Environmental Analytical and Ecotoxicological Aspects of *Bt* Maize in the Pannonian Biogeographical Region of the European Union

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Abstract Insect-resistant transgenic crops expressing toxins originated from *Bacillus thuringiensis* (*Bt*) appear advantageous by not requiring field applications of *Bt* bioinsecticides, and by prevention of efficacy losses due to improper application timing, wash-off or inactivation. Through preventing insect damage potentially transmitting infection by toxinogenic fungi, *Bt* plants may indirectly reduce mycotoxin contamination. Strong disadvantages are, however, that Cry1Ab toxin-based *Bt* bioinsecticides and *Bt* plants differ in their active ingredients: *MON 810 Bt* maize expresses a single truncated (preactivated) CrylAb toxin, while the corresponding bioinsecticide contains a Cry1Ab protoxin (with other Cry1, Cry2 and Vip protoxins). This can facilitate rapid insect resistance development not only against Cry1Ab (see cross-resistance). Cry1Ab toxin protected from decomposition in plant tissues shows environmental persistence in the stubble. Protected butterflies (Lepidoptera) in Hungary, showing higher sensitivity to Cry1Ab than the target pest, are exposed to Cry1Ab toxin through the dispersal of *Bt* maize pollen. *Bt* maize showed moderate but statistically significant effects on parasitoid or predator beneficial insects in tritrophic studies. Finally, *Bt* plants produce *Cry* toxin during their entire vegetation period. Thus, toxin administration cannot be limited to the occurrence of the pest insect that contradicts the threshold-based treatment timing principle of integrated pest management.

Keywords Cry proteins · Protoxin · Preactivated toxin · Immunoassay · Pest resistance · Protected insects · Tritrophic assessment · *MON 810* · *DAS-59122-7*

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Abbreviations

AM	arbuscular mycorrhizal
Bt	*Bacillus thuringiensis*
CR	the cross-reactivity
CRP	Co-operative Research Programmes
Cry	crystal *Bt* endotoxin
Cyt	cytolytic *Bt* endotoxin
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ERA	environmental risk assessment
EU	European Union
GM	genetically modified
GMO	genetically modified organism
HT	herbicide-tolerant
IPM	integrated pest management
IR	insect-resistant
IRM	insect resistance management
ITU	international toxic unit
OECD	Organisation for Economic Co-operation and Development
PPP	plant protection products
RNAi	ribonucleic acid interference
SAB	Scientific Advisory Body
UN	United Nations
US	United States (of America)
Vip	vegetative insecticidal proteins

Introduction

The European Union (EU) legislation specifies a genetically modified organism (GMO) as "*an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination*" (EC 1990). The corresponding Hungarian law is even more specific defining a GMO (in the original text an organism modified by gene technology) as "*a natural organism in which the genetic material has been altered by genetic modification, including the progeny of such organisms carrying the properties appearing as a result of these modifications*", and a genetic modification as "*a method defined by the relevant law issued under the authorisation of this Act which extracts a gene or any part thereof from the cells and transplants it into another cell, or introduces synthetic genes or gene fragments into a natural organism to alter the genetic material of the recipient*" (Government of Hungary 1998). Recognising potential risks of unintended

releases and reproduction of GMOs in the environment and possible irreversible consequences, commercial use of genetically modified (GM) crops is authorised only upon assessment of human health and environmental risks on the basis of the precautionary principle (EC 2001). Among currently registered GM plants, the vast majority (approximately 80%) are represented by crops that have been genetically modified for plant protection purposes (so-called first-generation GM plants). Of these first-generation GM plants, according to their acreage in 2017 (Clive 2017), 47% are herbicide-tolerant (HT) plant varieties, 12% are insect-resistant (IR) and 41% contain stacked events (HT and/or IR).

The only genetic event approved in the EU for cultivation for food and feed purposes is IR maize event *MON 810*, cultivated in two EU Member States, Spain and Portugal altogether on 131,535 hectares in 2017 representing a 4% decrease compared to the previous year (Clive 2017). This is a marginal level, representing 0.07%, 0.22% and approximately 2.2% of the global cultivation area of GM crops, GM maize and IR maize, respectively. It has to be also noted that the ratio of stacked events has been rapidly increasing lately. Reported global cultivation areas are, however, somewhat misleading: acreages of stacked event GM crops are biased as are considered as "trait hectares", i.e. actual crop acreage multiplied by the number of traits to "confer multiple benefits in a single biotech variety".

Insect resistance in GM crops is achieved by the incorporation of a transgene encoding an endotoxin protein (or its variety) from *Bacillus thuringiensis* (*Bt*), a well-known insect pathogenic, endospore-forming, soil-borne, Gram-positive bacterium. *B. thuringiensis*, first reported in 1901 in Japan, described in 1915, and proven to have numerous strains worldwide, forms characteristic parasporal bodies during sporulation containing crystal (Cry) and cytolytic (Cyt) endotoxins that are known to exert pore-forming effects in the insect midgut (Palma et al. 2014). Cry endotoxins produced by various *B. thuringiensis* strains are lectin-like proteins with a characteristic three-domain structure consisting of an α-helix subunit (domain 1) facilitating the incorporation of the toxin in membranes; as well as two β-sheets (domains 2 and 3) participating in binding to lectin receptors of the cell membranes in the midgut epithelium and upon oligomerisation-forming pores in the insect midgut (Schnepf et al. 1998). These pores disturb the ion channel functions in the cell membranes; the insect ceases feeding, its digestion stops, and subsequently dies of internal sepsis due to the microwounds created on the midgut wall. Commercial topical microbial *Bt*-based insecticides, containing Cry toxins as their active ingredients, have long been registered and applied in integrated pest management and in ecological farming, and have been found to be effective to control selected insect pests, more benign environmentally than broad-spectrum insecticides and safe for birds and mammals (Kaur 2000; Sanchis 2011; Sanahuja et al. 2011; Gatehouse et al. 2011; Székács and Darvas 2012a; Palma et al. 2014; Bravo et al. 2018). Factors limiting their applicability, however, include low field stability, narrow activity spectrum, and recently an assessment by the European Food Safety Authority (EFSA) raising concern regarding the ability to *Bt* strains to possibly infect humans via food (EFSA 2016) impugned later by *Bt* occurrence, epidemiological and phylogenic data (Raymond and Federici 2017).

Cry toxins have been classified by their primary protein structure (amino acid sequence) into 54 types (Cry1 to Cry54) and several subtypes (e.g. Cry1Aa, Cry1Ba). Different subtypes exert toxicity to different insect orders (Lepidoptera, Coleoptera, Diptera, Hemiptera, Hymenoptera), as well as to nematodes (Rhabditida) and snails (Gastropoda), or even to human cancer cells (Palma et al. 2014). They are typically produced in the microorganism as protoxins that require activation in the alkaline pH of the insect midgut and are stabilised by disulphide bonds in the parasporal protein crystals.

Bt Crop Varieties

Since the cloning of *Bt* strains producing various Cry toxins and the introduction and expression of their genes responsible for Cry toxin production into other microorganisms and into plants, various *Bt* crops have emerged and are being cultivated worldwide (Clive 2017). Transgenic *Bt* potato (Cry3A) against Colorado potato beetle (*Leptinotarsa decemlineata*) and *Bt* cotton (Cry1Ac) against the American bollworm (*Helicoverpa armigera*), the spotted bollworm (*Earias vittella*) and the pink bollworm (*Pectinophora gossypiella*) became commercialised in the USA in 1995, followed by *Bt* maize (Cry1Ab) against the European corn borer (*Ostrinia nubilalis*) in 1996, and another *Bt* maize variety (Cry3) against the Western corn rootworm (*Diabrotica virgifera virgifera*) in 2003. The range of *Bt* plants rapidly increased worldwide in different additional crops including soybean and other field cultures (rice, alfalfa, canola), vegetables (tomato, chickpea), tobacco, sugarcane and poplar with transgenes encoding different Cry and secretable Vip (vegetative insecticidal proteins) *Bt* toxins (10 *Bt* toxins used in transgenic crops against 15 insect pests) in single and combined (stacked) genetic events (single and multiple traits) using the, in the case of "SmartStax" varieties using six stacked Cry genes, three toxins (Cry34Ab1/Cry35Ab1 and Cry3Bb) against coleopteran pests and three other (Cry1A.1.05, Cry2Ab and Cry1F) against Lepidopteran pests, in addition to two traits conferring HR. Although IR GM crops represent the lesser proportion of first-generation GM plants (see above), the overall worldwide cultivation area of *Bt* crops reached over 100 million hectares by 2017 (Clive 2017).

