



Genetically engineered crops help support conservation biological control

Jörg Romeis^{a,*}, Steven E. Naranjo^b, Michael Meissle^a, Anthony M. Shelton^c

^a Research Division Agroecology and Environment, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland

^b USDA-ARS, Arid-Land Agricultural Research Center, 21881 N. Cardon Lane, Maricopa, AZ 85138, USA

^c Department of Entomology, Cornell University, 630 W. North St., New York State Agricultural Experiment Station, Geneva, NY 14456, USA

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ABSTRACT

Genetically engineered (GE) crops producing insecticidal proteins from *Bacillus thuringiensis* (Bt) (mainly Cry proteins) have become a major control tactic for a number of key lepidopteran and coleopteran pests, mainly in maize, cotton, and soybean. As with any management tactic, there is concern that using GE crops might cause adverse effects on valued non-target species, including arthropod predators and parasitoids that contribute to biological control. Such potential risks are addressed prior to the commercial release of any new GE plant. Over the past 20+ years, extensive experience and insight have been gained through laboratory and field-based studies of the non-target effects of crops producing Cry proteins. Overall, the vast majority of studies demonstrates that the insecticidal proteins deployed today cause no unintended adverse effects to natural enemies. Furthermore, when Bt crops replace synthetic chemical insecticides for target pest control, this creates an environment supportive of the conservation of natural enemies. As part of an overall integrated pest management (IPM) strategy, Bt crops can contribute to more effective biological control of both target and non-target pests. The growing use of insecticidal seed treatments in major field crops (Bt or not) may dampen the positive gains realized through reductions in foliar and soil insecticides. Nonetheless, Bt technology represents a powerful tool for IPM.

1. Introduction

Biological control is a cornerstone of Integrated Pest Management (IPM) and plays an important role in the sustainable and economic suppression of arthropod pest populations (Bale et al., 2008; Naranjo et al., 2015). The global value of biological control (trophic regulation of populations) has been estimated at \$617/ha (2018 dollars) (Constanza et al., 1997). For pest control provided by natural enemies in the USA alone, a value of \$5.9 billion (2018 dollars) has been estimated (Losey and Vaughn, 2006), a value that is regarded as extremely conservative (Landis et al., 2008). The importance of biological control for sustainable agricultural production is widely recognized and biological control is regarded as an important regulating service in the Millennium Ecosystem Assessment (MEA, 2005).

Biological pest control comprises different “tactics” including augmentative or inundative control, which requires an initial or repeated release of natural enemies, and classical biological control in which exotic natural enemies are introduced, mainly to manage invasive pests (Heimpel and Mills, 2017). Conservation biological control, in contrast, takes advantage of resident natural enemies and involves management strategies to conserve their populations and the services they provide.

Two general approaches are followed. One involves habitat manipulations to increase the abundance and activity of natural enemies (Landis et al., 2000), because natural enemies have been shown to benefit from increased landscape complexity (Bianchi et al., 2006; Chaplin-Kramer et al., 2011; Veres et al., 2013). The second focuses on reducing use of control tactics, such as insecticides, that may harm natural enemies. New molecular tools provide opportunities for the development of genetically engineered (GE) pest-resistant crops that control key pests and require less input of foliar and soil insecticides (Brookes and Barfoot, 2018; Gurr and You, 2016; Klümper and Qaim, 2014; Naranjo, 2011). The first GE crops developed in the late 1980s expressed insecticidal proteins (Cry) from the bacterium *Bacillus thuringiensis* Berliner (Bt) because of their known specificity and the excellent safety record of microbial Bt formulations (Fischhoff et al., 1987; Perlak et al., 1990; Meadows, 1993). Pest-resistant Bt plants are now widely used on a global scale (ISAAA, 2017). There is evidence that Bt crops can reduce target pest populations over broad scales (Carrière et al., 2003; Dively et al., 2018; Hutchison et al., 2010; Wan et al., 2012; Wu et al., 2008; Zhang et al., 2018) resulting in reduced damage on both GE and non-GE crops in the region. In addition, they have been shown to promote biological pest control in the system, if foliar insecticides are reduced

* Corresponding author.

E-mail address: joerg.romeis@agroscope.admin.ch (J. Romeis).

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(Lu et al., 2012; Zhang et al., 2018).

Host-plant resistance, whether developed through traditional breeding practices or genetic engineering, is an important tactic to protect crops against arthropod pests (Smith, 2005). Together with biological control, host plant resistance forms the foundation of sustainable IPM programs (Kennedy, 2008; Smith, 2005; Tingey and Steffens, 1991). Mechanisms of resistance can be categorized as constitutive or inducible and direct or indirect defenses. Direct defenses can be chemical (e.g., toxicants) or physical attributes (e.g., trichomes) and are defined by having a direct impact on the herbivore by negatively affecting its important life-history parameters or by deterring adult oviposition (Hagenbucher et al., 2013; Price et al., 1980; Sabelis et al., 1999). In contrast, indirect defenses act by enhancing the effectiveness of natural enemies of the attacking herbivore. Examples are the emission of volatiles that are used by natural enemies to find their hosts/prey and the provision of food (e.g., extrafloral nectaries) (Turlings and Wäckers, 2004).

Plant characteristics that affect herbivores may also directly or indirectly affect their natural enemies. Studies on plant-herbivore-natural enemy interactions reveal that plant defense traits can have negative, positive, or neutral effects on natural enemies (Boethel and Eikenbary, 1986; Hare, 2002; Ode, 2006; Peterson et al., 2016b; Price et al., 1980). The tools of genetic engineering have provided a novel and powerful means of transferring insect-resistance genes to crops, and there is evidence that those resistance traits have similar effects on natural enemies than resistance achieved by conventional breeding (Kennedy and Gould, 2007; Romeis et al. 2008c). GE insect resistant crops have been grown on a large scale for more than 20 years, and there is considerable experience and knowledge on how they can affect natural enemies and how their risks can be assessed prior to commercialization.

As a highly effective form of host plant resistance, insecticidal GE crops are a foundational tactic in IPM. They work synergistically with other tactics such as conservation biological control to achieve more sustainable pest control. This review will present basic information on the adoption and use of GE crops, discuss the impact of GE crops on

natural enemies through the lens of risk assessment and provide evidence on how GE crops can enable biological control to become a more effective component of IPM.

2. GE plant cultivation

Since the first GE plant was commercialized in 1996, the area grown with GE varieties has steadily increased. The two major traits that are deployed are herbicide-tolerance (HT) and resistance to insects. Here, we will focus primarily on insect-resistant GE crops (but see Box 1 for HT crops). In 2017, GE varieties expressing one or several insecticidal genes from Bt were grown on a total of 101 million hectares worldwide, reaching adoption levels above 80% in some regions (ISAAA, 2017; Fig. 1). Thus, Bt plants have turned what was once a minor foliar insecticide into a major control strategy (Shelton et al., 2008) and their role in IPM has received considerable attention (Downes et al., 2017; Gray, 2011; Meissle et al., 2011; Naranjo, 2011; Romeis et al., 2008b; Wilson et al., 2018).

The majority of today's insect-resistant GE plants produce crystallized (Cry) proteins from Bt. However, this bacterium possesses another class of insecticidal proteins, the vegetative insecticidal proteins (Vips), which are synthesized during the vegetative growth phase (Estruch et al., 1996) and have a different mode of action than Cry proteins (Lee et al., 2003). Vips are already deployed in some commercial maize hybrids (e.g., MIR162, Raybould and Vlachos, 2011) and cotton (e.g., COT102 in Bollgard III, Whitehouse et al., 2014). While the early generation of Bt crops expressed single cry genes, current varieties typically express two or more insecticidal genes. These so-called pyramid events are more effective in controlling the target pests and help to slow down the evolution of resistance (Gressel et al., 2017; Huang, 2015; Que et al., 2010; Zhao et al. 2005). Currently, SmartStax® maize produces the most combined GE traits of any currently commercially cultivated GE crop, i.e., six different cry genes to control lepidopteran and coleopteran pests and two genes for herbicide tolerance (Head et al., 2017).

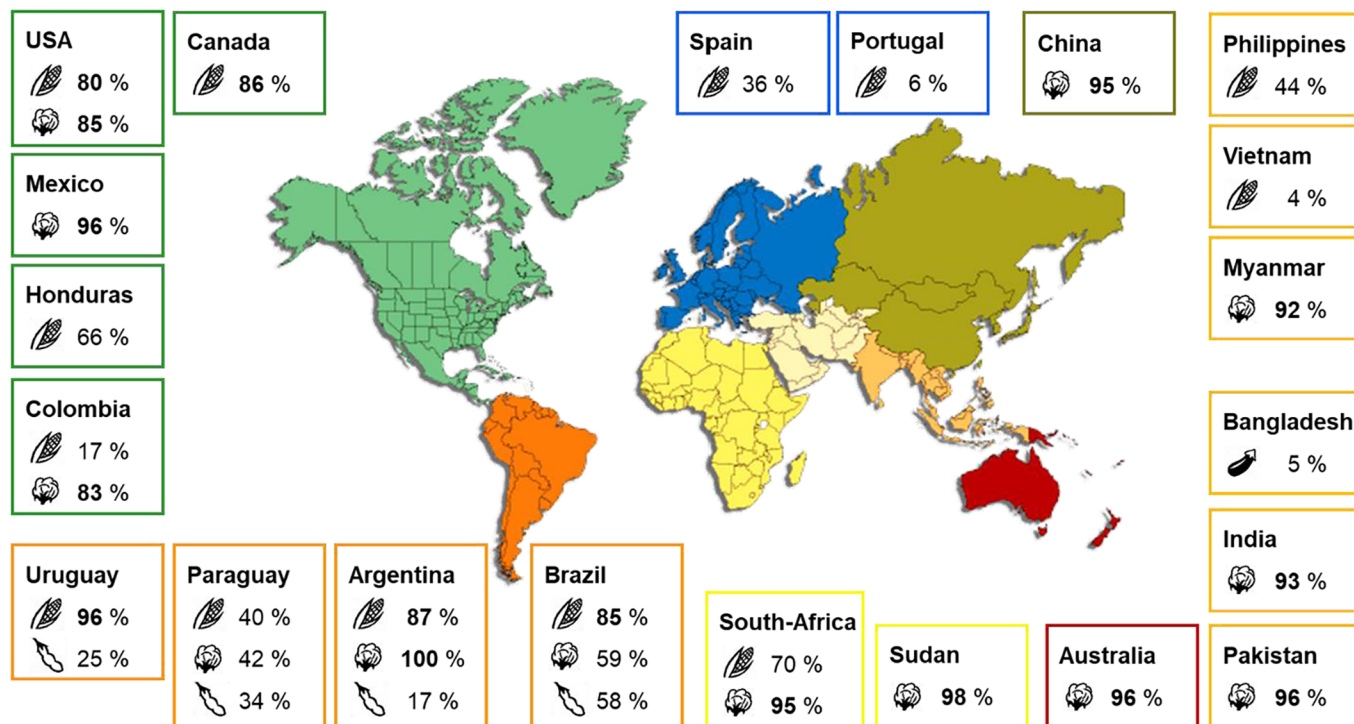


Fig. 1. Global adoption (in %) of GE crops (maize, cotton, soybean, eggplant) with insect-resistance traits (either alone or stacked with HT traits) in 2017 (data source: ISAAA, 2017). Only countries are listed where the biotech crop was grown on > 1000 ha. Adoption levels > 80% are highlighted in bold. In the case of Vietnam and Spain, adoption levels were calculated based on data from the USDA Foreign Agricultural Service (www.fas.usda.gov).

Box 1

Herbicide-tolerant GE crops and biological control.

Tolerance to broad-spectrum herbicides such as glyphosate, glufosinate or dicamba is the most widely deployed trait in GE crops (soybean, maize, cotton, canola, alfalfa and sugar beet). In 2017, GE varieties carrying the herbicide tolerance (HT) trait either alone or stacked with insecticidal traits were grown on a total of 166.4 million hectares worldwide (ISAAA, 2017). The benefits of this technology include highly effective weed control, greater flexibility in applying the herbicide, reduced phytotoxicity to the crop, and savings in time and costs (Bigler and Albajes, 2011). Herbicides generally have low toxicity to arthropods and this is evaluated during the approval process for each new product. However, changes in weed management affect weed diversity and abundance and also might indirectly affect the abundance, diversity and effectiveness of biological control (Bigler and Albajes, 2011; Lundgren et al., 2009; Sanvido et al., 2007).

It is well established that weeds interact with both arthropod pests and their natural enemies (Norris and Kogan, 2000, 2005). Weeds can provide food such as pollen and nectar, harbor (alternative) prey/hosts, provide shelter and refuge, alter the microclimate and structure in the field, and interact with the crop affecting its morphology, phenology and physiology with consequences for natural enemies. Because these interactions are very complex and our understanding remains incomplete, making predictions on how changes in weed abundance and diversity affect arthropods is extremely difficult.

Several studies in Europe have addressed the impact of HT crops and their management on arthropod biodiversity. The most publicized project was the UK Farm Scale Evaluations (FSE) conducted in different crops (spring-sown beet, maize and oilseed rape, and winter-sown oilseed rape). The project used split fields where one half was sown with a conventional crop variety and managed conventionally, while the other half was grown with a HT variety and only the associated herbicide was applied. As expected, the change in the weed management scheme affected both the composition of weed species and the invertebrate taxa in the field. Most importantly, however, crop type and sowing seasons had a far larger impact on the weed and invertebrate composition than the herbicide regime (Hawes et al., 2003; Smith et al., 2008). Subsequent field studies conducted with HT maize in both Spain and the Czech Republic and with HT cotton in Spain have shown that the response of arthropods to altered weed abundance and diversity was variable and differed among taxa (Albajes et al., 2009, 2011; García-Ruiz et al., 2018; Svobodová et al., 2018). For example, Albajes et al. (2009) compared the weed and arthropod abundance in HT maize treated with glyphosate to untreated maize plots. Both the abundance and composition of weeds differed significantly between the treatments. Among the herbivores collected, aphids and leafhoppers were more abundant in the glyphosate-treated HT plots, while the opposite was observed for thrips (Thysanoptera). In the case of predators for example, *Orius* spp. (Hemiptera: Anthracoridae) and spiders (Araneae) were more abundant in the glyphosate treated plots, while the opposite was observed for *Nabis* spp. (Hemiptera: Nabidae) and Carabidae (Coleoptera) (Albajes et al., 2009). A follow-up study indicated that the differences in *Orius* spp. densities were more linked to prey availability than weed abundance per se (Albajes et al., 2011).

One of the biggest changes with HT weed management is flexibility in the timing of herbicide application, which has a marked effect on the population dynamics of weeds. This has been demonstrated for HT sugar beet, which provides opportunities to alter weed management, including enhancing weed biomass while protecting the crop from pests (Dewar et al., 2000). Additionally, it is possible to enhance arthropod biomass and weed seed banks to provide food for farmland birds (Dewar et al., 2003; May et al., 2005).

In addition to changes in weed management, the use of HT varieties has also been found to have impacts on tillage practice. No-tillage and conservation tillage regimes have become more widely adopted with the introduction of HT crops (Givens et al., 2009; Smyth et al., 2011). Reduced tillage or no-tillage minimizes the disruption of the soil structure, composition and biodiversity with positive impacts on arthropods and biological control (Holland, 2004; Stinner and House, 1990; Tamburini et al., 2016; Triplett and Dick, 2008). Furthermore, reduced tillage and fewer tillage passes contribute significantly to carbon sequestration and reduce the amount of greenhouse gas emissions (Brookes and Barfoot, 2018; Smyth et al., 2011), which in turn could help mitigate climate change.

Overall, the experience available to date shows that the effects caused by a shift from a conventional weed management scheme to a HT crop system on arthropods and biological control are difficult to predict. Depending on the crop, the arthropod taxa and the actual changes in crop management (types of herbicides, application timing, tillage practice, etc.) effects can be positive or negative. Because of this complexity, assessing potential risk of HT technology compared to conventional cropping systems is difficult. Such an assessment, however, is a regulatory requirement in the European Union (Lamichhane et al., 2017). The European Food Safety Authority (EFSA) assessed the environmental impact of HT maize and soybean and concluded that their cultivation is unlikely to raise additional environmental safety concerns compared to conventional maize or soybean in most conditions (EFSA, 2011, 2012).

The application of the Bt technology, however, is currently largely limited to the three field crops maize, cotton, and soybean. Most of the Bt varieties target lepidopteran pests (Hellmich et al., 2008; Naranjo et al., 2008). This includes stemborers, such as *Ostrinia nubilalis* (Lepidoptera: Crambidae) in maize, the pink bollworm *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) in cotton and the budworm/bollworm complex in cotton and soybean, including *Helicoverpa/Heliothis* spp. (Lepidoptera: Noctuidae) and other caterpillar pests. In the case of maize, traits are available that target the larvae of corn rootworms *Diabrotica* spp. (Coleoptera: Chrysomelidae). Recently, the technology has been applied to eggplant for protection against the eggplant fruit and shoot borer (*Leucinodes orbonalis*, Lepidoptera: Crambidae) (Hautea et al., 2016). Following years of field trials in Bangladesh, Bt eggplant was grown by 20 farmers in 2014 and over 27,000 in 2018 (ISAAA, 2017; Shelton et al., 2018). Bt-transgenic poplar trees producing

lepidopteran-active Cry proteins have been grown in China since 2002, covering 450 ha in 2011 (Wang et al., 2018).

The adoption of the Bt technology differs among continents. While Bt-transgenic varieties are widely used in the Americas and in Asia, only few countries in Europe and Africa grow these crops. Bt maize is very popular in the Americas, often reaching > 80% adoption. Bt cotton is also widely grown in the USA and Mexico, while Bt soybean remains at relatively low adoption levels (17 to 34%) in South America with the exception of Brazil (58% adoption). In Chile, stacked Bt/HT maize and in Costa Rica, stacked Bt/HT cotton have been grown for seed export only (ISAAA, 2017). In several Asian countries and Australia, the technology is used to control lepidopteran pests (mainly *Helicoverpa* spp.) in cotton with adoption levels > 90%. Bt maize is grown at a significant level in the Philippines to control the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae) while Vietnam only

introduced Bt-transgenic varieties in 2015 and their use is still limited. In Europe, the only product currently approved for cultivation is the Bt maize event MON810 that produces the Cry1Ab protein and protects the plants from corn borers. The largest cultivation area is in Spain with an overall adoption level of 36% in 2017 (ISAAA, 2017). In Africa, Bt crops are currently cultivated in only two countries. South Africa grows Bt maize to control stem borers and Bt cotton to control *Helicoverpa armigera* (Lepidoptera: Noctuidae). Sudan deploys Bt cotton targeting the same pest. Use of Bt cotton has been temporarily halted in Burkina Faso after eight years (Pertry et al., 2016; Sanou et al., 2018). With the recent invasion of the fall armyworm in Africa (Goergen et al., 2016), there is increased interest in using Bt maize as part of a management program (Prasanna et al., 2018).

