reader can find greater depth of coverage in the following books, articles and websites:

Anderson, J. A., Ellsworth, P. C., Faria, J. C., Head, G. P., Owen, M. D. K., Pilcher, C. D., Shelton, A. M. and Meissle, M. 2019. Genetically engineered crops: importance of diversified Integrated Pest Management for agricultural sustainability. *Frontiers in Bioengineering and Biotechnology* 7, 24. doi:10.3389/fbioe.2019.00024.

Cooper, A. M., Silver, K., Zhang, J., Park, Y. and Zhu, K. Y. 2019. Molecular mechanisms influencing efficiency of RNA interference in insects. *Pest Management Science* 75, 18–28. doi:10.1002/ps.5126.

Naranjo, S. E. 2009. Impacts of Bt crops on non-target invertebrates and insecticide use patterns. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 4(11), 11. doi:10.1079/PAVSNNR20094011.

Price, D. R. G. and Gatehouse, J. A. 2008. RNAi-mediated crop protection against insects. *Trends in Biotechnology* 26(7), 393–400. doi:10.1016/j.tibtech.2008.04.004.

Ricroch, A. Chopra, S. and Fleischer, S. J. (Eds). 2014. *Plant Biotechnology: Experiences and Future Prospects*. Springer, Cham, Heidelberg, New York, Dordrecht and London.

Romeis, J., Naranjo, S. E., Meissle, M. and Shelton, A. M. 2019. Genetically engineered crops help support conservation biological control. *Biological Control* 130, 136–54. doi:10.1016/j.biocontrol.2018.10.001.

Romeis, J., Shelton, A. M. and Kennedy, G. G. (Eds). 2008. *Integration of Insect-Resistant Genetically Modified Crops with IPM Systems*. Springer, Berlin, Germany.

The USA-Environmental Protection Agency (US-EPS) maintains a website that provides details on the insecticide resistance management plans for the deployment of GE crops with insecticidal traits within the USA (https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/insect-resistance-management-bt-plant-incorporated).

A recent Organization for Economic Cooperation and Development (OECD) Conference focused on the development and use of RNAi-based pest management technologies. The presentations can be viewed and a proceedings publication is forthcoming (http://www.oecd.org/chemicalsafety/pesticides-biocides/conference-on-rnai-based-pesticides.htm).

The International Service for the Acquisition of Agri-biotech Applications (ISAAA) is a non-profit international organization that maintains an extensive website providing a wealth of information on the latest developments of GE crops, with particular focus on the developing world. They publish and make available a number of reports, spearhead and support a number of programs in biotech literacy and application, and maintain a useful database that tracks the regulatory approval and use of all GE crops in adopting countries (http://www.isaaa.org/).

9 References

Ahmad, A., Negri, I., Oliveira, W., Brown, C., Asiimwe, P., Sammons, B., Horak, M., Jiang, C. and Carson, D. 2016. Transportable data from non-target arthropod field studies for the environmental risk assessment of genetically modified maize expressing an insecticidal double-stranded RNA. *Transgenic Research* 25(1), 1–17. doi:10.1007/s11248-015-9907-3.

Ahmed, A., Hoddinott, J. F., Abedin, N. and Hossain, N. 2019. Impacts of Bt brinjal (eggplant) technology in Bangladesh (Draft Report). International Food Policy Research Institute, Bangladesh Policy Research and Strategy Support Program. February 2019.

Ali, M. I. and Luttrell, R. G. 2007. Susceptibility of bollworm and tobacco budworm (Lepidoptera: Noctuidae) to Cry2Ab2 insecticidal protein. *Journal of Economic Entomology* 100(3), 921–31. doi:10.1603/0022-0493(2007)100[921:sobatb]2.0.co;2.

Allen, M. L. and Walker, W. B. 2012. Saliva of *Lygus lineolaris* digests double stranded ribonucleic acids. *Journal of Insect Physiology* 58(3), 391–6. doi:10.1016/j.jinsphys.2011.12.014.

Allen, K. C., Lutrell, R. G., Sappington, T. W., Hesler, L. S. and Papiernik, S. K. 2018. Frequency and abundance of selected early-season insect pests of cotton. *Journal of Integrated Pest Management* 9, 20.

Anderson, J. A., Ellsworth, P. C., Faria, J. C., Head, G. P., Owen, M. D. K., Pilcher, C. D., Shelton, A. M. and Meissle, M. 2019. Genetically engineered crops: importance of diversified Integrated Pest Management for agricultural sustainability. *Frontiers in Bioengineering and Biotechnology* 7, 24. doi:10.3389/fbioe.2019.00024.

Ba, M. N., Huesing, J. E., Tamò, M., Higgins, T. J. V., Pittendrigh, B. R. and Murdock, L. L. 2018. An assessment of the risk of Bt-cowpea to non-target organisms in West Africa. *Journal of Pesticide Science* 91, 1165–79.

Bachman, P. M., Bolognesi, R., Moar, W. J., Mueller, G. M., Paradise, M. S., Ramaseshadri, P., Tan, J. G., Uffman, J. P., Warren, J., Wiggins, B. E. and Levine, S. L. 2013. Characterization of the spectrum of insecticidal activity against western corn rootworm (*Diabrotica virgifera virgifera*). *Transgenic Research* 22(6), 1207–22. doi:10.1007/s11248-013-9716-5.

Bachman, P. M., Hizinga, K. M., Jensen, P. D., Muller, G., Tan, J., Uffman, J. P. and Levine, S. L. 2016. Ecological risk assessment for *DvSnf7* RNA: a plant-incorporated protectant with targeted activity against western corn rootworm. *Regulatory Toxicology and Pharmacology: RTP* 81, 77–88. doi:10.1016/j.yrtph.2016.08.001.

Bachman, P. M., Ahmad, A., Ahrens, J. E., Akbar, W., Baum, J. A., Brown, S., Clark, T. L., Fridley, J. M., Gowda, A., Greenplate, J. T., Jensen, P. D., Mueller, G. M., Odegaard, M. L., Tan, J., Uffman, J. P. and Levine, S. L. 2017. Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry51Aa2.834_16. *PLoS ONE* 12(1), e0169409. doi:10.1371/journal.pone.0169409.

Bally, J., McIntyre, G. J., Doran, R. L., Lee, K., Perez, A., Jung, H., Naim, F., Larrinua, I. M., Narva, K. E. and Waterhouse, P. M. 2016. In-plant protection against *Helicoverpa armigera* by production of long hpRNA in chloroplasts. *Frontiers in Plant Science* 7, 1453. doi:10.3389/fpls.2016.01453.

Bates, S. L., Zhao, J. Z., Roush, R. T. and Shelton, A. M. 2005. Insect resistance management in GM crops: past, present and future. *Nature Biotechnology* 23(1), 57–62. doi:10.1038/nbt1056.

Baum, J. A. and Roberts, J. K. 2014. Progress towards RNAi-mediated insect pest management. In: Dhadialla, T. S. and Gill, S. S. (Eds), *Insect Midgut and Insecticidal Proteins, Vol. 47 of Advances in Insect Physiology*. Academic Press, London, pp. 249–95. doi:10.1016/B978-0-12-800197-4.00005-1.

Baum, J. A., Bogaert, T., Clinton, W., Heck, G. R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T. and Roberts, J. 2007. Control of coleopteran insect pests through RNA interference. *Nature Biotechnology* 25(11), 1322–6. doi:10.1038/nbt1359.

Baum, J. A., Sukuru, U. R., Penn, S. R., Meyer, S. E., Subbarao, S., Shi, X., Flasinski, S., Heck, G. R., Brown, R. S. and Clark, T. L. 2012. Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. *Journal of Economic Entomology* 105(2), 616–24. doi:10.1603/ec11207.

Bergman, J. M. and Tingey, W. M. 1979. Aspects of interaction between plant genotypes and biological control. *Bulletin of the Entomological Society of America* 25(4), 275–9. doi:10.1093/besa/25.4.275.

Bhattacharya, R. C., Viswakarma, N., Bhat, S. R., Kirti, P. B. and Chopra, V. L. 2002. Development of insect-resistant cabbage plants expressing a synthetic *cry1Ab* gene from *Bacillus thuringiensis*. *Current Science* 83, 146–50.

Blanco, C. A., Chiaravalle, W., Dalla-Rizza, M., Farias, J. R., Garcia-Degano, M. F., Gastaminza, G., Mota-Sanchez, D., Murua, M. G., Omoto, C., Pieralisi, B. K., Rodriguez, J., Rodriguez-Maciel, J. C., Teran-Santofimio, H., Teran-Vargas, A. P., Valencia, S. J. and Willink, E. 2016. Current situation of pests targeted by Bt crops in Latin America. *Current Opinion in Insect Science* 15, 131–8. doi:10.1016/j.cois.2016.04.012.

Boeckman, C. J., Huang, E., Sturtz, K., Walker, C., Woods, R. and Zhang, J. 2019. Characterization of the spectrum of insecticidal activity for IPD072Aa: a protein derived from *Pseudomonas chlororaphis* with activity against *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 112(3), 1190–6. doi:10.1093/jee/toz029.

Bolognesi, R., Ramaseshadri, P., Anderson, J., Bachman, P., Clinton, W., Flannagan, R., Ilagan, O., Lawrence, C., Levine, S., Moar, W., Mueller, G., Tan, J., Uffman, J., Wiggins, E., Heck, G. and Segers, G. 2012. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *PLoS ONE* 7(10), e47534. doi:10.1371/journal.pone.0047534.

Bourguet, D., Desquilbet, M. and Lemarié, S. 2005. Regulating insect resistance management: the case of non-Bt corn refuges in the US. *Journal of Environmental Management* 76(3), 210–20. doi:10.1016/j.jenvman.2005.01.019.

Bravo, A., Gill, S. S. and Soberón, M. 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon: Official Journal of the International Society on Toxinology* 49(4), 423–35. doi:10.1016/j.toxicon.2006.11.022.

Brookes, G. and Barfoot, P. 2017. *GM Crops: Global Socio-economic and Environmental Impacts 1996-2015*. PG Economics Ltd, UK.

Brookes, G. and Barfoot, P. 2018. Environmental impacts of genetically modified (GM) crop use 1996–2016: impacts on pesticide use and carbon emissions. *GM Crops and Food* 9(2), 59–89. doi:10.1080/21645698.2018.1464866.