Technological, economic and social benefits of *Bt* crops have been reviewed extensively in the scientific literature (US National Research Council 2010; Hutchison et al. 2010; Royal Society 2016; Brookes and Barfoot 2017; Clive 2017; Carzoli et al. 2018; Dively et al. 2018; Zilberman et al. 2018; Brookes 2019; Catarino et al. 2019) and are reflected in their substantial utilisation in intensive agriculture. Thus, *Bt* crops certainly realise a considerable profit for the variety of owners and have been claimed to produce economic benefits for farmers. In addition, relying on environmentally favourable active ingredients, Cry toxin proteins, *Bt* crops represent environmental benefits relative to broad-spectrum insecticides (some even claim (Carzoli et al. 2018), practically no risks are associated with these crops), as well as communal benefits due to area-wide suppression of pests

(Tabashnik 2010), and increases in beneficial insect populations in around fields of *Bt* crops compared to conventional management (Gatehouse et al. 2011). Environmental risks potentially associated with Cry proteins may differ dramatically among various Cry proteins and by their various expression levels in different crops or different genetic events in the same crop (e.g. maize) (Clark et al. 2005; Székács and Darvas 2012a; Chátalová 2019). Yet, the amount of Cry toxin produced by these crops, increased in the case of stacked Bt events, is only rarely considered, when reduced insecticide applications due to GM technology are estimated (Benbrook 2012; Hilbeck and Otto 2015), and in the case of *Bt* crops the comparator plant protection products (PPPs) should not be the broad-spectrum insecticide agrochemicals, but bioinsecticide of the same type of active ingredient, Cry toxin-based bioinsecticides. This report does not intend to summarise agrotechnological, economical and societal aspects of *Bt* crops, but focuses on its environmental and ecotoxicological impacts as having been considered in their regulation in the EU and particularly in its Pannonian Biogeographical Region within the Natura 2000 protected area network.

In Hungary, as in other EU Member States, a single *Bt* maize variety registered in the EU has been filed for authorisation for public cultivation, genetic event *MON 810* (Monsanto Corp.), and therefore this variety has been assessed by environmental analysis and in ecotoxicity tests. It has to be emphasised that a safeguard clause moratorium on the cultivation of *MON 810* GM maize is effective in Hungary (Ministry of Agriculture, Hungary 2005) on the basis, to a great extent, of the studies summarised here. Therefore, just like in neighbouring Austria, deliberate releases of *MON 810* have been carried out in Hungary only for experimental purposes. In addition, a different *Bt* maize variety *DAS 59122–7* (Pioneer Hi-Bred International, Inc.) was assessed in analytical and tritrophic biological studies.

Transgenic Cry Proteins Expressed, Methodological and Conceptual Problems in Their Analysis

To assess possible effects (main or side-effects) of *Bt* maize, the amount of *Bt* toxins (Cry or Vip) produced needs to be determined. This is of importance not only for the efficacy of the technology, but also for assessing unintended effects on non-target organisms depending on the level and distribution of the transgenic Cry or Vip toxin produced. It is a requirement for the registration of all PPPs that the active ingredient has to be quantitatively detectable, for which appropriate analytical method has to be available; moreover, analytical standards of the purified active substance of relevant metabolites have to be provided by the applicant or producer upon request (EC 2009, 2013). In accordance, analytical methods for detecting Cry toxin residues in commerce, as well as expression level data of the toxin (termed "plant-incorporated protectant") in various plant organs were requested by the US Environmental Protection Agency (EPA) for the reassessment of *Bt* crops (Mendelsohn et al. 2003).

Cry and Vip toxins are commonly analysed by enzyme-linked immunosorbent assays (ELISAs) in a so-called sandwich ELISA setup. In these immunoassays, Cry-specific antibodies are immobilised on the wall of 96-well microplates, and toxin protein captured from the sample is allowed to react with a second Cry-specific antibody labelled with a reporter enzyme, or this second antibody is further reacted with an immunoglobulin-specific antibody labelled with a reporter enzyme. Immunoassays are readily used in research and development, but are less frequently accepted for regulatory, surveillance or enforcement purposes by control authorities, particularly if chromatographic instrumental analytical methods are available. Exceptions to this include the use of immunoassay as a means of determination of the amount of a pesticidal *Bt* protein in GM crops and commodities, where ELISAs or lateral flow devices are the method of choice (Grothaus et al. 2006). These immunoassays appeared to be of good reproducibility, yet reported Cry toxin concentrations in GM plants highly vary among different laboratories, cultivation sites or even with the same GM variety at a given location. To test quantitative detectability of transgenic Cry toxins by the ELISA method approved by Monsanto Corp. for toxin determination, to corroborate its analytical features, and to follow Cry toxin production in the crop during vegetation, we tested the performance of Cry toxin-specific immunoassay method in detail. We have assessed the analytical performance of the immunoanalytical determination of Cry toxins, and have identified various sources of analytical variation and error (Takács et al. 2012a; Székács 2013). Such errors included discrepancy in the identity of the analyte, consequent inaccuracies in Cry toxin content reported, as well as tissue-specific and seasonal variabilities in Cry toxin levels produced in *Bt* maize (see below).

Protein Forms of Given Cry Toxins

As mentioned above, the Cry toxin content in *B. thuringiensis* endotoxin crystals are mostly protoxins, from which the active toxin form is liberated by alkaline hydrolysis. In the case of Cry1Ab toxin, the molecular mass of the protoxin is 131 kDa, and the protein forms bipyramidal crystals stabilised by a maximum of 16 disulphide bonds per molecule. Upon reduction of the disulphide bonds and hydrolytic cleavage of the protoxin, an activated toxin with a molecular mass of approximately 63–65 kDa is formed. The transgene in *MON 810* encodes neither the protoxin, nor the activated toxin, but a protein form in between, a partially hydrolysed Cry1Ab protoxin of 91 kDa molecular mass; therefore, it produces this so-called preactivated toxin. As seen from the above, the active ingredients of the microorganism-based *Bt* bioinsecticide and of *MON 810* maize are different, being the Cry1Ab protoxin (131 kDa) and the preactivated Cry1Ab toxin (91 kDa), respectively, both hydrolysed in the insect to form the activated Cry1Ab toxin (63–65 kDa) responsible for insecticidal activity (Székács and Darvas 2012a, b).

An important analytical consequence of the above is that ELISA kits manufactured for the determination of bacterial Cry endotoxins (using Cry protoxin as an

immunogen) will provide biased results when detecting the preactivated Cry toxin: antibodies directed to the protoxin are expected to show lower affinity to the truncated preactivated toxin protein, therefore, virtual signals sensed in quantitative immunoassays validated to detect protoxin molecules will correspond to higher concentrations of the preactivated toxin than those of the protoxin (as the antibody has lower immunoaffinity to the former than to the latter). The extent of the bias is described by the cross-reactivity (CR) between these toxin forms, defined as the percentage ratio of their IC_{50} values in the ELISA test.

CR values determined for Cry1Ab protoxin/preactivated toxin ranged 0.41–0.56, indicating that the ELISA kits are suitable to detect Cry1Ab protoxin (in microbial samples), but require correction with the CR values determined when used on *MON 810* maize samples containing preactivated Cry1Ab toxin (Székács et al. 2010a). Actual preactivated Cry1Ab toxin concentrations in these *Bt* maize samples are 1.8–2.3-fold higher than detected by the Cry1Ab protoxin-specific ELISA kits. This applies to Cry1Ab values in *MON 810* maize reported to date in the scientific literature, including data by the variety owner, Monsanto Corp.

The other *Bt* maize studied was variety *DAS 59122-7* producing Cry34Ab1 (14 kDa) and Cry35Ab1 (44 kDa) binary toxins. Similar, but less substantial differences exist for these toxins between their microbial and plant-biosynthesised forms, where the maize-derived Cry34Ab1 and Cry35Ab1 proteins were found nearly identical to the microbe-expressed forms, with Cry34Ab1 having one amino acid missing at the N-terminal and exhibiting forms at 60, 50 and 42 kDa in addition to the expected 13.6 kDa protein (Latham et al. 2017). Therefore, significant differences in the CRs of these toxins originated from microbes and maize are not expected.

Matrix Effects in the Determination of Cry Toxin Levels

Maize leaf material is a sample matrix commercial ELISA kits have been validated for. Therefore, Cry toxin measurement in foliage is unproblematic, other than toxin level fluctuation in the leaves, but that is not a question of tissue matrix. In addition to that, the ELISA kits were straight forward applicable on stem, root and seed samples as tissues of plant origin (Székács et al. 2010a, b; Takács et al. 2012a). More marked matrix effects were observed with pollen that required higher sample extract dilution due to its high fat, protein and mineral contents (Székács et al. 2010a). In addition, the Cry1Ab ELISA test was assessed and used on animal tissues as well (Takács et al. 2015).