3. Non-target risk assessment of GE plants

Worldwide, GE plants are subject to an environmental risk assessment (ERA) before being released for cultivation (Craig et al., 2008). The ecosystem service of biological control is an important protection goal to be addressed in the ERA (Sanvido et al., 2012; Devos et al., 2015). Growing insecticidal GE plants could harm natural enemies and biological control in three ways. First, the plant transformation process could have introduced potential harmful unintended changes. In the ERA, this risk is typically addressed by a weight-of-evidence approach considering information from the molecular characterization of the particular GE events and from a comparison of the composition and agronomic and phenotypic characteristics of the GE plant with its conventional counterpart(s) (Garcia-Alonso, 2010; Garcia-Alonso and Raybould, 2014). There is increasing evidence that the process of genetic engineering generally has fewer effects on crop composition compared with traditional breeding methods (Herman and Price, 2013). The current approach is conservative, in particular because off-types are typically eliminated over the many years of breeding and selection that happen in the process of developing a new GE variety (Ladies et al., 2015; Privalle et al., 2012; Schnell et al., 2015; Weber et al., 2012). Second, the plant-produced insecticidal protein could directly affect natural enemies. Such potential toxicity is tested on a number of non-target species and these data are an important part of the regulatory dossier. Third, indirect effects could occur as a consequence of changes in crop management or arthropod food-webs. Such affects are addressed in the pre-market ERA but, because of the complexity of agro-ecosystems, potential impacts might only be visible once plants are grown in farmer fields.

For insecticidal proteins in GE plants to directly affect a natural enemy, the organism has to ingest the toxin (exposure, see Section 4) and be susceptible to it (toxicity, see Section 5). Toxicity of the insecticidal protein to natural enemies is typically evaluated in a tiered risk assessment approach that is conceptually similar to that used for pesticides. Testing starts with laboratory studies representing highly controlled, worst-case exposure conditions and progresses to bioassays with more realistic exposure to the toxin and semi-field or open field studies carried out under less controlled conditions (Garcia-Alonso et al., 2006; Romeis et al., 2008a). From a practical standpoint, because not all natural enemies potentially at risk can be tested, a representative subset of species (surrogates) is selected for assessment (Carstens et al., 2014; Romeis et al., 2013b; Wach et al., 2016). First, the species must be amenable and available for testing. This means that suitable life-stages of the test species must be obtainable in sufficient quantity and quality, and validated test protocols should be available that allow consistent detection of adverse effects on ecologically relevant parameters. Second, what is known about the spectrum of activity of the insecticidal protein and its mode of action should be taken into account to identify the species or taxa that are most likely to be sensitive. In the case of Bt proteins (and even more so in the case of insecticidal GE plants based on RNA interference) the phylogenetic relatedness of the natural enemy with the target pest species are of importance. Third, the

species tested should be representative of taxa or functional groups that contribute to biological control and that are most likely to be exposed to the insecticidal compound in the field (see Section 4). Knowledge on the natural enemies present in a particular crop, their biological control activity, and their biology and ecology is used to select representative test species. Databases containing this information have been established for various field crops in Europe (e.g., Meissle et al., 2012; Riedel et al., 2016) and for rice in China (Li et al., 2017b). The manner by which this information can be used to support the species selection process has been demonstrated for Bt maize in Europe (Romeis et al., 2014a) and for Bt rice in China (Li et al., 2017b). Attempts to construct arthropod food webs and use this information to select the most appropriate surrogate species for testing have also been developed for Bt cowpea in West Africa (Ba et al., 2018), Bt sweet potato in Uganda (Rukarwa et al., 2014), and Bt pine trees in New Zealand (Todd et al., 2008).

4. Exposure of natural enemies to insecticidal proteins

4.1. Concentration of Bt proteins in plant material

When Bt genes are incorporated into crops, they are usually combined with constitutive promoters, such as CaMV 35s or the maize ubiquitin promoter that are active in all tissues. Consequently, Bt proteins in current crops can be found in the whole plant including roots, stems, leaves, pollen, and fruits. However, concentrations can vary considerably in different plant tissues, across different developmental stages, and among different Bt proteins and transformation events (Eisenring et al., 2017; Knight et al., 2013; Nguyen and Jehle 2007, 2009; Obrist et al., 2006a; Svobodová et al., 2017). One example is the pollen of Bt maize producing Cry1Ab. Early cultivars with the transformation event 176 had high concentrations of Cry1Ab in pollen, which lead to concerns that valued butterfly populations may be affected when inadvertently ingesting insecticidal pollen deposited on their host plants. Modern Bt maize varieties based on other transformation events (e.g., MON810, Bt11) express very low levels of Cry1Ab in the pollen (Perry et al., 2010; Shelton and Sears, 2001).

In contrast to sprayed insecticides, which are applied at distinct time points, plant-produced Bt proteins are present constantly. Exposure to the pest and non-target organisms is therefore longer than it would be with most insecticides. Bt protein concentrations in younger tissue, however, are often higher than in mature tissue, which can lead to lower Bt protein concentrations towards the end of the growing season. This has, for example, been reported for Cry3Bb1 in maize event MON88017 (Nguyen and Jehle, 2009), but not for Cry1Ab in maize event MON810 (Nguyen and Jehle, 2007). In the case of Bt cotton, the Cry1Ac concentration typically declines when plants get older, while the Cry2ab protein remains relatively stable (Adamczyk et al. 2001; Knight et al., 2013; Olsen et al., 2005).

Bt plant material entering the decomposition process in the soil is degraded rapidly. When litter bags filled with senescent Bt maize leaves were buried in a maize field in autumn, almost no Bt protein was detectable eight months later (Zurbrügg et al., 2010). Similarly, residual root stalks collected eight months after harvest contained 100-fold less Cry1Ab than fresh root samples (Nguyen and Jehle, 2007).

Because Bt proteins are gut-active, they need to be ingested to reveal their insecticidal properties (Bravo et al., 2011). Natural enemies can be exposed to plant produced-Bt proteins when feeding directly on plant tissue, or via prey or host species that have consumed Bt plant material (Fig. 2, routes 1 and 2).

4.2. Exposure through GE plant material

Plant material is mainly consumed by herbivores, which include major pest species that are the targets of the Bt crop, but also a range of non-target species from different taxonomic orders that are not

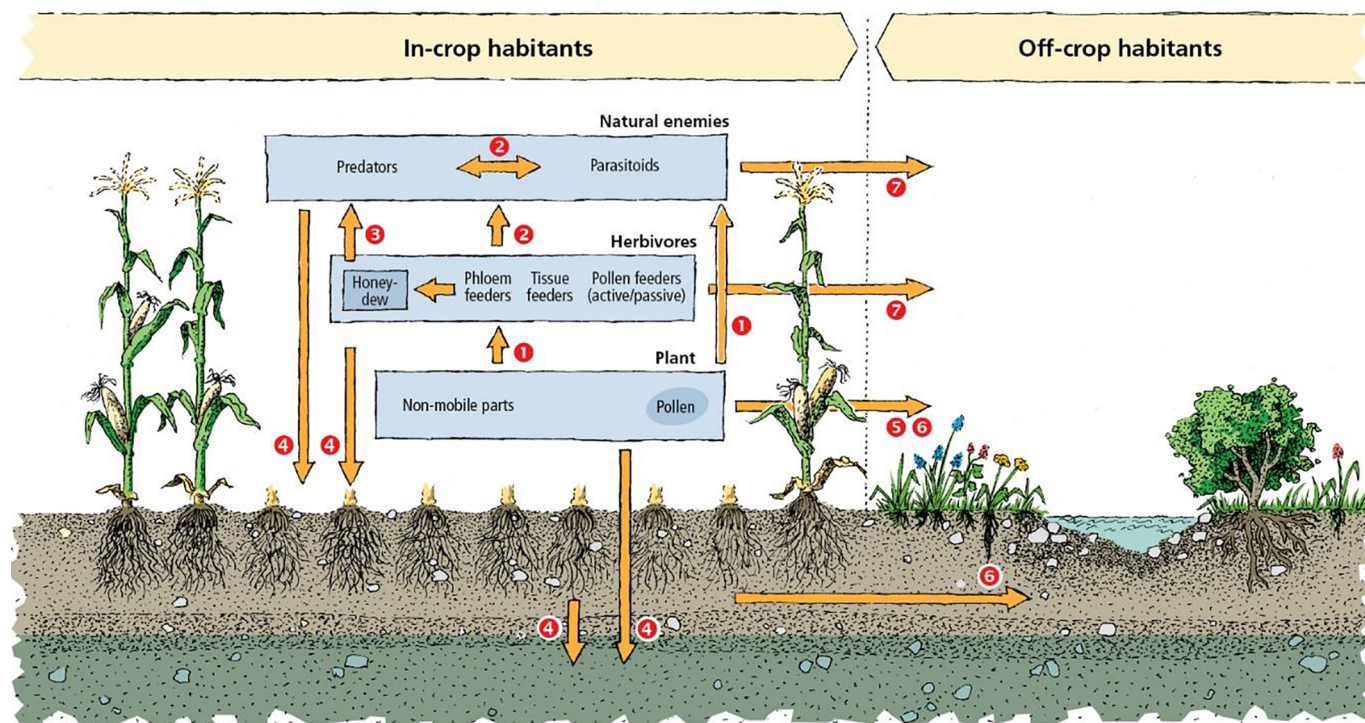


Fig. 2. Routes through which natural enemies could be exposed to plant-produced insecticidal compounds. Arthropods, including herbivores and natural enemies, can feed directly on non-mobile plant parts or pollen (1). Natural enemies can be exposed through prey or hosts when consuming other arthropods, such as herbivores or other members of higher trophic levels (2). Honeydew, sugary excretions of phloem-feeding Hemiptera, might expose natural enemies if the insecticidal compounds are present in the phloem (3). Insecticidal compounds may enter the soil via decaying plant tissue, root exudates, or dead herbivores or natural enemies, where soil living arthropods may get exposed (4). Arthropods living in off-crop habitats may also get exposed when insecticidal compounds leached or exuded from the plants are transported by ground water, or when pollen or plant debris are blown off the field (5, 6). Finally, herbivores and natural enemies leaving the crop may expose natural enemies in off-crop habitats (7). Drawing by Ursus Kaufmann, Agroscope.

susceptible to the produced Bt proteins. Many predators are also facultative herbivores, which feed on pollen and other plant tissue when prey is scarce (Fig. 2, route 1) (Lundgren, 2009; Meissle et al., 2014; Peterson et al., 2016a; Van Rijn et al., 2002). Pollen feeding has, for example, been reported for predatory bugs, such as *Orius* spp. (Hemiptera: Anthracoridae), for ladybird beetles, such as *Coleomegilla maculata* or *Harmonia axyridis* (Coleoptera: Coccinellidae), for spiders (Araneae), for ground beetles (Coleoptera: Carabidae), and for predatory mites (Acari: Phytoseiidae) (Lundgren, 2009; Meissle et al., 2014, Table S1). Field studies with Bt maize, which sheds large amounts of pollen, revealed that *Orius* spp. and ladybeetles contained higher levels of Bt protein during anthesis than before or after, indicating pollen consumption (Meissle and Romeis, 2009a; Obrist et al., 2006a). Green lacewings, *Chrysoperla carnea* (Neuroptera: Chrysopidae), feed exclusively on pollen and nectar in the adult stage (Li et al., 2010; Sheldon and MacLeod, 1971), while larvae are predators which can supplement their diet with pollen (Meissle et al., 2014). Predators may seek pollen as a food source actively. They may, however, also ingest it passively, e.g. when it sticks to their prey or, in the case of spiders, when they clean or recycle their web (Meissle and Romeis, 2009a; Peterson et al., 2016a). In Carabidae (Coleoptera), some species are mainly predators, some are considered omnivores, feeding on prey and plant tissue, and others live mainly as herbivores, e.g. on plant roots or seeds (Lundgren, 2009). Predatory bugs, such as *Geocoris* spp. (Hemiptera: Geocoridae) and *Nabis* spp. (Hemiptera: Nabidae), have also been reported to feed directly on green leaf tissue (Yu et al., 2014). Soil inhabiting natural enemies may feed on roots or on decaying plant or arthropod material occasionally, which might expose them to Bt protein. They may also encounter root exudates that contain Bt protein (Fig. 2, route 4) (Icoz and Stotzky, 2008).

Nectar is an important source of carbohydrates for adult parasitoids and some predators (Coll and Guershon, 2002; Lundgren, 2009), although there is no evidence that nectar contains Cry proteins. Parasitoids commonly don't consume plant tissue and adult parasitoids collected in Bt maize and Bt rice fields did not contain measurable Cry protein concentrations (Harwood et al., 2005; Li et al., 2017b). There is evidence, however, for direct plant feeding by *Pseudogonatopus flavifemur* (Hymenoptera: Dryinidae), a parasitoid of planthoppers, that contained Cry protein when caged with Bt rice plants devoid of hosts (Tian et al., 2017). While exposure through direct plant feeding might be a significant route of exposure for some natural enemy species or in particular situations (e.g. when no prey is available), the more common route of exposure to Bt proteins is through consumption of prey or hosts (Fig. 2, route 2).

4.3. Exposure through prey or hosts

Herbivores feeding on Bt plants may ingest the insecticidal protein (s) and expose their antagonists to these proteins. Feeding studies with sensitive insects have shown that Bt protein measured in herbivores (spider mites, caterpillars, rootworm adults) immunologically by ELISA is still biologically active, which indicates that ELISA data can be used to estimate levels of exposure to active Bt protein (Chen et al. 2008; Guo et al., 2016; Li et al., 2011; Meissle and Romeis, 2009b; Obrist et al., 2006b; Tian et al., 2012, 2013, 2018b). When consuming prey or hosts, bioactive Bt protein is thus transferred from herbivores to natural enemies.

For arthropods consuming Bt protein-containing food, the protein becomes undetectable after a few days when switched to non-Bt diet (Obrist et al., 2005; Romeis et al., 2004; Torres and Ruberson, 2008;

Torres et al., 2006; Wei et al., 2008; Zhao et al., 2016). This indicates that most of the ingested Bt protein is digested in the gut or excreted. However, Cry1Ac was also found in the body tissue outside the gut in cotton bollworms, *H. armigera* (Zhao et al., 2016). It has been claimed that Bt proteins may accumulate in a ladybird in a system using aphids and purified Bt proteins in an artificial diet (Paula and Andow, 2016). However, the body of literature from more realistic laboratory and field experiments does not provide any evidence for such an accumulation (Meissle and Romeis, 2012).

Many natural enemies use aphids as prey or hosts because aphids are abundant in most crops worldwide. Bt proteins, however, do not seem to enter the phloem sap, which is the food for aphids (Raps et al., 2001). Consequently, aphids contain, at best, trace amounts of Bt protein several orders of magnitude lower than the concentrations in green tissue (Romeis and Meissle, 2011; Tian et al., 2015). Natural enemies consuming mainly aphids are thus generally not exposed to significant concentrations of Bt protein. Consequently, aphid honeydew, which is an important source of energy for both predators and parasitoids (Wäckers, 2005), is a negligible route of exposure to plant-produced Bt proteins (Fig. 2, route 3). The same appears to be true for the honeydew produced by other sap-feeders. Only trace amounts of Cry proteins were detected in the honeydew produced by the brown planthopper (*Nilaparvata lugens*; Hemiptera: Delphacidae) on different Bt rice lines (Bernal et al., 2002; Tian et al., 2018a). However, other transgenic compounds (e.g., *Galanthus nivalis* agglutinin, Hogervorst et al., 2009) have been found in aphid honeydew. Consequently, this route of exposure could be important for insecticidal non-Bt plants.

Herbivores feeding on green plant tissue ingest relatively high amounts of Bt protein. Those include species with chewing mouthparts, e.g. caterpillars (Lepidoptera), and species with piercing sucking mouthparts, such as bugs (Hemiptera), thrips (Thysanoptera), or spider mites (Acari) (Meissle and Romeis, 2009a, 2018). Spider mites have been found to be among the herbivores with the highest concentrations of Bt protein because they suck out contents in mesophyll cells where the Bt protein is concentrated. Concentrations are in the same order of magnitude (or even higher) than those found in the leaf tissue (Álvarez-Alfageme et al., 2008, 2011; Dutton et al., 2002; Guo et al., 2016; Li and Romeis, 2010; Meissle and Romeis, 2009a, 2018; Obrist et al., 2006b,c; Torres and Ruberson, 2008; Svobodová et al., 2017).

Tritrophic studies with Bt plants, herbivorous prey, and predators have shown that ladybeetles ingest relatively high amounts of Bt protein, while concentrations in lacewings, predatory bugs, and spiders were lower (Álvarez-Alfageme et al., 2008, 2011; Eisenring et al., 2017; Li and Romeis, 2010; Meissle and Romeis, 2009a, 2018; Peterson et al., 2011, 2016a; Torres and Ruberson, 2008; Torres et al., 2006). Ground beetle larvae that live below-ground and feed mainly on other soil-inhabiting species, including decomposers, might contain Bt protein (Peterson et al., 2009). Adults of most carabid species are ground-dwelling predators, omnivores, or herbivores and are thus exposed to Bt proteins via plant tissue or prey (Zwahlen and Andow, 2005). Field collections of predators have shown that Bt protein concentrations also can vary considerably among species of the same taxonomic group, such as spiders (Peterson et al. 2016a), carabids (Zwahlen and Andow, 2005), or ladybird beetles (Harwood et al., 2005), which can be explained by differences in feeding habits.

Parasitoids are potentially exposed to Bt proteins when feeding on their hosts. Similar to predators, the Bt protein concentration in the host, as well as the feeding habit of the parasitoid, influence exposure. In general, parasitoids that consume the gut of their host, where most of the Bt protein is located, are expected to experience higher exposure than those leaving the host without consuming the gut (Meissle et al., 2004; Vojtech et al., 2005). In some species, adults also feed on the host, which might lead to exposure. For most parasitoid species, however, adults feed on nectar or honeydew and consequently do not ingest significant amounts of Bt protein (Harwood et al., 2005; Li et al., 2017b).

In conclusion, Bt proteins are generally transferred from plants to herbivores to natural enemies. But the amount of Bt protein ingested by natural enemies is highly variable and depends on the concentration of the Bt protein in the plant, the stability of the Bt protein, the time of the last meal, the mode of feeding of the herbivore and the natural enemy, and behavior (Dutton et al., 2003; Romeis et al., 2009). Furthermore, excretion and digestion at each trophic level leads to a dilution effect when Bt proteins move along the food chain. This is supported by evidence from ELISA measurements of field collected arthropods from Bt maize (Harwood et al., 2005; Meissle and Romeis, 2009a; Obrist et al., 2006a, Peterson et al., 2009), cotton (Eisenring et al., 2017; Torres et al., 2006), soybean (Yu et al., 2014), and rice (Li et al., 2017b).

