The role and use of genetically engineered insect-resistant crops in Integrated Pest Management systems

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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
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.

**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.
Use of genetically engineered insect-resistant crops in IPM systems

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 hectares of Bt maize, cotton and potato, and a total of 1.7 million hectares 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

<table>
<thead>
<tr>
<th>IRGE proteins/dsRNA</th>
<th>Bt Maize</th>
<th>Bt Cotton</th>
<th>Bt Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td>% adoption (first year)</td>
<td>Production (Ha × 1000)</td>
<td>% adoption (first year)</td>
<td>Production (Ha × 1000)</td>
</tr>
<tr>
<td><strong>North/Central America</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>1780</td>
<td>86 (1997)</td>
<td>Cry1Ab, Cry1F, Cry2Ab, Cry1A.105, ECry3.1Ab, Cry3/35Ab, Cry3Bb, mCry3A, Vip3A</td>
</tr>
<tr>
<td>Honduras</td>
<td>44</td>
<td>66 (2001)</td>
<td>Cry1Ab, Cry3Bb, Cry2Ab, Cry1A.105, Cry1F</td>
</tr>
<tr>
<td>Mexico</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>36790</td>
<td>80 (1996)</td>
<td>Cry1Ab, Cry1F, Cry2Ab, Cry1A.105, ECry3.1Ab, Cry3/35Ab, Cry3Bb, mCry3A, Vip3A, DvSnf7</td>
</tr>
<tr>
<td><strong>South America</strong></td>
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<tr>
<td>Argentina</td>
<td>5400</td>
<td>87 (1998)</td>
<td>Cry1Ab, Cry1F, Cry2Ab, Cry1A.105, ECry3.1Ab, Cry3Bb, mCry3A, Vip3A</td>
</tr>
<tr>
<td>Brazil</td>
<td>17550</td>
<td>85 (2008)</td>
<td>Cry1Ab, Cry1F, Cry2Ab, Cry1A.105, ECry3.1Ab, Cry3/35Ab, Cry3Bb, mCry3A, Vip3A</td>
</tr>
</tbody>
</table>

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* Production data and adoption rates are from the first year of commercialization for each region.
* ** indicates the most commonly adopted IRGE proteins in each region.
<table>
<thead>
<tr>
<th>Country</th>
<th>Production (Ha × 1000)</th>
<th>% Adoption (first year)</th>
<th>IRGE proteins/dsRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombia</td>
<td>375</td>
<td>20 (2007)</td>
<td>Cry1Ab, Cry1F, Vip3A, Cry1A.105b, Cry34/35Abd, Cry3Bb, mCry3A, Vip3A</td>
</tr>
<tr>
<td>Paraguay</td>
<td>640</td>
<td>40 (2012)</td>
<td>Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, Cry3Bb, Vip3A</td>
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<tr>
<td>Uruguay</td>
<td>50</td>
<td>96 (2003)</td>
<td>Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, Vip3A</td>
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<td><strong>North/Central America</strong></td>
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<td><strong>Canada</strong></td>
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<td>1780, 86 (1997) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, ECry3.1Abc, Cry34/35Abd, Cry3Bb, mCry3A, Vip3A</td>
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<td>44, 66 (2001) Cry1Ab, Cry3Bb, Cry2Ab, Cry1A.105, Cry1F</td>
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<td><strong>Mexico</strong></td>
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<td>210, 96 (1996) Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Vip3A</td>
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<td><strong>USA</strong></td>
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<td>36790, 80 (1996) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, ECry3.1Abc, Cry34/35Abd, Cry3Bb, mCry3A, Vip3A</td>
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<td><strong>4492, 85 (1996)</strong> Cry1Ac, Cry1Ab, Cry1F, Cry2Ae, Vip3A</td>
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<td><strong>South America</strong></td>
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<td><strong>Argentina</strong></td>
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<td>5400, 87 (1998) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, Cry3Bb, mCry3A</td>
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<td><strong>Brazil</strong></td>
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<td>17550, 85 (2008) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, ECry3.1Abc, Cry34/35Abd, Cry3Bb, mCry3A, Vip3A</td>
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<td><strong>Colombia</strong></td>
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<td>375, 20 (2007) Cry1Ab, Cry1F, Vip3A</td>
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<td><strong>Paraguay</strong></td>
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<td>640, 40 (2012) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, Cry3Bb, Vip3A</td>
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<td><strong>Uruguay</strong></td>
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<td>50, 96 (2003) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b, Vip3A</td>
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<td><strong>Europe</strong></td>
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<td><strong>Portugal</strong></td>
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<td></td>
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<td>116, 6 (1999) Cry1Ab</td>
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<td></td>
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<td></td>
<td><strong>Spain</strong></td>
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<td></td>
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<td>345, 6 (1999) Cry1Ab</td>
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<td></td>
<td></td>
<td></td>
<td><strong>Africa</strong></td>
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<td><strong>South Africa</strong></td>
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<td>2300, 70 (1997) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b</td>
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<td><strong>Sudan</strong></td>
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<td></td>
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<td>120, 98 (2012) Cry1Ac</td>
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<td><strong>Asia</strong></td>
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<td></td>
<td><strong>Bangladesh</strong></td>
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<td></td>
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<td></td>
<td>3400, 95 (1997) Cry1A, Cry1Ac, Cry1Ab-Ach, CpTI</td>
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<td></td>
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<td></td>
<td><strong>China</strong></td>
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<td></td>
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<td>12400, 93 (2002) Cry1Ac, Cry1Ab-Ach, Cry1C, Cry2Ab</td>
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<td></td>
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<td></td>
<td><strong>India</strong></td>
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<td></td>
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<td></td>
<td>249, 92 (2006) Cry1Ac</td>
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<td><strong>Pakistan</strong></td>
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<td></td>
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<td></td>
<td>1229, N/A (2018) Cry1Ab, Cry1F, Cry2Ab, Cry1A.105b</td>
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<td></td>
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<td></td>
<td><strong>Philippines</strong></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1378, 44 (2003) Cry1Ab, Cry1Fa2, Cry2Ab, Cry1A.105</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th></th>
<th>Bt Maize</th>
<th>Bt Cotton</th>
<th>Bt Soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Production (Ha × 1000)</td>
<td>% adoptiona</td>
<td>IRGE proteins/dsRNA</td>
</tr>
<tr>
<td>Vietnam</td>
<td>1151</td>
<td>4 (2015)</td>
<td>Cry1Ab, Cry2Ab, Cry1A.105b</td>
</tr>
<tr>
<td>Australasia</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Australia</td>
<td>530</td>
<td>96 (1996)</td>
<td>Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry2Ae, Vip3A</td>
</tr>
</tbody>
</table>