Assay Validation

Analytical characteristics and applicability of the ELISA kits were tested using Shewhart analytical control charts and quality control by internal standard reference samples to detect analytical goodness (precision, accuracy, stability) for the detection of both Cry1Ab (Takács et al. 2012a, 2015) and Cry34/35Ab1 toxins (Takács et al. 2010, 2012b). In addition, an inter-laboratory ring trial test has been carried out with the participation of specialised laboratories in Germany, Hungary, Norway and Switzerland to explore whether high variability in reported Cry toxin concentrations in the same *Bt* maize variety is due to the ELISA protocols, instrumentation, extraction methods, human error, sample reproducibility or plant variability (Székács et al. 2012). In turn, such ring tests have been proposed to be performed as a part of the standardised environmental risk assessment (ERA) of *Bt* maize effects on non-target insects as a means of external quality assurance (Lang et al. 2019). Reduction or elimination of sources of analytical variability allows feasible quality control of *Bt* plants and makes proper interpretation of differences or variability among published data from different laboratories possible, but the results underlined the importance of well-controlled reference materials, ELISA kits and protocol, particularly for reported concentrations of Cry toxins in pollen that render mathematical models for the environmental fate (Romeis et al. 2008) or biological effects (Perry et al. 2010) burdened with uncertainty.

Estimated Production and Bioavailability of Cry Proteins

Bt maize varieties, *MON 810* and *DAS 59122-7* cultivars, were demonstrated to produce the corresponding Cry toxins (Cry1Ab and Cry34/35Ab1) in a tissue- and time-specific manner (see below). Cry1Ab in *MON 810* provides protection against Lepidopteran pests, particularly against larvae of the European corn borer feeding in the stem. This pest may damage in two or three generations in a season, therefore, the highest level of expression of the transgenic protein should preferably occur in the stem from the VT growth stage on. In contrast, Cry34/35Ab1 in *DAS 59122-7* provides protection against Coleopterans, e.g. the corn rootworm that damages at the larval stage of the root. Thus, the highest level of toxin production would be desirable in the root in the V12-R3 growth stages. To assess compliance of toxin production dynamics with these required protection times, actual toxin production was experimentally systematically monitored throughout the entire vegetation periods for these *Bt* maize varieties. When available, Cry toxin production was compared to the availability of the corresponding Cry toxin from *Bt*-based bioinsecticides.

Tissue and Temporal Variability of Cry Toxin Production in Bt *Maize Varieties*

Levels of Cry1Ab toxin (corrected for active toxin content on the basis of cross-reactivities between the activated Cry1Ab toxin and the Cry1Ab protoxin) were found to fall between 9.6 ± 2.1 and 17.2 ± 1.7 μg/g in the leaves, 0.47 ± 0.03 and 5.0 ± 0.3 μg/g in the stem, 2.3 ± 0.3 and 5.3 ± 0.5 μg/g in the roots and 0.03 ± 0.01 and 0.5 ± 0.03 μg/g in the pollen of *MON 810* maize (plant material expressed in fresh weight) with characteristic patterns during the vegetation period tested in 3 different years within an 8-year period (Székács et al. 2005, 2010a). Since crop damage by the European corn borer (causing yield loss by decreased kernel number and weight due to disruption of plant growth, broken stalks and dropped ears) occurs mainly in the stem, it is a rather unfavourable feature of the *MON 810* maize variety (DK-440-BTY) that only 12–20% of the Cry1Ab toxin protein biosynthesised in the plant is produced in the stalk. This means the plant produces 7–8-times more toxin protein, than the amount being utilised in the crop protection mechanism.

Poor targeting of pesticide application is, however, not unique to *Bt* crops. Estimates of the efficacy of spray applications range from 1% (Pimentel 1995) to 30–40% (Matthews et al. 2014). The 12–20% accuracy of *Bt* crops regarding Cry toxin content in the target plant tissues also falls into this range. The accuracy of aerosol treatments, however, can be enhanced with targeted application and precision agricultural technologies (Pedersen and Lind 2017), while toxin production is determined by the genetic sequence of the GM crop. Therefore, GM crop development is strongly recommended to focus on varieties with target tissue-specific transgene expression.

The toxin content in pollen has been found strikingly different among different *MON 810* maize varieties provided by the variety owner, Monsanto Corp. Preactivated CryAb toxin quantity in the pollen of those varieties was determined to be 0.03 ± 0.01, 0.11 ± 0.02, and 0.47 ± 0.03 μg/g fresh weight, while pollen productivity was practically unchanged, 1.39 ± 0.33 g/plant among varieties and cultivation years. Pollen amount on the field was determined to be 3.5–5.5 × 10^{11} pollen/ha, which is only a fraction of the potentially produced pollen quantity (6.4–7.2 × 10^{11} pollen/ha).

Minor, but statistically significant variability was found in preactivated Cry1Ab toxin content in maize leaves diagonally, with approximately 20% higher levels (9.9 ± 0.9 μg/g fresh weight) near the leaf vein, than further towards at leaf edges. Longitudinal distribution of the preactivated toxin showed a much higher variability in the leaves, with the highest toxin concentration (8.9 ± 1.5 μg/g fresh weight) in the lamella middle between the base and the leaf tip, almost 5- and 2-fold higher than at the sheath and at the tip, respectively. Low levels at the sheath are explained by the leaf base being the most rapidly growing zone of the leaf, and at the tip with partial plant tissue necrotisation, as decreased toxin levels were seen only in slightly yellow leaf tips (Székács et al. 2010b). Necrotisation has been found a major cause of decrease in toxin concentrations among leaf levels (with outstandingly, 1.3- to

2.3-fold lower toxin concentrations 4.8 ± 1.0 μg/g fresh weight at the first leaf level than at all other leaf levels) and in the stem as well. Cry1Ab toxin in the plant tissue is protected from rapid decomposition, and can long remain in the stubble in maize roots (containing 7.7–9.7% of the overall toxin production of the plant) or as plant foliage biomass enters the soil unintentionally or intentionally during harvest. Results indicate that 1–8% of the toxin content in the stubble can be detected 1 year after harvest, indicating environmental persistence of the toxin protein in the stubble (Székács et al. 2005; Székács and Darvas 2012a).

Concentrations of Cry34Ab1 and Cry35Ab1 binary toxins in the leaves of *DAS 59122-7* maize were 81.1 ± 17.7 and 75.1 ± 11.9 μg/g dry weight, respectively. The longitudinal distribution of the toxin proteins showed a similar trend than seen for *MON 810* maize: Cry34Ab1 and Cry35Ab1 toxin levels were 3.1- and 2.7-fold higher, respectively, in the lamella middle of the leaf than in the leaf base. Because crop damage by the corn rootworm occurs in the root nodes, the efficacy of Cry34/35Ab1 toxin production of the *DAS 59122-7* maize variety is rather unfavourable as only 2–3% of the toxin protein biosynthesised in the plant is produced in the root, indicating that the plant produces 35–46-times more toxin protein, than the toxin amount providing the desired protective effect. The pollen contained 47.4 ± 12.3 and < 0.12 μg/g fresh weight (the latter being the limit of detection of the ELISA) of Cry34Ab1 and Cry35Ab1, respectively (Takács et al. 2011, 2012b).

Assessment of the production of preactivated Cry1Ab toxin in the tissues of *MON 810* and of Cry34Ab1 and Cry35Ab1 binary toxins in *DAS 59122-7* maize appeared to be proportional with chlorophyll content and therefore, photosynthetic activity in the tissue. This is of significance not only for plant physiology, but also for the exposure of herbivorous insects. Insects that prefer green plant tissues (unlike species that develop in the fruit (fructus), seed, root or saprophagous ones) will tend to become exposed to tissues with the highest toxin content. Such photosynthesis-related toxin production, however, is far not optimal for maize pest control: larvae of the European corn borer (*O. nubilalis*) feeds in the stem, where 3- to 17-fold lower preactivated Cry1Ab toxin production occurs than in the leaves of *MON 810* maize (Székács et al. 2010a), and larvae of the corn rootworm (*D. virgifera*) damages the root, where Cry34Ab1 and Cry35Ab1 toxin production is approximately 5- and 10-fold lower, respectively, than in the leaves of *DAS 59122-7* maize (Takács et al. 2011, 2012b).

Determination of Cry Toxin from Bt-*Based Bioinsecticides*

Conventional insecticides are officially characterised by their net active ingredient content, while such specification is unfortunately no longer required for endotoxin-based bacterial preparations, as these bioinsecticides are assessed by their efficacy (ITU/g, ITU referring to international toxic units). However, it is not the bacterium, but its endotoxins that are responsible for the biological effect; therefore, efficacy

should be attributed to the actual endotoxin content. To monitor active ingredient content, immunoanalytical (ELISA) determinations were applied to Cry1Ab and Cry4 endotoxin content in bioinsecticide preparations Dipel (Székács and Darvas 2012a) and Vectobac (Fejes et al. 2011), respectively. These studies with Dipel revealed that only a minor fraction of the toxin protein is immediately bioavailable (soluble) at neutral pH, the vast majority of the crystal mass is only bioaccessible (temporarily non-bioavailable), and a part of the entire endotoxin content is non-bioavailable due to decomposition during crystal digestion. Thus, the nominal concentration of a common formulation of Dipel, 3.2% (corresponding to 32 mg/g bacterial protein in the bioinsecticide) corresponded to average bioavailable Cry1Ab/Cry1Ac endotoxin content of 20.6 ± 2.6 μg/g and to bioaccessible Cry1Ab/Cry1Ac endotoxin content of 0.085–8.16 mg/g. In addition to a clarification of the active ingredient content, these measurements allowed to compare detected Cry1Ab production of *MON 810* maize to corresponding bioavailable and bioaccessible Cry1Ab/Cry1Ac endotoxin content in Dipel (Székács and Darvas 2012a). In the case of Vectobac, determination of the Cry4 endotoxin content by immunoassay has been correlated with efficacy measurement in dose–mortality bioassays (Fejes et al. 2011).