4.4. Exposure in off-crop habitats

Arthropods inhabiting or visiting Bt crop fields may be exposed to plant-produced Bt proteins. However, arthropods living in the field margins or other elements of the surrounding landscape may also encounter Bt proteins from fields where Bt plants are grown. The most prominent example is pollen from Bt maize that is deposited on food plants of butterflies in the field margins (Fig. 2, route 5). During the period of pollen shed, butterfly larvae are likely to ingest certain amounts of pollen grains together with their food plant (Perry et al., 2010; Schuppener et al., 2012; Shelton and Sears, 2001). This is also likely for other herbivores and potentially their natural enemies. Maize pollen is relatively heavy and deposited mainly within or in close proximity to the maize field, which limits exposure of arthropods off-crop, although certain wind conditions may lead to pollen drift over several kilometers (Sanvido et al., 2008). During harvest, in particular when only cobs are harvested and the remaining plant material is shredded and left on the field, parts of the plant debris might drift to neighboring habitats and expose decomposers and their natural enemies (Fig. 2, route 6). Pollen, plant debris, and also exudates from living roots or exudes from decaying plant material might enter small streams that often run close to agricultural fields (Rosi-Marshall et al., 2007; Tank et al., 2010). Those are potential routes of exposure for aquatic organisms, such as shredders, filter feeders, and their natural enemies. Bt protein concentrations in aquatic systems, however, are expected to be very low due to a huge dilution effect of the running water (Carstens et al., 2012; Tank et al., 2010). Finally, herbivores and other arthropods that have ingested Bt protein from the Bt crop may leave the field and expose natural enemies off-crop (Fig. 2, route 7). Because of the rapid excretion and digestion, however, this route of exposure is temporally very limited.

5. Toxicity of insecticidal proteins produced in GE plants

Studies to investigate the toxicity (hazard) of the insecticidal compounds produced by Bt plants to natural enemies include direct feeding studies in which the natural enemies are fed artificial diet containing purified Bt protein, bitrophic studies where natural enemies are fed Bt plant tissue (e.g., pollen), or tritrophic studies using a herbivore to expose the natural enemy to the plant-produced toxin. Numerous such studies have been conducted on a large number of Bt proteins, Bt crops and transformation events.

In summary, the available body of literature provides evidence that insecticidal proteins used in commercialized Bt crops cause no direct, adverse effects on non-target species outside the order (i.e., Lepidoptera for Cry1 and Cry2 proteins) or the family (i.e. Coleoptera, Chrysomelidae for Cry3 proteins) of the target pest(s). This also holds true for Bt plants that produce two or more different insecticidal proteins. The available data indicate that these pyramided insecticidal proteins typically act additively in sensitive species and cause no unexpected effects in species that are not sensitive to the individual toxins (Graser et al., 2017; Guo et al., 2016; Haller et al. 2017; Kumar et al.,

2014; Levine et al., 2016; Raybould et al., 2012; Shu et al., 2018; Su et al., 2015; Svobodová et al., 2017; Tian et al., 2014, 2018b; Walters et al., 2018). Recent studies have demonstrated that this is also true for a combination of Cry proteins and dsRNA (Khajuria et al., 2018; Levine et al., 2015; Ni et al., 2017).

While a few studies claim to have revealed unexpected non-target effects, none of those claims has been verified, i.e., confirmed in follow-up studies conducted by other research groups. It is thus likely that those results are artifacts, probably resulting from problems in study design (EFSA, 2009; Rauschen, 2010; Ricoch et al., 2010; Romeis et al., 2013a, 2014b). This emphasizes the need for risk assessment studies to be carefully designed to avoid erroneous results that include false negatives (i.e., the failure to detect adverse effects of toxins that are potentially harmful in the field) and positives (i.e., the detection of adverse effects when the toxin is unlikely to be harmful in the field) (De Schrijver et al., 2016; Li et al., 2014b; Romeis et al., 2011, 2013a).

5.1. Direct feeding studies

To support the regulatory risk assessment, non-target studies with natural enemies are typically conducted under worst-case exposure conditions in the laboratory. Recombinant insecticidal proteins produced in microorganisms are usually used as the test substance. It is often not feasible to use plant-expressed protein because sufficient mass cannot be reasonably purified from the plant source. As a consequence, those proteins must be well characterized to demonstrate a functional and biochemical equivalence with the plant-produced protein (Raybould et al., 2013). In general, studies with purified Bt proteins have not indicated any adverse effects on the tested non-target organisms. Reviews are available for a number of Bt proteins including the Coleoptera-active Cry34/35Ab1 (De Schrijver et al., 2016; Narva et al., 2017) and Cry3Bb1 (Devos et al., 2012) and the Lepidoptera-active Cry1Ab (Romeis et al., 2013a), Cry1Ac (CERA, 2011), Cry2Ab (CERA, 2013a), Cry1F (Baktavachalam et al., 2015; CERA, 2013b), and Vip3Aa (CERA, 2012; Raybould and Vlachos, 2011).

As noted above, more realistic routes of exposure for natural enemies include feeding directly on the plant or indirectly through their prey or hosts feeding on the plants. The following sections will focus on these types of studies

5.2. Bitrophic studies

To our knowledge, bitrophic studies, where natural enemies were directly fed with Bt plant material, have been conducted on a total of 20 species from 6 orders and 12 families (Table 1). The majority of studies tested material from Bt-transgenic maize, followed by rice, potato, and cotton. The most commonly used test substance was pollen. The studies recorded survival, but also sublethal parameters, e.g., developmental time or body mass. With two exceptions, exposure of the natural enemies to the plant produced Cry proteins has been confirmed or can be expected given the test system and the feeding mode of the test organism. The exceptions are studies conducted with adult egg parasitoids belonging to the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) which, due to their minute size, are not able to feed on maize pollen grains (Romeis et al., 2005). Studies conducted with Bt maize pollen from events MON810 and Bt11 (Meissle et al., 2014; Obrist et al., 2006c) also lacked exposure given the very low concentrations of Cry proteins in the pollen of this event (Dutton et al., 2003; Nguyen and Jehle, 2007). Thus, valid conclusions about Cry1Ab toxicity are not possible from those studies.

With the exception of four studies, none of the bitrophic studies has reported putative adverse effects of the Bt plant on the natural enemies when compared with the respective control plant. The first study concerns the impact of Bt rice (Cry1Ab, KMD1 and KMD2) pollen on *Propylea japonica* (Coleoptera: Coccinellidae). Out of several life-table parameters that were measured, the longevity of females was reduced

compared to the control in the KMD1 treatment, but not in the KMD2 treatment, despite similar exposure. In the second study, the impact of Bt maize (Cry1Ab, Bt11) pollen on the predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae) was tested and the authors reported a significant increase in female development time and a significant decrease in fecundity in the Bt treatment (Obrist et al. 2006c). The authors suggest that the observed effects were not related to the Cry1Ab protein since in a parallel study no effects were observed when the predator was fed with spider mites that contained much higher amounts of Cry1Ab compared to Bt maize pollen. Similarly, Mason et al. (2008) observed a reduced fecundity in lacewings fed pollen from Bt maize MON810 but not for pollen from event 176 which contains much higher concentrations of Cry1Ab. Adverse effects (increase in 4th instar developmental time) were reported in a third study where larvae of *C. maculata* were fed seedlings of Bt maize (Cry1Ab, MON810) (Mosser et al., 2008). In this study, however, a non-related non-Bt maize variety was used as the control. In summary, it is apparent, that the unexpected effects observed in these four studies were not caused by the expressed Cry protein but by some unidentified plant-related characteristics. Because several breeding steps are necessary to generate a stable GE line from the parental line, differences in the composition of plant tissues exist even between a GE line and the respective near-isoline (see Section 3). These differences are likely to increase when the transgenic event is conventionally crossed into a range of different genetic backgrounds to generate commercial varieties.

5.3. Tritrophic studies

Studies that have examined potential impacts of Bt plants on natural enemies in tritrophic test systems have deployed a variety of prey and host species as the Cry protein carrier. This has included prey or host species that are: 1) susceptible to the Cry proteins (lepidopteran and coleopteran species that are targets of Bt crops), 2) species that are not susceptible to the Cry proteins because of their taxonomic affiliation (aphids, leafhoppers, mites, thrips, etc.), and 3) target herbivores (exclusively lepidopterans) that have developed resistance to the Cry proteins. One challenge with tritrophic studies is that they can lead to erroneous results when sublethally affected Cry-sensitive herbivores are used as prey or hosts. This can lead to adverse effects on the natural enemy that are related to the reduced quality of the prey/host rather than to the insecticidal protein itself (Fig. 3) (Naranjo, 2009; Romeis et al., 2006). The importance of such prey/host-quality effects has been demonstrated experimentally for the parasitoids *Diadegma insulare* (Hymenoptera: Ichneumonidae) (Chen et al., 2008) and *Macrocentrus cingulum* (Hymenoptera: Braconidae) (Wang et al., 2017) and for the predators *C. carnea* (Lawo et al., 2010) and *C. maculata* (Li et al., 2011). Ignorance of prey/host-quality effects has led to erroneous claims that lepidopteran-active Cry proteins cause direct toxic effects on natural enemies (see Shelton et al., 2009 for detailed discussion).

One way of overcoming the effects of host/prey-quality is to use non-susceptible or resistant herbivores that can consume the Cry protein without being compromised and serve as prey or host for the predator or parasitoid. Through a literature review, we have retrieved 68 publications presenting the results from such tritrophic studies using Bt plant material as the test substance. This list includes phloem feeding insects like aphids, but there is increasing evidence in the literature that phloem feeders have extremely low or non-existent titers of Cry proteins in their bodies after feeding on Bt plants (Romeis and Meissle, 2011). While these studies offer realistic trophic scenarios, because aphids are common prey and hosts in crop fields, they are not suitable for testing the direct effects of Cry proteins on natural enemies. The same holds true for studies that have offered eggs to natural enemies from herbivores that developed on Bt-transgenic plants. We have thus separated the tritrophic studies into those where exposure to the plant-produced Cry proteins was confirmed or expected (Table S1), and those where exposure was not given or shown to be very low (Table S2) and

Table 1
Biotrophic studies exposing natural enemies to Cry proteins using Bt plant tissue as test substance.

Order: Family	Species	Tissue, plant, event/line/trade name (Cry protein) ^{a,b}
<i>Parasitoids</i>		
<i>Hymenoptera</i>		
Dryinidae	<i>Pseudogonatopus flavifemur</i>	Leaves, rice, T1C-19 (Cry1C) ¹ , T2A-1 (Cry2A) ¹
Trichogrammatidae	<i>Trichogramma chilonis</i>	Pollen, cotton, SGK321 (Cry1Ac + CpTI) ²
	<i>Trichogramma pretiosum</i>	Pollen, maize, MON89034 (Cry1A.105 + Cry2Ab2) ³
<i>Predators</i>		
<i>Coleoptera</i>		
Carabidae	<i>Harpalus caliginosus</i>	Pollen, maize, MON863 (Cry3Bb1) ⁴
	<i>Harpalus pensylvanicus</i>	Pollen, maize, MON863 (Cry3Bb1) ⁴
Coccinellidae	<i>Coleomegilla maculata</i>	Pollen, maize, event 176 (Cry1Ab) ⁵ , MON863 (Cry3Bb1) ^{4,6,7}
	<i>Harmonia axyridis</i>	Seedlings, maize, MON810 (Cry1Ab) ⁸ Flower, potato, Superior NewLeaf® (Cry3A) ⁹ Seedlings, maize, MON810 (Cry1Ab) ⁸ Pollen, maize, SmartStax® (Cry1A.105, Cry1F, Cry34Ab1, Cry35AB1, Cry2Ab2, Cry3Bb1) ¹⁰ , event N30 (Cry1Ab/Cry2Aj) ¹¹
	<i>Micraspis discolor</i>	Pollen, rice, Huahui 1 (Cry1Ac/Cry1Ab) ¹²
	<i>Propylea japonica</i>	Pollen, rice, KMD1 (Cry1Ab) ¹³ , KMD2 (Cry1Ab) ¹³ , T1C-19 (Cry1C) ¹⁴ , T2A-1 (Cry2A) ¹⁴ Pollen, maize, BT-799 (Cry1Ac) ¹⁵ , SK 12-5 (Cry1Ab/2Aj) ¹⁵ , IE09S034 (Cry1Ie) ¹⁶
<i>Neuroptera</i>		
<i>Chrysopidae</i>		
	<i>Chrysoperla carnea</i>	Pollen, maize, event 176 (Cry1Ab) ¹⁷⁻¹⁹ , MON810 (Cry1Ab) ¹⁹ , MON88017 (Cry3Bb1) ^{18,19} , SmartStax® (Cry1A.105, Cry1F, Cry34Ab1, Cry35AB1, Cry2Ab2, Cry3Bb1) ¹⁰
	<i>Chrysoperla nipponensis</i> (= <i>C. sinica</i>)	Pollen, rice, T1C-19 (Cry1C) ²⁰ , T2A-1 (Cry2Aa) ²¹
	<i>Chrysoperla plorabunda</i>	Pollen, maize, event 176 (Cry1Ab) ²² , MON810 (Cry1Ab) ²² , TC1507 (Cry1F) ²²
<i>Acari</i>		
Phytoseiidae	<i>Amblyseius</i> (= <i>Neoseiulus</i>) <i>cucumeris</i>	Pollen, maize, Bt11 (Cry1Ab) ²³
<i>Aranea</i>		
Araneidae	<i>Araneus diadematus</i>	Pollen, maize, event 176 (Cry1Ab) ²⁴
Theridiidae	<i>Phylloneta impressa</i> (= <i>Theridion impressum</i>)	Pollen, maize, MON88017 (Cry3Bb1) ²⁵ , SmartStax® (Cry1A.105, Cry1F, Cry34Ab1, Cry35AB1, Cry2Ab2, Cry3Bb1) ¹⁰
<i>Hemiptera</i>		
<i>Anthocoridae</i>		
	<i>Orius insidiosus</i>	Pollen, maize, event 176 (Cry1Ab) ¹⁷
	<i>Orius majusculus</i>	Pollen, maize, event 176 (Cry1Ab) ²⁶
	<i>Orius tristicolor</i>	Leaf, potato, Russet Burbank NewLeaf® (Cry3A) ²⁷
Geocoridae	<i>Geocoris</i> spp.	Leaf, potato, Russet Burbank NewLeaf® (Cry3A) ²⁷
Nabidae	<i>Nabis</i> spp.	Leaf, potato, Russet Burbank NewLeaf® (Cry3A) ²⁷

^a Wherever possible transformation events are provided: Exceptions include NewLeaf® potatoes where trade name covers different events (<http://www.isaaa.org/genemapapprovaldatabase/>) and SmartStax® that contains multiple events (MON89034 x TC1507 x MON88017 x DAS-59122-7).

^b References: ¹Tian et al. (2017), ²Geng et al. (2006), ³De Sousa et al. (2017), ⁴Ahmad et al. (2006), ⁵Pilcher et al. (1997), ⁶Lundgren and Wiedenmann (2002), ⁷Duan et al. (2002), ⁸Moser et al. (2008), ⁹Ferry et al. (2007), ¹⁰Svobodová et al. (2017), ¹¹Chang et al. (2017), ¹²Zhou et al. (2016), ¹³Bai et al. (2005), ¹⁴Li et al. (2015), ¹⁵Liu et al. (2016b), ¹⁶Li et al. (2017a), ¹⁷Pilcher et al. (1997), ¹⁸Li et al. (2008), ¹⁹Meissle et al. (2014), ²⁰Li et al. (2014a), ²¹Wang et al. (2012), ²²Mason et al. (2008), ²³Obrist et al. (2006c), ²⁴Ludy and Lang (2006), ²⁵Meissle and Romeis (2009a), ²⁶Lumbierres et al. (2012), ²⁷Armer et al. (2000).

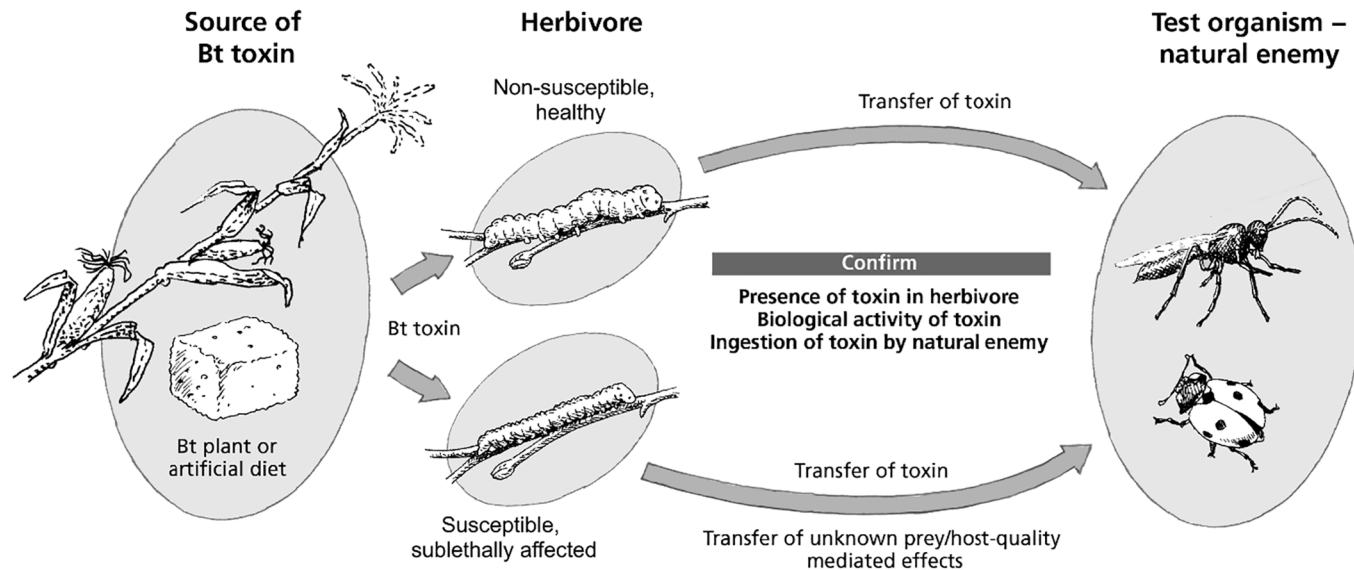


Fig. 3. Studying the direct toxic effects of insecticidal compounds on natural enemies through tritrophic exposure. Care must be taken to avoid the use of sensitive herbivores as prey/hosts to avoid the occurrence of prey-quality mediated effects that may be falsely interpreted as toxic effects. Drawing by Ursus Kaufmann, Agroscopie.