Burke, W. G., Klanoglu, E., Kilotilin, I., Menassa, R. and Donly, C. 2019. RNA Interference in the tobacco hornworm, *Manduca sexta*, using plastid-encoded long double-stranded RNA. *Frontiers in Plant Science* 10, 313. doi:10.3389/fpls.2019.00313.

Calles-Torrez, V., Knodel, J. J., Beauzay, P., Doetkott, C. D., Ransom, J. K., Podliska, K. K., French, B. W. and Fuller, B. W. 2017. Transgenic Bt corn, soil insecticide, and insecticidal seed treatment effects on corn rootworm (Coleoptera: Chrysomelidae) beetle emergence, larval feeding injury, and corn yield in North Dakota. *Journal of Economic Entomology* 111, 348–60.

Cao, J., Zhao, J. Z., Tang, J., Shelton, A. and Earle, E. 2002. Broccoli plants with pyramided Cry1Ac and Cry1C Bt genes control diamondback moth resistant to Cry1A and Cry1C proteins. *Theoretical and Applied Genetics* 105, 258–64.

Cao, J., Brants, A. and Earle, E. D. 2003. Cauliflower plants expressing a *cry1C* transgene control larvae of diamondback moths resistant or susceptible to Cry1A, and cabbage loopers. *Journal of New Seeds* 5(2–3), 193–207. doi:10.1300/J153v05n02_06.

Cao, J., Shelton, A. M. and Earle, E. D. 2005. Development of transgenic collards (*Brassica oleracea* L., var. *acephala*) expressing a *cry1Ac* or *cry1C* Bt gene for control of the diamondback moth. *Crop Protection* 24(9), 804–13. doi:10.1016/j.cropro.2004.12.014.

Caprio, M. A. 1998. Evaluating resistance management strategies for multiple toxins in the presence of external refuges. *Journal of Economic Entomology* 91(5), 1021–31. doi:10.1093/jee/91.5.1021.

Caprio, M. A., Martinez, J. C., Porter, P. A. and Bynum, E. 2016. The impact of inter-kernel movement in the evolution of resistance to dual-toxin bt-corn varieties in *Helicoverpa zea* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 109(1), 307–19. doi:10.1093/jee/tov295.

Carrière, Y., Ellers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehy, T. J. and Tabashnik, B. E. 2003. Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. *Proceedings of the National Academy of Sciences of the United States of America* 100(4), 1519–23. doi:10.1073/pnas.0436708100.

Carrière, Y., Crowder, D. W. and Tabashnik, B. E. 2010. Evolutionary ecology of insect adaptation to Bt crops. *Evolutionary Applications* 3(5–6), 561–73. doi:10.1111/j.1752-4571.2010.00129.x.

Carrière, Y., Crickmore, N. and Tabashnik, B. E. 2015. Optimizing pyramided transgenic Bt crops for sustainable pest management. *Nature Biotechnology* 33(2), 161–8. doi:10.1038/nbt.3099.

Carrière, Y., Brown, Z. S., Downes, S. J., Gujar, G., Epstein, G., Omoto, C., Storer, N. P., Mota-Sanchez, D., Jørgensen, P. S. and Carroll, S. P. 2019. Governing evolution: a socioecological comparison of resistance management for insecticidal transgenic Bt crops among four countries. *Ambio – a Journal of the Human Environment*. doi:10.1007/s13280-019-01167-0.

Carstens, K., Cayabyab, B., De Schrijver, A., Gadaleta, P. G., Hellmich, R. L., Romeis, J., Storer, N., Valicente, F. H. and Wach, M. 2014. Surrogate species selection for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. *GM Crops and Food* 5(1), 11–5. doi:10.4161/gmcr.26560.

Casacuberta, J. M., Devos, Y., du Jardin, P., Ramon, M., Vaucheret, H. and Nogué, F. 2015. Biotechnological uses of RNAi in plants: risk assessment considerations. *Trends in Biotechnology* 33(3), 145–7. doi:10.1016/j.tibtech.2014.12.003.

Castañera, P., Farinós, G. P., Ortego, F. and Andow, D. A. 2016. Sixteen years of Bt maize in the EU hotspot: why has resistance not evolved? *PLoS ONE* 11(5), e0154200. doi:10.1371/journal.pone.0154200.

Chilcutt, C. F. and Tabashnik, B. E. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. *Proceedings of the National Academy of Sciences of the United States of America* 101(20), 7526–9. doi:10.1073/pnas.0400546101.

Christiaens, O. and Smagghe, G. 2014. The challenge of RNAi-mediated control of hemipterans. *Current Opinion in Insect Science* 6, 15–21. doi:10.1016/j.cois.2014.09.012.

Christiaens, O., Swevers, L. and Smagghe, G. 2014. DsRNA degradation in the pea aphid (*Acyrthosiphon pisum*) associated with lack of response in RNAi feeding and injection assay. *Peptides* 53, 307–14. doi:10.1016/j.peptides.2013.12.014.

Chu, C. C., Sun, W., Spencer, J. L., Pittendrigh, B. R. and Seufferheld, M. J. 2014. Differential effects of RNAi treatments on field populations of the western corn rootworm. *Pesticide Biochemistry and Physiology* 110, 1–6. doi:10.1016/j.pestbp.2014.02.003.

Comas, C., Lumbierres, B., Pons, X. and Albajes, R. 2014. No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a

meta-analysis of 26 arthropod taxa. *Transgenic Research* 23(1), 135–43. doi:10.1007/s11248-013-9737-0.

Corrales Madrid, J. L., Martínez Carrillo, J. L., Osuna Martínez, M. B., Durán Pompa, H. A., Alonso Escobedo, J., Javier Quiñones, F., Garzón Tiznado, J. A., Castro Espinoza, L., Zavala García, F., Espinoza Banda, A., González García, J., Jiang, C., Brown, C. R., de la F. Martínez, J. M., Heredia Díaz, O., Whitsel, J. E., Asiimwe, P., Baltazar, B. M. and Ahmad, A. 2018. Transportability of non-target arthropod field data for the use in environmental risk assessment of genetically modified maize in Northern Mexico. *Journal of Applied Entomology* 142(5), 525–38. doi:10.1111/jen.12499.

Craig, W., Tepfer, M., Degrassi, G. and Ripandelli, D. 2008. An overview of general features of risk assessments of genetically modified crops. *Euphytica* 164(3), 853–80. doi:10.1007/s10681-007-9643-8.

Crawley, M. J., Brown, S. L., Hails, R. S., Kohn, D. D. and Rees, M. 2001. Transgenic crops in natural habitats. *Nature* 409(6821), 682–3. doi:10.1038/35055621.

Crowder, D. W., Onstad, D. W. and Gray, M. E. 2006. Planting transgenic insecticidal corn based on economic thresholds: consequences for integrated pest management and insect resistance management. *Journal of Economic Entomology* 99(3), 899–907. doi:10.1603/0022-0493-99.3.899.

Davis, P. M. and Onstad, D. W. 2000. Seed mixtures as a resistance management strategy for European corn borers (Lepidoptera: Crambidae) infesting transgenic corn expressing Cry1Ab protein. *Journal of Economic Entomology* 93(3), 937–48. doi:10.1603/0022-0493-93.3.937.

Devos, Y., Craig, W. and Schiemann, J. 2012. Transgenic crops, risk assessment and regulatory framework in the European Union. In: Meyer, R. A. (Ed.), *Encyclopedia of Sustainability Science and Technology*. Springer, New York, NY, pp. 10765–96.

Devos, Y., Ortiz-García, S., Hokanson, K. E. and Raybould, A. 2018. Teosinte and maize x teosinte hybrid plants in Europe – environmental risk assessment and management implications for genetically modified maize. *Agriculture, Ecosystems and Environment* 259, 19–27. doi:10.1016/j.agee.2018.02.032.

Dhurua, S. and Gujar, G. T. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science* 67(8), 898–903. doi:10.1002/ps.2127.

DiFonzo, C. D. and Hammond, R. 2008. Range expansion of western bean cutworm, *Striacosta albicosta* (Noctuidae), into Michigan and Ohio. *Crop Management* 7. doi:10.1094/CM-2008-0519-01-BR.

DiFonzo, C. D., Krupke, C., Shields, E., Tilmon, K. and Tooker, J. 2016. An open letter to the Seed Industry regarding the efficacy of Cry1F against western bean cutworm. *Cornell Field Crop News*, October. Available at: http://blogs.cornell.edu/ccefieldcropnews/2016/10/04/an-open-letter-to-the-seed-industry-regarding-the-efficacy-of-cry1f-bt-against-western-bean-cutworm-october-2016/ (accessed on 29 April 2019).

Disque, H. H., Hamby, K. A., Dubey, A., Taylor, C. and Dively, G. P. 2019. Effects of clothianidin-treated seed on the arthropod community in a mid-Atlantic no-till corn agroecosystem. *Pest Management Science* 75(4), 969–78. doi:10.1002/ps.5201.

Dively, G. P., Venugopal, P. D. and Finkenbinder, C. 2016. Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. *PLoS ONE* 11(12), e0169115. doi:10.1371/journal.pone.0169115.

Dively, G. P., Venugopal, P. D., Bean, D., Whalen, J., Holmstrom, K., Kuhar, T. P., Doughty, H. B., Patton, T., Cissel, W. and Hutchison, W. D. 2018. Regional pest suppression

associated with widespread Bt maize adoption benefits vegetable growers. *Proceedings of the National Academy of Sciences of the United States of America* 115(13), 3320–5. doi:10.1073/pnas.1720692115.

Dorhout, D. L. and Rice, M. E. 2004. First report of western bean cutworm, *Richia albicosta* (Noctuidae) in Illinois and Missouri. *Crop Management* 3. doi:10.1094/CM-2004-1229-01-BR.

Douglas, M. R. and Tooker, J. F. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. field crops. *Environmental Science and Technology* 49(8), 5088–97. doi:10.1021/es506141g.

Downes, S. 2016. 2015–16 End of season resistance monitoring report. Available at: http://www.cottoninfo.com.au/publications/end-season-resistance-monitoring-conventional-insecticide-testing-report (accessed on 29 April 2019).

Duan, J. J., Lundgren, J. G., Naranjo, S. E. and Marvier, M. 2010. Extrapolating non-target risk of Bt crops from laboratory to field. *Biology Letters* 6(1), 74–7. doi:10.1098/rsbl.2009.0612.