Resistance Development and Non-target Effects

Beyond the technological advantages of the *Bt* maize varieties, their potential technological drawbacks also need to be assessed. These include the problems of emergence of pest resistance, potential non-target effects and applicability of these *Bt* corps in integrated pest management (IPM) practice. The first two issues are discussed in this section, and the third one is covered under "Legislatory measures" (see below).

Some of the general problems of pest control discussed in the context of the assessment of *Bt* crops are in fact not unique solely to *Bt* crops. This particularly applies to pest resistance development, where problems emerged, but achievements in their mitigation have also been accomplished (see below). Moreover, clear advantages of the environmentally benign characteristics of Cry proteins compared to broad-spectrum insecticides need to be emphasised.

Resistant Populations

Pest resistance development is a practically inevitable natural response to intervention by agricultural technologies in the agro-ecosystem. Any method of pest control can potentially be overcome by the evolution of resistance in the pest population. Resistance to pesticides (synthetic and biological, including *Bt* sprays) is rife and is more a reflection of widespread and continual use of PPPs than the properties of

those products themselves. Moreover, even crop rotations to suppress build-up of pest populations can be defeated by those pests: corn rootworm has evolved extended diapause in response to attempts to control it in growing soybeans between successive maize crops.

The occurrence of resistance against a single substance is easier to emerge if a simple mutation in the pest population can result in biochemical changes that inactivate the site of action of the compound abolishing susceptibility of the mutant individual to the agent. A common approach to prevent resistance is the parallel use of different agents acting by somewhat or completely different modes of action. Resistance development against *Bt* microorganisms or their preparations is slow, as their numerous, related, but different Cry toxins act in concert at different lectin receptors. This resistance to Dipel rapidly declined in the highly resistant populations of the target insect (the diamondback moth, *Plutella xylostella*) upon halting administration of Dipel, but returned upon resumed treatments (Tabashnik et al. 1994). The study not only indicated reduced and restored *in vitro* binding to the receptors in the midgut of the affected insects being associated with emerging and declining resistance, and not only revealed the importance of maintaining a susceptible sub-population of the insect pest that later became the fundamental aspect of insect resistance management (IRM) programmes, but also warned that continuous cultivation of *Bt* crops may also cause resistance problems by eliminating temporal refuges for susceptible insect sub-populations. Indeed, field-evolved resistance against single Cry toxins in *Bt* crops has later been reported in different insect pests in various regions from the United States to Australia, India, South Africa and China (Tabashnik et al. 2013), yet a more recent survey indicates that such occurrences are narrowly distributed (Tabashnik and Carrière 2017). Although the incidence of practical resistance (resistance occurring in at least 50% of the pest insect population resulting in an observable decrease in crop insecticidal efficacy) has been on the rise in the past two decades, pest susceptibility was somewhat more frequently sustained. Practical resistance to *Bt* crops occurred mostly in maize, followed by cotton. Various approaches including the "high dose/refuge" strategy using non-*Bt* plants in the cultivation area to allow limited reproduction of the susceptible insects and the "pyramid" strategy of parallel use of two or more toxins with affinity to different lectin receptors have been applied for IRM. This is commendable – *Bt* crops at least have mandated IRM programmes, as requested by the US EPA (Mendelsohn et al. 2003), unlike many other pest control products, and these programmes have been successful in their own terms; for example, IRM attempts to delay resistance evolution, not to prevent it altogether (which would be impossible). Combined action and synergism of several toxins, however, not only provide advantages against pest resistance, but may also result in combined side-effects on non-target organisms (Then 2010; Hilbeck and Otto 2015), although such side effects are expected to be additive, as synergism has been claimed quite rare among the Cry proteins used in *Bt* crops (Walters et al. 2018). Field-evolved resistance in the corn rootworm and the European corn borer occurs in 6–7 years of application, particularly when singe Cry toxins are applied, and in the case of extended chemical pressure applied by preactivated Cry1Ab toxin (produced by *MON 810* maize),

resistance against Cry1Ab was found to be combined with cross-resistance to Cry2 toxins (Darvas 2011). In such cases, pest resistance triggered by *MON 810* maize renders the application of *Bt*-based bioinsecticides, such as Dipel, also ineffective.

Effects on Protected Insects (Lepidoptera)

Effects of *Bt* crops on non-target organisms, e.g. non-target insects, have to be considered in their ERA (Wolfenbarger and Phifer 2000; Marvier 2001; Darvas et al. 2004; Andow and Hilbeck 2004; O'Callaghan et al. 2005; Andow and Zwahlen 2006; Romeis et al. 2006, 2008; Lang et al. 2007; Lang and Otto 2010; Hilbeck et al. 2011). Practically all methods of pest control will have effects on non-target organisms as well, the most obvious of which being non-target toxicity of insecticides. The more specific an anti-insect agent is, the more favourable it potentially is in terms of non-target effects. Due to the insect specificity of Cry1Ab toxin produced by *MON 810* maize, only Lepidopteran insect species are at hazard. These species are, however, not limited to herbivorous insects feeding on *Bt* maize, as air-drifting maize pollen may settle on other plants, and insects feeding on those plants may become thus exposed by ingesting *Bt* maize pollen along with their food. Sublethal physiological symptoms (decreased larval, pupal and adult weight, delay in development) heighten mortality of the affected individuals and possibly their population.

Three ruderal weed species, frequently emerging on the perimeters of maize fields, the stinging nettle (*Urtica dioica*), the European dewberry (*Rubus caesius*) and Jimsonweed (*Datura stramonium*) were proven to have substantial pollen capture capacity of 328 ± 200, 431 ± 334 and 339 ± 266 pollen grains/cm^2, respectively. Protected Lepidopteran species in the Pannonian Biogeographical Region, potentially exposed to the pollen of *MON 810* maize were identified by comparing their habitat preferences with the pollen shedding period of maize. There exist 213 protected butterfly species in Hungary (the Pannonian Biogeographical Region), 50 of which occur in the perimeters of maize fields (Darvas et al. 2004). Thus, during pollination, larvae of the comma butterfly (*Polygonia c-album*), the peacock butterfly (*Nymphalis io*, earlier *Inachis io*), the red admiral (*Vanessa atalanta*) and the small tortoiseshell (*Aglais urticae*) feeding on stinging nettle; larvae of the cardinal (*Pandoriana pandora*), the lesser marbled fritillary (*Brenthis ino*), the niobe fritillary (*Argynnis niobe*) and the red underwing skipper (*Spialia sertorius*) feeding on the European dewberry; as well as larvae of the death's-head hawkmoth (*Acherontia atropos*) feeding on Jimsonweed were specified as species that suffer the greatest level of exposure to the pollen of *Bt* maize (Lauber 2011).

Cry1Ab toxin content in the pollen of certain *MON-810-6* varieties (DK-440-BTY) (0.5 ± 0.03 μg Cry1Ab preactivated toxin/g pollen, see above) caused mortality on the larvae of protected butterflies, including the peacock butterfly (*N. io*). Sensitivities (assessed by LC_{50} values against Dipel) of the larvae of the protected Lepidopteran species investigated to Cry1 toxin ranged between 1.9 and 15.1 μg/ml:

4.4 µg/ml of *N. io* in stage L1, significantly higher, 1.9 µg/ml in stage L2, 3.0–5.7 µg/ml in stages L3–L4 and slightly but significantly higher, 6.2 µg/ml in stage L5. Sensitivities of *N. c-album* and in *V. atalanta* in stage L1 were 1.7- and 3.5-fold lower than *N. io* in the same stage. Lepidopteran maize pest insects, the American bollworm (*H. armigera*) and the European corn borer (*O. nubilalis*) were 3.4–26.5-fold and 4.1–7.4-fold less sensitive in stages L1 and L2, respectively, than *N. io* in the same stages (Lauber 2011). The increased sensitivity of *N. io* was shown to be related to group behaviour of stage L1 larvae: mortality of lone larvae increase to 25–75% due to suppressed feeding in the absence of group stimuli (Lauber and Darvas 2009; Székács and Darvas 2012b), therefore, larval mortality due to consuming pollen containing Cry1Ab toxin triggers an avalanche-like effect that exaggerates mortality in larvae not lethally affected by Cry1Ab toxin but remaining solitary by the mortality of their groupmates. The exact extent of this effect could be ascertained by a detailed risk assessment as performed for the monarch butterfly (*Danaus plexippus*) in the USA (Sears et al. 2001). Major differences to the monarch butterfly case are, however, that the peacock butterfly, unlike the monarch butterfly, is a protected species, the habitat of which is safeguarded by law; and that the European corn borer is not a major pest in the Pannonian Biogeographical Region. On the basis of its outstanding sensitivity to Cry1Ab toxin, *N. io* was suggested as a model species for ERA of Cry1Ab (Lauber and Darvas 2009), which has later been implemented (Holst et al. 2013a, b; Fahse et al. 2018). As seen from the sensitivity data discussed, almost an order of magnitude difference in sensitivity to Cry1Ab occurs among larvae in various stages of protected butterflies, and larvae of pest insects are even less sensitive.

