

BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Biopesticides for sustainable agriculture

Edited by Professor Nick Birch, formerly The James Hutton Institute, UK

Professor Travis Glare, Lincoln University, New Zealand

E-CHAPTER FROM THIS BOOK



Advances in the use of Bt genes in insect-resistant crops

Salvatore Arpaia, ENEA Research Centre Trisaia-Rotondella (MT), Italy

- 1 Introduction
- 2 *Bacillus thuringiensis* (Bt) insecticidal toxins
- 3 Incorporating Bt-expressing GM plants in integrated pest management (IPM)
- 4 Insecticide resistance
- 5 Conclusion and future trends
- 6 Acknowledgements
- 7 Where to look for further information
- 8 References

1 Introduction

Genetically modified (GM) crops have often been referred to as one of the agricultural innovations with the fastest adoption rate by farmers worldwide. Indeed in 2018, about 17 million farmers in 26 countries planted 191.7 million ha with GM crops, an increase of 1% or 1.9 million hectares from 2017 (ISAAA, 2018). This fast pace of adoption has led in the last two decades to the unchallenged primacy of GM varieties on worldwide production of cotton (80% of the invested surface) and soybean (77%), while about one-third of cultivated maize and canola is currently GM. This large adoption of GM varieties, especially in the Americas, has created several problems in international trading (Parisi et al., 2016), since there are major importers of staple food and feed, for example the European Union, that still have a precautionary attitude towards GM products and, therefore, require imported GM food and feed to be specifically authorized and their processed products on the market, labelled. The negative attitude of the EU towards GM crops is even more obvious in case of cultivation and as a consequence the current practice of growing GM crops in Europe is limited to the Iberian peninsula where the 'historical' GM event maize MON810 resistant to the corn borers *Ostrinia nubilalis* and *Sesamia nonagrioides* was grown on ca 121 000 ha in 2018. The gene conferring resistance to Lepidoptera

in the MON810 maize is a *cry1Ab* gene derived by the soil bacterium *Bacillus thuringiensis* (Bt) and which is selectively toxic to Lepidoptera. This gene can be considered the founder of a series of synthetically reproduced traits mimicking the natural array of genes codifying for insecticidal toxins present in the genome of different strains of *B. thuringiensis*, each characterized by a rather narrow range of target organisms (Palma et al., 2014). These genes still constitute the large majority of genes expressed by GM crops resistant to insects, alone or in combination with other traits expressed in the so-called stacked events, which represent to date the majority of GM crops used for cultivation. Even though insect-resistant traits are considered as belonging to the first generation of GM crops, the role of Bt-derived genes is still very relevant in the currently grown biotech crops worldwide. In fact, the only available commercial event expressing a gene to induce resistance to insects not derived from Bt is the MON87411 maize developed by Monsanto (authorized for cultivation by the US Environmental Protection Agency in 2015) which, in addition to a Cry3Bb delta-endotoxin, encodes for a double-stranded RNA transcript containing a 240 bp fragment of the *Diabrotica virgifera virgifera* *Snf7* gene to confer resistance to this beetle. This maize event has been then crossed to several other GM events and it is now included in several stacked GM maize hybrids.

2 *Bacillus thuringiensis* (Bt) insecticidal toxins

The list of Bt-derived genes currently contains 858 entries (<http://www.btnomenclature.info/>; accessed 5 July 2019), although only less than a dozen are currently expressed in commercially available GM crops (<http://www.isa.aa.org/gmapprovaldatabase/geneslist/default.asp>; accessed on 5 July 2019). The majority of the proteins produced by these genes are δ -endotoxin formed during the sporulating phase of the bacterium life cycle and can be grouped in two families: cry and cyt. The different cry toxins are specifically noxious to insects of the orders Lepidoptera, Coleoptera, Hymenoptera and Diptera. In contrast, cyt toxins are mostly found in *B. thuringiensis* strains active against Diptera, although a few exceptions of coleopteran active strains containing Cyt proteins have been documented (Bravo and Soberon, 2010). In addition, strains of *B. thuringiensis* active against insect orders of Hymenoptera, Homoptera, Orthoptera and Mallophaga and to other invertebrates such as nematodes and mites have also been identified (<http://www.btnomenclature.info/>; accessed on 5 July 2019). Another class of toxins is synthesized by this bacterium during the vegetative phase of the life cycle (Vip toxins) and their use has been exploited recently for obtaining GM plants resistant to insects (e.g. MIR162 maize, Bollgard III cotton). Bt toxins are known to be quite specific, though some cross-order activity has been reported (Van Frankenhuyzen, 2013). Bt-based microbial pesticides have been used for the biological control

of insect pests for more than 80 years, and are currently included in over 100 commercial products (Glare and O'Callaghan, 2000). Bt microbial pesticides represent one of the main pillars of pest management for organic farmers, since their remarkable selectivity, their short persistence in the environment and their harmlessness for mammals make this class of pesticides quite innocuous for non-target organisms.

Due to all the positive characteristics of these molecules, it is not surprising that Bt genes have been one of the first practical applications of genetic engineering in agriculture, when the successful transformation of crop plants was achieved in the 1980s. The efficiency of these GM plants in tolerating the attack of insect pests spurred seed companies to produce new varieties which reached the market in the late 1990s upon the first approval by the US EPA of the StarLink maize expressing the toxin Cry9C as a plant incorporating pesticide (https://archive.epa.gov/pesticides/biopesticides/web/html/starlink_corn.html; accessed 23 July 2019).