Dubelman, S., Fischer, J., Zapata, F., Huizinga, K., Jiang, C., Uffman, J., Levine, S. and Carson, D. 2014. Environmental fate of double-stranded RNA in agricultural soils. *PLoS ONE* 9(3), e93155. doi:10.1371/journal.pone.0093155.

Duensing, N., Sprink, T., Parrott, W. A., Fedorova, M., Lema, M. A., Wolt, J. D. and Bartsch, D. 2018. Novel features and considerations for ERA and regulation of crops produced by genome editing. *Frontiers in Bioengineering and Biotechnology* 6, 79. doi:10.3389/fbioe.2018.00079.

Eastick, R. J. and Hearnden, M. N. 2006. Potential for weediness of Bt cotton in northern Australia. *Weed Science* 54(6), 1142–51. doi:10.1614/WS-06-077R.1.

Ellsworth, P. C., Fournier, A., Frisvold, G. and Naranjo, S. E. 2018. Chronicling the socio-economic impact of integrating biological control, technology, and knowledge over 25 years of IPM in Arizona. In: Mason, P. G., Gillespie, D. R. and Vincent, C. (Eds), *Proceedings of the 5th International Symposium on Biological Control of Arthropods*. CAB International, pp. 214–16. Available at: http://www.isbca-2017.org/download/ISBCA-Proceedings.web.pdf (accessed on 29 April 2019).

Estruch, J. J., Warren, G. W., Mullins, M. A., Nye, G. J., Craig, J. A. and Koziel, M. G. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences of the United States of America* 93(11), 5389–94. doi:10.1073/pnas.93.11.5389.

European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms (GMO), Naegeli, H., Birch, A. N., Casacuberta, J., De Schrijver, A., Gralak, M. A., Guerche, P., Jones, H., Manachini, B. and Messéan, A., Nielsen, E. E. 2017. Annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2015 from Monsanto Europe SA. *EFSA Journal* 15, e04805.

Farias, J. R., Andow, D. A., Horikoshi, R. J., Sorgatto, R. J., Fresia, P., dos Santos, A. C. and Omoto, C. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. *Crop Protection* 64, 150–8. doi:10.1016/j.cropro.2014.06.019.

Farias, J. R., Andow, D. A., Horikoshi, R. J., Sorgatto, R. J., Santos, A. C. D. and Omoto, C. 2016. Dominance of Cry1F resistance in *Spodoptera frugiperda* (Lepidoptera:

Noctuidae) on TC1507 Bt maize in Brazil. *Pest Management Science* 72(5), 974–9. doi:10.1002/ps.4077.

Farinós, G. P., Hernández-Crespo, P., Ortego, F. and Castañera, P. 2018. Monitoring of *Sesamia nonagrioides* resistance to MON 810 maize in the European Union: lessons from a long-term harmonized plan. *Pest Management Science* 74(3), 557–68. doi:10.1002/ps.4735.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391(6669), 806–11. doi:10.1038/35888.

Fischer, J. R., Zapata, F., Dubelman, S., Mueller, G. M., Uffman, J. P., Jiang, C., Jensen, P. D. and Levine, S. L. 2017. Aquatic fate of a double-stranded RNA in a sediment water system following an over-water application. *Environmental Toxicology and Chemistry* 36(3), 727–34. doi:10.1002/etc.3585.

Fischhoff, D. A., Bowdish, K. S., Perlak, F. J., Marrone, P. G., McCormick, S. M., Niedermeyer, J. G., Dean, D. A., Kusano-Kretzmer, K., Mayer, E. J., Rochester, D. E., Rogers, S. G. and Fraley, R. T. 1987. Insect tolerant transgenic tomato plants. *Nature Biotechnology* 5(8), 807–13. doi:10.1038/nbt0887-807.

Fishilevich, E., Bowling, A. J., Frey, M. L. F., Wang, P. H., Lo, W., Rangasamy, M., Worden, S. E., Pence, H. E., Gandra, P., Whitlock, S. L., Schulenberg, G., Knorr, E., Tenbusch, L., Lutz, J. R., Novak, S., Hamm, R. L., Schnelle, K. D., Vilcinskas, A. and Narva, K. E. 2019. RNAi targeting of rootworm Troponin I transcripts confers root protection in maize. *Insect Biochemistry and Molecular Biology* 104, 20–9. doi:10.1016/j.ibmb.2018.09.006.

Fitzpatrick, J., Cheavegatti-Gianotto, A., Ferro, J. A., Grossi-de-Sa, M. F., Keese, P., Layton, R., Lima, D., Nickson, T., Raybould, A., Romano, E., Romeis, J., Ulian, E. and Berezovsky, M. 2009. Problem formulation in environmental risk assessment of genetically modified crops: a Brazilian workshop report. *Bioassay* 4, 5. Available at: www.bioassay.org.br/ojs/index.php/bioassay/article/view/65 (accessed on 29 April 2019).

Ganiger, P. C., Yeshwanth, H. M., Muralimohan, K., Vinay, N., Kumar, A. R. V. and Chandrashekara, K. 2018. Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. *Current Science* 115, 621–3.

Garbutt, J. S., Belles, X., Richards, E. H. and Reynolds, S. E. 2013. Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: evidence from *Manduca sexta* and *Blattella germanica*. *Journal of Insect Physiology* 59(2), 171–8. doi:10.1016/j.jinsphys.2012.05.013.

Garcia-Alonso, M., Jacobs, E., Raybould, A., Nickson, T. E., Sowig, P., Willekens, H., van der Kouwe, P., Layton, R., Amijee, F., Fuentes, A. M. and Tencalla, F. 2006. A tiered system for assessing the risk of genetically modified plants to nontarget organisms. *Environmental Biosafety Research* 5(2), 57–65. doi:10.1051/ebr:2006018.

Garcia-Alonso, M., Hendley, P., Bigler, F., Meyeregger, E., Parker, R., Rubinstein, C., Satorre, E., Solari, F. and McLean, M. A. 2014. Transportability of confined field trial data for environmental risk assessment of genetically engineered plants: a conceptual framework. *Transgenic Research* 23(6), 1025–41. doi:10.1007/s11248-014-9785-0.

Gassmann, A. J., Carrière, Y. and Tabashnik, B. E. 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 54, 147–63. doi:10.1146/annurev.ento.54.110807.090518.

Gassmann, A. J., Petzold-Maxwell, J. L., Keweshan, R. S. and Dunbar, M. W. 2011. Field-evolved resistance to Bt maize by western corn rootworm. *PLoS ONE* 6(7), e22629. doi:10.1371/journal.pone.0022629.

Gassmann, A. J., Petzold-Maxwell, J. L., Clifton, E. H., Dunbar, M. W., Hoffmann, A. M., Ingber, D. A. and Keweshan, R. S. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. *Proceedings of the National Academy of Sciences of the United States of America* 111(14), 5141–6. doi:10.1073/pnas.1317179111.

Gassmann, A. J., Shrestha, R. B., Jakka, S. R., Dunbar, M. W., Clifton, E. H., Paolino, A. R., Ingber, D. A., French, B. W., Masloski, K. E., Dounda, J. W. and St. Clair, C. R. 2016. Evidence of resistance to Cry34/35Ab1 corn by western corn rootworm (Coleoptera: Chrysomelidae): root injury in the field and larval survival in plant-based bioassays. *Journal of Economic Entomology* 109(4), 1872–80. doi:10.1093/jee/tow110.

Georghiou, G. P. and Taylor, C. E. 1977. Genetic and biological influences in the evolution of insecticide resistance. *Journal of Economic Entomology* 70(3), 319–23. doi:10.1093/jee/70.3.319.

Glare, T. R. and O'Callaghan, M. 2000. *Bacillus thuringiensis: Biology, Ecology and Safety*. John Wiley and Sons, New York.

Glaser, J. A. and Matten, S. R. 2003. Sustainability of insect resistance management strategies for transgenic Bt corn. *Biotechnology Advances* 22(1–2), 45–69. doi:10.1016/j.biotechadv.2003.08.016.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A. and Tamò, M. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE* 11(10), e0165632. doi:10.1371/journal.pone.0165632.

Gonzalez, D., Patterson, B. R., Leigh, T. F. and Wilson, L. T. 1982. Mites, a primary food source for two predators in San Joaquin cotton. *California Agriculture* 36, 18–20.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* 43, 701–26. doi:10.1146/annurev.ento.43.1.701.

Gowda, A., Rydel, T. J., Wollacott, A. M., Brown, R. S., Akbar, W., Clark, T. L., Flasinski, S., Nageotte, J. R., Read, A. C., Shi, X., Werner, B. J., Pleau, M. J. and Baum, J. A. 2016. A transgenic approach for controlling *Lygus* in cotton. *Nature Communications* 7, 12213. doi:10.1038/ncomms12213.

Grafius, E. J. and Douches, D. S. 2008. The present and future role of insect-resistant genetically modified potato cultivars in IPM. In: Romeis, J., Shelton, A. M. and Kennedy, G. G. (Eds), *Integration of Insect-Resistant Genetically Modified Crops with IPM Systems*. Springer, Berlin, Germany, pp. 195–221.

Graham, S. H. and Stewart, S. D. 2018. Field study investigating Cry51Aa2.834_16 in cotton for control of thrips (Thysanoptera: Thripidae) and tarnished plant bugs (Hemiptera: Miridae). *Journal of Economic Entomology* 111(6), 2717–26. doi:10.1093/jee/toy250.

Gray, M. E. 2011. Relevance of traditional integrated pest management (IPM) strategies for commercial corn producers in a transgenic agroecosystem: a bygone era? *Journal of Agricultural and Food Chemistry* 59(11), 5852–8. doi:10.1021/jf102673s.

Grimi, D. A., Ocampo, F., Martinelli, S. and Head, G. P. 2015. *Detection and Characterization of Diatraea saccharalis Resistant to Cry1A.105 Protein in a Population of Northeast*

San Luis Province in Argentina. Congreso Argentino de Entomología, Posadas, Misiones, Argentina.

Gustafson, D. I., Head, G. P. and Caprio, M. A. 2006. Modeling the impact of alternative hosts on *Helicoverpa zea* adaptation to Bollgard cotton. *Journal of Economic Entomology* 99(6), 2116–24. doi:10.1603/0022-0493-99.6.2116.

Hagen, A. F. 1962. The biology and control of the western bean cutworm in dent corn in Nebraska. *Journal of Economic Entomology* 55(5), 628–31. doi:10.1093/jee/55.5.628.