A strange sequel in light of the above has been that a mathematical model, authored by some of the members of the EFSA GMO Panel at that time (Perry et al. 2010), that analysed exposure of larvae of non-target species, e.g. *N. io* and *V. atalanta* to Cry toxins in four European countries, assumed larvae of *V. atalanta* and *N. io* equally susceptible to Cry1Ab. They cited Darvas et al. (2004) as a reference for such equitoxicity, even though the cited paper contains no data about species sensitivity. Lang et al. (2011) found that the incomplete and uncertain input data cause a higher uncertainty than indicated by Perry et al. (2010). In the mathematical model extended to non-target effects of Cry1F toxin in *Bt* maize pollen (Perry et al. 2012), the sensitivity of non-target insects has been considered purely on a theoretical basis, meanwhile the predictive power of a mathematical model rests on the certainty of its input data (species sensitivity in the current case), which cannot be speculative. Another flaw of the model is that it defines acceptable mortality thresholds, while no such thresholds apply to protected species in ERA. Pollen drifting from maize fields modifies habitat characteristics of protected species, which contradicts the Habitat Directive of the EU (EC 1992). The EFSA model (Perry et al. 2010, 2012; EFSA 2015) was later developed into the BtButTox model (Holst et al. 2013a, b) and the LepiX model (Fahse et al. 2018), but all these models, although lately became quite elaborated, rely on extrapolated data, while the only solid data measured on *N. io* are ours.

Effects on Soil-Borne Insects

A collembollan species (*Folsomia candida*) showed avoidance of stubble residues of *MON 810* maize (DK-440 BTY, "YieldGard"), but adapted to it upon longer exposure, and no relationship was found between physiological parameters and feeding history, except that insects feeding on *MON 810* maize stubble had lower egg and faecal pellet production, demonstrating that food selection is a key factor in population dynamics (Bakonyi et al. 2006, 2011). The results indicate that long-term feeding on maize containing Cry1Ab toxin does not appear to be harmful to this collembolan, and therefore, avoidance of *MON 810* maize as a food source may have been a result of the modified composition of the maize variety. *Bt* maize appears to be a less preferred and therefore probably a less usable food source for *F. candida* than the corresponding isogenic maize variety (DK-440). The data also illustrate that effects on soil-forming, decomposing microorganisms have not yet been sufficiently explored.

Effects on Toxinogenic and Arbuscular Fungi

Cry toxins may affect the production intensity of certain *Fusarium* mycotoxins by suppressing damage by insects serving as vectors for fungal infestation, with favourable health and economic consequences due to the hindrance of mycotoxin production (Wu 2006; Ostry et al. 2010; Folcher et al. 2010). The occurrence of *Fusarium* species, however, is only partially related to insect pest damage. Our corresponding studies also revealed that damage on *MON 810* maize cobs was caused predominantly by the cotton bollworm (*H. armigera*), where occasionally there occur insects surviving Cry1Ab toxin exposure, although *Fusarium* infestation is not transmitted in all cases (Darvas et al. 2011). By suppressing fungal infection by insect damage, the production of fumonisin B1 substantially decreased in *DAS-59122-7* maize (Bánáti et al. 2017).

The effect of Cry34/35Ab1 binary toxins produced by *DAS-59122-7* maize on the mycorrhizal colonisation on the roots by arbuscular mycorrhizal (AM) fungi was studied during the entire vegetation period (Seres et al. 2014). Statistically significantly (27–37%) reduced initial hyphal, arbuscule and arbuscular mycorrhizal colonisation was recorded on the root of the *DAS-59122-7* maize variety than in the control for up to 60 days after planting under field cropping conditions, but the effect vanished later (80–140 days), as the intensity of the arbuscular infection increased over time during plant maturation. In contrast, no reduction in vesicle colonisation was seen. The influence of GM crops on AM fungi is further discussed in chapter "Impact of Genetically Modified Crops on the Biodiversity of Arbuscular Mycorrhizal Fungi" of this book.

Tritrophic Assessment of *Bt* Maize

To test non-target effects by Cry toxins exerted through the food chain, physiological parameters of a parasitoid and a predator insect preying on non-target herbivores were tested in tritrophic bioassays. The assay design allowed assessment of the effects of indirect exposure of the non-target parasitoid or predator to Cry toxins through prey. In the case of the tritrophic study with a predator insect, direct exposure though pollen could also be evaluated.

In a tritrophic assessment setup upon exposure to *MON 810* maize, survival and development parameters of a product storage pest, the maize weevil (*Sitophilus zeamais*) and its natural enemy the ectoparasitoid pteromalid wasp *Lariophagus distinguendus*, used in biological control of weevils, were assessed (Hansen et al. 2013). Preactivated Cry1Ab toxin content in the maize did not significantly affect emergence rates or development time of the maize weevil, but the body mass of the adult females that fed on *MON 810* maize was moderately (2–6%), but statistically significantly higher than the control (isogenic line) in the absence of the parasitoid. This can result in increasing reproduction rate of the weevil population through increased fecundity of the larger females. The presence of the parasitoid with a preference to larger females as hosts for oviposition can counterbalance this effect. No significant differences were observed in the development time, body size, sex ratio or wing length of the emerging adult parasitoids; however, significantly (approximately 40%) fewer female parasitoids emerged from the treatment with *MON 810* maize than the control. Thus, tritrophic effects of transgenic maize on this parasitoid were demonstrated.

In another study with *DAS-59122-7* maize, long-term effects on the fecundity and fertility of the seven-spotted ladybird (*Coccinella septempunctata*) preying on the bird cherry-oat aphid (*Rhopalosiphum padi*) was assessed (Takács et al. 2010, 2012b). No significant differences were observed in the sex ratio, fecundity or fertility parameters of the predator, but the average weight of adult *C. septempunctata* that developed and preyed on *R. padi* feeding on DAS-59122-7 maize was significantly (11–29%) lower than in the control (isogenic line). This has been seen separately for both females and males, females being uniformly 20–24% larger than males both in the treatment and the control groups. When, however, three other commercial maize hybrids were also considered (beyond the isogenic line) in the control, this significant difference disappeared in the standard deviation of the four controls (isogenic line + three commercial hybrids).

Similarly to direct non-target toxicity of insecticides, tritrophic effects are also inevitable outcomes of pest control. Tritrophic effects in the first study on the ectoparasitoid wasp *L. distinguendus* are not necessarily direct consequences of the composition or property of the *Bt* crop itself, but may be attributed to the effective pest control resulting in a decrease in the prey population. Nonetheless, in the second study on *C. septempunctata*, the effect appears to be more related to crop composition as the predator insect had access to ad libitum feeding.

Legislatory Measures

On the basis of the early results of the above studies, a safeguard clause moratorium was announced in Hungary on the cultivation of *MON 810* GM maize (Ministry of Agriculture, Hungary 2005; Darvas and Székács 2011). This has met the criticism of EFSA, and the Hungarian environmental authority (along with corresponding authorities of Greece and Austria) was requested to a hearing by the GMO Panel of EFSA. Delegated by the Hungarian Ministry of the Environment and Water, three researchers, Prof. András Székács (author of this summary), Prof. Béla Darvas and Prof. Gábor Bakonyi presented results of their research groups on 11 June 2008 in Parma, Italy on environmental analysis, protected lepidopteran species and soil biology, respectively. No substantial rebuttal was expressed by the GMO Panel on the hearing and afterwards, yet no acceptance occurred, either. In contrast, EFSA maintained its position regarding ERA of *MON 810*, and Hungary renewed its moratorium on *MON 810*, and the number of European countries announcing such moratoria, joining GMO-free regions or opting out (at least regionally) of GM crop cultivation gradually rose to 19.

Legislation of *MON 810* maize gained recent actuality in the EU, where products containing this *Bt* maize variety have valid authorisation for food and feed purposes until 2027 (EC 2017), but re-registration for public cultivation of this genetic event is pending, while EFSA's position is supportive both in its scientific opinion statement (EFSA 2012) and assessment of its post-market environmental monitoring (EFSA 2019). In this context, ERA of *Bt* crops by EFSA has been criticised for underestimating exposure via pollen deposition (Maren Kruse-Plass et al. 2017) and for relying in some cases on experimental data of deficient or improper ecological relevance in impact assessment on honeybees and earthworms (Chátalová 2019).