There are some differences in the functioning of Bt toxins between microbial pesticides and GM plant-expressing Bt toxins (Arpaia et al., 2017) that trigger specifically targeted risk assessment in the two different cases. Some practical implications for the different action of Bt toxins in the two different delivery methods are summarized in Table 1.

The mechanism of action of cry toxins originating from *B. thuringiensis* occurring in the environment requires the cleavage of the protoxin (the natural form of cry proteins) upon ingestion, under favourable pH conditions in the midgut of target insects. This activation is not necessary in GM plants, since the

Table 1 Practical differences between the characteristics of Bt toxins in microbial pesticides and in GM plants due to the different delivery method

Microbial pesticides	GM plants
Release controlled by applicator: timing, point location, and so on	Under the regulation of constitutive promoters (e.g. 35s) toxin is released continuously and in all plant parts, independently from the infestation level
Product is evenly distributed over whole plants	There is a different tissue-specific toxin production (usually highest in leaf tissues), which also varies along the cropping season
Degradation begins immediately after application	Degradation only occurs when plant-expressed compounds are released in the environment (i.e. via root exudates and cultivation residues)
Possible dispersal by spray drift at the time of application	Possible dispersal by plant tissue transport during (e.g. with pollen) and after (e.g. via plant residues) the cropping season
Limited interactions with plant or other naturally occurring metabolites in the environment	Possible interactions with other plant produced compounds

newly expressed genes codify for the shorter form, already active, toxin. The way the toxic effect can be enhanced in microbial pesticides or in plants is also sensibly different. Laboratory studies have demonstrated that when grown in specific media, *B. thuringiensis* colonies produce higher amount of chitinases, which act synergistically with cry toxins (Vu et al., 2009); the toxic effect under these conditions can be 1.5–2 times higher as demonstrated in laboratory experiments targeting the larvae of *Aedes aegypti* (Ramirez-Suero et al., 2011).

The range of organisms and the modality of exposure to Bt toxins induced by the two delivery methods can also be quite different. For example, aerial transportation of Bt-pollen can expose non-target caterpillar larvae living on wild plants at some distance from the GM-cultivated areas (Perry et al., 2010; Holst et al., 2013), while this pathway seems more remote for microbial pesticides. The use of microbial pesticides against corn borers is usually recommended before maize flowering, thus pollen feeding organisms are normally not exposed to the toxins in this form. On the contrary, cry toxins are commonly present in pollen of GM plants, although they are less abundant compared to other tissues, therefore exposure of flower visiting organisms is still possible. Another difference, with potentially relevant biosafety implications, is due to the exposure pathway, which is expected to be largely bitrophic when Bt toxins are distributed on plant canopy with sprays since they will have a limited persistence. Possibilities of exposure for non-target organisms can instead follow several direct and indirect paths when these toxins are expressed in plants (Andow et al., 2006).

3 Incorporating Bt-expressing GM plants in integrated pest management (IPM)

The report 'Agriculture at a Crossroads' (IAASTD, 2009) indicated that agricultural knowledge, science and technology are fundamental to meeting the Millennium development goals. The biggest challenge of modern agriculture is ensuring enough food and feed production for a fast-growing world population while safeguarding economic, social and environmental sustainability of cropping practices. This scenario is further complicated by the evidence that climate change is altering the distribution, incidence and intensity of animal and plant pests and diseases (Taylor et al., 2018; Deutsch et al., 2018). GM crops represent a valuable new tool to support these actions and their new traits have the potential to improve crop production especially under heavy pest, disease and weed pressure (Gomez-Barbero et al., 2008; Areal et al., 2013). In particular, insecticidal and virus-resistant crops could help to keep pests and diseases in check, to lower the chemical pesticide load in the environment, to support complementary IPM tactics such as the active use of biocontrol agents and, therefore, to increase reliance on natural pest

control. However, the reduction of pesticide use alone does not guarantee that natural pest control in the presence of GM crops is maintained or enhanced, and the need to assess the role that GM plants might play in different IPM systems was highlighted by Menozzi et al. (2017). In order to verify how likely Bt crops might fit well in IPM programmes, we need to consider if these varieties can be successfully managed in the context of the main principles of IPM (i.e. monitoring population density, establishing action thresholds, selection of the most appropriate method(s) of pest control). Particularly, it is paramount to investigate if the ecological functions provided by the organisms living in an agro-ecosystem (e.g. pollinators, natural enemies, detritivores) are maintained to the same extent as in 'traditional' non-GM-based agro-ecosystems.

GM crops resistant to insects, as well as traditionally bred insect-resistant plants, work independently of any action threshold established for target pests, since they produce a kind of preventive defense even before insects attack cropped plants. Monitoring population levels of the target insects will, therefore, not be necessary when GM varieties are being cultivated, though monitoring for possible onset of resistance is regularly conducted by the applicants. On the other hand, the monitoring of other insect pests and natural enemies (which remains a necessary activity in any IPM programme) is not hampered in any way by the presence of GM plants. Therefore, the main issue to be considered is the role of this germplasm in combination with other means of pest control in the agro-ecosystem, including the use of chemical pesticides.