Hails, R. S. and Morley, K. 2005. Genes invading new populations: a risk assessment perspective. *Trends in Ecology and Evolution* 20(5), 245–52. doi:10.1016/j.tree.2005.02.006.

Haller, S., Widmer, F., Siegfried, B. D., Zhuo, X. and Romeis, J. 2019. Responses of two ladybird beetle species (Coleoptera: Coccinellidae) to dietary RNAi. *Pest Management Science*. doi:10.1002/ps.5370.

Han, Q., Wang, Z., He, Y., Xiong, Y., Lv, S., Li, S., Zhang, Z., Qiu, D. and Zeng, H. 2017. Transgenic cotton plants expressing the *HaHR3* gene conferred enhanced resistance to *Helicoverpa armigera* and improved cotton yield. *International Journal of Molecular Sciences* 18(9), 1874. doi:10.3390/ijms18091874.

Head, G. P. and Greenplate, J. 2012. The design and implementation of insect resistance management programs for Bt crops. *GM Crops and Food* 3(3), 144–53. doi:10.4161/gmcr.20743.

Head, G. P., Carroll, M. W., Evans, S. P., Rule, D. M., Willse, A. R., Clark, T. L., Storer, N. P., Flannagan, R. D., Samuel, L. W. and Meinke, L. J. 2017. Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. *Pest Management Science* 73(9), 1883–99. doi:10.1002/ps.4554.

Head, G. P., Jackson, R. E., Adamczyk, J., Bradley, J. R., Van Duyn, J., Gore, J., Hardee, D. D., Leonard, B. R., Luttrell, R., Ruberson, J., Mullins, J. W., Orth, R. G., Sivasupramaniam, S. and Voth, R. 2010. Spatial and temporal variability in host use by *Helicoverpa zea* as measured by analyses of stable carbon isotope ratios and gossypol residues. *Journal of Applied Ecology* 47(3), 583–92. doi:10.1111/j.1365-2664.2010.01796.x.

Hellmich, R. L., Calvin, D. D., Russo, J. M. and Lewis, L. C. 2005. Integration of Bt maize in IPM systems: U.S. perspective. In: *Proceedings 2nd International Symposium on Biological Control of Arthropods*. U.S. Dept. Agriculture, Forest Service, FHTET-2005-08, pp. 356–61.

Hellmich, R. L., Albajes, R., Bergvinson, D., Prasifka, J. R., Wang, Z. Y. and Weiss, M. J. 2008. The present and future role of insect-resistant genetically modified maize in IPM. In: Romeis, J., Shelton, A. M. and Kennedy, G. G. (Eds), *Integration of Insect-Resistant Genetically Modified Crops with IPM Systems*. Springer, Berlin, Germany, pp. 119–58.

Hokanson, K. E. 2019. When policy meets practice: the dilemma for guidance on risk assessment under the Cartagena Protocol on Biosafety. *Frontiers in Bioengineering and Biotechnology* 7, 82. doi:10.3389/fbioe.2019.00082.

Hoy, C. W., Vaughn, T. T. and East, D. A. 2000. Increasing the effectiveness of spring trap crops for *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata* 96(3), 193–204. doi:10.1046/j.1570-7458.2000.00697.x.

Hu, X., Richtman, N. M., Zhao, J. Z., Duncan, K. E., Niu, X., Procyk, L. A., Oneal, M. A., Kernodle, B. M., Steimel, J. P., Crane, V. C., Sandahl, G., Ritland, J. L., Howard, R. J., Presnail, J. K., Lu, A. L. and Wu, G. 2016. Discovery of midgut genes for the RNA

interference control of corn rootworm. *Scientific Reports* 6, 30542. doi:10.1038/srep30542.

Hu, J., Zhang, J., Chen, X., Lv, J., Jia, H., Zhao, S. and Lu, M. 2017. An empirical assessment of transgene flow from a Bt transgenic poplar plantation. *PLoS ONE* 12(1), e0170201. doi:10.1371/journal.pone.0170201.

Hurley, T. M., Babcock, B. A. and Hellmich, R. L. 2001. Bt corn and insect resistance: an economic assessment of refuges. *Journal of Agricultural and Resource Economics* 26, 176–94.

Hutchison, W. D., Burkness, E. C., Mitchell, P. D., Moon, R. D., Leslie, T. W., Fleischer, S. J., Abrahamson, M., Hamilton, K. L., Steffey, K. L., Gray, M. E., Hellmich, R. L., Kaster, L. V., Hunt, T. E., Wright, R. J., Pecinovsky, K., Rabaey, T. L., Flood, B. R. and Raun, E. S. 2010. Areawide suppression of European Corn Borer with Bt maize reaps savings to non-Bt maize growers. *Science* 330(6001), 222–5. doi:10.1126/science.1190242.

Hutchison, W. D., Hunt, T. E., Hein, G. L., Steffey, K. L., Pilcher, C. D. and Rice, M. E. 2011. Genetically engineered Bt corn and range expansion of the western bean cutworm (Lepidoptera: Noctuidae) in the United States: A response to Greenpeace Germany. *Journal of Integrated Pest Management* 2(3), B1–8. doi:10.1603/IPM11016.

International Institute for Tropical Agriculture (IITA). 2019. Major breakthrough for farmers and scientists as Nigerian biotech body approves commercial release of genetically modified cowpea. Press Release, 9 February 2019. Available at: http://www.iita.org/news-item/major-breakthrough-for-farmers-and-scientists-as-nigerian-biotech-body-approves-commercial-release-of-genetically-modified-cowpea/ (accessed on 29 April 2019).

ISAAA. 2017. Global status of commercialized Biotech/GM crops in 2017: biotech crop adoption surges as economic benefits accumulate in 22 years. ISAAA Brief No. 53. ISAAA, Ithaca, NY.

Islam, S. M. F. and Norton, G. W. 2007. Bt eggplant for fruit and shoot borer resistance in Bangladesh. In: Ramasamy, C., Selvaraj, K. N., Norton, G. W. and Vijayaraghavan, K. (Eds), *Economic and Environmental Benefits and Costs of Transgenic Crops: Ex-Ante Assessment*. Tamil Nadu Agricultural University, Coimbatore, India, pp. 91–106.

Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S. and Venkataraman, G. 2018. CRISPR for crop improvement: an update review. *Frontiers in Plant Science* 9, 985. doi:10.3389/fpls.2018.00985.

Jakka, S. R., Shrestha, R. B. and Gassmann, A. J. 2016. Broad-spectrum resistance to *Bacillus thuringiensis* toxins by western corn rootworm (*Diabrotica virgifera virgifera*). *Scientific Reports* 6, 27860. doi:10.1038/srep27860.

James, C. 1997. Global status of transgenic crops in 1997. ISAAA Briefs No. 5. ISAAA, Ithaca, NY.

Jarosch, A. and Moritz, R. F. A. 2012. RNA interference in honeybees: off-target effects caused by dsRNA. *Apidologie* 43(2), 128–38. doi:10.1007/s13592-011-0092-y.

Jiao, Y., Hu, X., Peng, Y., Wu, K., Romeis, J. and Li, Y. 2018. Bt rice plants may protect neighbouring non-Bt rice plants against the striped stem borer *Chilo suppressalis*. *Proceedings of the Royal Society. Series B, Biological Sciences* 285, 20181283.

Jin, L., Zhang, H., Lu, Y., Yang, Y., Wu, K., Tabashnik, B. E. and Wu, Y. 2015a. Large-scale test of the natural refuge strategy for delaying insect resistance to transgenic Bt crops. *Nature Biotechnology* 33(2), 169–74. doi:10.1038/nbt.3100.

Jin, S., Singh, N. D., Li, L., Zhang, X. and Daniell, H. 2015b. Engineered chloroplast dsRNA silences cyto-chrome p450 monooxygenase, *V-ATPase* and *chitin synthase* genes in

the insect gut and disrupts *Helicoverpa armigera* larval development and pupation. *Plant Biotechnology Journal* 13(3), 435–46. doi:10.1111/pbi.12355.

Keese, P. K., Robold, A. V., Myers, R. C., Weisman, S. and Smith, J. 2014. Applying a weed risk assessment approach to GM crops. *Transgenic Research* 23(6), 957–69. doi:10.1007/s11248-013-9745-0.

Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M., Miller, K., Zhang, Y., Ramaseshadri, P., Jiang, C., Hodge, T., Jensen, P., Chen, M., Gowda, A., McNulty, B., Vazquez, C., Bolognesi, R., Haas, J., Head, G. and Clark, T. 2018. Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. *PLoS ONE* 13(5), e0197059. doi:10.1371/journal.pone.0197059.

Klümper, W. and Qaim, M. 2014. A meta-analysis of the impacts of genetically modified crops. *PLoS ONE* 9(11), e111629. doi:10.1371/journal.pone.0111629.

Kranthi, K. R. 2015. Pink bollworm strikes Bt-cotton. *Cotton Statistics and News* 35, 1–6. Available at: http://caionline.in/download_publication/424.

Kranthi, S., Satija, U., Pusadkar, P., Kumar, R., Shastri, C. S., Ansari, S., Santosh, H. B., Monga, D. and Kranthi, K. R. 2017. Non-Bt seeds provided by seed companies in India - are they suitable as refuge for Bt-cotton? *Current Science* 112, 1992–3.

Kruger, M., Van Rensburg, J. B. J. and Van den Berg, J. 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. *Crop Protection* 28(8), 684–9. doi:10.1016/j.cropro.2009.04.001.

Kruger, M., Van Rensburg, J. B. J. and Van den Berg, J. 2012. Transgenic Bt maize: farmers' perceptions, refuge compliance and reports of stem borer resistance in South Africa. *Journal of Applied Entomology* 136(1–2), 38–50. doi:10.1111/j.1439-0418.2011.01616.x.

Krysan, J. L., Foster, D. E., Branson, T. F., Ostlie, K. R. and Cranshaw, W. S. 1986. Two years before the hatch: rootworms adapt to crop rotation. *Bulletin of the Entomological Society of America* 32(4), 250–3. doi:10.1093/besa/32.4.250.

Kulkarni, M. M., Booker, M., Silver, S. J., Friedman, A., Hong, P., Perrimon, N. and Mathey-Prevot, B. 2006. Evidence of off-target effects associated with long dsRNAs in *Drosophila melanogaster* cell-based assays. *Nature Methods* 3(10), 833–8. doi:10.1038/nmeth935.