A long-discussed issue in the scientific literature has been whether *Bt* crops comply with the principles of IPM. The use of crop cultivars tolerant or resistant to plant diseases, pests or stress factors has is definite preventive approach in IPM practices, and *Bt* plants as IR crop varieties have been argued on this basis to be compatible with IPM. *Bt* crops produce foreign substances that (or close derivatives of which) are registered insecticide active ingredients, therefore, their protection mechanism against the pest does not differ fundamentally from chemical pest control. Instead, these crops can be considered as "pesticides" formulated in the biological plant material. This has been reflected in the reassessment of *Bt* crops by US EPA, where the transgenic toxin was termed "plant-incorporated protectant" (Mendelsohn et al. 2003). There is, however, an essential element in IPM *Bt* crops cannot fulfil: the main ecological principle of IPM is that any protection measure should be initiated and timed only to periods, when pest damage exceeds a critical threshold, and *Bt* crops cannot comply with this requirement as they produce the toxin protein in their entire vegetation period, regardless of the pest population density. In addition, Cry toxin production and the corresponding (bio)chemical load on the environment is quite unfavourable in both *MON 810* and *DAS 59122–7* maize varieties, as the toxin proteins are produced in the highest concentration and amount in the foliage (leaves)

of the plant, and not in the organs, where they produce crop protective effect (stem and root, respectively). Thus, *MON 810* and *DAS 59122–7* maize varieties produce their corresponding transgenic Cry toxin proteins in 7–8- and 35–46-fold higher amount, than the technologically utilised quantity, respectively.

One could argue that some (but not all) potential hazards or risks associated with *Bt* bioinsecticides or *Bt* crops are not specific to these technologies or regulated products, but are posed by all forms of pest control. This reasoning would be valid from the aspect that, indeed, all technologies affect their working environment, and the question is whether those effects would still allow sustainability. Such a notion could even lead to a number of philosophical questions. One of these is that ideally, regulation should be technology-neutral: equivalent safety regulation criteria would preferably be applied in different segments of industrial activities. This expectation is, however, currently unrealistic as perceptibly different safety requirements apply to various sectors, due to societal consensus, allowing certain technologies that would be considered hazardous operation in other industrial segments. Another fundamental question clearly reaching far beyond the scope of this report is how essentially the principles of agroecology should be considered in assessing sustainability of intensive agriculture. Yet another basic question could be whether the precautionary principle implemented in risk assessment in the EU is reasonable, as excessive precaution prevents the benefits of the technology (Zilberman et al. 2018). Beyond my conviction that it is reasonable, as established in its concept, implementation and normative standardisation (Myhr 2010), this is certainly not a point to be considered at the level discussed here. Hazard identification and risk assessment relates to given technologies, and should not depend on the safety of other technologies: decision-making on the basis of comparative analysis of various technologies is a part of risk management.

As seen from the above, although *Bt* toxins in insect control are environmentally more benign than broad-spectrum insecticides, and economic and social benefits of *Bt* crops have been highlighted (US National Research Council 2010; Dively et al. 2018), concerns regarding environmental effects of *Bt* crops have also been raised, and the lack of consensus on their safety has been published (Hilbeck et al. 2015) and has also been evidenced by the UN Cartagena Biosafety Protocol and the Guidelines of the *Codex Alimentarius*. To address the environmental and socio-economic risk assessment interface, a European Network for systematic GMO impact assessment (ENSyGMO) has been proposed to enhance ERA and post-market environmental monitoring of GM (including *Bt*) crops (Graef et al. 2011). Nonetheless, such concerns, accentuated by the precautionary principle of the EU, apply not only to transgenic GMOs, but also in case-by-case assessment to *Bt* technology applied in combination with RNA interference (RNAi) (Heinemann et al. 2013; Head et al. 2017) and to products of emerging biotechnologies including genome editing (Székács 2016), and it remains questionable whether currently dominant bioeconomy solutions do indeed represent a step towards the ultimate development goal of truly sustainable ecocycles (Székács 2017).

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References

Andow DA, Hilbeck A (2004) Science-based risk assessment for nontarget effects of transgenic crops. Bioscience 54(7):637–649. https://academic.oup.com/bioscience/article-abstract/54/7/637/223525

Andow DA, Zwahlen C (2006) Assessing environmental risks of transgenic plants. Ecol Lett 9(2):196–214. https://doi.org/10.1111/j.1461-0248.2005.00846.x

Bakonyi G, Szira F, Kiss I, Villányi I, Seres A, Székács A (2006) Preference tests with collembolas on isogenic and Bt-maize. Eur J Soil Biol 42:S132–S135. https://doi.org/10.1016/j.ejsobi.2006.06.005

Bakonyi G, Dolezsai A, Mátrai N, Székács A (2011) Long-term effects of Bt-maize (*MON810*) consumption on the collembolan *Folsomia candida*. Insects 2(2):243–252. https://doi.org/10.3390/insects2020243

Bánáti H, Székács A, Fehér-Tóth S, Czéh Á, Darvas B (2017) Determination of mycotoxin production of *Fusarium graminearum* and *Fusarium verticillioides* in genetically modified maize varieties by quantitative flow immunocytometry. Toxins 9(2):70. https://doi.org/10.3390/toxins9020070

Benbrook CM (2012) Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Environ Sci Eur 24:24. https://doi.org/10.1186/2190-4715-24-24

Bravo A, Soberón M, Gill SS (2018) *Bacillus thuringiensis*: mechanisms and use. Comp Mol Insect Sci 6:175–205. https://doi.org/10.1016/B978-0-12-809633-8.04071-1

Brookes G (2019) Twenty-one years of using insect resistant (GM) maize in Spain and Portugal: farm-level economic and environmental contributions. GM Crops Food 10(2):90–101. https://doi.org/10.1080/21645698.2019.1614393

Brookes G, Barfoot P (2017) Farm income and production impacts of using GM crop technology 1996–2015. GM Crops Food 8(3):156–193. https://doi.org/10.1080/21645698.2017.1317919

Carzoli AK, Aboobucker SI, Sandall LL, Lübberstedt TT, Suza WP (2018) Risks and opportunities of GM crops: Bt maize example. Glob Food Secur 19:84–91. https://doi.org/10.1016/j.gfs.2018.10.004

Catarino R, Areal F, Park J, Parisey N (2019) Spatially explicit economic effects of non-susceptible pests' invasion on Bt maize. Agric Syst 175:22–33. https://doi.org/10.1016/j.agsy.2019.05.008

Chátalová V (2019) A critical evaluation of EFSA's environmental risk assessment of genetically modified maize MON810 for honeybees and earthworms. Environ Sci Eur 31:52. https://doi.org/10.1186/s12302-019-0238-5

Clark BW, Phillips TA, Coats JR (2005) Environmental fate and effects of *Bacillus thuringiensis* (Bt) proteins from transgenic crops: a review. J Agric Food Chem 53:4643–4653. https://doi.org/10.1021/jf040442k

Clive J (2017) Global status of commercialized biotech/GM crops in 2017. ISAAA briefs no. 53. International Service for the Acquisition of Agri-biotech Applications (ISAAA), New York. https://www.isaaa.org/resources/publications/briefs/53/download/isaaa-brief-53-2017.pdf

Darvas B (2011) GM plants and resistance – resistance-management. In: Darvas B, Székács A (eds) Hungarian background on views of 1st generation genetically modified plants. Agricultural Committee of the Hungarian Parliament, Budapest, pp 140–141. http://www.kormany.hu/download/2/9d/20000/GenetEM.pdf

Darvas B, Székács A (eds) (2011) Hungarian background on views of 1st generation genetically modified plants. Agricultural Committee of the Hungarian Parliament, Budapest, pp 108–213. ISBN:978-963-9848-44-3. http://mek.oszk.hu/09900/09933/index.phtml

Darvas B, Csóti A, Gharib A, Peregovits L, Ronkay L, Lauber É, Polgár AL (2004) Some data to the risk analysis of Bt -corn pollen and protected Lepidoptera species in Hungary. Növényvédelem 40:441–449. (in Hungarian)

Darvas B, Bánáti H, Takács E, Lauber É, Szécsi Á, Székács A (2011) Relationships of *Helicoverpa armigera*, *Ostrinia nubilalis* and *Fusarium verticillioides* on *MON 810* maize. Insects 2(1):1–11. https://doi.org/10.3390/insects2010001

Dively GP, Venugopal PD, Bean D, Whalen J, Holmstrom K, Kuhar TP, Doughty HB, Patton T, Cissel W, Hutchison WD (2018) Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. Proc Natl Acad Sci U S A 115(13):3320–3325. https://doi.org/10.1073/pnas.1720692115

EC (European Commission) (2013) Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Text with EEA relevance. Off J Eur Community L93(3.4.2013):1–84. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0283

EC (European Commission) (2017) Commission implementing regulation (EU) 2017/1207 of 4 July 2017 of renewing the authorisation for the placing on the market of genetically modified maize MON 810 (MON-ØØ81Ø-6) products pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council. Off J Eur Community L173:18–22. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017D1207

EC (European Council) (1990) Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. Off J Eur Community L117(8.5.1990):15–27. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31990L0220

EC (European Council) (1992) Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Off J Eur Community L206(22):1–7. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A31992L0043

EC (European Council) (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Off J Eur Community L106(17.4.2001):1–39. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32001L0018