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%.

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 hectares overall with over 101 million hectares 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 hectares 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
### Table 2 Summary of target pests of Bt crops in adopting countries

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

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<th>Species</th>
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<th>Bt Maize</th>
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Compiled from various sources including: 
- Hellmich et al. (2008) 
- Naranjo et al. (2008) 
- Blanco et al. (2016) 
- Rocha-Munive et al. (2018), personal communications.
<table>
<thead>
<tr>
<th>Species Family</th>
<th>Common name</th>
<th>Bt Species</th>
<th>Use in IPM Systems</th>
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<td>Noctuidae</td>
<td>Soybean looper</td>
<td>Pseudoplusia includens</td>
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<td>Noctuidae</td>
<td>Mediterranean corn borer</td>
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<td>Noctuidae</td>
<td>Beet armyworm</td>
<td>Spodoptera exigua</td>
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<tr>
<td>Noctuidae</td>
<td>Armyworm</td>
<td>Spodoptera frugiperda</td>
<td>1, 2, 4, 6, 7, 9, 10, 11, 12, 13, 14, 17, 19, 21</td>
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<td>Western bean cutworm</td>
<td>Striacosta albicosta</td>
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<td>Pyralidae</td>
<td>Cotton leaf roller</td>
<td>Sylepta derogata</td>
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<td>Noctuidae</td>
<td>Cabbage looper</td>
<td>Trichoplusia ni</td>
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<td>Chrysomelidae</td>
<td>Corn rootworms</td>
<td>Diabrotica spp.</td>
<td>1, 4, 5, 8, 21</td>
</tr>
</tbody>
</table>

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
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 noted above (see 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 multinational 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 shown, 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* 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).
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 $2,151/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 that pest
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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 (see below).

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).
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 (see below). 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.
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. virgifera virgifera* and *D. barberi* in the USA (Gassmann et al., 2011).
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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.

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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 chloraphis, 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. chloraphis 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 *Diabrotica* 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).
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. septempuctata* 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 \textit{CYP71A1} gene that eliminates the production of serotonin, allowing for higher salicylic acid levels, making the plant resistant to the brown planthopper, \textit{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 European Union, 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 hectares 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:


Use of genetically engineered insect-resistant crops in IPM systems


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-rnaibased-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


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


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:


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


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


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...


Published by Burleigh Dodds Science Publishing Limited, 2019.


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


Assessment of risk of insect-resistant transgenic crops to nontarget arthropods.


USA Environmental Protection Agency (USA-EPA). 2017. Pesticide product with new active ingredients (dsRNA transcript comprising a \textit{DvSnf7} inverted repeat sequence derived from \textit{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.


Use of genetically engineered insect-resistant crops in IPM systems