Lee, M. K., Walters, F. S., Hart, H., Palekar, N. and Chen, J. S. 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. *Applied and Environmental Microbiology* 69(8), 4648–57. doi:10.1128/aem.69.8.4648-4657.2003.

Levine, E., Oloumi-Sadeghi, H. and Fisher, J. R. 1992. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. *Journal of Economic Entomology* 85(1), 262–7. doi:10.1093/jee/85.1.262.

Levine, E., Spencer, J. L., Isard, S. A., Onstad, D. W. and Gray, M. E. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. *American Entomologist* 48(2), 94–107. doi:10.1093/ae/48.2.94.

Li, Y., Hallerman, E. M., Liu, Q., Wu, K. and Peng, Y. 2016. The development and status of Bt rice in China. *Plant Biotechnology Journal* 14(3), 839–48. doi:10.1111/pbi.12464.

Li, Y., Zhang, Q., Liu, Q., Meissle, M., Yang, Y., Wang, Y., Hua, H., Chen, X., Peng, Y. and Romeis, J. 2017. Bt rice in China—focusing the non-target risk assessment. *Plant Biotechnology Journal* 15(10), 1340–5. doi:10.1111/pbi.12720.

Liu, J., Smagghe, G. and Swevers, L. 2013. Transcriptional response of BmToll9-1 and RNAi machinery genes to exogenous dsRNA in the midgut of *Bombyx mori*. *Journal of Insect Physiology* 59(6), 646–54. doi:10.1016/j.jinsphys.2013.03.013.

Lu, Y., Wu, K., Jiang, Y., Xia, B., Li, P., Feng, H., Wyckhuys, K. A. G. and Guo, Y. 2010. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* 328(5982), 1151–4. doi:10.1126/science.1187881.

Lu, Y., Wu, K., Jiang, Y., Guo, Y. and Desneux, N. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* 487(7407), 362–5. doi:10.1038/nature11153.

Lu, H. P., Luo, T., Fu, H. W., Wang, L., Tan, Y. Y., Huang, J. Z., Wang, Q., Ye, G. Y., Gatehouse, A. M. R., Lou, Y. G. and Shu, Q. Y. 2018. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. *Nature Plants* 4(6), 338–44. doi:10.1038/s41477-018-0152-7.

Luo, Y., Wang, X., Wang, X., Yu, D., Chen, B. and Kang, L. 2013. Differential responses of migratory locusts to systemic RNA interference via double-stranded RNA injection and feeding. *Insect Molecular Biology* 22(5), 574–83. doi:10.1111/imb.12046.

Ma, Y., Creanga, A., Lum, L. and Beachy, P. A. 2006. Prevalence of off-target effects in *Drosophila* RNA interference screens. *Nature* 443(7109), 359–63. doi:10.1038/nature05179.

Mahon, R. J., Olsen, K. M., Downes, S. and Addison, S. 2007. Frequency of alleles conferring resistance to the Bt toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 100(6), 1844–53. doi:10.1603/0022-0493(2007)100[1844:foacrt]2.0.co;2.

Mallet, J. and Porter, P. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *Proceedings of the Royal Society of London. Series B: Biological Sciences* 250(1328), 165–9. doi:10.1098/rspb.1992.0145.

Mao, Y. B., Cai, W. J., Wang, J. W., Hong, G. J., Tao, X. Y., Wang, L. J., Huang, Y. P. and Chen, X. Y. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature Biotechnology* 25(11), 1307–13. doi:10.1038/nbt1352.

Martinez, J. C., Caprio, M. A. and Friedenberg, N. A. 2018. Density dependence and growth rate: evolutionary effects on resistance development to Bt (*Bacillus thuringiensis*). *Journal of Economic Entomology* 111(1), 382–90. doi:10.1093/jee/tox323.

Matten, S. M., Hellmich, R. L. and Reynolds, A. 2004. Current resistance management strategies for Bt corn in the United States. In: Koul, O. and Dhaliwal, G. S. (Eds), *Transgenic Crop Production: Concepts and Strategies*. Science Publishers, Inc., Plymouth, UK, pp. 261–88.

McHughen, A. and Smyth, S. 2008. US regulatory system for genetically modified [genetically modified organism (GMO), rDNA or transgenic] crop cultivars. *Plant Biotechnology Journal* 6(1), 2–12. doi:10.1111/j.1467-7652.2007.00300.x.

Michel, A. P., Krupke, C. H., Baute, T. S. and Difonzo, C. D. 2010. Ecology and management of the western bean cutworm (Lepidoptera: Noctuidae) in corn and dry beans. *Journal of Integrated Pest Management* 1(1), A1–A10. doi:10.1603/IPM10003.

Millennium Ecosystem Assessment (MEA). 2005. *Ecosystems and Human Well-Being: Synthesis*. MEA, Washington DC. Available at: http://www.millenniumassessment.org/documents/document.356.aspx.pdf.

Mohan, K. S., Ravi, K. C., Suresh, P. J., Sumerford, D. and Head, G. P. 2016. Field resistance to the *Bacillus thuringiensis* protein Cry1Ac expressed in Bollgard® hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders), populations in India. *Pest Management Science* 72(4), 738–46. doi:10.1002/ps.4047.

Nagoshi, R. N., Goergen, G., Tounou, K. A., Agboka, K., Koffi, D. and Meagher, R. L. 2018. Analysis of strain distribution, migratory potential, and invasion history of fall armyworm populations in northern sub-Saharan Africa. *Scientific Reports* 8(1), 3710. doi:10.1038/s41598-018-21954-1.

Naranjo, S. E. 2001. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Protection* 20(9), 835–52. doi:10.1016/S0261-2194(01)00115-6.

Naranjo, S. E. 2009. Impacts of Bt crops on non-target invertebrates and insecticide use patterns. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 4(11), 11. doi:10.1079/PAVSNNR20094011.

Naranjo, S. E. and Ellsworth, P. C. 2009. Fifty years of the integrated control concept: moving the model and implementation forward in Arizona. *Pest Management Science* 65(12), 1267–86. doi:10.1002/ps.1861.

Naranjo, S. E. and Ellsworth, P. C. 2010. Fourteen years of Bt cotton advances IPM in Arizona. *Southwestern Entomologist* 35(3), 437–44. doi:10.3958/059.035.0329.

Naranjo, S. E., Ruberson, J. R., Sharma, H. C., Wilson, L. and Wu, K. M. 2008. The present and future role of insect-resistant genetically modified cotton in IPM. In: Romeis, J., Shelton, A. M. and Kennedy, G. G. (Eds), *Integration of Insect-Resistant Genetically Modified Crops with IPM Systems*. Springer, Berlin, Germany, pp. 159–94.

Ni, M., Ma, W., Wang, X., Gao, M., Dai, Y., Wei, X., Zhang, L., Peng, Y., Chen, S., Ding, L., Tian, Y., Li, J., Wang, H., Wang, X., Xu, G., Guo, W., Yang, Y., Wu, Y., Heuberger, S., Tabashnik, B. E., Zhang, T. and Zhu, Z. 2017. Next-generation transgenic cotton: pyramiding RNAi and Bt counters insect resistance. *Plant Biotechnology Journal* 15(9), 1204–13. doi:10.1111/pbi.12709.

Niu, X., Kassa, A., Hu, X., Robeson, J., McMahon, M., Richtman, N. M., Steimel, J. P., Kernodle, B. M., Crane, V. C., Sandahl, G., Ritland, J. L., Presnail, J. K., Lu, A. L. and Wu, G. 2017. Control of western corn rootworm (*Diabrotica virgifera virgifera*) reproduction through plant-mediated RNA interference. *Scientific Reports* 7(1), 12591. doi:10.1038/s41598-017-12638-3.

Niu, J., Taning, C. N. T., Christiaens, O., Smagghe, G. and Wang, J. J. 2018. Rethink RNAi in insect pest control: challenges and perspectives. *Advances in Insect Physiology* 55, 1–17. doi:10.1016/bs.aiip.2018.07.003.

North, J. H., Gore, J., Catchot, A. L., Stewart, S. D., Lorenz, G. M., Musser, F. R., Cook, D. R., Kerns, D. L. and Dodds, D. M. 2018. Value of neonicotinoid insecticide seed treatments in Mid-South cotton (*Gossypium hirsutum* [Malvales: Malvaceae]) production systems. *Journal of Economic Entomology* 111(1), 10–5. doi:10.1093/jee/tox324.

Nyrop, J. P., Foster, R. E. and Onstad, D. W. 1986. Value of sample information in pest control decision making. *Journal of Economic Entomology* 79(6), 1421–9. doi:10.1093/jee/79.6.1421.

Nyrop, J. P., Shelton, A. M. and Theunissen, J. 1989. Value of a control decision rule for leek moth infestations in leek. *Entomologia Experimentalis et Applicata* 53(2), 167–76. doi:10.1111/j.1570-7458.1989.tb01301.x.

Omoto, C., Bernardi, O., Salmeron, E., Sorgatto, R. J., Dourado, P. M., Crivellari, A., Carvalho, R. A., Willse, A., Martinelli, S. and Head, G. P. 2016. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. *Pest Management Science* 72(9), 1727–36. doi:10.1002/ps.4201.

Onstad, D. W. 1987. Calculation of economic-injury levels and economic thresholds for pest management. *Journal of Economic Entomology* 80(2), 297–303. doi:10.1093/jee/80.2.297.

Onstad, D. W. (Ed.). 2013. *Insect Resistance Management: Biology, Economics, and Prediction*. Academic Press, Waltham, MA.

Onstad, D. W., Crespo, A. L. B., Pan, Z., Crain, P. R., Thompson, S. D., Pilcher, C. D. and Sethi, A. 2018. Blended refuge and insect resistance management for insecticidal corn. *Environmental Entomology* 47(1), 210–9. doi:10.1093/ee/nvx172.

O'Rourke, P. K. and Hutchison, W. D. 2000. First report of the western bean cutworm, *Richia albicosta* (Smith) (Lepidoptera: Noctuidae) in Minnesota corn. *Journal of Agricultural and Urban Entomology* 17, 213–7.

Ostlie, K. R., Hutchison, W. D. and Hellmich, R. L. 1997. Bt corn and European corn borer. North Central Regional Publication 602. Available at: https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1633&context=entomologyfacpub.