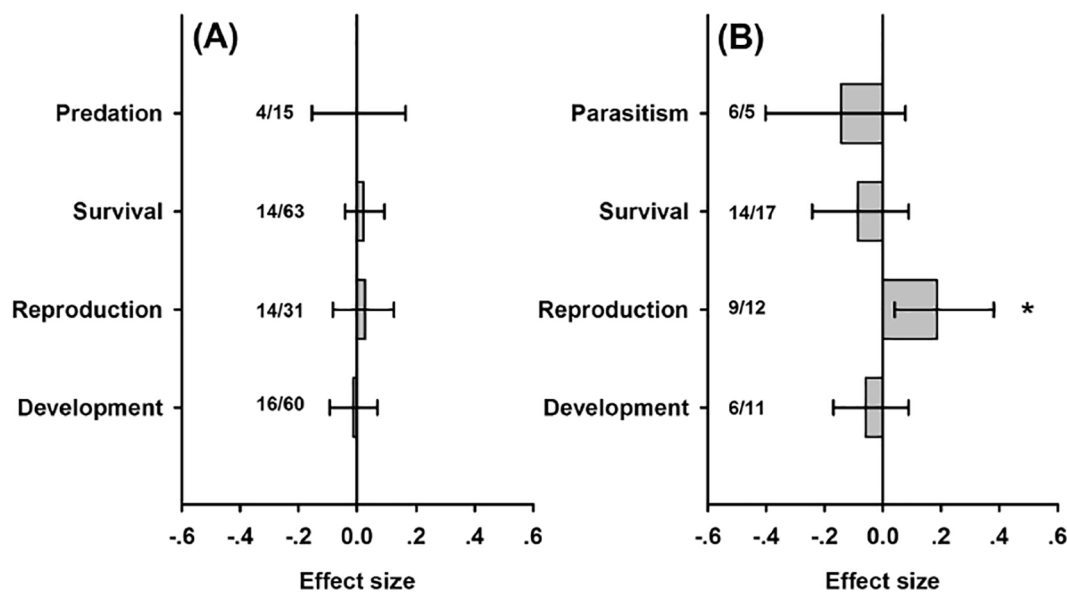


Fig. 4. Meta-analyses of the effect of Bt Cry proteins on the life history characteristic and biological control function of A) arthropod predators and B) insect parasitoids through laboratory tritrophic exposure studies (plant—herbivore—natural enemy). Studies used either prey or hosts resistant to the Cry proteins or prey or hosts that are not susceptible to the proteins based on taxonomic affiliation (Tables S1 and S2). Asterisks denote effect sizes significantly different from zero; sample sizes denote observations of resistant/non-susceptible prey or hosts and error bars are 95% bias corrected confidence intervals.

where consequently no conclusions about the toxicity of the Cry proteins could be drawn. Tritrophic studies where natural enemies were exposed to plant-produced Cry proteins were conducted with 6 hymenopteran parasitoids from 4 families, 32 predators from 12 families in 5 orders, and one entomopathogenic nematode (Table S1). Studies with no or negligible exposure were conducted with 7 hymenopteran parasitoids from 4 families and 12 predators from 6 families in 5 orders (Table S2).

Relevant data were extracted from the identified studies (Tables S1 and S2) for various life history traits that have a bearing on population dynamics and biological control function (immature development, immature and adult survival, fecundity, parasitism and predation). These data are summarized using meta-analysis (Fig. 4). Care was taken to preserve independence in observations from any one study and to use metrics that reflected the longest exposure to the Cry protein. For example, if individual stage development time and total immature development time were measured for a natural enemy species, only total development time was retained. Likewise, if both fecundity and fertility (% hatch) were measured, only fecundity was retained because the former was generally measured over the life of the adult but the latter was often measured for only a brief period. A similar strategy was used for all studies so that only a single independent metric of a given life history trait was retained for each species studied. More detail on general screening methods can be found in Naranjo (2009). We further retained only data from studies in which the plant was used as the source of the Cry protein, although this plant material could have been incorporated into an artificial diet. For studies that cumulatively exposed the natural enemy over multiple generations we used the results from the final exposed generation based on the rationale that this would represent the most extreme exposure to Cry proteins. The non-Bt plants used were generally isolines or near-isolines of the Bt plants (> 90% of studies); the remaining studies did not provide sufficient information. We used Hedge's *d* as the effect size estimator. This metric measures the difference between respective means from each treatment (Bt or non-Bt) divided by a pooled variance and further corrected for small sample size (Rosenberg et al., 2000). A random effects model was used for analyses to enable a broad inference of effects and bias-corrected, bootstrapped 95% confidence intervals were used to determine if the effect size differed from zero. The effect size was calculated such that a

positive value indicates a more favorable response from the Bt compared with the non-Bt treatment. All analyses were conducted using MetaWin v2.1 (Rosenberg et al., 2000).

Results mirror those found in prior meta-analyses with fewer studies (Naranjo, 2009) in showing that a variety of Bt plants and Cry proteins have no negative effects on a broad range of natural enemy species when the non-target species were exposed in an ecologically realistic manner (Fig. 4). Effect sizes were generally larger for parasitoids and analyses indicated that reproduction (number of progeny) was actually higher when their hosts had fed on Bt plants compared with non-Bt plants. This result was driven by a single study where parasitoids (*Cotesia plutellae*; Hymenoptera: Braconidae) were offered a choice between Bt resistant *Plutella xylostella* (Lepidoptera: Plutellidae) caterpillars on Bt compared with non-Bt oilseed rape in field simulators in the laboratory (Schuler et al., 2003). Eliminating this study reduced the effect size to a non-significant 0.0633. For predators, the majority of studies used non-susceptible prey and the results were exactly the same whether using non-susceptible or Bt-resistant prey (not shown). For parasitoids, studies tended to use Bt-resistant hosts more, but again the results were the same regardless of the type of host. We re-ran the analyses eliminating all studies that used herbivores as host or prey that did not contain Cry proteins (Table S1). The results were similar.

The analyses of the tritrophic studies provide further substantiation of the lack of effects of Bt plants and different Cry proteins on the biology or function of natural enemies. This together with the results from the bitrophic studies (Section 5.2) also confirms that transformation-related, unintended effects do not appear to impair natural enemy performance. Thus, the data available do not support the proposal by some scientists (Arpaia et al., 2017) and the European Food Safety Authority (EFSA) (EFSA, 2010) that *in-planta* studies are needed to fully assess the Bt-plant effects on natural enemies (see Devos et al., 2016 for detailed discussion).

Two tritrophic laboratory studies compared non-target effects of Bt plants to those of conventional insecticides. Herbivore strains were deployed that were non-susceptible to either a particular Bt Cry protein or insecticides. The first study used a strain of Cry1C-resistant diamondback moth (*P. xylostella*) or strains that were resistant to four different insecticides (Chen et al., 2008). Caterpillars were treated with their respective toxins by feeding on leaf disks from Bt (Cry1C) broccoli

or disks treated with the insecticides and then exposed to the parasitoid *D. insulare*. Adult parasitoids only emerged from the Cry1C-resistant larvae. This provided clear evidence that the commonly used insecticides harmed the internal parasitoid while Cry1C did not. Similar results were reported in a second study where non-susceptible strains of aphids (*Myzus persicae*; Hemiptera: Aphididae) were used in tritrophic studies with Bt (Cry1Ab or Cry1C) broccoli or pyrethroid-treated broccoli and the predators *C. maculata* and *Eupeodes americanus* (Diptera: Syrphidae) or the parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) (Tian et al., 2015). Again, adverse effects on the natural enemies were observed in the pyrethroid treatment but not in the case of Bt broccoli.

6. Impacts of Bt crops on arthropod natural enemy abundance and function in the field

As noted, there has been considerable laboratory research demonstrating the safety of Bt proteins to a suite of important natural enemies. Further, it has been suggested that such early tier laboratory studies can conservatively predict non-target effects expected in the field (Duan et al., 2010). Thus, Bt crops represent a highly selective control tactic that should conserve natural enemies and contribute to enhanced management of pests, especially if Bt crops replace the application of broad-spectrum insecticides for control of Bt targeted pests. Bt maize and Bt cotton have been grown commercially for more than 20 years and provide an opportunity to assess their role in conservation biological control.

As of late 2008, over 63 field studies had been conducted to assess the potential impacts of Bt crops on non-target arthropods encompassing six classes, > 21 orders and > 185 species, with the vast majority of these being natural enemies important to providing biological control services (Naranjo, 2009). Dozens of studies have since been added, especially in the rice and soybean systems, but also with continued focus on cotton and maize. These studies have been discussed and summarized in narrative reviews (Romeis et al., 2006; Sanvido et al., 2007; Lundgren et al., 2009) and several quantitative syntheses (Comas et al., 2014; Dang et al., 2017; Marvier et al., 2007; Naranjo, 2009; Pellegrino et al., 2018; Peterson et al., 2011; Wolfenbarger et al., 2008). Overall, these studies have collectively concluded that non-target effects of Bt crops are minimal or negligible, especially in comparison to the negative effects of the use of insecticides for control of the Bt targeted pest (Fig. 5). A notable exception is the abundance of parasitoids for Bt maize. Many studies in this crop have been dominated by *Macrocentrus grandii* (Hymenoptera: Braconidae), an exotic parasitoid introduced to the USA for control of *O. nubilalis*, which is in turn the main target of Bt maize. Not surprisingly, the abundance of such specialist parasitoids and the biological control services they provide may decline in Bt maize once their host insects are effectively controlled (Bourguet et al., 2002; Manachini, 2003; Manachini and Lozzia, 2004; Siegfried et al., 2001). However, reductions in target host abundance do not always lead to reductions in biological control function (Dhillon and Sharma, 2013; Fernandes et al., 2007; Lumbierres et al., 2011; Orr and Landis, 1997; Rose, 2005; Thomazoni et al., 2010). In contrast, the use of insecticides for Bt targeted pests in non-Bt crops can significantly reduce biological control function (Musser and Shelton, 2003; Rose, 2005).

The impact of Bt crops on the biological control services supplied by generalist arthropod predators have been uniformly neutral in Bt maize (Ahmad et al., 2006) and Bt cotton (Head et al., 2005; Naranjo, 2005b; Olson and Ruberson, 2012; Sisterson et al., 2004). Only one study observed small reductions in several arthropod predator taxa in Bt cotton in long term field studies in Arizona that were likely associated with reductions in caterpillar prey (Naranjo, 2005a). However, using predator:prey ratios, sentinel prey and life tables of natural populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae), it was shown that these small

reductions in predator abundance were not associated with any change in the overall biological control services provided by the natural enemy community (Naranjo, 2005b). Overall, such changes in the target herbivore community are not unique to Bt crops, but would arise from the deployment of any effective pest management tactic or overall IPM strategy. Nonetheless, extant data suggests that Bt crops do not alter the function of the natural enemy community and may provide for enhanced biological control services if they prevent or reduce the alternative use of broader-spectrum insecticides for control of the Bt targeted pest. Several case studies in cotton and maize are presented below that demonstrate the potential role of Bt crops in conservation biological control.

7. Role of GE plants in integrated production systems

7.1. Bt cotton

The compatibility of Bt crops and biological control has been well documented with Bt cotton in Arizona as part of their overall IPM program. In 1996, Cry1Ac-cotton was introduced into Arizona to control the pink bollworm, *P. gossypiella*, a notorious pest of cotton in the southwestern US and northern regions of Mexico, as well as many other parts of the world including India. In Arizona, Bt cotton led to dramatic reductions in the use of foliar insecticides for the target pest, all of them broad-spectrum in nature (Henneberry and Naranjo, 1998). The quickly increased adoption of Bt cotton led to broad, areawide control of the pest (Carrière et al., 2003) and opened the door for an opportunity to eradicate this invasive pest. Bt cotton became a cornerstone element in the pink bollworm eradication program initiated in 2006 in Arizona, and insecticide use for this pest ceased entirely by 2008 (Naranjo and Ellsworth, 2010). Concurrently in 1996, a new IPM program was introduced for *B. tabaci* (MEAM1), another invasive pest that had quickly developed resistance to pyrethroids by 1995. Several new selective insect growth regulators were introduced leading to further reductions in broad spectrum insecticide use (Naranjo and Ellsworth, 2009a). With the introduction in 2006 of a selective insecticide for *Lygus hesperus* (Hemiptera: Miridae) the package was complete and overall insecticide use statewide for cotton was dramatically reduced. This pattern was associated with a disproportionately larger reduction in broad-spectrum insecticides resulting in a situation where most of the few insecticides now applied are those that more selectively target the pests and conserve natural enemies.

These progressive reductions in insecticide use provided an environment that allowed biological control by a diverse community of native natural enemies to flourish. Extensive experimental work documented the role of natural enemies generally and their conservation specifically in the suppression and economic management of *B. tabaci* (Asiimwe et al., 2016; Naranjo and Ellsworth, 2005, 2009b; Vandervoet et al., 2018). Overall, the Arizona cotton IPM strategy has cumulatively saved growers over \$500 million since 1996 in yield protection and control costs (\$274/ha/year), while preventing over 25 million pounds of active ingredient from being used in the environment (Ellsworth et al., 2018). While many components contributed to this transformative change that allowed conservation biological control to function at a high capacity in Arizona cotton production, Bt cotton was a keystone technology that eliminated the early season use of broad-spectrum insecticides for pink bollworm. Without this capstone event, it is unlikely this success would have been possible.

In China, a large-scale study demonstrated that the decline in insecticide sprays in Bt cotton resulted in an increased abundance of important natural enemies and an associated decline in aphid populations (Lu et al., 2012). More importantly, these effects were not only observed in the Bt crop itself but also in other (non-GE) crops within the region. Overall, Brookes and Barfoot (2018) estimate massive reductions in foliar insecticide use in Bt cotton production globally, pointing

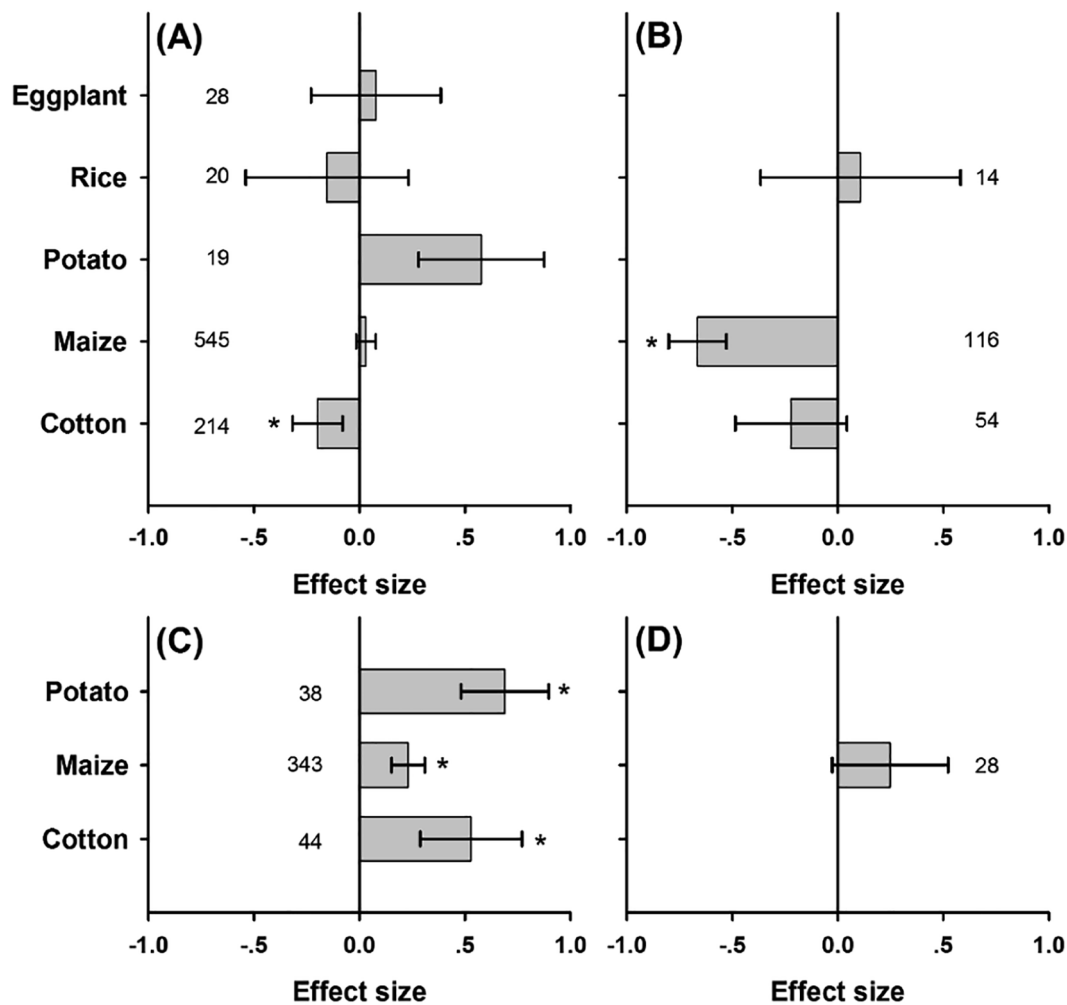


Fig. 5. Meta-analyses of studies that measured the abundance of predators and parasitoids in five Bt crops. A) Predators and B) parasitoids in Bt crops compared with non-Bt crops, neither treated with insecticides. C) Predators and D) parasitoids in Bt crops compared with non-Bt crops treated with insecticides. Asterisks denote effect sizes significantly different from zero; sample sizes denote total observations and error bars are 95% confidence intervals. (Modified from Naranjo, 2009 with permission).

strongly to the potential for conservation biological control to play an important and ever increasing role in IPM more broadly in this crop system.

The use of seed treated with various neonicotinoids has become pervasive in several field crops in the USA (Douglas and Tooker, 2015; Papiernik et al., 2018) and potentially negates to some degree the reduction in insecticides possible through the deployment of Bt crops. In the USA, neonicotinoid seed-treatments for cotton is common in some production regions (Allen et al., 2018; North et al., 2018; Toews et al., 2010), where it can provide economic control of thrips during the seedling establishment period (North et al., 2018). The impacts of such usage on arthropod natural enemies is not well understood in cotton, but some data suggest minimal effects at recommended doses (Saeed et al. 2016). Unlike most of the cotton production region in the US, the use of treated seed in Arizona is relatively rare (< 13% of acreage, P. Ellsworth, personal communication), mainly because plants in this production environment can quickly outgrow any minor thrips damage and some species such as *Frankliniella occidentalis* (Thysanoptera: Thripidae) are actually considered beneficial (Gonzalez et al., 1982; Trichilo and Leigh, 1986).

7.2. Bt maize

As for cotton, studies have shown that using Bt maize (field corn) has

resulted in large global reductions in the use of foliar insecticides for control of Lepidoptera (Klümper and Qaim, 2014; Brookes and Barfoot, 2018). Studies on the widespread adoption of Bt maize in the Midwestern USA corn belt have demonstrated a dramatic decline in populations of *O. nubilalis*, and thus the need for insecticide treatments for this key lepidopteran pest. Furthermore, this decline occurred not only for those who adopted Bt maize, but also for surrounding maize farmers that did not (Hutchinson et al., 2010). A similar ‘halo’ effect of lepidopteran suppression by the widespread adoption of Bt maize in the eastern USA has also been documented, as well as the benefits of pest declines in surrounding vegetable fields (Dively et al., 2018). While these studies document lower pest pressure because of wide spread adoption of Bt maize and less need for insecticidal sprays, by implication they also suggest that widespread conservation of natural enemies may be occurring. However, as noted, there has been a trend in the USA to add neonicotinoid seed treatments and to date virtually all maize seeds sold are treated (Douglas and Tooker, 2015; Sappington et al., 2018). This insurance approach is targeting a number of early-season pests that occur only sporadic but for some of which rescue treatments are not available (Gray, 2011; Sappington et al., 2018). Recent work suggests that seed treatments in maize can negatively impact some natural enemy populations early season even though there is recovery later on (Disque et al., 2018). Thus, such treatments have the potential to erase some of the very positive gains in foliar insecticide reduction in maize.