A meta-analysis conducted by Klümper and Qaim (2014) has revealed that on average GM technology adoption has reduced chemical pesticide use by 37%, and increased crop yields by 22%. The extent of these effects vary especially according to the modified crop traits and geographic regions, both yield gains and pesticide reductions are larger for insect-resistant crops than for other foreign traits expressed in GM plants. Not surprisingly, cotton – a crop on which the use of insecticides is quite heavy – scores as the agro-ecosystem where reduction of pesticide load is highest when GM varieties are adopted. A reduced use of insecticides will obviously favour the activity of natural enemies, especially generalist predators that can find secondary preys among non-target herbivores in the agro-ecosystem and maintain their valuable contribution to ecosystem services (Arpaia et al., 2007). Even in some cases where particular species showed a reduced abundance in Bt stands compared to the untransformed control plants, the redundancy in the agro-ecosystem allowed other species of the same guild to concur in maintaining the level of natural pest control, therefore guaranteeing the ecological service (Naranjo, 2005). However, ecological relationships regulating food webs in the field are most commonly not linear ones and the possible issue of secondary pests needs to be carefully monitored (Wang et al., 2008; Hagenbucher et al., 2013; Catarino et al., 2016).

Several meta-analyses have investigated comparatively the impact of different pest control approaches, including Bt-expressing GM crops, on natural enemies (e.g. Marvier et al., 2007; Naranjo, 2009). The general outcomes of these studies indicate that non-target invertebrates are more abundant in Bt cotton and Bt maize fields compared with non-transgenic fields managed with insecticides. However, in comparison with insecticide-free control fields, certain non-target taxa are less abundant in Bt fields. The organisms which experience a negative effect when reared on Bt-based diets are mostly specialized parasitoids linked to target pests, which suffer most likely for the poor quality of available hosts (e.g. Ramirez-Romero et al., 2007; Walker et al., 2007; Naranjo, 2009). This is not surprising, considering the strict link with the host species that this group of natural enemies requires for successfully completing their life cycle. Even though direct toxic effects are not expected to occur, the reduced abundance or quality of hosts in Bt fields may hamper the efficacy of parasitoid-based natural biological control in some agro-ecosystems.

The concepts of IPM are usually successfully realized over an extended spatial and temporal scale. Approaching the problem from an agro-ecosystems angle is best to design less vulnerable cropping systems, moving towards longer-term prevention and suppression of key pests in the system over multiple fields, farms and seasons (Barzman et al., 2015). To analyse the effects of Bt maize adoption on European corn borer (ECB), *O. nubilalis* populations, Hutchison et al. (2010) estimated the annual per capita growth rates in maize-growing areas in the Midwest of the United States where maize constitutes the most relevant cropping system. Possible confounding factors, such as previous population levels in different regions, and density-dependent mortality were accounted for in the model when time series were analysed. Reduction of populations of the ECB ranged from 23% to 73% when historical data from the pre-Bt era (i.e. 1963–1996) were compared with data collected from 1996 to 2009. This clearly indicates that benefits from the adoption of Bt maize were extended to all maize-growing farmers, including non-adopters of GM maize. A more recent paper (Dively et al., 2018) addressed the issue of area-wide pest management at a regional scale, considering also mixed cropping systems where several crops share relevant surfaces in the area. The case study concerns the Mid-Atlantic United States where crops such as peppers, green beans and sweet corn are cultivated on large acreages and are all susceptible to the attack of ECB and *Helicoverpa zea*. Using data spanning from 1976 to 2016 through a regional network of blacklight traps in three states, Dively et al. (2018) demonstrated that vegetable growers benefit via decreased crop damage and insecticide applications due to the large decline of populations of the two pests. Their model also includes new features and it is able to account for different concurring biological phenomena linked to climate change. The number of

recommended insecticide sprays to control ECB in peppers, green beans and sweet corn, and *H. zea* in sweet corn, significantly decreased as a function of average Bt corn adoption rates. Time series regarding field damage reports for ECB in the area are available, and the comparisons from the pre-Bt maize introduction and the current surveys indicate their decline in pepper reached 78%, and 70% in sweet corn.

Positive examples of area-wide pest management achieved with the contribution of Bt crops are not limited to maize. For instance, adoption of Bt cotton in China suppressed pink bollworm *Pectinophora gossypiella* and cotton bollworm *Helicoverpa armigera* populations in non-Bt cotton as well as in other crops (Wu et al., 2008). Regional reductions in the pink bollworm, which is fairly specialized to cotton, have also been reported from the use of Bt cotton in the United States (Carrière et al., 2003).

Another positive aspect regarding the action of natural enemies and GM crops was elucidated by using population models which indicated that natural enemies can support the effectiveness of GM cultivations, by delaying the possible adaptation of target pests to Bt toxins (Gould 1994; Arpaia et al., 1997). The possible effect is linked to the behaviour of the natural enemies occurring in the agro-ecosystem, namely if natural enemies prey on the pest in a density-dependent fashion, this could lead to faster adaptation by the pest to the toxin, since the difference in fitness between resistant and susceptible individuals will be further enhanced in favour of the former. In contrast, inverse density-dependent predation is expected to slow the rate of adaptation by imposing more fitness costs to the few expected resistant survivors.

Theoretical considerations and some experimental evidence suggest that Bt-expressing GM crops might profitably be included in an IPM framework (Lundgren et al., 2009; Romeis et al., 2019); however, Hokkanen (2015) warned that in reality these benefits seldom seem to be achieved, as GM crops are still perceived by the growers as a stand-alone technology without any real attempts to integrate them as a component in IPM. For example, not conducting field sampling for secondary pests and natural enemies may cause a failure in estimating predator-prey relationships in the agro-ecosystem with the consequence that intense insecticide use might become necessary to control secondary pests, which are not sensitive to Bt toxins.