Ostrem, J. S., Pan, Z., Flexner, J. L., Owens, E., Binning, R. and Higgins, L. S. 2016. Monitoring susceptibility of western bean cutworm (Lepidoptera: Noctuidae) field populations to *Bacillus thuringiensis* Cry1F protein. *Journal of Economic Entomology* 109(2), 847–53. doi:10.1093/jee/tov383.

Painter, R. H. 1951. *Insect Resistance in Crop Plants*. University of Kansas Press, Lawrence, KS.

Pan, H., Xu, L., Noland, J. E., Li, H., Siegfried, B. D. and Zhou, X. 2016. Assessment of potential risks of dietary RNAi to a soil micro-arthropod, *Sinella curviseta* Brook (Collembola: Entomobryidae). *Frontiers in Plant Science* 7, 1028. doi:10.3389/fpls.2016.01028.

Pan, H., Yang, X., Bidne, K., Hellmich, R. L., Siegfried, B. D. and Zhou, X. 2017. Dietary risk assessment of *v-ATPase A* dsRNAs on monarch butterfly larvae. *Frontiers in Plant Science* 8, 242. doi:10.3389/fpls.2017.00242.

Papiernik, S. K., Sappington, T. W., Luttrell, R. G., Hesler, L. S. and Allen, K. C. 2018. Overview: risk factors and historic levels of pressure from insect pests of seedling corn, cotton, soybean, and wheat in the United States. *Journal of Integrated Pest Management* 9, 18.

Parker, K. M., Barragán Borrero, V., van Leeuwen, D. M., Lever, M. A., Mateescu, B. and Sander, M. 2019. Environmental fate of RNA interference pesticides: adsorption and degradation of double-stranded RNA molecules in agricultural soils. *Environmental Science and Technology* 53(6), 3027–36. doi:10.1021/acs.est.8b05576.

Paula-Moraes, S., Hunt, T. E., Wright, R. J., Hein, G. L. and Blankenship, E. E. 2013. Western bean cutworm survival and the development of economic injury levels and economic thresholds in field corn. *Journal of Economic Entomology* 106(3), 1274–85. doi:10.1603/ec12436.

Pereira, A. E., Carneiro, N. and Siegfried, B. D. 2016. Comparative susceptibility of southern and western corn rootworm adults and larvae to *vATPase-A* and *Snf7* dsRNAs. *Journal of RNAi and Gene Silencing* 12, 528–35.

Poppy, G. M. and Wilkinson, M. J. (Eds). 2005. *Gene Flow from GM Plants*. Blackwell Publishing Ltd, Oxford, UK.

Poston, F. L., Pedigo, L. P. and Welch, S. M. 1983. Economic injury levels: reality and practicality. *Bulletin of the Entomological Society of America* 29(1), 49–53. doi:10.1093/besa/29.1.49.

Price, D. R. G. and Gatehouse, J. A. 2008. RNAi-mediated crop protection against insects. *Trends in Biotechnology* 26(7), 393–400. doi:10.1016/j.tibtech.2008.04.004.

Prodhan, M. Z. H., Hasan, M. T., Chowdhury, M. M. I., Alam, M. S., Rahman, M. L., Azad, A. K., Hossain, M. J., Naranjo, S. E. and Shelton, A. M. 2018. Bt eggplant (*Solanum melongena* L.) in Bangladesh: fruit production and control of eggplant fruit and shoot borer (*Leucinodes Orbonalis* Guenee), effects on non-target arthropods and economic returns. *PLoS ONE* 13(11), e0205713. doi:10.1371/journal.pone.0205713.

Quisenberry, S. S. and Schotzko, D. J. 1994. Integration of plant resistance with pest management methods in crop production systems. *Journal of Agricultural Entomology* 11, 279–90.

Ramaseshadri, P., Segers, G., Flannagan, R., Wiggins, E., Clinton, W., Ilagan, O., McNulty, B., Clark, T. and Bolognesi, R. 2013. Physiological and cellular responses caused by RNAi-mediated suppression of Snf7 orthologue in western corn rootworm (*Diabrotica virgifera virgifera*) larvae. *PLoS ONE* 8(1), e54270. doi:10.1371/journal.pone.0054270.

Rashid, M. A., Hasan, M. K. and Matin, M. A. 2018. Socio-economic performance of Bt eggplant cultivation in Bangladesh. *Bangladesh Journal of Agricultural Research* 43(2), 187–203. doi:10.3329/bjar.v43i2.37313.

Raybould, A. and Quemada, H. 2010. Bt crops and food security in developing countries: realized benefits, sustainable use and lowering barriers to adoption. *Food Security* 2(3), 247–59. doi:10.1007/s12571-010-0066-3.

Raybould, A. and Vlachos, D. 2011. Non-target organism effects tests on Vip3A and their application to the ecological risk assessment for cultivation of MIR162 maize. *Transgenic Research* 20(3), 599–611. doi:10.1007/s11248-010-9442-1.

Raybould, A., Higgins, L. S., Horak, M. J., Layton, R. J., Storer, N. P., de la Fuente, J. M. and Herman, R. A. 2012. Assessing the ecological risks from the persistence and spread of feral populations of insect-resistant transgenic maize. *Transgenic Research* 21(3), 655–64. doi:10.1007/s11248-011-9560-4.

Reisig, D. D. 2017. Factors associated with willingness to plant non-Bt maize refuge and suggestions for increasing refuge compliance. *Journal of Integrated Pest Management* 8(1), doi:10.1093/jipm/pmx002.

Roberts, A. F., Devos, Y., Lemgo, G. N. Y. and Zhou, X. 2015. Biosafety research for non-target organism risk assessment of RNAi-based GE plants. *Frontiers in Plant Science* 6, 958. doi:10.3389/fpls.2015.00958.

Rocha-Munive, M. G., Soberon, M., Castaneda, S., Niaves, E., Scheinvar, E., Eguiarte, L. E., Mota-Sanchez, D., Rosales-Robles, E., Nava-Camberos, U., Martinez-Carrillo, J. L., Blanco, C. A., Bravo, A. and Souza, V. 2018. Evaluation of the impact of genetically modified cotton after 20 years of cultivation in Mexico. *Frontiers in Bioengineering and Biotechnology* 6, 82. doi:10.3389/fbioe.2018.00082.

Romeis, J., Meissle, M. and Bigler, F. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology* 24(1), 63–71. doi:10.1038/nbt1180.

Romeis, J., Bartsch, D., Bigler, F., Candolfi, M. P., Gielkens, M. M. C., Hartley, S. E., Hellmich, R. L., Huesing, J. E., Jepson, P. C., Layton, R., Quemada, H., Raybould, A., Rose, R. I., Schiemann, J., Sears, M. K., Shelton, A. M., Sweet, J., Vaituzis, Z. and Wolt, J. D. 2008.

Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nature Biotechnology* 26(2), 203–8. doi:10.1038/nbt1381.

Romeis, J., Lawo, N. C. and Raybould, A. 2009. Making effective use of existing data for case-by-case risk assessments of genetically engineered crops. *Journal of Applied Entomology* 133(8), 571–83. doi:10.1111/j.1439-0418.2009.01423.x.

Romeis, J., Hellmich, R. L., Candolfi, M. P., Carstens, K., De Schrijver, A., Gatehouse, A. M. R., Herman, R. A., Huesing, J. E., McLean, M. A., Raybould, A., Shelton, A. M. and Waggoner, A. 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. *Transgenic Research* 20(1), 1–22. doi:10.1007/s11248-010-9446-x.

Romeis, J., Raybould, A., Bigler, F., Candolfi, M. P., Hellmich, R. L., Huesing, J. E. and Shelton, A. M. 2013a. Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. *Chemosphere* 90(3), 901–9. doi:10.1016/j.chemosphere.2012.09.035.

Romeis, J., McLean, M. A. and Shelton, A. M. 2013b. When bad science makes good headlines: Bt maize and regulatory bans. *Nature Biotechnology* 31(5), 386–7. doi:10.1038/nbt.2578.

Romeis, J., Meissle, M., Álvarez-Alfageme, F., Bigler, F., Bohan, D. A., Devos, Y., Malone, L. A., Pons, X. and Rauschen, S. 2014. Potential use of an arthropod database to support the non-target risk assessment and monitoring of transgenic plants. *Transgenic Research* 23(6), 995–1013. doi:10.1007/s11248-014-9791-2.

Romeis, J., Naranjo, S. E., Meissle, M. and Shelton, A. M. 2019. Genetically engineered crops help support conservation biological control. *Biological Control* 130, 136–54. doi:10.1016/j.biocontrol.2018.10.001.

Roush, R. T. 1997. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pesticide Science* 51(3), 328–34. doi:10.1002/(SICI)1096-9063(199711)51:3<328::AID-PS650>3.0.CO;2-B.

Roush, R. T. 1998. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 353(1376), 1777–86. doi:10.1098/rstb.1998.0330.

Rukarwa, R. J., Mukasa, S. B., Odongo, B., Ssemakula, G. and Ghislain, M. 2014. Identification of relevant non-target organisms exposed to weevil-resistant Bt sweetpotato in Uganda. *3 Biotech* 4(3), 217–26. doi:10.1007/s13205-013-0153-1.

Russell, D., Uijtewaal, B., Shelton, A. M., Chen, M., Srinivasan, R., Gujar, G., Rauf, A., Grzywacz, D. and Gregory, P. 2008. Bt brassicas for DBM control: the CIMBAA public/private partnership. In: Shelton, A. M., Collins, H. L., Zhang, Y. J. and Wu, Q. J. (Eds), *Management of Diamondback Moth and Other Crucifer Pests*. China Agricultural Science and Technology Press, Beijing, pp. 272–9.

Saeed, R., Razaq, M. and Hardy, I. C. W. 2016. Impact of neonicotinoid seed treatment of cotton on the cotton leafhopper, *Amrasca devastans* (Hemiptera: Cicadellidae), and its natural enemies. *Pest Management Science* 72(6), 1260–7. doi:10.1002/ps.4146.

Sanahuja, G., Banakar, R., Twyman, R. M., Capell, T. and Christou, P. 2011. *Bacillus thuringiensis*: a century of research, development and commercial applications. *Plant Biotechnology Journal* 9(3), 283–300. doi:10.1111/j.1467-7652.2011.00595.x.

Sanou, E. I. R., Gheysen, G., Koulibaly, B., Roelofs, C. and Speelman, S. 2018. Farmers' knowledge and opinions towards Bollgard II® implementation in cotton

production in western Burkina Faso. *New Biotechnology* 42, 33–41. doi:10.1016/j.nbt.2018.01.005.