Studies in sweet corn, which is routinely treated with foliar insecticides far more than field corn, have been able to document that the conservation of natural enemies with Bt plants results in enhanced biological control. In the northeastern US where a considerable amount of sweet corn is grown, studies have shown that Bt sweet corn is far less toxic to the major predators in the system (ladybeetles *C. maculata* and *H. axyridis* and the minute pirate bug, *Orius insidiosus* [Hemiptera: Anthocoridae]), than the commonly used pyrethroid lambda cyhalothrin, spinosad, and indoxacarb (Musser and Shelton, 2003). Furthermore, this study demonstrated that Bt sweet corn provided better control of lepidopteran pests, and did not negatively affect the predation rates of sentinel egg masses of the European corn borer, as did lambda cyhalothrin and indoxacarb. A follow-up study proposed a model that integrated biological and chemical control into a decision-making tool and highlighted the benefit of conserving natural enemies so they could play a role in suppressing not only the lepidopteran pests but secondary pests such as aphids that infest the ears and affect marketability (Musser et al., 2006).

7.3. Importance of natural enemies for resistance management

Work by Stern and colleagues in California in the 1950s demonstrated that use of selective insecticides could be used to control the spotted alfalfa aphid without disrupting an important parasitoid that helped keep it in check (Smith and Hagen, 1959; Stern and van den Bosch, 1959; Stern et al., 1959). They noted that when biological control was disrupted, it often led to an ‘insecticide treadmill’ for the pests which, in turn, led to their eventual resistance to the insecticides. This key finding on the importance of conserving biological control agents was instrumental in the development of the Integrated Control Concept (Smith and Hagen, 1959; Stern and van den Bosch, 1959; Stern et al., 1959), the precursor of the IPM concept.

As described previously, multiple studies have shown that Cry1 proteins expressed in plants control targeted Lepidoptera but do not harm important natural enemies, thus conserving them to function as biological control agents. With the threat of targeted pests evolving resistance to Bt proteins expressed in plants (Tabashnik and Carrière, 2017), investigations have been undertaken to determine whether natural enemies may help delay resistance to Bt proteins in the targeted pest.

Using a system composed of Bt broccoli (Cry1Ac), the diamondback moth (*P. xylostella*), the predator, *C. maculata*, and the parasitoid, *D. insulare*, the interaction of resistance evolution and biological control was explored. In a greenhouse study over multiple generations, use of *C. maculata* and Bt broccoli provided excellent control of *P. xylostella* while delaying resistance in *P. xylostella* to Bt broccoli (Liu et al., 2014). Using this same system, a model was created to study the influence of *D. insulare* on the long-term pest management and evolution of resistance in *P. xylostella* (Onstad et al., 2013). Simulations demonstrated that parasitism by *D. insulare* provided the most reliable long-term control of *P. xylostella* within this system and always delayed the evolution of resistance to Bt broccoli. This latter finding agrees with previous studies using this experimental system that demonstrated the lack of harm to the parasitoid by Cry1Ac, compared to other commonly used insecticides for control of *P. xylostella* (Chen et al., 2008). These findings suggest that biological control, in addition to other factors including refuges and gene expression (Tabashnik et al., 2013), may play a significant role in limiting the number of cases of resistance to Bt plants to date, especially compared to the ever-increasing cases of resistance to broad-spectrum insecticides (Sparks and Nauen, 2015).

8. Outlook

In the near future, we are likely to see currently used as well as new Bt (Cry and Vip) proteins deployed in additional (including minor) crops. For example, in China dozens of rice lines with resistance to

various lepidopteran pests have already been developed that are highly resistant to stem borers such as *Chilo suppressalis* (Crambidae) (Li et al., 2016; Liu et al. 2016a). While two lines expressing a *cry1Ab/Ac* fusion gene have received biosafety certificates by the Ministry of Agriculture already in 2009, no Bt rice is commercialized yet (Li et al., 2016). Another example is that of cowpea (*Vigna unguiculata* ssp. *unguiculata*) that contains Cry1Ab to protect the plant from damage by *Maruca testulalis* (Lepidoptera: Crambidae) (Ba et al., 2018; Huesing et al., 2011; Mohammed et al., 2014). While the plant has not yet been approved, it has the potential to significantly reduce the yield loss caused by this major pest in sub-Saharan Africa, where cowpea is the most important grain legume (Murdock et al., 2008). In addition to cowpea, field experiments with various Bt crops are ongoing in different countries in Africa (ISAAA, 2017). Genes for new Bt proteins may include modifications to improve efficacy or to facilitate expression in plants (Lucena et al., 2014). An example is modified Cry51Aa2 protein (Cry51Aa2.834_16) that protects cotton against feeding damage caused by hemipteran and thysanopteran pests (Baum et al., 2012; Gowda et al., 2016; Bachman et al., 2017). Furthermore, we can expect to see novel combinations of Cry and Vip proteins in pyramided GE crops.

In today’s Bt-transgenic plants, the expression of the insecticidal genes is driven by constitutive promoters (i.e., CaMV 35s) and the proteins are constantly produced in most plant tissues. Scientists thus search for effective wound-inducible promoters that ensure that the insecticidal compound is only produced when and where it is required. The feasibility of this approach has been documented in the glasshouse and in the field for rice where *cry* genes were driven by the wound-inducible *mpi* promoter from maize (Breitler et al., 2001, 2004). Another example is the successful use of the wound-inducible AoPR1 promoter isolated from *Asparagus officinalis* in cotton and potato (Ahmed et al., 2017; Anayol et al., 2016). Other examples of non-constitutive promoters include tissue-specific and inducible promoters that may help not only limit exposure to natural enemies but can be used for resistance management (Bates et al., 2005).

In addition to Bt, effective toxins have also been isolated from other bacteria including species of *Pseudomonas* (Anderson et al., 2018) and *Chromobacter* (Sampson et al. 2017) that might be expressed in future insect-resistant GE plants. Much research has also been devoted to protease and alpha-amylase inhibitors and lectins to target lepidopteran, coleopteran, and hemipteran pests (Malone et al., 2008). A compound that is of particular interest is the alpha-amylase inhibitor α AI-1 from the green bean that has been introduced into various other legumes and shown to provide very high levels of protection from certain (susceptible) bruchid (Coleoptera: Bruchidae) species (Lüthi et al., 2013a; Morton et al., 2000). Despite the fact that the alpha-amylases of hymenopteran parasitoids of bruchids are also susceptible to this particular inhibitor (Álvarez-Alfageme et al., 2012), tritrophic studies have shown that the α AI-1 containing GE seeds cause no harm to their parasitoids (Lüthi et al., 2013b, 2018). In any case, to our knowledge, none of those insecticidal compounds is close to reaching the market stage anytime soon.

Another promising new development is the use of RNA interference (RNAi) to control arthropod pests by developing plants to produce double-stranded RNA (dsRNA) that silences an essential gene in the target species after ingestion (Burand and Hunter, 2013; Zhang et al., 2017). RNAi effects caused by ingested dsRNA have been shown in various insect orders but with highly variable success rates in the down regulation of the target genes (Baum and Roberts, 2014; Huvenne and Smagghe, 2010). In general, dietary RNAi works very well in Coleoptera but less so in Lepidoptera (Baum and Roberts, 2014). What makes the technology interesting is the fact that one can also target hemipteran pests (including phloem-feeders) that have not yet been targeted by Bt proteins (Baum and Roberts, 2014; Ibrahim et al., 2017). The potential of RNAi for pest control has first been demonstrated in 2007 for *H. armigera* and *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) (Baum et al., 2007; Mao et al., 2007). Later, Zhang

et al. (2015) reported control of the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) by expressing dsRNA in chloroplasts of potato. The first insect-resistant dsRNA-expressing GE crop was registered by the US Environmental Protection Agency (US EPA) in June 2017 (<https://www.epa.gov/newsreleases/epa-registers-innovative-tool-control-corn-rootworm>). This GE maize event (MON87411) produces a dsRNA targeting the *Snf7* protein in *D. v. virgifera*, which is crucial for the transport of transmembrane proteins (Bachman et al., 2013; Bolognesi et al., 2012). Suppression of *Snf7* has been reported to cause increased *D. v. virgifera* larval mortality leading to reduced root damage (Bolognesi et al., 2012). Because the RNAi effect is sequence specific, the dsRNA can be designed to specifically target the gene in the target pest insect. Studies on numerous non-target species using the dsRNA targeting *Snf7* in *D. v. virgifera* have demonstrated this specificity (Bachmann et al., 2016). Combining Bt Cry proteins with RNAi has great potential to delay resistance development (Khajuria et al., 2018; Ni et al., 2017). As expected, development of resistance will also be a concern in respect to RNAi-based GE crops and thus needs to be managed. A recent study demonstrated that insects can develop resistance to dsRNA (Khajuria et al., 2018). Interestingly, resistance was not sequence-specific but caused by an impaired luminal uptake, indicated by cross resistance to other dsRNAs tested.

New plant breeding techniques, such as genome editing that are protein-mediated or based on sequence-specific nucleases are continuously being developed (Baltes and Voytas, 2015). These techniques allow the knock-out of a specific gene. Of those, CRISPR-Cas9 (Doudna and Charpentier, 2014) has gained the highest importance. The technique has already been successfully applied to crop plants to alter agriculturally important traits such as disease resistance (Wang et al., 2014) and drought tolerance (Shi et al., 2017). To our knowledge, there is only one example where the technology was used to develop an insect-resistant plant. By knocking out the cytochrome P450 gene *CYP71A1*, rice plants became resistant against rice brown planthopper (*N. lugens*) and striped stem borer (*C. suppressalis*) (Lu et al., 2018). The gene encodes for an enzyme that catalyzes the conversion of tryptamine to serotonin. The suppression of the serotonin biosynthesis resulted in enhanced insect resistance.

As these new technologies develop it will be important that research be conducted to ensure that any unacceptable non-target effects be identified and mitigated before commercialization so that GE crops will continue to be useful tools in the context of IPM and sustainable pest control.

9. Conclusions

The efficacy of Bt-transgenic crops in controlling important target pests has been very high. Furthermore, the large-scale adoption of Bt crops in some parts of the world has led to area-wide suppressions of target pest populations benefitting both farmers that adopted the technology and those that did not. As expected and intended, the insecticidal proteins deployed today have a narrow spectrum of activity and cause no detrimental unintended effects to natural enemies. The use of Bt crops typically replaces chemical broad-spectrum insecticides (foliar sprays and soil insecticides). However, in the USA, and possibly other parts of the world, this benefit is to some extent counteracted by the increasing application of insecticidal seed treatments (to both Bt and non-Bt crops) for the management of early season pests and as insurance against sporadic pests (Allen et al., 2018; Sappington et al., 2018).

Overall, the change in insecticide use has benefitted non-target species in general and biological control in particular. In respect to Bt-transgenic crops, the National Academies of Sciences, Engineering, and Medicine (NASEM, 2016) recently concluded: “On the basis of the available data, the committee found that planting of Bt crops has tended to result in higher insect biodiversity on farms than planting similar varieties without the Bt trait that were treated with synthetic insecticides.” Earlier,

the European Academies have stated that “There is compelling evidence that GM crops can contribute to sustainable development goals with benefits to farmers, consumers, the environment and the economy.” (EASAC, 2013). Consequently, such insect-resistant GE varieties can not only help to increase yields and provide economic benefits to farmers but also improve environmental and human health. The large body of evidence supporting such outcomes should be considered when developing and introducing new insecticidal GE plants in new countries and cropping systems.

Author statement

All authors compiled, wrote and approved this review article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2018.10.001>.

References

- Adamczyk Jr., J.J., Hardee, D.D., Adams, L.C., Sumerford, D.V., 2001. Correlating differences in larval survival and development of bollworms (Lepidoptera: Noctuidae) and fall armyworms (Lepidoptera: Noctuidae) to differential expression of Cry1Ac(c) δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94, 284–290.
- Ahmad, A., Wilde, G.E., Whitworth, R.J., Zolnerowich, G., 2006. Effect of corn hybrids expressing the coleopteran-specific Cry3Bb1 protein for corn rootworm control on aboveground insect predators. *J. Econ. Entomol.* 99, 1085–1095.
- Ahmed, H.A.A., Onarici, S., Bakhsh, A., Akdoğan, G., Karakoç, Ö.C., Özcan, S.F., Aydın, G., Aasim, M., Ünlü, L., Sancak, C., Naimov, S., Özcan, S., 2017. Targeted expression of insecticidal hybrid SN19 gene in potato leads to enhanced resistance against Colorado potato beetle (*Leptinotarsa decemlineata* Say) and tomato leafminer (*Tuta absoluta* Meyrick). *Plant Biotechnol. Rep.* 11, 315–329.
- Albajes, R., Lumbierres, B., Pons, X., 2009. Responsiveness of arthropod herbivores and their natural enemies to modified weed management in corn. *Environ. Entomol.* 38, 944–954.
- Albajes, R., Lumbierres, B., Pons, X., 2011. Two heteropteran predators in relation to weed management in herbicide-tolerant corn. *Biol. Control* 59, 30–36.
- Allen, K.C., Luttrell, R.G., Sappington, T.W., Hesler, L.S., Papiernik, S.K., 2018. Frequency and abundance of selected early-season insect pests of cotton. *J. Integr. Pest Manage.* 9, 20.
- Álvarez-Álfageme, F., Ferry, N., Castañera, P., Ortego, F., Gatehouse, A.M.R., 2008. Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). *Transgenic Res.* 17, 943–954.
- Álvarez-Álfageme, F., Bigler, F., Romeis, J., 2011. Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design. *Transgenic Res.* 20, 467–479.
- Álvarez-Álfageme, F., Lüthi, C., Romeis, J., 2012. Characterization of digestive enzymes of bruchid parasitoids – initial steps for environmental risk assessment of genetically modified legumes. *PLoS One* 7, e36862.
- Anayol, E., Bakhsh, A., Karakoç, Ö.C., Onarici, S., Köm, D., Aasim, M., Özcan, S.F., Barpete, S., Khabbazi, S.D., Önel, B., Sancak, C., Khawar, K.M., Ünlü, L., Özcan, S., 2016. Towards better insect management strategy: restriction of insecticidal gene expression to biting sites in transgenic cotton. *Plant Biotechnol. Rep.* 10, 83–94.
- Anderson, J.A., Staley, J., Challender, M., Heuton, J., 2018. Safety of *Pseudomonas chlororaphis* as a gene source for genetically modified crops. *Transgenic Res.* 27, 103–113.
- Armer, C.A., Berry, R.E., Kogan, M., 2000. Longevity of phytophagous heteropteran predators feeding on transgenic Bt-potato plants. *Entomol. Exp. Appl.* 95, 329–333.
- Arpaia, S., Birch, A.N.E., Kiss, J., van Loon, J.J.A., Messéan, A., Nuti, M., Perry, J.N., Sweet, J.B., 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, 123–132.
- Asimwe, P., Ellsworth, P.C., Naranjo, S.E., 2016. Natural enemy impacts on *Bemisia tabaci* (MEAM1) dominate plant quality effects in the cotton system. *Ecol. Entomol.* 41, 642–652.
- Ba, M.N., Huesing, J.E., Tamò, M., Higgins, T.J.V., Pittendrigh, B.R., Murdock, L.L., 2018. An assessment of the risk of Bt-cowpea to non-target organisms in West Africa. *J. Pest Sci.* 91, 1165–1179.
- Bachman, P.M., Bolognesi, R., Moar, W.J., Mueller, G.M., Paradise, M.S., Ramaseshadri, P., Tan, J., Uffman, J.P., Warren, J., Wiggins, B.E., Levine, S.L., 2013.

- Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Transgenic Res.* 22, 1207–1222.
- Bachmann, P.M., Huizinga, K.M., Jensen, P.D., Mueller, G., Tan, J., Uffman, J.P., Levine, S.L., 2016. Ecological risk assessment for DvSnf7 RNA: A plant-incorporated protectant with targeted activity against western corn rootworm. *Regul. Toxicol. Pharm.* 81, 77–88.
- Bachmann, P.M., Ahmad, A., Ahrens, J.E., Akbar, W., Baum, J.A., Brown, S., Clark, T.L., Fridley, J.M., Gowda, A., Greenplate, J.T., Jensen, P.D., Mueller, G.M., Odegaard, M.L., Tan, J., Uffman, J.P., Levine, S.L., 2017. Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry1Aa2.834.16. *PLoS One* 12 e0169409.
- Bai, Y.Y., Jiang, M.X., Cheng, J.A., 2005. Effects of transgenic *cry1Ab* rice pollen on fitness of *Propylea japonica* (Thunberg). *J. Pest Sci.* 78, 123–128.
- Baktavachalam, G.B., Delaney, B., Fisher, T.L., Ladics, G.S., Layton, R.J., Locke, M.E.H., Schmidt, J., Anderson, J.A., Weber, N.N., Herman, R.A., Evans, S.L., 2015. Transgenic maize event TC1507: Global status of food, feed, and environmental safety. *GM Crops Food* 6, 80–102.
- Bale, J.S., van Lenteren, J.C., Bigler, F., 2008. Biological control and sustainable food production. *Philos. Trans. R. Soc. B* 363, 761–776.
- Baltes, N.J., Voytas, D.F., 2015. Enabling plant synthetic biology through genome engineering. *Trends Biotechnol.* 33, 120–131.
- Bates, S.L., Zhao, J.-Z., Roush, R.T., Shelton, A.M., 2005. Insect resistance management in GM crops: past present and future. *Nat. Biotechnol.* 23, 57–62.
- Baum, J.A., Roberts, J.K., 2014. Progress towards RNAi-mediated insect pest management. *Adv. Insect Physiol.* 47, 249–295.
- Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., Vaughn, T., Roberts, J., 2007. Control of coleopteran insect pests through RNA interference. *Nat. Biotechnol.* 25, 1322–1326.
- Baum, J.A., Sukuru, U.R., Penn, S.R., Meyer, S.E., Subbarao, S., Shi, X., Flasiniski, S., Heck, G.R., Brown, R.S., Clark, T.L., 2012. Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. *J. Econ. Entomol.* 105, 616–624.
- Bernal, C.C., Aguda, R.M., Cohen, M.B., 2002. Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis*. *Entomol. Exp. Appl.* 102, 21–28.
- Bianchi, F.J.J.A., Booij, C.J.H., Tscharntke, T., 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proc. R. Soc. B* 273, 1715–1727.
- Bigler, F., Albajes, R., 2011. Indirect effects of genetically modified herbicide tolerant crops on biodiversity and ecosystem services: the biological control example. *J. Consum. Prot. Food Saf.* 6 (Suppl. 1), S79–S84.
- Boethel, D.J., Eikenbary, R.D., 1986. Interactions of Plant Resistance and Parasitoids and Predators of Insects. John Wiley and Sons, New York.
- Bolognesi, R., Ramaseshadri, P., Anderson, J., Bachman, P., Clinton, W., Flannagan, R., Illagan, O., Lawrence, C., Levine, S., Moar, W., Mueller, F., Tan, J., Uffman, J., Wiggins, E., Heck, G., Segers, G., 2012. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *PLoS One* 7, e47534.
- Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N., Pagliari, C., 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environ. Biosaf. Res.* 1, 49–60.
- Bravo, A., Likitvatanavong, S., Gill, S.S., Soberón, M., 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. *Insect Biochem. Mol. Biol.* 41, 423–431.
- Breidler, J.C., Cordero, M.J., Royer, M., Meynard, D., San Segundo, B., Guiderdoni, E., 2001. The –689/+197 region of the maize protease inhibitor gene directs high level, wound inducible expression of the *cry1B* gene which protects transgenic rice plants from stemborer attack. *Mol. Breed.* 7, 259–274.
- Breidler, J.C., Vassal, J.M., del Mar Catala, M., Meynard, D., Marfà, V., Melé, E., Royer, M., Murillo, I., Segundo, B.S., Guiderdoni, E., Messegue, J., 2004. *Bt* rice harbouring *cry* genes controlled by a constitutive or wound-inducible promoter: protection and transgene expression under Mediterranean field conditions. *Plant Biotechnol. J.* 2, 417–430.
- Brookes, G., Barfoot, P., 2018. Environmental impacts of genetically modified (GM) crop use 1996–2016: Impacts on pesticide use and carbon emissions. *GM Crops Food*. <https://doi.org/10.1080/21645698.2018.1476792>.
- Burand, J.P., Hunter, W.B., 2013. RNAi: future in insect management. *J. Invertebr. Pathol.* 112 (Suppl. 1), S68–S74.
- Carrière, Y., Ellers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehy, T.J., Tabashnik, B.E., 2003. Long-term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. *Proc. Nat. Acad. Sci. U.S.A.* 100, 1519–1523.
- Carstens, K., Anderson, J., Bachman, P., De Schrijver, A., Dively, G., Federici, B., Hamer, M., Gielkens, M., Jensen, P., Lamp, W., Rauschen, S., Ridley, G., Romeis, J., Waggoner, A., 2012. Genetically modified crops and aquatic ecosystems: considerations for environmental risk assessment and non-target organism testing. *Transgenic Res.* 21, 813–842.
- Carstens, K., Cayabyab, B., De Schrijver, A., Gadaleta, P.G., Hellmich, R.L., Romeis, J., Storer, N., Valicente, F.H., Wach, M., 2014. Surrogate species selection for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. *GM Crops Food* 5, 11–15.
- CERA, 2011. A review of the environmental safety of the Cry1Ac protein. *Environ. Biosaf. Res.* 10, 27–49.
- CERA, 2012. A Review of the Environmental Safety of Vip3Aa. Center for Environmental Risk Assessment, ILSI Research Foundation, Washington DC, USA. Available online: < http://ilsi.org/wp-content/uploads/sites/5/2016/06/vip3Aa_EN.pdf > .
- CERA, 2013a. A Review of the Environmental Safety of the Cry2Ab Protein. Center for Environmental Risk Assessment 2013 ILSI Research Foundation Washington DC, USA Available online: < <http://ilsi.org/researchfoundation/wp-content/uploads/sites/5/2016/05/Cry2Ab-monograph.pdf> > .
- CERA, 2013b. A Review of the Environmental Safety of the Cry1F Protein. Center for Environmental Risk Assessment, ILSI Research Foundation, Washington DC, USA. Available online: < <http://ilsi.org/researchfoundation/wp-content/uploads/sites/5/2016/05/Cry1F-monograph.pdf> > .
- Chang, X., Lu, Z., Shen, Z., Peng, Y., Ye, G., 2017. Biotrophic and tritrophic effects of transgenic *cry1Ab/cry2Aj* maize on the beneficial, nontarget *Harmonia axyridis* (Coleoptera: Coccinellidae). *Environ. Entomol.* 46, 1171–1176.
- Chaplin-Kramer, R., O'Rourke, M.E., Blitzer, E.J., Kremen, C., 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecol. Lett.* 14, 922–932.
- Chen, M., Zhao, J.-Z., Collins, H.L., Earle, E.D., Cao, J., Shelton, A.M., 2008. A critical assessment of the effects of Bt transgenic plants on parasitoids. *PLoS One* 3, e2284.
- Coll, M., Guershon, M., 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annu. Rev. Entomol.* 47, 267–297.
- Comas, C., Lumbierres, B., Pons, X., Albajes, R., 2014. No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. *Transgenic Res.* 23, 135–143.
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260.
- Craig, W., Tepfer, M., Degraffi, G., Ripandelli, D., 2008. An overview of general features of risk assessments of genetically modified crops. *Euphytica* 164, 853–880.
- Dang, C., Lu, Z., Wang, L., Chang, X., Wang, F., Yao, H., Peng, Y., Stanley, S., Ye, G., 2017. Does *Bt* rice pose risks to non-target arthropods? Results of a meta-analysis in China. *Plant Biotechnol. J.* 15, 1047–1053.
- De Schrijver, A., Devos, Y., De Clercq, P., Gathmann, A., Romeis, J., 2016. Quality of laboratory studies assessing effects of Bt-proteins on non-target organisms: minimal criteria for acceptability. *Transgenic Res.* 25, 395–411.
- De Sousa, M.F., Fernandes, M.G., Mota, T.A., 2017. Biology of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) fed transgenic maize pollen. *Florida Entomol.* 100, 653–656.
- Devos, Y., De Schrijver, A., De Clercq, P., Kiss, J., Romeis, J., 2012. *Bt*-maize event MON88017 expressing Cry3Bb1 does not cause harm to non-target organisms. *Transgenic Res.* 21, 1191–1214.
- Devos, Y., Romeis, J., Luttki, R., Maggiore, A., Perry, J.N., Schoonjans, R., Streissl, F., Tazazona, J.V., Brock, T.C.M., 2015. Optimising environmental risk assessments. accounting for ecosystem services helps to translate broad policy protection goals into specific operational ones for environmental risk assessments. *EMBO Rep.* 16, 1060–1063.
- Devos, Y., Álvarez-Alfageme, F., Gennaro, A., Mestdag, S., 2016. Assessment of unanticipated unintended effects of genetically modified plants on non-target organisms: a controversy worthy of pursuit? *J. Appl. Entomol.* 140, 1–10.
- Dewar, A.M., Haylock, L.A., Bean, K.M., May, M.J., 2000. Delayed control of weeds in glyphosate-tolerant sugar beet and the consequences on aphid infestation and yield. *Pest Manage. Sci.* 56, 345–350.
- Dewar, A.M., May, M.J., Woiwod, I.P., Haylock, L.A., Champion, G.T., Garner, B.H., Sands, R.J.N., Qi, A., Pidgeon, J.D., 2003. A novel approach to the use of genetically modified herbicide tolerant crops for environmental benefit. *Proc. R. Soc. B* 270, 335–340.
- Dhillon, M., Sharma, H., 2013. Comparative studies on the effects of Bt-transgenic and non-transgenic cotton on arthropod diversity, seedcotton yield and bollworms control. *J. Environ. Biol.* 34, 67–73.
- Disque, H.H., Hamby, K.A., Dubey, A., Taylor, C., Dively, G.P., 2018. Effects of clothianidin-treated seed on the arthropod community in a mid-Atlantic no-till corn agroecosystem. *Pest Manage. Sci.* <https://doi.org/10.1002/ps.5201>.
- Dively, G.P., Venugopala, P.D., Bean, D., Whalen, J., Holmstrom, K., Kuhar, T.P., Doughty, H.B., Patton, T., Cissel, W., Hutchison, W.D., 2018. Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. *Proc. Natl. Acad. Sci. U.S.A.* 115, 3320–3325.
- Doudna, J.A., Charpentier, E., 2014. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346, 1258096.
- Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. field crops. *Environ. Sci. Technol.* 49, 5088–5097.
- Downes, S., Kriticos, D., Parry, H., Paull, C., Schellhorn, N., Zalucki, M.P., 2017. A perspective on management of *Helicoverpa armigera*: transgenic Bt cotton, IPM, and landscapes. *Pest Manage. Sci.* 73, 485–492.
- Duan, J.J., Head, G., McKee, M.J., Nickson, T.E., Martin, J.W., Sayegh, F.S., 2002. Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*. *Entomol. Exp. Appl.* 104, 271–280.
- Duan, J.J., Lundgren, J.G., Naranjo, S.E., Marvier, M., 2010. Extrapolating non-target risk of *Bt* crops from laboratory to field. *Biol. Lett.* 6, 74–77.
- Dutton, A., Klein, H., Romeis, J., Bigler, F., 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecol. Entomol.* 27, 441–447.
- Dutton, A., Romeis, J., Bigler, F., 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. *BioControl* 48, 611–636.
- EASAC, 2013. Planting the future: Opportunities and challenges for using crop genetic improvement technologies for sustainable agriculture. European Academies Science Advisory Council, EASAC Policy Report 21. Available online: < <https://easac.eu/> >

- publications/details/planting-the-future-opportunities-and-challenges-for-using-crop-genetic-improvement-technologies-fo/ > .
- EFSA, 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA J. 8, 1879.
- EFSA Panel on Genetically Modified Organisms (GMO), 2009. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMORX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA J. 1149, 1–84. Available online: <https://www.efsa.europa.eu/de/efsajournal/pub/1149>.
- EFSA Panel on Genetically Modified Organisms (GMO), 2011. Scientific Opinion on application (EFSAGMO-UK-2008-60) for placing on the market of genetically modified herbicide tolerant maize GA21 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA J. 9 (12), 2480. Available online: <https://www.efsa.europa.eu/de/efsajournal/pub/2480>.
- EFSA Panel on Genetically Modified Organisms (GMO), 2012. Scientific Opinion on an application (EFSAGMO-NL-2005-24) for the placing on the market of the herbicide tolerant genetically modified soybean 40–3–2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA J. 10 (6), 2753. Available online: <https://www.efsa.europa.eu/de/efsajournal/pub/2753>.
- Eisenring, M., Romeis, J., Naranjo, S.E., Meissle, M., 2017. Multitrophic toxin flow in a Bt-cotton field. *Agric. Ecosyst. Environ.* 247, 283–289.
- Ellsworth, P.C., Fournier, A., Frisvold, G., Naranjo, S.E., 2018. Chronicling the socio-economic impact of integrating biological control, technology, and knowledge over 25 years of IPM in Arizona. Available online In: Mason, P.G., Gillespie, D.R., Vincent, C. (Eds.), Proceedings of the 5th International Symposium on Biological Control of Arthropods. CAB International, pp. 214–216.
- Estruch, J.J., Warren, G.W., Mullins, M.A., Nye, G.J., Craig, J.A., Koziel, M.G., 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc. Natl. Acad. Sci. U.S.A.* 93, 5389–5394.
- Fernandes, O.A., Faria, M., Martinelli, S., Schmidt, F., Carvalho, V.F., Moro, G., 2007. Short-term assessment of Bt maize on non-target arthropods in Brazil. *Sci. Agric.* 64, 249–255.
- Ferry, N., Mulligan, E.A., Majerus, M.E.N., Gatehouse, A.M.R., 2007. Biotrophic and tri-trophic effects of Bt Cry3A transgenic potato on beneficial, non-target, beetles. *Transgenic Res.* 16, 795–812.
- Fischhoff, D.A., Bowditch, K.S., Perlak, F.J., Marrone, P.G., McCormick, S.M., Niedermeyer, J.G., Dean, D.A., Kusano-Kretzmer, K., Mayer, E.J., Rochester, D.E., Rogers, S.G., Fraley, R.T., 1987. Insect tolerant transgenic tomato plants. *Bio/Technology* 5, 807–813.
- García-Alonso, M., 2010. Current challenges in environmental risk assessment: the assessment of unintended effects of GM crops on non-target organisms. *IOBC/WPRS Bull.* 52, 57–63.
- García-Alonso, M., Raybould, A., 2014. Protection goals in environmental risk assessment: a practical approach. *Transgenic Res.* 23, 945–956.
- García-Alonso, M., Jacobs, E., Raybould, A., Nickson, T.E., Sowig, P., Willekens, H., van der Kouwe, P., Layton, R., Amijee, F., Fuentes, A.M., Tencalla, F., 2006. A tiered system for assessing the risk of genetically modified plants to nontarget organisms. *Environ. Biosaf. Res.* 5, 57–65.
- García-Ruiz, E., Loureiro, I., Fariño, G.P., Gómez, P., Gutiérrez, E., Sánchez, F.J., Concepción Escorial, M., Ortego, F., Cristina Chueca, M., Castañera, P., 2018. Weeds and ground-dwelling predators' response to two different weed management systems in glyphosate-tolerant cotton: a farmscale study. *PLoS One* 13, e0191408.
- Geng, J.-H., Shen, Z.-R., Song, K., Zheng, L., 2006. Effect of pollen of regular cotton and transgenic Bt+ CpTI cotton on the survival and reproduction of the parasitoid wasp *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) in the Laboratory. *Environ. Entomol.* 35, 1661–1668.
- Givens, W.A., Shaw, D.R., Kruger, G.R., Johnson, W.G., Weller, S.C., Young, B.G., Wilson, R.G., Owen, M.D.K., Jordan, D., 2009. Survey of tillage trends following the adoption of glyphosate-resistant crops. *Weed Technol.* 23, 150–155.
- Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., Tamò, M., 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One* 11, e0165632.
- Gonzalez, D., Patterson, B.R., Leigh, T.F., Wilson, L.T., 1982. Mites, a primary food source for two predators in San Joaquin cotton. *Calif. Agric.* 36, 18–20.
- Gowda, A., Rydel, T.J., Wollacott, A.M., Brown, R.S., Akbar, W., Clark, T.J., Flasiniski, S., Nageotte, J.R., Read, A.C., Shi, X., Werner, B.J., Leau, M.J., Baum, J.A., 2016. A transgenic approach for controlling *Lygus* in cotton. *Nat. Commun.* 7, 12213.
- Graser, G., Walters, F.S., Burns, A., Sauve, A., Raybould, A., 2017. A general approach to test for interaction among mixtures of insecticidal proteins which target different orders of insect pests. *J. Insect Sci.* 17, 39.
- Gray, M.E., 2011. Relevance of traditional integrated pest management (IPM) strategies for commercial corn producers in a transgenic agroecosystem: a bygone era? *J. Agric. Food Chem.* 59, 5852–5858.
- Gressel, J., Gassmann, A.J., Owen, M.D.K., 2017. How well will stacked transgenic pest/herbicide resistances delay pests from evolving resistance? *Pest Manage. Sci.* 73, 22–34.
- Guo, Y.Y., Tian, J.C., Shi, W.P., Dong, X.H., Romeis, J., Naranjo, S.E., Hellmich, R.L., Shelton, A.M., 2016. The interaction of two-spotted spider mites, *Tetranychus urticae* Koch, with Cry protein production and predation by *Amblyseius andersoni* (Chant) in Cry1Ac/Cry2Ab cotton and Cry1F maize. *Transgenic Res.* 25, 33–44.
- Gurr, G.M., You, M., 2016. Conservation biological control of pests in the molecular era: new opportunities to address old constraints. *Front. Plant Sci.* 6, 1255.
- Hagenbucher, S., Olson, D.M., Ruberson, J.R., Wäckers, F.L., Romeis, J., 2013. Resistance mechanisms against arthropod herbivores in cotton and their interactions with natural enemies. *Crit. Rev. Plant Sci.* 32, 458–482.
- Haller, S., Romeis, J., Meissle, M., 2017. Effects of purified or plant-produced Cry proteins on *Drosophila melanogaster* (Diptera: Drosophilidae) larvae. *Sci. Rep.* 7, 11172.
- Hare, J.D., 2002. Plant genetic variation in tritrophic interactions. In: Tschirntke, T., Hawkins, B.A. (Eds.), *Multitrophic Level Interactions*. Cambridge University Press, Cambridge, UK, pp. 8–43.
- Harwood, J.D., Wallin, W.G., Obycki, J.J., 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. *Mol. Ecol.* 14, 2815–2823.
- Hautea, D.M., Taylo, L.D., Masanga, A.P.L., Sison, M.L.J., Narciso, J.O., Quilloy, R.B., Hautea, R.A., Shotkoski, F.A., Shelton, A.M., 2016. Field performance of Bt eggplants (*Solanum melongena* L.) in the Philippines: Cry1Ac Expression and control of the eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee). *PLoS One* 11, e0157498.
- Hawes, C., Houghton, A.J., Osborne, J.L., Roy, D.B., Clark, S.J., Perry, J.N., Rothery, P., Bohan, D.A., Brooks, D.R., Champion, G.T., Dewar, A.M., Herad, M.S., Woivod, I.P., Daniels, R.E., Young, M.W., Parish, A.M., Scott, R.J., Firbank, L.G., Squire, G.R., 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos. Trans. R. Soc. London B* 358, 1899–1913.
- Head, G.P., Carroll, M.W., Evans, S.P., Rule, D.W., Willse, A.R., Clark, T.L., Storer, N.P., Flannagan, R.D., Samuel, L.W., Meinel, L.J., 2017. Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. *Pest Manage. Sci.* 73, 1883–1899.
- Head, G., Moar, M., Eubanks, M., Freeman, B., Ruberson, J., Hagerty, A., Turnipseed, S., 2005. A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. *Environ. Entomol.* 34, 1257–1266.
- Heimpel, G.E., Mills, N.J., 2017. *Biological Control. Ecology and Applications*. Cambridge University Press, Cambridge, UK.
- Hellmich, R.L., Albajes, R., Bergvinson, D., Prasifka, J.R., Wang, Z.-Y., Weiss, M.J., 2008. The present and future role of insect-resistant genetically modified maize in IPM. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Business Media B.V., pp. 119–158.
- Henneberry, T.J., Naranjo, S.E., 1998. Integrated management approaches for pink bollworm in the southwestern United States. *Integ. Pest Manage. Rev.* 3, 31–52.
- Herman, R.A., Price, W.D., 2013. Unintended compositional changes in genetically modified (GM) crops: 20 years of research. *J. Agric. Food Chem.* 61, 11695–11701.
- Hogervorst, P.A.M., Wäckers, F.L., Woodring, J., Romeis, J., 2009. Snowdrop lectin (*Galanthus nivalis* agglutinin) in aphid honeydew negatively affects survival of a honeydew-consuming parasitoid. *Agric. For. Entomol.* 11, 161–173.
- Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. *Agric. Ecosyst. Environ.* 103, 1–25.
- Huang, F., 2015. Resistance management for Bt maize and above-ground lepidopteran targets in the USA. From single gene to pyramided traits. In: Soberón, M., Gao, Y., Bravo, A. (Eds.), *Bt Resistance*. CAB International, Wallingford, UK, pp. 173–185.
- Huesing, J.E., Romeis, J., Ellstrand, N.C., Raybould, A., Hellmich, R.L., Wolt, J.D., Ehlers, J.D., Dabiré-Binso, L.C., Fatokun, C.A., Hokanson, K.E., Ishiyaku, M.F., Margam, V.M., Obokoh, N., Mignouna, J.D., Nang'ayo, F., Ouedraogo, J.T., Pasquet, R.S., Pittendrigh, B.R., Schaal, B.A., Stein, J., Tamò, M., Murdock, L.L., 2011. Regulatory considerations surrounding the deployment of Bt-expressing cowpea in Africa. *GM Crops* 2, 211–224.
- 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., Rabeya, T.O., Flood, B.R., Raun, E.S., 2010. Area-wide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* 330, 222–225.
- Huvenne, H., Smaghe, G., 2010. Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. *J. Insect Physiol.* 56, 227–235.
- Ibrahim, A.B., Monteiro, T.R., Cabral, G.B., Aragão, F.J.L., 2017. RNAi-mediated resistance to whitefly (*Bemisia tabaci*) in genetically engineered lettuce (*Lactuca sativa*). *Transgenic Res.* 26, 613–624.
- Icoz, I., Stotzy, G., 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. *Soil Biol. Biochem.* 40, 559–586.
- ISAAA, 2017. Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 years. ISAAA Brief No. 53. ISAAA, Ithaca, NY.
- Kennedy, G.G., 2008. Integration of insect-resistant genetically modified crops within IPM programs. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Business Media B.V., pp. 1–26.
- Kennedy, G.G., Gould, F., 2007. Ecology of natural enemies and genetically engineered host plants. In: Kogan, M., Jepson, P. (Eds.), *Perspectives in Ecological Theory and Integrated Pest Management*. Cambridge University Press, Cambridge, UK, pp. 269–300.
- Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M., Miller, K., Zhang, Y., Ramaseshadri, P., Jiang, C., Hodge, T., Jensen, P., Chen, M., Gowda, A., McNulty, B., Vazquez, C., Bolognesi, R., Haas, J., Head, G., Clark, T., 2018. Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. *PLoS One* 13, e0197059.
- Klümper, W., Qaim, M., 2014. A meta-analysis of the impacts of genetically modified crops. *PLoS One* 9, e111629.