4 Insecticide resistance

Insecticide resistance is a well-known phenomenon regarding almost 600 insect species and more than 300 active ingredients (Sparks and Nauen, 2015). Population models indicate that the higher the selection pressure against a trait (i.e. susceptibility to a chemical compound), the quicker the spread of a resistant allele will be in the population. This principle applies equally to

exposed insect populations when a Bt toxin is expressed in a GM plant in the field. The possible build-up of resistance to Bt toxins can be mainly considered as an agronomic and commercial problem; however, the main regulatory agencies (e.g. EPA in the USA, EFSA in the EU) demand that developers of GM varieties also monitor in a proactive manner the risk of insurgence of resistance in commercial cultivations, due to its possible environmental consequences (e.g. spread of resistant strains that could damage one or several crop species and then require additional use of pesticides). That's why insecticide resistance management measures have been deemed necessary from the very beginning of commercialization of Bt crops.

Current insect resistance management strategies rely on the combined use of GM events with high expression levels of cry toxins, able to kill all susceptible and heterozygote individuals and implementing refuge areas (i.e. non-GM stands) close to GM cultivations to favour mating between susceptible and resistant individuals to further dilute resistance allele frequency in the population. Indeed, to date Bt crops have remained effective against most pest populations after many years of commercial cultivation (Castañera et al., 2016; Tabashnik and Carrière, 2017); however, cases of field-evolved resistance with consequent increased crop damage to GM events have been reported in some populations of major target insect pests (Luttrell et al., 2004; Van Rensburg, 2007; Tabashnik et al., 2008, 2009; Bagla, 2010; Storer et al., 2010; Dhurua and Gujar, 2011; Gassmann et al., 2011; Kruger et al., 2012). The main cause of these failures has been the non-compliance to the high expression-refuge strategy, but lately a few newly occurring cases of previously susceptible pests damaging Bt crops also when compliance to the strategy was ensured (US EPA, FIFRA Scientific Advisory Panel, 2018), reopened the debate on how to improve monitoring methods and continue to develop strategies on how to preserve resistant germplasm over time.

Mathematical models have been largely used by regulatory authorities to establish a system of early warning to prevent the onset of resistance by the target pests. Though with some differences all models concur in indicating that increase of allele frequency over time is a natural, unavoidable, phenomenon that in the long run will lead to the onset of resistance. To proactively prevent the insurgence of resistance, common approaches suggest the possible introduction of novel toxins against the target pest either replacing those originally used or by pyramiding more toxins in a single stacked GM event. The latter tactic is now quite popular among biotech companies who have on the market GM events expressing several insect resistance and herbicide-tolerant genes in the same varieties (<http://www.isaaa.org/gmapprovaldatabase>). However, the strong asynchrony in the approval of GM cultivations between countries renders this approach not currently feasible in some areas, especially in Europe.

5 Conclusion and future trends

The possible environmental impacts of GM crops and the way to assess the relative risks have been quite extensively debated (Arpaia, 2010). A recent survey indicated that biodiversity preservation has been the predominant sub-topic (58%) in the projects studying implications of GM crop cultivations in Europe (Menozzi et al., 2017). The effect on non-target organisms was specifically analysed in 25% of those projects. In spite of the large body of data accumulating on this subject, the dichotomy regarding the approval of commercial cultivations of GM crops between geographical regions remains quite obvious. In order to support the production of scientifically sound data during environmental risk assessment (ERA), the European Food Safety Authority (EFSA) has renewed its guidelines for environmental risk assessment of GM plants (EFSA, 2010), making clear the need for sound ecological data on which ERA of GM crops should be rooted. The practical application of such guidelines has been implemented in a EU-funded research project (Arpaia et al., 2014) which has collected relevant baseline ecological data regarding active food webs in maize and potato agro-ecosystems and the surrounding landscapes in different European areas. Such datasets represent an important benchmark against which ERA for introduction of new GM germplasm could be realistically built. Surveys conducted in commercial fields in the framework of this project also triggered the recommendation that region-specific non-target indicator species should be included in ERA protocols to better account for the relevance of the receiving environment where Bt crops are to be released (van Capelle et al., 2016). The rationale on which the ERA is conducted by several regulatory agencies involve the monitoring of cultivated land (on-crop area), but sometimes of the surrounding landscape (off-crop area) and their interactions in terms of ecological functionality. This type of monitoring should include possibilities to study combined effects of different stressors on selected focal species and the extent of ecosystem services they provide (e.g. pollination, natural pest control). In the context of IPM, natural enemies represent obviously the most relevant category of non-target organisms to be studied; however, developing pest control systems, which are compatible with the normal activities of other functional guilds and which are very relevant for agriculture (e.g. pollinators, detritivores), should be a primary goal for the sustainability of modern agriculture.

The maintenance of ecosystem services is a declared principle of ERA in Europe, not only in the field of GM organisms (EFSA, 2016). However, analysing ecosystem services is not a simple exercise since it involves the measurement of the contribution of service-providing species, the scale at which they operate and determine the possible effects of stressors on these species (Kremen and Ostfeld, 2005).

GM crops expressing *B. thuringiensis* toxins represent a considerable part of the first generation of GM crops, characterized by a single newly expressed trait (either insect resistance or herbicide tolerance). After more than two decades of cultivation of GM crops worldwide, the current trend is represented by stacked events, containing more than one transgene. Additionally, some new characteristics are being introduced by seed companies into elite varieties (e.g. drought resistance, improved quality, etc.). Bt genes have been so far almost the only source of resistance to insects engineered into plant varieties which were brought to the market by industry; approved GM events contain only a handful of them: *Cry1Ab*, *Cry1Ac*, *Cry1F*, *Cry3Bb*. In spite of the progress made in discovering new Bt toxins in the last few years (Adang et al., 2014), only more recently new Bt-derived genes have been exploited for the production of Cry34/Cry35 binary toxins which act in combination against Coleoptera, or vip3A active against a broad range of lepidopteran pests and they have been expressed in a few GM commercialized events.