EC (European Council) (2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Off J Eur Community L309(24):11.2009):1–11.2009)50. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009R1107

EFSA (European Food Safety Authority) (2012) Scientific opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. EFSA J 10(12):3017. https://doi.org/10.2903/j.efsa.2012.3017

EFSA (European Food Safety Authority) (2015) Updating risk management recommendations to limit exposure of non-target Lepidoptera of conservation concern in protected habitats to Bt-maize pollen. EFSA J 13(7):4127. https://doi.org/10.2903/j.efsa.2015.4127

EFSA (European Food Safety Authority) (2016) Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA J 14(7):4524. https://doi.org/10.2903/j.efsa.2016.4524

EFSA (European Food Safety Authority) (2019) Assessment of the 2017 post-market environmental monitoring report on the cultivation of genetically modified maize MON 810. EFSA J 17(6):5742. https://doi.org/10.2903/j.efsa.2019.5742

Fahse L, Papastefanou P, Otto M (2018) Estimating acute mortality of Lepidoptera caused by the cultivation of insect-resistant Bt maize – the LepiX model. Ecol Model 371:50–59. https://doi.org/10.1016/j.ecolmodel.2018.01.006

Fejes Á, Takács E, Fekete G, Darvas B, Ferguson BS, Saxena D, Székács A (2011) Aquatic effect study of Cry4 toxin with immunoassay and *Aedes aegypti* larval biotest. Aquat Insects 34(Suppl.1):207–222. https://doi.org/10.1080/01650424.2012.643062

Folcher L, Delos M, Marengue E, Jarry M, Weissenberger A, Eychenne N, Regnault-Roger C (2010) Lower mycotoxin levels in Bt maize grain. Agron Sustain Dev 30(4):711–719. https://doi.org/10.1051/agro/2010005

Gatehouse AMR, Ferry N, Edwards MG, Bell HA (2011) Insect-resistant biotech crops and their impacts on beneficial arthropods. Philos Trans R Soc Lond Ser B Biol Sci 366(1569):1438–1452. https://doi.org/10.1098/rstb.2010.0330

Government of Hungary (1998) Act XXVII of 1998 on gene technology activities. https://gmo.kormany.hu/download/6/36/70000/1998-27-gentorveny-2013-6-28-tol_EN.pdf

Graef F, Römbke J, Binimelis R, Myhr AI, Hilbeck A, Breckling B, Dalgaard T, Stachow U, Catacora-Vargas G, Bøhn T, Quist D, Darvas B, Dudel G, Oehen B, Meyer H, Henle K, Wynne B, Metzger MJ, Knäbe S, Settele J, Székács A, Wurbs A, Bernard J, Murphy-Bokern D, Buiatti M, Giovanetti M, Debeljak M, Andersen E, Paetz A, Dzeroski S, Tappeser B, van Gestel CAM, Wosniok W, Séralini G-E, Aslaksen J, Pesch R, Maly S, Doerpinghaus A, Werner A (2011) A framework for a European Network for a systematic environmental impact assessment of genetically modified organisms (GMO). BioRisk 7:73–97. https://doi.org/10.3897/biorisk.7.1969

Grothaus GD, Bandla M, Currier T, Giroux R, Jenkins GR, Lipp M, Shan G, Stave JW, Pantella V (2006) Immunoassay as an analytical tool in agricultural biotechnology. J AOAC Int 89(4):913–928. https://aoac.publisher.ingentaconnect.com/contentone/aoac/jaoac/2006/00000089/00000004/art00002

Hansen LS, Lövei GL, Székács A (2013) Survival and development of a stored product pest, *Sitophilus zeamais* (Coleoptera: Curculionidae) and its natural enemy, the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) on transgenic Bt-maize. Pest Manag Sci 69(5):602–606. https://doi.org/10.1002/ps.3410

Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, Storer NP, Flannagan RD, Samuel LW, Meinke LJ (2017) Evaluation of SmartStax and SmartStaxPRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest Manag Sci 73(9):1883–1899. https://doi.org/10.1002/ps.4554

Heinemann JA, Agapito-Tenfen SZ, Carman JA (2013) A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Int 55:43–55. https://doi.org/10.1016/j.envint.2013.02.010

Hilbeck A, 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. https://doi.org/10.3389/fenvs.2015.00071

Hilbeck A, Meier M, Römbke J, Jänsch S, Teichmann H, Tappeser B (2011) Environmental risk assessment of genetically modified plants – concepts and controversies. Environ Sci Eur 23:13. https://doi.org/10.1186/2190-4715-23-13

Hilbeck A, Binimelis R, Defarge N, Steinbrecher R, Székács A, Wickson F, Antoniou M, Bereano PL, Clark EA, Hansen M, Novotny E, Heinemann J, Meyer H, Shiva V, Wynne B (2015) No scientific consensus on GMO safety. Environ Sci Eur 27:4. https://doi.org/10.1186/s12302-014-0034-1

Holst N, Lang A, Lövei GL, Otto M (2013a) Corrigendum to "Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland". Ecol Model 265:126–133. 250. https://doi.org/10.1016/j.ecolmodel.2013.06.023

Holst N, Lang A, Lövei GL, Otto M (2013b) Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland. Ecol Model 250:126–133. https://doi.org/10.1016/j.ecolmodel.2012.11.006

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt TE, Wright RJ, Pecinovsky K, Rabaey TL, Flood BR, Raun ES (2010) Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330(6001):222–225. https://doi.org/10.1126/science.1190242

Kaur S (2000) Molecular approaches towards development of novel *Bacillus thuringiensis* biopesticides. World J Microbiol Biotechnol 16:781–793. https://doi.org/10.1023/A:1008931207374

Kruse-Plass M, Hofmann F, Kuhn U, Otto M, Schlechtriemen U, Schröder B, Vögel R, Wosniok W (2017) Reply to the EFSA (2016) on the relevance of recent publications (Hofmann et al. 2014, 2016) on environmental risk assessment and management of Bt-maize events (MON810, Bt11 and 1507). Environ Sci Eur 29:12. https://doi.org/10.1186/s12302-017-0106-0

Lang A, Otto M (2010) A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target Lepidoptera. Entomol Exp Appl 135:121–134. https://doi.org/10.1111/j.1570-7458.2010.00981.x

Lang A, Lauber É, Darvas B (2007) Early tier tests insufficient for GMO risk assessment. Nat Biotechnol 25:35–36. https://doi.org/10.1038/nbt0107-35

Lang A, Brunzel S, Dolek M, Otto M, Theißen B (2011) Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of Bt -maize effects. Proc R Soc B 278:980–981. https://doi.org/10.1098/rspb.2010.2085

Lang A, Lee M, Dolek M, Berchtold J, Otto M (2019) Laboratory tests with Lepidoptera to assess non-target effects of *Bt* maize pollen: analysis of current studies and recommendations for a standardised design. Environ Sci Eur 31:39. https://doi.org/10.1186/s12302-019-0220-2

Latham JR, Love M, Hilbeck A (2017) The distinct properties of natural and GM cry insecticidal proteins. Biotechnol Genet Eng Rev 33(1):62–96. https://doi.org/10.1080/02648725.2017.1357295

Lauber É (2011) Cry1 toxin content of *MON 810* Bt-corn and the effect of its pollen on protected butterfly species in Hungary. PhD thesis, Corvinus University, Budapest, Hungary. http://phd.lib.uni-corvinus.hu/598

Lauber É, Darvas B (2009) Increased mortality of isolated first instar larvae of *Inachis io* (Lepidoptera). Acta Phytopath Entomol Hung 44:111–117. https://doi.org/10.1556/APhyt.44.2009.1.11

Marvier M (2001) Ecology of transgenic crops. Am Sci 89(2):160–167. https://www.jstor.org/stable/27857439

Matthews GA, Bateman R, Miller P (2014) Pesticide application methods. Wiley, Chichester, p 24. ISBN:978-1-118-35130-7

Mendelsohn M, Kough J, Vaituzis Z, Matthews K (2003) Are Bt crops safe? Nat Biotechnol 21(9):1003–1009. https://doi.org/10.1038/nbt0903-1003

Ministry of Agriculture, Hungary (2005) The safeguard clause on the cultivation of *MON810* genetically modified maize. https://gmo.kormany.hu/safeguard-clauses-currently-in-effect-in-hungary

Myhr AI (2010) A precautionary approach to genetically modified organisms: challenges and implications for policy and science. J Agric Environ Ethics 23(6):501–525. https://doi.org/10.1007/s10806-010-9234-x

O'Callaghan M, Glare TR, Burgess EPJ, Malone LA (2005) Effects of plants genetically modified for insect resistance on nontarget organisms. Annu Rev Entomol 50:271–292. https://doi.org/10.1146/annurev.ento.50.071803.130352

Ostry V, Ovesna J, Skarkova J, Pouchova V, Ruprich J (2010) A review on comparative data concerning Fusarium mycotoxins in Bt maize and non-Bt isogenic maize. Mycotoxin Res 26:141–145. https://doi.org/10.1007/s12550-010-0056-5