Sanvido, O., Romeis, J. and Bigler, F. 2007. Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. *Advances in Biochemical Engineering/Biotechnology* 107, 235–78. doi:10.1007/10_2007_048.

Sappington, T. W., Hesler, L. S., Allen, K. C., Luttrell, R. G. and Papiernik, S. K. 2018. Prevalence of sporadic insect pests of seedling corn and factors affecting risk of infestation. *Journal of Integrated Pest Management* 9, 16.

Schellenberger, U., Oral, J., Rosen, B. A., Wei, J. Z., Zhu, G., Xie, W., McDonald, M. J., Cerf, D. C., Diehn, S. H., Crane, V. C., Sandahl, G. A., Zhao, J. Z., Nowatzki, T. M., Sethi, A., Liu, L., Pan, Z., Wang, Y., Lu, A. L., Wu, G. and Liu, L. 2016. A selective insecticidal protein from *Pseudomonas* for controlling rootworms. *Science* 354(6312), 634–7. doi:10.1126/science.aaf6056.

Schiemann, J., Dietz-Pfeilstetter, A., Hartung, F., Kohl, C., Romeis, J. and Sprink, T. 2019. Risk assessment and regulation of plants modified by modern biotechniques: current status and future challenges. *Annual Review of Plant Biology* 70, 699–726. doi:10.1146/annurev-arplant-050718-100025.

Senthil-Kumar, M. and Mysore, K. S. 2011. Caveat of RNAi in plants: the off-target effect. In: Kodama, H. and Komamine, A. (Eds), *RNAi and Plant Gene Function Analysis, Methods in Molecular Biology (Methods and Protocols)* (vol. 744). Humana Press, New York, NY, pp. 13–25.

Shelton, A. M. 2012. Genetically engineered vegetables expressing proteins from *Bacillus thruringiensis* for insect resistance: successes, disappointments, challenges and ways to move forward. *GM Crops and Food* 3(3), 175–83. doi:10.4161/gmcr.19762.

Shelton, A. M. and Badenes-Perez, F. R. 2006. Concept and applications of trap cropping in pest management. *Annual Review of Entomology* 51, 285–308. doi:10.1146/annurev.ento.51.110104.150959.

Shelton, A. M. and Nault, B. A. 2004. Dead-end trap cropping: a technique to improve management of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Crop Protection* 23(6), 497–503. doi:10.1016/j.cropro.2003.10.005.

Shelton, A. M., Tang, J. D., Roush, R. T., Metz, T. D. and Earle, E. D. 2000. Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnology* 18(3), 339–42. doi:10.1038/73804.

Shelton, A. M., Zhao, J. Z. and Roush, R. T. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annual Review of Entomology* 47, 845–81. doi:10.1146/annurev.ento.47.091201.145309.

Shelton, A. M., Hatch, S. L., Zhao, J. Z., Chen, M., Earle, E. D. and Cao, J. 2008a. Suppression of diamondback moth using Bt-transgenic plants as a trap crop. *Crop Protection* 27(3–5), 403–9. doi:10.1016/j.cropro.2007.07.007.

Shelton, A. M., Romeis, J. and Kennedy, G. G. 2008b. IPM and insect-protected transgenic plants: thoughts for the future. In: Romeis, J., Shelton, A. M. and Kennedy, G. G. (Eds), *Integration of Insect-Resistant Genetically Modified Crops with IPM Systems*. Springer, Berlin, Germany, pp. 419–29.

Shelton, A. M., Hossain, M. J., Paranjape, V., Azad, A. K., Rahman, M. L., Khan, A. S. M. M. R., Prodhan, M. Z. H., Rashid, M. A., Majumder, R., Hossain, M. A., Hussain, S. S., Huesing, J. E. and McCandless, L. 2018. Bt eggplant project in Bangladesh: history, present status, and future direction. *Frontiers in Bioengineering and Biotechnology* 6, 106. doi:10.3389/fbioe.2018.00106.

Showers, W. B., Hellmich, R. L., Derrick-Robinson, M. E. and Hendrix III, W. H. 2001. Aggregation and dispersal behavior of marked and released European corn borer (Lepidoptera: Crambidae) adults. *Environmental Entomology* 30(4), 700–10. doi:10.1603/0046-225X-30.4.700.

Shukla, J. N., Kalsi, M., Sethi, A., Narva, K. E., Fishilevich, E., Singh, S., Mogilicherla, K. and Palli, S. R. 2016. Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects. *RNA Biology* 13(7), 656–69. doi:10.1080/15476286.2016.1191728.

Siegfried, B. D. and Hellmich, R. L. 2012. Understanding successful resistance management: the European corn borer and Bt corn in the United States. *GM Crops and Food* 3(3), 184–93. doi:10.4161/gmcr.20715.

Siegfried, B. D., Spencer, T., Crespo, A. L., Storer, N. P., Head, G. P., Owens, E. D. and Guyer, D. 2007. Ten years of Bt resistance monitoring in the European corn borer: what we know, what we don't know, and what we can do better. *American Entomologist* 53(4), 208–14. doi:10.1093/ae/53.4.208.

Singh, S., Kumar, N. R., Maniraj, R., Lakshmikanth, R., Rao, K. Y. S., Muralimohan, N., Arulprakash, T., Karthik, K., Shashibhushan, N. B., Vinutha, T., Pattanayak, D., Dash, P. K., Kumar, P. A. and Sreevathsa, R. 2018. Expression of Cry2Aa, a *Bacillus thuringiensis* insecticidal protein in transgenic pigeon pea confers resistance to gram pod borer, *Helicoverpa armigera*. *Scientific Reports* 8(1), 8820. doi:10.1038/s41598-018-26358-9.

Smith, C. M. 2005. *Plant Resistance to Arthropods: Molecular and Conventional Approaches*. Springer, Dordrecht, The Netherlands.

Smith, J. L., Lepping, M. D., Rule, D. M., Farhan, Y. and Schaafsma, A. W. 2017. Evidence for field-evolved resistance of *Striacosta albicosta* (Lepidoptera: Noctuidae) to Cry1F *Bacillus thuringiensis* protein and transgenic corn hybrids in Ontario, Canada. *Journal of Economic Entomology* 110(5), 2217–28. doi:10.1093/jee/tox228.

Snow, A. A., Pilson, D., Rieseberg, L. H., Paulsen, M. J., Pleskac, N., Reagon, M. R., Wolf, D. E. and Selbo, S. M. 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflower. *Ecological Applications* 13(2), 279–86. doi:10.1890/1051-0761(2003)013[0279:ABTRHA]2.0.CO;2.

Stern, V. M., Smith, R. F., vanden Bosch, R. and Hagen, K. S. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. *Hilgardia* 29(2), 81–101. doi:10.3733/hilg.v29n02p081.

Sternberg, S. H. and Doudna, J. A. 2015. Expanding the biologist's toolkit with CRISPR-Cas9. *Molecular Cell* 58(4), 568–74. doi:10.1016/j.molcel.2015.02.032.

Stewart, C. N., Halfhill, M. D. and Warwick, S. I. 2003. Transgene introgression from genetically modified crops to their wild relatives. *Nature Reviews. Genetics* 4(10), 806–17. doi:10.1038/nrg1179.

Storer, N. P., Babcock, J. M., Schlenz, M., Meade, T., Thompson, G. D., Bing, J. W. and Huckaba, R. M. 2010. Discovery and characterization of field resistance to Bt Maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. *Journal of Economic Entomology* 103(4), 1031–8. doi:10.1603/ec10040.

Tabashnik, B. E. 1989. Managing resistance with multiple pesticide tactics: theory, evidence, and recommendations. *Journal of Economic Entomology* 82(5), 1263–9. doi:10.1093/jee/82.5.1263.

Tabashnik, B. E. 2008. Delaying insect resistance to transgenic crops. *Proceedings of the National Academy of Sciences of the United States of America* 105(49), 19029–30. doi:10.1073/pnas.0810763106.

Tabashnik, B. E. and Carrière, Y. 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. *Nature Biotechnology* 35(10), 926–35. doi:10.1038/nbt.3974.

Tabashnik, B. E. and Croft, B. A. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environmental Entomology* 11(6), 1137–44. doi:10.1093/ee/11.6.1137.

Tabashnik, B. E., Brévault, T. and Carrière, Y. 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology* 31(6), 510–21. doi:10.1038/nbt.2597.

Tabashnik, B. E., Sisterson, M. S., Ellsworth, P. C., Dennehy, T. J., Antilla, L., Liesner, L., Whitlow, M., Staten, R. T., Fabrick, J. A., Unnithan, G. C., Yelich, A. J., Ellers-Kirk, C., Harpold, V. S., Li, X. and Carrière, Y. 2010. Suppressing resistance to Bt cotton with sterile insect releases. *Nature Biotechnology* 28(12), 1304–7. doi:10.1038/nbt.1704.

Tan, J., Levine, S. L., Bachman, P. M., Jensen, P. D., Mueller, G. M., Uffman, J. P., Meng, C., Song, Z., Richards, K. B. and Beevers, M. H. 2016. No impact of *DvSnf7* RNA on honey bee (*Apis mellifera* L.) adults and larvae in dietary feeding tests. *Environmental Toxicology and Chemistry* 35(2), 287–94. doi:10.1002/etc.3075.

Tang, J. D., Collins, H. L., Metz, T. D., Earle, E. D., Zhao, J. Z., Roush, R. T. and Shelton, A. M. 2001. Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. *Journal of Economic Entomology* 94(1), 240–7. doi:10.1603/0022-0493-94.1.240.

Tay, W. T., Soria, M. F., Walsh, T., Thomazoni, D., Silvie, P., Behere, G. T., Anderson, C. and Downes, S. 2013. A brave new world for an old world pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *PLoS ONE* 8(11), e80134. doi:10.1371/journal.pone.0080134.