Even within this limited group of insecticidal toxins, some aspects of their mode of action are not completely understood (Hernández-Martínez et al., 2017). When some of these new traits were expressed in GM events aimed at commercial cultivation, the European Food Safety Authority argued that to resolve the remaining scientific uncertainty on the potential toxicity of the binary Cry34Ab1/Cry35Ab1 proteins on some predators, for example ladybirds, additional laboratory studies had to be performed prior to authorization (EFSA, 2013). A potential drastic change of this scenario may occur when more insect-resistant GM events expressing dsRNA will become available (Christiaens et al., 2018), if the request for authorization of these events will increase, therefore the possibilities of using genetically engineered plants for insect pest control will become less dependent on Bt toxins.

The possible onset of resistance to insecticidal toxins expressed in plants might also constitute a driving force towards searching new traits or combining more than one trait in a single variety to conduct a successful 'arms race' against new insect populations locally. These new scenarios will also trigger priority to new research questions in order to assess the possible effects due to the interactions of more than one toxin used in combination, on non-target organisms. The possible effects of multiple stressors, especially in valued species active as service providers in agro-ecosystems, is certainly a topic which has received limited attention so far. Combinatorial effects of Cry toxins with other proteins or chemicals are actually widely recognized and reported in the literature (Hilbeck and Otto, 2015). In Europe, ERA requirement for stacked GM varieties could be less extensive than the assessment of the parental GM events if the latter have already been considered to be safe, additional environmental studies are deemed relevant on a case-by-case basis (De Schrijver et al., 2007). Especially stacked events combining more than one insect-resistant toxin

should be risk assessed on non-target organisms, since potential for additive interactions between different Bt toxin classes exists (Pérez et al., 2005; Sharma et al., 2010).

A final consideration should be given to the potentially different environmental impacts in different receiving environments. Non-target organisms are known to show quite a different sensitivity to Bt toxins even within the same insect order (Van Frenkenhuizen, 2009). Review of laboratory studies have shown that experiments aimed at evaluating activity of different cry toxins on natural enemies are still performed on a relatively small number of surrogate species mostly in America and Europe and many relevant taxonomic groups which are involved in providing important ecological services are poorly represented (Lövei et al., 2009). In the last few years, however, efforts are ongoing in collecting new information in countries where only recently GM crops have been authorized. Environmental risk assessment needs, at a certain stage of the process, to collect information on regional ecologically relevant focal species that have been judged to have an important role in providing ecological services in a particular agro-ecosystem. Conducting monitoring to develop enough information for a thorough estimate of ecological services may represent a massive effort, which goes well beyond the scope and possibilities of an ERA specifically aimed at the approval of a new variety or a new insecticide to be used in agriculture. Local resources and expertise should then be involved, also after the approval of varieties resistant to insects, in order to confirm that biotech products are exploiting their potential and are managed in a way to support the sustainability of agriculture.

6 Acknowledgements

I am indebted to Nicholas A. E. Birch for his useful comments on an earlier version of this manuscript.

7 Where to look for further information

Regulatory agencies provide information on the legal framework, the current status of GMO authorized for commercialization and details about the opinions given on each dossier for risk assessment of GM crops in their territories.

- United States Environmental Protection Agency – <https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/epas-regulation-biotechnology-use-pest-management>.
- European Food Safety Authority – <http://www.efsa.europa.eu/en/topics/topic/gmo>.

The ICGEB has a web-based collection of reviews on different aspects of biosafety of GM organisms:

- International Centre for genetic Engineering and Biotechnology, Collection of Biosafety Reviews – <https://www.icgeb.org/biosafety-publications-collections/>.

The ISAAA web site publishes annual reports concerning worldwide use of GM crops:

- International Service for the Acquisition of Agri-biotech Applications – www.isaaa.org.

The following website is a good source of teaching materials for students wishing to improve their knowledge on the scientific aspects and the socio-economic implications of growing GM crops:

- University of Reading, teaching module on GMOs – <http://www.reading.ac.uk/AMIGA/ScienceofGM/ami-WhatareGMCrops.aspx>.

8 References

- Adang, M. J., Crickmore, N. and Jurat-Fuentes, J. L. 2014. Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. *Adv. Insect Physiol.* 47, 39-87. doi:10.1016/B978-0-12-800197-4.00002-6.
- Andow, D. A., Lovei, G. L. and Arpaia, S. 2006. Ecological risk assessment for Bt crops. *Nat. Biotechnol.* 24(7), 749-51; author reply 751. doi:10.1038/nbt0706-749.
- Areal, F. J., Riesgo, L. and Rodriguez-Cerezo, E. 2013. Economic and agronomic impact of commercialized GM crops: a meta-analysis. *J. Agric. Sci.* 151(1), 7-33. doi:10.1017/S0021859612000111.
- Arpaia, S. 2010. Genetically modified plants and “non-target” organisms: analysing the functioning of the agro-ecosystem. *Coll. Biosafety Rev.* 5, 12-80.
- Arpaia, S., Gould, F. and Kennedy, G. G. 1997. Potential impact of *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) predation on adaptation of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) to Bt-transgenic potatoes. *Entomol. Exper. Appl.* 82(1), 91-100. doi:10.1046/j.1570-7458.1997.00117.x.
- Arpaia, S., Di Leo, G. M., Fiore, M. C., Schmidt, J. E. U. and Scardi, M. 2007. Composition of arthropod species assemblages in Bt-expressing and near isogenic eggplants in experimental fields. *Environ. Entomol.* 36(1), 213-27. doi:10.1603/0046-225x(2007)36[213:coasai]2.0.co;2.
- Arpaia, S., Messéan, A., Birch, N. A., Hokannen, H., Härtel, S., Van Loon, J. J., Lovei, G. L., Park, J., Spreafico, H., Squire, G. R., Steffan-Dewenter, I., Tebbe, C. and Van Der Voet, H. 2014. Assessing and monitoring impacts of genetically modified plants on agro-ecosystems: the approach of AMIGA project. *Entomologia* 2, 154. doi:10.4081/entomologia.2014.154.