Palma L, Muñoz D, Berry C, Murillo J, Caballero P (2014) *Bacillus thuringiensis* toxins: an overview of their biocidal activity. Toxins 6(12):3296–3325. https://doi.org/10.3390/toxins6123296

Pedersen SM, Lind KM (2017) Precision agriculture – from mapping to site-specific application. In: Pedersen SM, Lind KM (eds) Precision agriculture: technology and economic perspectives. Springer, Cham, pp 9–18. ISBN:978-3-319-68713-1

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB (2010) A mathematical model of exposure of nontarget Lepidoptera to *Bt* -maize pollen expressing Cry1Ab within Europe. Proc R Soc B 277:1417–1425. https://doi.org/10.1098/rspb.2009.2091

Perry JN, Devos Y, Arpaia S, Bartsch D, Ehlert C, Gathmann A, Hails RS, Hendriksen NB, Kiss J, Messéan A, Mestdagh S, Neemann G, Nuti M, Sweet JB, Tebbe CC (2012) Estimating the effects of Cry1F *Bt* -maize pollen on non-target Lepidoptera using a mathematical model of exposure. J Appl Ecol 49:29–37. https://doi.org/10.1111/j.1365-2664.2011.02083.x

Pimentel D (1995) Amounts of pesticides reaching target pests: environmental impacts and ethics. J Agric Environ Ethics 8(1):17–29. https://doi.org/10.1007/BF02286399

Raymond B, Federici BA (2017) In defence of *Bacillus thuringiensis*, the safest and most successful microbial insecticide available to humanity – a response to EFSA. FEMS Microbiol Ecol 93(7):fix084. https://doi.org/10.1093/femsec/fix084

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

Romeis J, Bartsch D, Bigler F, Candolfi MP, Gielkens MMC, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD (2008) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat Biotechnol 26:203–208. https://doi.org/10.1038/nbt1381

Royal Society (2016) GM plants. Questions and answers. Royal Society, London. https://royalsociety.org/~/media/policy/projects/gm-plants/gm-plant-q-and-a.pdf

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

Sanchis V (2011) From microbial sprays to insect-resistant transgenic plants: history of the biospesticide *Bacillus thuringiensis*. A review. Agron Sustain Dev 31:217–231. https://doi.org/10.1051/agro/2010027

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean HD (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev 62:775–806. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC98934

Sears MK, Hellmich RL, Stanley-Horn DE, Oberhauser KS, Pleasants JM, Mattila HR, Siegfried BD, Dively GP (2001) Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment. Proc Natl Acad Sci U S A 98(21):11937–11942. https://doi.org/10.1073/pnas.211329998

Seres A, Kiss I, Nagy P, Sály P, Darvas B, Bakonyi G (2014) Arbuscular mycorrhizal fungi colonisation of *Cry3* toxin-producing *Bt* maize and near isogenic maize. Plant Soil Environ 60(12):569–573. https://doi.org/10.17221/674/2014-PSE

Székács A (2013) Cry1Ab toxin quantification in MON 810 maize. In: Breckling B, Verhoeven R (eds) GM-crop cultivation – Ecological effects on a landscape scale. Theorie in der Ökologie, vol 17. Peter Lang GmbH, Frankfurt, pp 123–124. ISBN:9783631628706

Székács A (2016) New technologies in agricultural biotechnology. Ecocycles 2(2):54–56. https://doi.org/10.19040/ecocycles.v2i2.65

Székács A (2017) Environmental and ecological aspects in the overall assessment of bioeconomy. J Agric Environ Ethics 30(1):153–170. https://doi.org/10.1007/s10806-017-9651-1

Székács A, Darvas B (2012a) Comparative aspects of Cry toxin usage in insect control. In: Ishaaya I, Palli SR, Horowitz R (eds) Advanced technologies for managing insect pests. Springer, Berlin, pp 195–230. https://doi.org/10.1007/978-94-007-4497-4_10

Székács A, Darvas B (2012b) Environmental assessment of *MON 810* maize in the Pannonian Biogeographical Region. Acta Phytopathol Entomol Hung 47(2):307–320. https://doi.org/10.1556/APhyt.47.2012.2.11

Székács A, Juracsek J, Polgar LA, Darvas B (2005) Levels of expressed Cry1Ab toxin in genetically modified corn DK-440-BTY (YIELDGARD) and stubble. FEBS J 272(Suppl.1):508

Székács A, Lauber É, Juracsek J, Darvas B (2010a) Cry1Ab toxin production of MON 810 transgenic maize. Environ Toxicol Chem 29(1):182–190. https://doi.org/10.1002/etc.5

Székács A, Lauber É, Takács E, Darvas B (2010b) Detection of Cry1Ab toxin in the leaves of *MON 810* transgenic maize. Anal Bioanal Chem 396(6):2203–2211. https://doi.org/10.1007/s00216-009-3384-6

Székács A, Weiss G, Quist D, Takács E, Darvas B, Meier M, Swain T, Hilbeck A (2012) Interlaboratory comparison of Cry1Ab toxin quantification in *MON 810* maize by enzyme-immunoassay. Food Agric Immunol 23(2):99–121. https://doi.org/10.1080/09540105.2011.604773

Tabashnik BE (2010) Communal benefits of transgenic corn. Science 330(6001):189–190. https://doi.org/10.1126/science.1196864

Tabashnik BE, Carrière Y (2017) Surge in insect resistance to transgenic crops and prospects for sustainability. Nat Biotechnol 35(10):926–935. https://doi.org/10.1038/nbt.3974

Tabashnik BE, Finson N, Groeters FR, Moar WJ, Johnson MW, Luo K, Adang MJ (1994) Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. Proc Natl Acad Sci U S A 91(10):4120–4124. https://doi.org/10.1073/pnas.91.10.4120

Tabashnik BE, Brévault T, Carrière Y (2013) Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotechnol 31(6):510–521. https://doi.org/10.1038/nbt.2597

Takács E, Bánáti H, Fónagy A, Darvas B (2010) Short term study of DAS-59122-7 maize on L1 and L2 larvae of seven-spotted ladybird (*Coccinella septempunctata*) feeding on the bird cherry-oat aphid (*Rhopalosiphum padi*). Cereal Res 59(Suppl):625–628. https://doi.org/10.1556/Novenyterm.59.2010.Suppl.6

Takács E, Juracsek J, Fónagy A, Székács A (2011) Monitoring Cry34Ab1 and Cry35Ab1 protein concentrations in transgenic maize (DAS-59122-7). In: Abstract book of the 5th EIGMO meeting. Ecological impact of genetically modified organisms – IOBC/wprs Working Group meeting, Česke Budějovice, Czech Republic, July 22–25, 2011. Biology Centre ASCR v.v.i., Prague, Czech Republic. ISBN:978-80-86668-14-7. https://www.iobc-wprs.org/expert_groups/Abstract-book-EIGMO-2011.pdf

Takács E, Darvas B, Székács A (2012a) Analytical difficulties and certain biological aspects of Cry1Ab toxin determination in *MON 810* genetically modified maize. Acta Phytopathol Entomol Hung 47(2):293–306. https://doi.org/10.1556/APhyt.47.2012.2.10

Takács E, Fónagy A, Juracsek J, Kugler N, Székács A (2012b) Characterisation of tritrophic effects of *DAS-59122-7* maize on seven-spotted ladybird (*Coccinella septempunctata*) feeding on the bird cherry-oat aphid (*Rhopalosiphum padi*). IOBC/wprs Bull 73:121–134. ISBN:978-92-9067-250-0

Takács E, Nagy A, Gelencsér É, Székács A (2015) Internal quality control of an enzyme-linked immunoassay for Cry1Ab toxin detection applied in animal tissues. Acta Aliment Hung 44(4):593–600. https://doi.org/10.1556/066.2015.44.0032

Then C (2010) Risk assessment of toxins derived from *Bacillus thuringiensis* – synergism, efficacy, and selectivity. Environ Sci Pollut Res 17(3):791–797. https://doi.org/10.1007/s11356-009-0208-3

US National Research Council (2010) The impact of genetically engineered crops on farm sustainability in the United States. National Academies Press, Washington, DC. ISBN:978-0-309-14708-8. https://www.nap.edu/catalog/12804/the-impact-of-genetically-engineered-crops-on-farm-sustainability-in-the-united-states

Walters FS, Graser G, Burns A, Raybould A (2018) When the whole is not greater than the sum of the parts: a critical review of laboratory bioassay effects testing for insecticidal protein interactions. Environ Entomol 47(2):484–497. https://doi.org/10.1093/ee/nvx207

Wolfenbarger LL, Phifer PR (2000) The ecological risks and benefits of genetically engineered plants. Science 290:2088–2093. https://doi.org/10.1126/science.290.5499.2088

Wu F (2006) Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. Transgenic Res 15:277–289. https://doi.org/10.1007/s11248-005-5237-1

Zilberman D, Holland TG, Trilnick I (2018) Agricultural GMOs – what we know and where scientists disagree. Sustainability 10(5):1514. https://doi.org/10.3390/su10051514