Terenius, O., Papanicolaou, A., Garbutt, J. S., Eleftherianos, I., Huvenne, H., Kanginakudru, S., Albrechtsen, M., An, C., Aymeric, J. L., Barthel, A., Bebas, P., Bitra, K., Bravo, A., Chevalier, F., Collinge, D. P., Crava, C. M., de Maagd, R. A., Duvic, B., Erlandson, M., Faye, I., Felfoldi, G., Fujiwara, H., Futahashi, R., Gandhe, A. S., Gatehouse, H. S., Gatehouse, L. N., Giebultowicz, J. M., Gomez, I., Grimmelikhuijzen, C. J., Groot, A. T., Hauser, F., Heckel, D. G., Hegedus, D. D., Hrycaj, S., Huang, L., Hull, J. J., Iatrou, K., Iga, M., Kanost, M. R., Kotwica, J., Li, C., Li, J., Liu, J., Lundmark, M., Matsumoto, S., Meyering-Vos, M., Millichap, P. J., Monteiro, A., Mrinal, N., Niimi, T., Nowara, D., Ohnishi, A., Oostra, V., Ozaki, K., Papakonstantinou, M., Popadic, A., Rajam, M. V., Saenko, S., Simpson, R. M., Soberon, M., Strand, M. R., Tomita, S., Toprak, U., Wang, P., Wee, C. W., Whyard, S., Zhang, W., Nagaraju, J., Ffrench-Constant, R. H., Herrero, S., Gordon, K., Swevers, L. and Smagghe, G. 2011. RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. *Journal of Insect Physiology* 57(2), 231–45. doi:10.1016/j.jinsphys.2010.11.006.

Todd, J. H., Ramankutty, P., Barraclough, E. I. and Malone, L. A. 2008. A screening method for prioritizing non-target invertebrates for improved biosafety testing of transgenic crops. *Environmental Biosafety Research* 7(1), 35–56. doi:10.1051/ebr:2008003.

Toews, M. D., Tubbs, R. S., Wann, D. Q. and Sullivan, D. 2010. Thrips (Thysanoptera: Thripidae) mitigation in seedling cotton using strip tillage and winter cover crops. *Pest Management Science* 66(10), 1089–95. doi:10.1002/ps.1983.

Tooker, J. F. and Fleischer, S. J. 2010. First report of western bean cutworm (*Striacosta albicosta*) in Pennsylvania. *Crop Management* 9. doi:10.1094/CM-2010-0616-01-RS.

Trichilo, P. J. and Leigh, T. F. 1986. Predation on spider mite eggs by the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), an opportunist in a cotton agroecosystem. *Environmental Entomology* 15(4), 821–5. doi:10.1093/ee/15.4.821.

USA Environmental Protection Agency (USA-EPA). 1998. Scientific Advisory panel, Subpanel on Bacillus thuringiensis (Bt) Plant-Pesticides and Resistance Management, 9–10 February 1998. Docket Number: OPP 00231.

USA Environmental Protection Agency (USA-EPA). 2007. Pesticide News Story: EPA Approves Natural Refuge for Insect Resistance Management in Bollgard II Cotton. Available at: https://archive.epa.gov/oppfead1/cb/csb_page/updates/2007/web/html/bollgard-cotton.html.

USA Environmentl Protection Agency (USA-EPA). 2017. Pesticide product with new active ingredients (dsRNA transcript comprising a *DvSnf7* inverted repeat sequence derived from *Diabrotica virgifera virgifera* and Bt Cry3Bb1 protein and the genetic material necessary for their production (vector PV-ZMIR10871) in MON 87411 corn (OECD unique identifier MON-87411-9) – FIFRA.

USDA. 2018. Secretary Perdue issues USDA statement on plant breeding innovation. Available at: https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation.

Vaeck, M., Reynaerts, A., Höfte, H., Jansens, S., De Beuckeleer, M., Dean, C., Zabeau, M., Montagu, M. V. and Leemans, J. 1987. Transgenic plants protected from insect attack. *Nature* 328(6125), 33–7. doi:10.1038/328033a0.

Van Rensburg, J. B. J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *South African Journal of Plant and Soil* 24(3), 147–51. doi:10.1080/02571862.2007.10634798.

Vélez, A. M., Jursenski, J., Matz, N., Zhou, X., Wang, H., Ellis, M. and Siegfried, B. D. 2016a. Developing an in vivo toxicity assay for RNAi risk assessment in honey bees, *Apis mellifera* L. *Chemosphere* 144, 1083–90. doi:10.1016/j.chemosphere.2015.09.068.

Vélez, A. M., Vellichirammal, N. N., Jurat-Fuentes, J. L. and Siegfried, B. D. 2016b. Cry1F resistance among lepidopteran pests: a model for improved resistance management? *Current Opinion in Insect Science* 15, 116–24. doi:10.1016/j.cois.2016.04.010.

Vélez, A. M., Khajuria, C., Wang, H., Narva, K. E. and Siegfried, B. D. 2016c. Knockdown of RNA interference pathway genes in western corn rootworms (*Diabrotica virgifera virgifera* Le Conte) demonstrates a possible mechanism of resistance to lethal dsRNA. *PLoS ONE* 11(6), e0157520. doi:10.1371/journal.pone.0157520.

Wach, M., Hellmich, R. L., Layton, R., Romeis, J. and Gadaleta, P. G. 2016. Dynamic role and importance of surrogate species for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. *Transgenic Research* 25(4), 499–505. doi:10.1007/s11248-016-9945-5.

Wan, P., Huang, Y., Tabashnik, B. E., Huang, M. and Wu, K. 2012. The halo effect: suppression of pink bollworm on non-Bt cotton by Bt cotton in China. *PLoS ONE* 7(7), e42004. doi:10.1371/journal.pone.0042004.

Wan, P., Xu, D., Cong, S. B., Jiang, Y. Y., Huang, Y. X., Wang, J. T., Wu, H. H., Wang, L., Wu, K. M., Carriere, Y., Mathias, A., Li, X. C. and Tabashnik, B. E. 2017. Hybridizing transgenic Bt cotton with non-Bt cotton counters resistance in pink bollworm. *Proceedings of the National Academy of Sciences of the United States of America* 114(21), 5413–8. doi:10.1073/pnas.1700396114.

Wang, X., Liu, Q., Meissle, M., Peng, Y., Wu, K., Romeis, J. and Li, Y. 2018. Bt rice could provide ecological resistance to non-target planthoppers. *Plant Biotechnology Journal* 16(10), 1748–55. doi:10.1111/pbi.12911.

Wangila, D. S., Leonard, B. R., Ghimire, M. N., Bai, Y., Zhang, L., Yang, Y., Emfinger, K. D., Head, G. P., Yang, F., Niu, Y. and Huang, F. 2013. Occurrence and larval movement of *Diatraea saccharalis* (Lepidoptera: Crambidae) in seed mixes of non-Bt and Bt pyramid corn. *Pest Management Science* 69(10), 1163–72. doi:10.1002/ps.3484.

Western Farm Press. 2018. Cotton industry celebrates pink bollworm eradication. Available at: https://www.farmprogress.com/cotton/cotton-industry-celebrates-pink-bollworm-eradication.

Whelan, A. I. and Lema, M. A. 2015. Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. *GM Crops and Food* 6(4), 253–65. doi:10.1080/21645698.2015.1114698.

Whitehouse, M. E. A., Wilson, L. J., Davies, A. P., Cross, D., Goldsmith, P., Thompson, A., Harden, S. and Baker, G. 2014. Target and nontarget effects of novel "triple-stacked" Bt-transgenic cotton 1: canopy arthropod communities. *Environmental Entomology* 43(1), 218–41. doi:10.1603/EN13167.

Whyard, S., Singh, A. D. and Wong, S. 2009. Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochemistry and Molecular Biology* 39(11), 824–32. doi:10.1016/j.ibmb.2009.09.007.

Wolfenbarger, L. L., Naranjo, S. E., Lundgren, J. G., Bitzer, R. J. and Watrud, L. S. 2008. Bt crops effects on functional guilds of non-target arthropods: a meta-analysis. *PLoS ONE* 3(5), e2118. doi:10.1371/journal.pone.0002118.

Wu, K. M., Lu, Y. H., Feng, H. Q., Jiang, Y. Y. and Zhao, J. Z. 2008. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. *Science* 321(5896), 1676–8. doi:10.1126/science.1160550.

Wynant, N., Santos, D., Verdonck, R., Spit, J., Van Wielendaele, P. and Vanden Broeck, J. 2014. Identification, functional characterization and phylogenetic analysis of double stranded RNA degrading enzymes present in the gut of the desert locust, *Schistocerca gregaria*. *Insect Biochemistry and Molecular Biology* 46, 1–8. doi:10.1016/j.ibmb.2013.12.008.

Yoon, J. S., Gurusamy, D. and Palli, S. R. 2017. Accumulation of dsRNA in endosomes contributes to inefficient RNA interference in the fall armyworm, *Spodoptera frugiperda*. *Insect Biochemistry and Molecular Biology* 90, 53–60. doi:10.1016/j.ibmb.2017.09.011.

Zhang, J., Khan, S. A., Hasse, C., Ruf, S., Heckel, D. G. and Bock, R. 2015. Pest control. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science* 347(6225), 991–4. doi:10.1126/science.1261680.

Zhang, J., Khan, S. A., Heckel, D. G. and Bock, R. 2017. Next-generation insect-resistant plants: RNAi-mediated crop protection. *Trends in Biotechnology* 35(9), 871–82. doi:10.1016/j.tibtech.2017.04.009.

Zhao, J. Z., Cao, J., Collins, H. C., Bates, S. L., Roush, R. T., Earle, E. D. and Shelton, A. M. 2005. Concurrent use of transgenic plants expressing a single and two Bt genes speeds insect adaptation to pyramided plants. *Proceedings of the National Academy of Sciences of the United States of America* 102, 8426–30.

Zhao, J. Z., Cao, J., Li, Y., Collins, H. L., Roush, R. T., Earle, E. D. and Shelton, A. M. 2003. Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nature Biotechnology* 21(12), 1493–7. doi:10.1038/nbt907.

Zhu, Y. C., Yao, J. and Luttrell, R. 2016. Identification of genes potentially responsible for extra-oral digestion and overcoming plant defense from salivary glands of the tarnished plant bug (Hemiptera: Miridae) using cDNA sequencing. *Journal of Insect Science* 16(1), 60. doi:10.1093/jisesa/iew041.

Zukoff, S. N., Ostlie, K. R., Potter, B., Meihls, L. N., Zukoff, A. L., French, L., Ellersieck, M. R., Wade French, B. and Hibbard, B. E. 2016. Multiple assays indicate varying levels of cross resistance in Cry3Bb1-selected field populations of the western corn rootworm to mCry3A, eCry3. 1Ab, and Cry34/35Ab1. *Journal of Economic Entomology* 109, 1387–98. doi:10.1093/jee/tow073.

Published by Burleigh Dodds Science Publishing Limited, 2020.