- Arpaia, S., Birch, A. N. E., Kiss, J., Van Loon, J. J. A., Messéan, A., Nuti, M., Perry, J. N., Sweet, J. B. and Tebbe, C. C. 2017. Assessing environmental impacts of genetically modified plants on non-target organisms: the relevance of in planta studies. *Sci. Total Environ.* 583, 123–32. doi:10.1016/j.scitotenv.2017.01.039.
- Bagla, P. 2010. Hardy cotton-munching pests are latest blow to GM crops. *Science* 327, 1439.
- Barzman, M., Bàrberi, P., Birch, A. N. E., Boonekamp, P., Dachbrodt-Saaydeh, S., Graf, B., Hommel, B., Jensen, J. E., Kiss, J., Kudsk, P., Lamichhane, J. R., Messéan, A., Moonen, A. C., Ratnadass, A., Ricci, P., Sarah, J. L. and Sattin, M. 2015. Eight principles of integrated pest management. *Agron. Sustain. Dev.* 35(4), 1199–215. doi:10.1007/s13593-015-0327-9.
- Bravo, A. and Soberon, M. 2010. *Bacillus thuringiensis*: mechanisms and use. In: Gilbert, L. I. and Gill, S. S. (Eds), *Insect Control Biological and Synthetic Agents*. Academic Press, Amsterdam.
- Carrière, Y., Eilers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehy, T. J. and Tabashnik, B. E. 2003. Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. *Proc. Natl. Acad. Sci. U.S.A.* 100(4), 1519–23. doi:10.1073/pnas.0436708100.
- Castañera, P., Farinós, G. P., Ortego, F. and Andow, D. A. 2016. Sixteen years of Bt maize in the EU hotspot: why has resistance not evolved? *PLoS ONE* 11(5), e0154200. doi:10.1371/journal.pone.0154200.
- Catarino, R., Ceddia, G., Areal, F., Parisey, N. and Park, J. 2016. Managing maize under pest species competition: is Bt (*Bacillus thuringiensis*) maize the solution? *Ecosphere* 7(6), e01340. doi:10.1002/ecs2.1340.
- Christiaens, O., Dzhambova, T., Kostov, K., Arpaia, S., Joga, M. R., Urru, I., Sweet, J. J. and Smagghe, G. 2018. Literature review of baseline information on RNAi to support the environmental risk assessment of RNAi-based GM plants. EFSA supporting publication 2018: EN-1424, 173pp. doi:10.2903/sp.efsa.2018.EN-1424.
- De Schrijver, A., Devos, Y., Van den Bulcke, M., Cadot, P., De Loose, M., Reheul, D. and Sneyers, M. 2007. Risk assessment of GM stacked events obtained from crosses between GM events. *Trends Food Sci. Technol.* 18(2), 101–9. doi:10.1016/j.tifs.2006.09.002.
- Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B. and Naylor, R. L. 2018. Increase in crop losses to insect pests in a warming climate. *Science* 361(6405), 916–9. doi:10.1126/science.aat3466.
- Dhurua, S. and Gujar, G. T. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) from India. *Pest Manag. Sci.* 67, 898–903.
- Dively, G. P., Venugopal, P. D., Bean, D., Whalen, J., Holmstrom, K., Kuhar, T. P., Doughty, H. B., Patton, T., Cissel, W. and Hutchison, W. D. 2018. Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. *Proc. Natl. Acad. Sci. U.S.A.* 115(13), 3320–5. doi:10.1073/pnas.1720692115.
- EFSA Panel on Genetically Modified Organisms (GMOs). 2010. Guidance on the environmental risk assessment of genetically modified plants. *EFSA J.* 8(11), 1879. doi:10.2903/j.efsa.2010.1879.
- EFSA Panel on Genetically Modified Organisms (GMOs). 2013. Statement supplementing the environmental risk assessment conclusions and risk management recommendations on genetically modified insect-resistant maize 59122 for