- Knight, K., Head, G., Rogers, J., 2013. Season-long expression of Cry1Ac and Cry2Ab proteins in Bollgard II cotton in Australia. *Crop Prot.* 44, 50–58.
- Kumar, R., Tian, J., Naranjo, S., Shelton, A., 2014. Effects of Bt cotton on *Thrips tabaci* (Thysanoptera: Thripidae) and its predator, *Orius insidiosus* (Hemiptera: Anthocoridae). *J. Econ. Entomol.* 107, 927–932.
- Ladics, G.S., Bartholomaeus, A., Bregitzer, P., Doerr, N.G., Gray, A., Holzhauser, T., Jordan, M., Keese, P., Kok, E., Macdonald, P., Parrott, W., Privalle, L., Raybould, A., Rhee, S.Y., Rice, E., Romeis, J., Vaughn, J., Wal, J.-M., Glenn, K., 2015. Genetic basis and detection of unintended effects in genetically modified crop plants. *Transgenic Res.* 24, 587–603.
- Lamichhane, J.R., Devos, Y., Beckie, H.J., Owen, M.D.K., Tillie, P., Messéan, A., Kudsk, P., 2017. Integrated weed management systems with herbicide-tolerant crops in the European Union: lessons learnt from home and abroad. *Crit. Rev. Biotech.* 37, 459–475.
- Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu. Rev. Entomol.* 45, 175–201.
- Landis, D.A., Gardiner, M.W., van der Werf, W., Swinton, S.M., 2008. Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20552–20557.
- Lawo, N.C., Wäckers, F.L., Romeis, J., 2010. Characterizing indirect prey-quality mediated effects of a Bt crop on predatory larvae of the green lacewing, *Chrysoperla carnea*. *J. Insect Physiol.* 56, 1702–1710.
- Lee, M.K., Walters, F.S., Hart, H., Palekar, N., Chen, J.-S., 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ -endotoxin. *Appl. Environ. Microbiol.* 69, 4648–4657.
- Levine, S.L., Tan, J., Mueller, G.M., Bachman, P.M., Jensen, P.D., Uffman, J.P., 2015. Independent action between DvSnf7 RNA and Cry3Bb1 protein in southern corn rootworm, *Diabrotica undecimpunctata howardi* and Colorado potato beetle, *Leptinotarsa decemlineata*. *PLoS One* 10, e0118622.
- Levine, S.L., Mueller, G.M., Uffman, J.P., 2016. Assessing the potential for interaction between the insecticidal activity of two genetically engineered cotton events combined by conventional breeding: an example with COT102 x MON 15985. *Regul. Toxicol. Pharmacol.* 79, 35–41.
- Li, Y., Romeis, J., 2010. Bt maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae*, or its ladybird beetle predator, *Stethorus punctillum*. *Biol. Control* 53, 337–344.
- Li, Y., Meissle, M., Romeis, J., 2008. Consumption of Bt maize pollen expressing Cry1Ab or Cry3Bb1 does not harm adult green lacewings, *Chrysoperla carnea* (Neuroptera: Chrysopidae). *PLoS One* 3, e2909.
- Li, Y., Meissle, M., Romeis, J., 2010. Use of maize pollen by adult *Chrysoperla carnea* (Neuroptera: Chrysopidae) and fate of Cry proteins in Bt-transgenic varieties. *J. Insect Physiol.* 56, 157–164.
- Li, Y., Romeis, J., Wang, P., Peng, Y., Shelton, A.M., 2011. A comprehensive assessment of the effects of Bt cotton on *Coleomegilla maculata* demonstrates no detrimental effects by Cry1Ac and Cry2Ab. *PLoS One* 6, e22185.
- Li, Y., Hu, L., Romeis, J., Chen, X., Peng, Y., 2014a. Bt rice producing Cry1C protein does not have direct detrimental effects on the green lacewing *Chrysoperla sinica* (Tjeder). *Environ. Toxicol. Chem.* 33, 1391–1397.
- Li, Y.-H., Romeis, J., Wu, K.-M., Peng, Y.-F., 2014b. Tier-1 assays for assessing the toxicity of insecticidal proteins produced by genetically engineered plants to non-target arthropods. *Insect Sci.* 21, 125–134.
- Li, Y., Zhang, X., Chen, X., Romeis, J., Yin, X., Peng, Y., 2015. Consumption of Bt rice pollen containing Cry1C or Cry2A does not pose a risk to *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae). *Sci. Rep.* 5, 7679.
- Li, Y., Hallerman, M.E., Liu, Q., Wu, K., Peng, Y., 2016. The development and status of Bt rice in China. *Plant Biotechnol. J.* 14, 839–848.
- Li, Y., Liu, Y., Yin, Y., Romeis, J., Chen, X., Song, X., Peng, Y., Li, Y., 2017a. Consumption of Bt maize pollen containing Cry1Ie does not negatively affect *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae). *Toxins* 9, 108.
- Li, Y., Zhang, Q., Liu, Q., Meissle, M., Yang, Y., Wang, Y., Hua, H., Chen, X., Peng, Y., Romeis, J., 2017b. Bt rice in China—focusing the non-target risk assessment. *Plant Biotechnol. J.* 15, 1340–1345.
- Liu, X.X., Chen, M., Collins, H.L., Onstad, D.W., Roush, R.T., Zhang, Q., Earle, E.D., Shelton, A.M., 2014. Natural enemies delay insect resistance to Bt plants. *PLoS One* 9, e90366.
- Liu, Q., Hallermann, E., Peng, Y., Li, Y., 2016a. Development of Bt rice and Bt maize in China and their efficacy in target pest control. *Int. J. Mol. Sci.* 17, 1561.
- Liu, Y., Liu, Q., Wang, Y., Chen, X., Song, X., Romeis, J., Li, Y., Peng, Y., 2016b. Ingestion of Bt corn pollen containing Cry1Ab/2Aj or Cry1Ac does not harm *Propylea japonica* larvae. *Sci. Rep.* 6, 23507.
- Losey, J.E., Vaughn, M., 2006. The economic value of ecological services provided by insects. *BioScience* 56, 311–323.
- Lu, H.-P., Luo, T., Fu, H.-W., Wang, L., Tan, Y.-Y., Huang, J.-Z., Wang, Q., Ye, G.-Y., Gatehouse, A.M.R., Lou, Y.-G., Shu, Q.-Y., 2018. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. *Nat. Plants* 4, 338–344.
- Lu, Y., Wu, K., Jiang, Y., Guo, Y., Desneux, N., 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* 487, 362–365.
- Lucena, W.A., Pelegrini, P.B., Martins-de-Sa, D., Fonseca, F.C.A., Gomes Jr., J.E., de Macedo, L.L.P., da Silva, M.C.M., Oliveira, R.S., Grossi-de-Sa, M.F., 2014. Molecular approaches to improve the insecticidal activity of *Bacillus thuringiensis* Cry toxins. *Toxins* 6, 2393–2423.
- Ludy, C., Lang, A., 2006. Bt maize pollen exposure and impact on the garden spider, *Araneus diadematus*. *Entomol. Exp. Appl.* 118, 145–156.
- Lumbierres, B., Stary, P., Pons, X., 2011. Effect of Bt maize on the plant-aphid-parasitoid tritrophic relationships. *BioControl* 56, 133–143.
- Lumbierres, B., Albajes, R., Pons, X., 2012. Positive effect of Cry1Ab-expressing Bt maize on the development and reproduction of the predator *Orius majusculus* under laboratory conditions. *Biol. Control* 63, 150–156.
- Lundgren, J.G., 2009. Relationship of Natural Enemies and Non-prey Foods. Springer Science + Business Media B.V.
- Lundgren, J.G., Wiedenmann, R.N., 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). *Environ. Entomol.* 31, 1213–1218.
- Lundgren, J.G., Gassmann, A.J., Bernal, J., Duan, J.J., Ruberson, J., 2009. Ecological compatibility of GM crops and biological control. *Crop Prot.* 28, 1017–1030.
- Lüthi, C., Álvarez-Alfageme, F., Ehlers, J.D., Higgins, T.J.V., Romeis, J., 2013a. Resistance of α AI-1 transgenic chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) dry grains to bruchid beetles (Coleoptera: Chrysomelidae). *Bull. Entomol. Res.* 103, 373–381.
- Lüthi, C., Álvarez-Alfageme, F., Romeis, J., 2013b. Impact of α AI-1 expressed in genetically modified cowpea on *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae) and its parasitoid, *Dinarmus basalis* (Hymenoptera: Pteromalidae). *PLoS One* 8, e67785.
- Lüthi, C., Álvarez-Alfageme, F., Romeis, J., 2018. The bean α -amylase inhibitor α AI-1 in genetically modified chickpea seeds does not harm parasitoid wasps. *Pest Manage. Sci.* 74, 2444–2449.
- Malone, L.A., Gatehouse, A.M.R., Barratt, B.L.P., 2008. Beyond Bt: Alternative strategies for insect-resistant genetically modified crops. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Business Media B.V., pp. 357–417.
- Manachini, B., 2003. Effect of transgenic corn on *Lydella thompsoni* Hertig (Diptera: Tachinidae) parasitoid of *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae). *Boll. Zool. Agric. Bachtic.* 35, 111–125.
- Manachini, B., Lozza, G.C., 2004. Studies on the effects of Bt corn expressing Cry1Ab on two parasitoids of *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae). *IOBC-WPRS Bull.* 27, 109–116.
- Mao, Y.-B., Cai, W.-J., Wang, J.-W., Hong, G.-J., Tao, X.-Y., Wang, L.-J., Huang, Y.-P., Chen, X.-Y., 2007. Silencing a cotton bollworm P450 monoxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nat. Biotechnol.* 25, 1307–1313.
- Marvier, M., McCreedy, C., Regetz, J., Kareiva, P., 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science* 316, 1475–1477.
- Mason, C.E., Sheldon, J.K., Pesek, J., Bacon, H., Gallusser, R., Radke, G., Slabaugh, B., 2008. Assessment of *Chrysoperla plorabunda* longevity, fecundity, and egg viability when adults are fed transgenic Bt corn pollen. *J. Agric. Urban Entomol.* 25, 265–278.
- May, M.J., Champion, G.T., Dewar, A.M., Qi, A., Pidgeon, J.D., 2005. Management of genetically modified herbicide-tolerant sugar beet for spring and autumn environmental benefit. *Proc. R. Soc. B* 272, 111–119.
- Meadows, M.P., 1993. *Bacillus thuringiensis* in the environment: ecology and risk assessment. In: Entwistle, P.F., Cory, J.S., Bailey, M.J., Higgs, S. (Eds.), *Bacillus thuringiensis*, an Environmental Biopesticide: Theory and Practice. John Wiley & Sons, Chichester, UK, pp. 193–220.
- Meissle, M., Romeis, J., 2009a. The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. *Plant Biotechnol. J.* 7, 645–656.
- Meissle, M., Romeis, J., 2009b. Insecticidal activity of Cry3Bb1 expressed in Bt maize on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Entomol. Exp. Appl.* 131, 308–319.
- Meissle, M., Romeis, J., 2012. No accumulation of Bt protein in *Phylloneta impressa* (Araneae: Theridiidae) and prey arthropods in Bt maize. *Environ. Entomol.* 41, 1037–1042.
- Meissle, M., Romeis, J., 2018. Transfer of Cry1Ac and Cry2A proteins from genetically engineered Bt cotton to herbivores and predators. *Insect Sci.* 25, 823–832.
- Meissle, M., Vojtech, E., Poppy, G.M., 2004. Implications for the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) when developing in Bt maize-fed *Spodoptera littoralis* larvae (Lepidoptera: Noctuidae). *IOBC/WPRS Bull.* 27 (3), 117–123.
- Meissle, M., Romeis, J., Bigler, F., 2011. Bt maize and integrated pest management – a European perspective. *Pest Manage. Sci.* 67, 1049–1058.
- Meissle, M., Álvarez-Alfageme, F., Malone, L.A., Romeis, J., 2012. Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU. Supporting Publication 2012:EN-334. European Food Safety Authority (EFSA), Parma, Italy. Available online: < <http://www.efsa.europa.eu/en/supporting/pub/334e.htm> > .
- Meissle, M., Zünd, J., Waldburger, M., Romeis, J., 2014. Development of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on pollen from Bt-transgenic and conventional maize. *Sci. Rep.* 4, 5900.
- Millennium Ecosystem Assessment (MEA), 2005. *Ecosystems and Human Well-Being: Synthesis*. Washington, DC. Available online: < <http://www.millenniumassessment.org/documents/document.356.aspx.pdf> > .
- Mohammed, B.S., Ishiyaku, M.F., Abdullahi, U.S., Katung, M.D., 2014. Response of transgenic Bt cowpea lines and their hybrids under field conditions. *J. Plant Breed. Crop Sci.* 6, 91–96.
- Morton, R.L., Schroeder, H.E., Bateman, K.S., Chrispeels, M.J., Armstrong, E., Higgins, T.J.V., 2000. Bean α -amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proc. Nat. Acad. Sci. U.S.A.* 97, 3820–3825.
- Moser, S.E., Harwood, J.D., Obrycki, J.J., 2008. Larval feeding on Bt hybrid and non-Bt corn seedlings by *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ. Entomol.* 37, 525–533.
- Murdock, L.L., Coulbaly, O., Higgins, T.J.V., Huesing, J.E., Ishiyaku, M., Sithole-Niang, I., 2008. Cowpea. In: Kole, C., Hall, T.C. (Eds.), *Compendium of Transgenic Crop Plants: Transgenic Legume Grains and Forages*. Blackwell Publishing, Oxford, pp. 23–56.