- cultivation in the light of new scientific information on non-target organisms and regionally sensitive areas. *EFSA J.* 11(11), 3443.
- EFSA Scientific Committee. 2016. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. *EFSA J.* 14(6), e04499.
- Gassmann, A. J., Petzold-Maxwell, J. L., Keweshan, R. S. and Dunbar, M. W. 2011. Field-evolved resistance to Bt maize by western corn rootworm. *PLoS One* 6, e22629.
- Glare, T. R. and O'Callaghan, M. 2000. *Bacillus thuringiensis: Biology, Ecology and Safety*. John Wiley and Sons Ltd, Chichester UK.
- Gomez-Barbero, M., Berbel, J. and Rodriguez-Cerezo, E. 2008. Bt corn in Spain—the performance of the EU's first GM crop. *Nat. Biotechnol.* 26(4), 384–6. doi:10.1038/nbt0408-384.
- Gould, F. 1994. Potential and problems with high dose strategies for pesticidal, engineered crops. *Biocontr. Sci. Technol.* 4, 451–61.
- Hagenbucher, S., Wäckers, F. L., Wettstein, F. E., Olson, D. M., Ruberson, J. R. and Romeis, J. 2013. Pest trade-offs in technology: reduced damage by caterpillars in Bt cotton benefits aphids. *Proc. Royal Soc. B: Biol. Sci.* 280(1758), 20130042. doi:10.1098/rspb.2013.0042.
- Hernández-Martínez, P., Gomis-Cebolla, J., Ferré, J. and Escriche, B. 2017. Changes in gene expression and apoptotic response in *Spodoptera exigua* larvae exposed to sublethal concentrations of Vip3 insecticidal proteins. *Sci. Rep.* 7(1), 16245.
- Hilbeck, A. and Otto, M. 2015. Specificity and combinatorial effects of *Bacillus thuringiensis* Cry toxins in the context of GMO environmental risk assessment. *Front. Environ. Sci.* 3, 71. doi:10.3389/fenvs.2015.00071.
- Hokkanen, H. M. 2015. Integrated pest management at the crossroads: science, politics, or business (as usual)? *Arthrop. Plant Interact.* 9(6), 543–5.
- Holst, N., Lang, A., Lövei, G. and Otto, M. 2013. Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland. *Ecol. Modell.* 250, 126–33. doi:10.1016/j.ecolmodel.2012.11.006.
- Hutchison, W. D., Burkness, E. C., Mitchell, P. D., Moon, R. D., Leslie, T. W., Fleischer, S. J., Abrahamson, M., Hamilton, K. L., Steffey, K. L., Gray, M. E., Hellmich, R. L., Kaster, L. V., Hunt, T. E., Wright, R. J., Pecinovsky, K., Rabaey, T. L., Flood, B. R. and Raun, E. S. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* 330(6001), 222–5. doi:10.1126/science.1190242.
- IAASTD. 2009. Agriculture at a crossroads. Synthesis Report. Island Press, Washington DC.
- ISAAA. 2018. Global Status of Commercialized Biotech/GM Crops in 2018: Biotech Crops Continue to Help Meet the Challenges of Increased Population and Climate Change. ISAAA Brief No. 54. ISAAA, Ithaca, NY.
- Klümper, W. and Qaim, M. 2014. A meta-analysis of the impacts of genetically modified crops. *PLoS ONE* 9(11), e111629. doi:10.1371/journal.pone.0111629.
- Kremen, C. and Ostfeld, R. S. 2005. A call to ecologists: measuring, analyzing, and managing ecosystem services. *Front. Ecol. Environ.* 3(10), 540–8. doi:10.1890/1540-9295(2005)003[0540:ACTEMA]2.0.CO;2.
- Kruger, M., Van Rensburg, J. B. J. and Van den Berg, J. 2012. Transgenic Bt maize: farmers perceptions, refuge compliance and reports of stem borer resistance in South Africa. *J. Appl. Entomol.* 136, 38–50.

- Lövei, G. L., Andow, D. A. and Arpaia, S. 2009. Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. *Environ. Entomol.* 38(2), 293–306. doi:10.1603/022.038.0201.
- Lundgren, J. G., Gassmann, A. J., Bernal, J., Duan, J. J. and Ruberson, J. 2009. Ecological compatibility of GM crops and biological control. *Crop Protect.* 28(12), 1017–30. doi:10.1016/j.cropro.2009.06.001.
- Luttrell, R. G., Ali, I., Allen, K. C., Young III, S. Y., Szalanski, A., Williams, K., Lorenz, G., Parker Jr, C. D. and Blanco, C. 2004. Resistance to Bt in Arkansas populations of cotton bollworm. In: Richter, D. A. (Ed.), *Proceedings of the 2004 Beltwide Cotton Conferences*, San Antonio, Texas, 5–9 January 2004, pp. 1373–83.
- Marvier, M., McCreedy, C., Regetz, J. and Kareiva, P. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science* 316(5830), 1475–7. doi:10.1126/science.1139208.
- Menozi, D., Kostov, K., Sogari, G., Arpaia, S., Moyankova, D. and Mora, C. 2017. A stakeholder engagement approach for identifying future research directions in the evaluation of current and emerging applications of GMOs. *Bio-Based Appl. Econom. J.* 6, 57–79.
- Naranjo, S. E. 2005. Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. *Environ. Entomol.* 34(5), 1211–23. doi:10.1093/ee/34.5.1211.
- Naranjo, S. E. 2009. Impacts of Bt crops on non-target invertebrates and insecticide use patterns. *CAB Rev.* 4(11), 011. doi:10.1079/PAVSNNR20094011.
- Palma, L., Muñoz, D., Berry, C., Murillo, J. and Caballero, P. 2014. *Bacillus thuringiensis* toxins: an overview of their biocidal activity. *Toxins* 6(12), 3296–325. doi:10.3390/toxins6123296.
- Parisi, C., Tillie, P. and Rodriguez-Cerezo, E. 2016. The global pipeline of GM crops out to 2020. *Nat. Biotechnol.* 34(1), 31–6. doi:10.1038/nbt.3449.
- Pérez, C., Fernandez, L. E., Sun, J., Folch, J. L., Gill, S. S., Soberón, M. and Bravo, A. 2005. *Bacillus thuringiensis* subsp. israelensis Cyt1Aa synergizes Cry11Aa toxin by functioning as a membrane-bound receptor. *Proc. Natl. Acad. Sci. U.S.A.* 102(51), 18303–8. doi:10.1073/pnas.0505494102.
- Perry, J. N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R. S., Kiss, J., Lheureux, K., Manachini, B., Mestdag, S., Neemann, G., Ortego, F., Schiemann, J. and Sweet, J. B. 2010. A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. *Proc. R. Soc. B.* 277(1686), 1417–25.
- Ramirez-Romero, R., Bernal, J. S., Chaufaux, J. and Kaiser, L. 2007. Impact assessment of Bt-maize on a moth parasitoid, *Cotesia marginiventris* (Hymenoptera: Braconidae), via host exposure to purified Cry1Ab protein or Bt-plants. *Crop Protect.* 26(7), 953–62. doi:10.1016/j.cropro.2006.09.001.
- Ramírez-Suero, M., Valerio-Alfaro, G., Bernal, J. S. and Ramírez-Lepe, M. 2011. Synergistic effect of chitinases and *Bacillus thuringiensis* israelensis spore-toxin complex against *Aedes aegypti* larvae. *Can. Entomol.* 143(2), 157–64. doi:10.4039/n10-051.
- Romeis, J., Naranjo, S. E., Meissle, M. and Shelton, A. M. 2019. Genetically engineered crops help support conservation biological control. *Biol. Contr.* 130, 136–54. doi:10.1016/j.biocontrol.2018.10.001.

- Sharma, P., Nain, V., Lakhanpaul, S. and Kumar, P. A. 2010. Synergistic activity between *Bacillus thuringiensis* Cry1Ab and Cry1Ac toxins against maize stem borer (*Chilo partellus* Swinhoe). *Lett. Appl. Microbiol.* 51(1), 42-7. doi:10.1111/j.1472-765X.2010.02856.x.
- Sparks, T. C. and Nauen, R. 2015. IRAC: mode of action classification and insecticide resistance management. *Pestic. Biochem. Physiol.* 121, 122-8. doi:10.1016/j.pestbp.2014.11.014.
- Storer, N. P., Babcock, J. M., Schlenz, M., Meade, T., Thompson, G. D., Bing, J. W. and Huckaba, R. M. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. *J. Econom. Entomol.* 103, 1031-8.
- Tabashnik, B. E. and Carrière, Y. 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. *Nat. Biotechnol.* 35(10), 926.
- Tabashnik, B. E., Gassmann, A. J., Crowder, D. W. and Carrière, Y. 2008. Insect resistance to Bt crops: evidence versus theory? *Nat. Biotechnol.* 26, 199-202.
- Tabashnik, B. E., Van Rensburg, J. B. J. and Carrière, Y. 2009. Field-evolved insect resistance to Bt crops: definition, theory, and data. *J. Econom. Entomol.* 102, 2011-25.
- Taylor, R., Herms, D., Cardina, J. and Moore, R. 2018. Climate change and pest management: unanticipated consequences of trophic dislocation. *Agronomy* 8(1), 7. doi:10.3390/agronomy8010007.
- US EPA, FIFRA Scientific Advisory Panel. 2018. Meeting Minutes and Final Report No. 2018-06, 17-19 July 2018. Available at: <https://www.regulations.gov/document?D=EPA-HQ-OPP-2017-0617-0032>.
- Van Capelle, C., Schrader, S. and Arpaia, S. 2016. Selection of focal earthworm species as non-target soil organisms for environmental risk assessment of genetically modified plants. *Sci. Total Environ.* 548-549, 360-9.
- Van Frankenhuyzen, K. 2009. Insecticidal activity of *Bacillus thuringiensis* crystal proteins. *J. Invertebr. Pathol.* 101(1), 1-16. doi:10.1016/j.jip.2009.02.009.
- Van Frankenhuyzen, K. 2013. Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. *J. Invertebr. Pathol.* 114(1), 76-85. doi:10.1016/j.jip.2013.05.010.
- Van Rensburg, J. B. J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt-transgenic maize. *S. A. J. Plant Soil* 24(3), 147-51.
- Vu, K. D., Yan, S., Tyagi, R. D., Valéro, J. R. and Surampalli, R. Y. 2009. Induced production of chitinase to enhance entomotoxicity of *Bacillus thuringiensis* employing starch industry wastewater as a substrate. *Bioresour. Technol.* 100(21), 5260-9. doi:10.1016/j.biortech.2009.03.084.
- Walker, G. P., Cameron, P. J., MacDonald, F. M., Madhusudhan, V. V. and Wallace, A. R. 2007. Impacts of *Bacillus thuringiensis* toxins on parasitoids (Hymenoptera: Braconidae) of *Spodoptera litura* and *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Biol. Contr.* 40(1), 142-51. doi:10.1016/j.biocontrol.2006.09.008.
- Wang, S., Just, D. R. and Pinstrup-Andersen, P. P. 2008. Bt-cotton and secondary pests. *Int. J. Biotechnol.* 10(2/3), 113. doi:10.1504/IJBT.2008.018348.
- Wu, K. M., Lu, Y. H., Feng, H. Q., Jiang, Y. Y. and Zhao, J. Z. 2008. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. *Science* 321(5896), 1676-8. doi:10.1126/science.1160550.