- Musser, F.R., Shelton, A.M., 2003. Bt sweet corn and selective insecticides: Impacts on pests and predators. *J. Econ. Entomol.* 96, 71–80.
- Musser, F.R., Nyrop, J.P., Shelton, A.M., 2006. Integrating biological and chemical controls in decision making: European corn borer control in sweet corn as an example. *J. Econ. Entomol.* 99, 1538–1549.
- Naranjo, S.E., 2005a. Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of nontarget arthropod natural enemies. *Environ. Entomol.* 34, 1193–1210.
- Naranjo, S.E., 2005b. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. *Environ. Entomol.* 34, 1211–1223.
- Naranjo, S.E., 2009. Impacts of *Bt* crops on non-target invertebrates and insecticide use patterns. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 4, 11.
- Naranjo, S.E., 2011. Impacts of *Bt* transgenic cotton on integrated pest management. *J. Agric. Food Chem.* 59, 5842–5851.
- Naranjo, S.E., Ellsworth, P.C., 2005. Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomol. Exp. Appl.* 116, 93–108.
- Naranjo, S.E., Ellsworth, P.C., 2009a. Fifty years of the integrated control concept: moving the model and implementation forward in Arizona. *Pest Manage. Sci.* 65, 1267–1286.
- Naranjo, S.E., Ellsworth, P.C., 2009b. The contribution of conservation biological control to integrated control of *Bemisia tabaci* in cotton. *Biol. Control* 51, 458–470.
- Naranjo, S.E., Ellsworth, P.C., 2010. Fourteen years of *Bt* cotton advances IPM in Arizona. *Southw. Entomol.* 35, 437–444.
- Naranjo, S.E., Ruberson, J.R., Sharma, H.C., Wilson, L., Wu, K., 2008. The present and future role of insect-resistant genetically modified cotton in IPM. In: Romeis, J., Shelton, A.M.S., Kennedy, G.G. (Eds.), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Media B.V., pp. 159–194.
- Naranjo, S.E., Ellsworth, P.C., Frisvold, G.B., 2015. Economic value of biological control in integrated pest management of managed plant systems. *Annu. Rev. Entomol.* 60, 621–645.
- Narva, K.E., Wang, N.X., Herman, R., 2017. Safety considerations derived from Cry34Ab1/Cry35Ab1 structure and function. *J. Invert. Pathol.* 142, 27–33.
- National Academies of Sciences, Engineering, and Medicine (NASEM), 2016. *Genetically Engineered Crops: Experiences and Prospects*. The National Academies Press, Washington, DC, USA.
- Nguyen, H.T., Jehle, J.A., 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize MON810. *J. Plant Dis. Prot.* 114, 82–87.
- Nguyen, H.T., Jehle, J.A., 2009. Expression of Cry3Bb1 in transgenic corn MON88017. *J. Agric. Food Chem.* 57, 9990–9996.
- Ni, M., Ma, W., Wang, X., Gao, M., Dai, Y., Wei, X., Zhang, L., Peng, Y., Chen, S., Ding, L., Tian, Y., Li, J., Wang, H., Wang, X., Xu, G., Guo, W., Yang, Y., Wu, Y., Heuberger, S., Tabashnik, B.E., Zhang, T., Zhu, Z., 2017. Next-generation transgenic cotton: pyramiding RNAi and Bt counters insect resistance. *Plant Biotechnol. J.* 15, 1204–1213.
- Norris, R.F., Kogan, M., 2000. Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. *Weed Sci.* 48, 94–158.
- Norris, R.F., Kogan, M., 2005. Ecology of interactions between weeds and arthropods. *Annu. Rev. Entomol.* 50, 479–503.
- North, J.H., Gore, J., Catchot, A.L., Stewart, S.D., Lorenz, G.M., Musser, F.R., Cook, D.R., Kerns, D.L., Dodds, D.M., 2018. Value of neonicotinoid insecticide seed treatments in Mid-South cotton (*Gossypium hirsutum* [Malvales: Malvaceae]) production systems. *J. Econ. Entomol.* 111, 10–15.
- Obrist, L.B., Klein, H., Dutton, A., Bigler, F., 2005. Effects of Bt maize on *Frankliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. *Entomol. Exp. Appl.* 115, 409–416.
- Obrist, L.B., Dutton, A., Albajes, R., Bigler, F., 2006a. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. *Ecol. Entomol.* 31, 143–154.
- Obrist, L.B., Dutton, A., Romeis, J., Bigler, F., 2006b. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *BioControl* 51, 31–48.
- Obrist, L.B., Klein, H., Dutton, A., Bigler, F., 2006c. Assessing the effects of Bt maize on the predatory mite *Neoseiulus cucumeris*. *Exp. Appl. Acarol.* 38, 125–139.
- Ode, P.J., 2006. Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* 51, 163–185.
- Olsen, K.M., Daly, J.C., Holt, H.E., Finnegan, E.J., 2005. Season-long variation in expression of Cry1Ac gene and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 98, 1007–1017.
- Olson, D., Ruberson, J., 2012. Crop-specific mortality of southern green stink bug eggs in Bt- and non-Bt cotton, soybean and peanut. *Biocontrol Sci. Technol.* 22, 1417–1428.
- Onstad, D., Liu, X., Chen, M., Roush, R., Shelton, A.M., 2013. Modeling the integration of parasitism, insecticide and transgenic insecticidal crops for the long-term control of an insect pest. *J. Econ. Entomol.* 106, 1103–1107.
- Orr, D.B., Landis, D.A., 1997. Oviposition of European corn borer and impact of natural enemy populations in transgenic versus isogenic corn. *J. Econ. Entomol.* 90, 905–909.
- Papiernik, S.K., Sappington, T.W., Luttrell, R.G., Hesler, L.S., Allen, K.C., 2018. Overview: Risk factors and historic levels of pressure from insect pests of seedling corn, cotton, soybean, and wheat in the United States. *J. Integr. Pest Manag.* 9, 18.
- Paula, D.P., Andow, D.A., 2016. Uptake and bioaccumulation of Cry toxins by an aphidophagous predator. *Environ. Poll.* 209, 164–168.
- Pellegrino, E., Bedini, S., Nuti, M., Ercoli, L., 2018. Impact of genetically engineered maize on agronomic, environmental and toxicological traits: a meta-analysis of 21 years of field data. *Sci. Rep.* 8, 3113.
- Perlak, F.J., Deaton, R.W., Armstrong, T.A., Fuchs, R.L., Sims, S.R., Greenplate, J.T., Fischhoff, D.A., 1990. Insect resistant cotton plants. *Bio/Technology* 8, 939–943.
- Perry, J.N., Devos, Y., Arpaia, S., Bartsch, D., Gathmann, A., Hails, R.S., Kiss, J., Lheureux, K., Manachini, B., Mestdagh, S., Neemann, G., Ortego, F., Schiemann, J., Sweet, J.B., 2010. A mathematical model of exposure of nontarget Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. *Proc. R. Soc. B* 277, 1417–1422.
- Pertry, I., Sanou, E.I.R., Speelman, S., Ingelbrecht, I., 2016. The success story of Bt cotton in Burkina Faso: a role model for sustainable cotton production in other cotton growing countries? In: De Buck, S., Ingelbrecht, I., Hejide, M., Van Montagu, M. (Eds.), *Innovative Farming and Forestry Across the Emerging World: The Role of Genetically Modified Crops and Trees*. International Industrial Biotechnology Network (IIBN), e-book, pp. 81–93. Available online: < <https://biblio.ugent.be/publication/8500337/file/8501700> > .
- Peterson, J.A., Obyrck, J.J., Harwood, J.D., 2009. Quantification of Bt-endotoxin exposure pathways in carabid food webs across multiple transgenic events. *Biocontr. Sci. Technol.* 19, 613–625.
- Peterson, J.A., Lundgren, J.G., Harwood, J.D., 2011. Interactions of transgenic *Bacillus thuringiensis* insecticidal crops with spiders (Araneae). *J. Arachnol.* 39, 1–21.
- Peterson, J.A., Obyrck, J.J., Harwood, J.D., 2016a. Spiders from multiple functional guilds are exposed to Bt-endotoxins in transgenic corn fields via prey and pollen consumption. *Biocontr. Sci. Technol.* 26, 1230–1248.
- Peterson, J.A., Ode, P.J., Oliveira-Hofman, C., Harwood, J.D., 2016b. Integration of plant defense traits with biological control of arthropod pests: challenges and opportunities. *Front. Plant Sci.* 7, 1794.
- Pilcher, C.D., Obyrck, J.J., Rice, M.E., Lewis, L.C., 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. *Environ. Entomol.* 26, 446–454.
- Prasanna, B.M., Huesing, J.E., Eddy, R., Peschke, V.M. (Eds.), 2018. *Fall Armyworm in Africa: A Guide for Integrated Pest Management*, first ed. CIMMYT, Mexico, CDMX. Available at: https://www.usaid.gov/sites/default/files/documents/1867/Fall-Armyworm-IPM-Guide-for-Africa-Jan_30-2018.pdf.
- Price, P.W., Bouton, C.E., Gross, P., McPherson, B.A., Thompson, J.N., Weis, A.E., 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11, 41–65.
- Privalle, L.S., Chen, J., Clapper, G., Hunst, P., Spiegelhalter, F., Zhong, C.X., 2012. Development of an agricultural biotechnology crop product: testing from discovery to commercialization. *J. Agric. Food Chem.* 60, 10179–10187.
- Que, Q., Chilton, M.-D.M., de Fontes, C.M., He, C., Nuccio, M., Zhu, T., Wu, Y., Chen, J.S., Shi, L., 2010. Trait stacking in transgenic crops: Challenges and opportunities. *GM Crops* 1, 220–229.
- Raps, A., Kehr, J., Gugerli, P., Moar, W.J., Bigler, F., Hilbeck, A., 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the non-target herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Mol. Ecol.* 10, 525–533.
- Rauschen, S., 2010. A case of “pseudo science”? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is reminiscent of the case of the green lacewing. *Transgenic Res.* 19, 13–16.
- Raybould, A., Vlachos, D., 2011. Non-target organism effects tests on Vip3A and their application to the ecological risk assessment for cultivation of MIR162 maize. *Transgenic Res.* 20, 599–611.
- Raybould, A., Graser, G., Hill, K., Ward, K., 2012. Ecological risk assessment for transgenic crops with combined insect-resistance traits: the example of Bt11 x MIR604 maize. *J. Appl. Entomol.* 136, 27–37.
- Raybould, A., Kilby, P., Graser, G., 2013. Characterising microbial protein test substances and establishing their equivalence with plant-produced proteins for use in risk assessments of transgenic crops. *Transgenic Res.* 22, 445–460.
- Ricroch, A., Bergé, J.P., Kuntz, M., 2010. Is the German suspension of MON810 maize cultivation scientifically justified? *Transgenic Res.* 19, 1–12.
- Riedel, J., Romeis, J., Meissle, M., 2016. Update and expansion of the database of biological information on non-target arthropod species established to support the environmental risk assessment of genetically modified crops in the EU. *Supporting Publication 2016:EN-956*. European Food Safety Authority (EFSA), Parma, Italy. Available online: < <http://www.efsa.europa.eu/en/supporting/pub/956e> > .
- Romeis, J., Meissle, M., 2011. Non-target risk assessment of Bt crops – Cry protein uptake by aphids. *J. Appl. Entomol.* 135, 1–6.
- Romeis, J., Dutton, A., Bigler, F., 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *J. Insect Physiol.* 50, 175–183.
- Romeis, J., Babendreier, D., Wäckers, F.L., Shanower, T.G., 2005. Habitat and plant specificity of *Trichogramma* egg parasitoids – underlying mechanisms and implications. *Basic Appl. Ecol.* 6, 215–236.
- Romeis, J., Meissle, M., Bigler, F., 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol.* 24, 63–71.
- Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E., Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould, A., Rose, R.L., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z., Wolt, J.D., 2008a. Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nat. Biotechnol.* 26, 203–208.
- Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), 2008. *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Business Media B. V.
- Romeis, J., Van Driesche, R.G., Barratt, B.I.P., Bigler, F., 2008c. Insect-resistant transgenic crops and biological control. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Business Media B.V., pp. 87–117.
- Romeis, J., Meissle, M., Raybould, A., Hellmich, R.L., 2009. Impact of insect-resistant transgenic crops on above-ground non-target arthropods. In: Ferry, N., Gatehouse, A.M.R. (Eds.), *Environmental Impact of Genetically Modified Crops*. CABI, Wallingford, UK, pp. 165–198.
- Romeis, J., Hellmich, R.L., Candolfi, M.P., Carstens, K., De Schrijver, A., Gatehouse,

- A.M.R., Herman, R.A., Huesing, J.E., McLean, M.A., Raybould, A., Shelton, A.M., Waggoner, A., 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. *Transgenic Res.* 20, 1–22.
- Romeis, J., McLean, M.A., Shelton, A.M., 2013a. When bad science makes good headlines: Bt maize and regulatory bans. *Nat. Biotechnol.* 31, 386–387.
- Romeis, J., Raybould, A., Bigler, F., Candolfi, M.P., Hellmich, R.L., Huesing, J.E., Shelton, A.M., 2013b. Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. *Chemosphere* 90, 901–909.
- Romeis, J., Meissle, M., Álvarez-Alfageme, F., Bigler, F., Bohan, D.A., Devos, Y., Malone, L.A., Pons, X., Rauschen, S., 2014a. Potential use of an arthropod database to support the non-target risk assessment and monitoring of transgenic plants. *Transgenic Res.* 23, 995–1013.
- Romeis, J., Meissle, M., Naranjo, S., Li, Y., Bigler, F., 2014b. The end of a myth – Bt (Cry1Ab) maize does not harm green lacewings. *Front. Plant Sci.* 5, 391.
- Rose, R., 2005. An Ecological Risk Assessment of Bt Transgenic Sweet corn on Non-Target Arthropod Communities. Ph.D. Dissertation. University of Maryland, College Park, Maryland.
- Rosenberg, M.S., Adams, D.C., Gurevitch, J., 2000. MetaWin Version 2: Statistical Software for Meta-Analysis. Sinauer Associates Inc, Sunderland, Massachusetts.
- Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokorski, J., Stephen, M.L., 2007. Toxins in transgenic crop by-products may affect headwater stream ecosystems. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16204–16208.
- Rukarwa, R.J., Mukasa, S.B., Odongo, B., Ssemakula, G., Ghislain, M., 2014. Identification of relevant non-target organisms exposed to weevil-resistant Bt sweetpotato in Uganda. *3 Biotech* 4, 217–226.
- Sabelis, M.W., van Baalen, M., Bakker, F.M., Bruin, J., Drukker, B., Egas, M., Jansser, A.R.M., Lesna, I.K., Pels, B., van Rijn, O.C.J., Scutareanu, P., 1999. The evolution of direct and indirect plant defence against herbivorous arthropods. In: Olff, H., Brown, V.K., Drent, R.H. (Eds.), *Herbivores: Between Plants and Predators*. Blackwell Science, Oxford, UK, pp. 109–166.
- Saeed, R., Razaq, M., Hardy, I.C.W., 2016. Impact of neonicotinoid seed treatment of cotton on the cotton leafhopper, *Amrasca devastans* (Hemiptera: Cicadellidae), and its natural enemies. *Pest Manage. Sci.* 72, 1260–1267.
- Sampson, K., Zaitseva, J., Stauffer, M., Vande Berg, B., Guo, R., Tomso, D., McNulty, B., Desai, N., Balasubramanian, D., 2017. Discovery of a novel insecticidal protein from *Chromobacterium piscinae*, with activity against Western Corn Rootworm, *Diabrotica virgifera virgifera*. *J. Invertebr. Pathol.* 142, 34–43.
- Sanou, E.I.R., Gheysens, G., Koulibaly, B., Roelofs, C., Speelman, S., 2018. Farmers' knowledge and opinions towards Bollgard II[®] implementation in cotton production in western Burkina Faso. *New Biotechnol.* 42, 33–41.
- Sanvido, O., Romeis, J., Bigler, F., 2007. Ecological impacts of genetically modified crops: Ten years of field research and commercial cultivation. *Adv. Biochem. Eng. Biotechnol.* 107, 235–278.
- Sanvido, O., Widmer, F., Winzler, M., Streit, B., Szerencsits, E., Bigler, F., 2008. Definition and feasibility of isolation distances for transgenic maize cultivation. *Transgenic Res.* 17, 317–335.
- Sanvido, O., Romeis, J., Gathmann, A., Gielkens, M., Raybould, A., Bigler, F., 2012. Evaluating environmental risks of genetically modified crops – ecological harm criteria for regulatory decision-making. *Environ. Sci. Policy* 15, 82–91.
- Sappington, T.W., Hesler, L.S., Allen, K.C., Luttrell, R.G., Papiernik, S.K., 2018. Prevalence of sporadic insect pests of seedling corn and factors affecting risk of infestation. *J. Integr. Pest Manage.* 9, 16.
- Schnell, J., Steele, M., Bean, J., Neuspiel, M., Girard, C., Dormann, N., Pearson, C., Savoie, A., Bourbonnière, L., Macdonald, P., 2015. A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. *Transgenic Res.* 24, 1–17.
- Schuler, T., Potting, R., Denholm, I., Clark, S., Clark, A., Stewart, C.P., 2003. Tritrophic choice experiments with Bt plants, the diamond moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*. *Transgenic Res.* 12, 351–361.
- Schuppener, M., Mühlhause, J., Müller, A.-K., Rauschen, S., 2012. Environmental risk assessment for the small tortoiseshell *Aglais urticae* and a stacked Bt-maize with combined resistances against Lepidoptera and Chrysomelidae in central European agrarian landscapes. *Mol. Ecol.* 21, 4646–4662.
- Sheldon, J.K., MacLeod, E.G., 1971. Studies on the biology of the Chrysopidae. II. the feeding behaviour of the adult of *Chrysopa carnea* (Neuroptera). *Psyche* 78, 107–121.
- Shelton, A.M., Sears, M.K., 2001. The monarch butterfly controversy: scientific interpretations of a phenomenon. *Plant J.* 27, 483–488.
- Shelton, A.M., Romeis, J., Kennedy, G.G., 2008. IPM and insect-protected transgenic plants: thoughts for the future. In: Romeis, J., Shelton, A.M., Kennedy, G.G. (Eds.), *Integration of Insect-Resistant Genetically Modified Crops within IPM Programs*. Springer Science + Business Media B.V., pp. 419–429.
- Shelton, A.M., Naranjo, S.E., Romeis, J., Hellmich, R.L., Wolt, J.D., Federici, B.A., Albajes, R., Bigler, F., Burgess, E.P.J., Dively, G.P., Gatehouse, A.M.R., Malone, L.A., Roush, R., Sears, M., Sehnael, F., 2009. Setting the record straight: a rebuttal to an erroneous analysis on transgenic insecticidal crops and natural enemies. *Transgenic Res.* 18, 317–322.
- Shelton, A.M., Hossain, M.J., Paranjape, V., Azad, A.K., Rahman, M.L., Khan, A.S.M.M.R., Prodhon, M.Z.H., Rashid, M.A., Majumder, R., Hossain, M.A., Hussain, S.S., Huesing, J.E., McCandless, L., 2018. Bt eggplant project in Bangladesh: history, present status, and future direction. *Front. Bioeng. Biotechnol.* 6, 106.
- Shi, J., Gao, H., Wang, H., Lafitte, H.R., Archibald, R.L., Yang, M., Hakimi, S.M., Mo, H., Habben, J.E., 2017. ARGOS variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotech. J.* 15, 207–216.
- Shu, Y., Romeis, J., Meissle, M., 2018. No interactions of stacked Bt maize with the non-target aphid *Rhopalosiphum padi* and the spider mite *Tetranychus urticae*. *Front. Plant Sci.* 9, 39.
- Siegfried, B.D., Zoerb, A.C., Spencer, T., 2001. Development of European corn borer larvae on Event 176 Bt corn: Influence on survival and fitness. *Entomol. Exp. Appl.* 100, 15–20.
- Sisterson, M.S., Biggs, R.W., Olson, C., Carrière, Y., Dennehy, T.J., Tabashnik, B.E., 2004. Arthropod abundance and diversity in Bt and non-Bt cotton fields. *Environ. Entomol.* 33, 921–929.
- Smith, C.M., 2005. *Plant Resistance to Arthropods*. Springer, Dordrecht, The Netherlands.
- Smith, R.F., Hagen, K.S., 1959. The integration of chemical and biological control of the spotted alfalfa aphid: Impact of commercial insecticide treatments. *Hilgardia* 29, 131–154.
- Smith, V., Bohan, D.A., Clark, S.J., Haughton, A.J., Bell, J.R., Heard, M.S., 2008. Weed and invertebrate community compositions in arable farmland. *Arthropod-Plant Interact.* 2, 21–30.
- Smyth, S.J., Gusta, M., Belcher, K., Phillips, P.W.B., Castle, D., 2011. Environmental impacts from herbicide tolerant canola production in Western Canada. *Agric. Syst.* 104, 403–410.
- Sparks, T.C., Nauen, R., 2015. IRAC: Mode of action classification and insecticide resistance management. *Pestic. Biochem. Physiol.* 121, 122–128.
- Stern, V.M., van den Bosch, R., 1959. The integration of chemical and biological control of the spotted alfalfa aphid: Field experiments on the effects of insecticides. *Hilgardia* 29, 103–130.
- Stern, V.M., Smith, R., van Bosch, R., Hagen, K.S., 1959. The integration of chemical and biological control of the spotted alfalfa aphid: The integrated control concept. *Hilgardia* 29, 81–101.
- Stinner, B.R., House, G.J., 1990. Arthropods and other invertebrates in conservation-tillage agriculture. *Annu. Rev. Entomol.* 35, 299–318.
- Su, H.-H., Tian, J.-C., Naranjo, S.E., Romeis, J., Hellmich, R.L., Shelton, A.M., 2015. *Bacillus thuringiensis* plants expressing Cry1Ac, Cry2Ab and Cry1F are not toxic to the assassin bug, *Zelus renardii*. *J. Appl. Entomol.* 139, 23–30.
- Svobodová, Z., Shu, Y., Skoková Habuštová, O., Romeis, J., Meissle, M., 2017. Stacked Bt maize and arthropod predators – Exposure to insecticidal Cry proteins and potential hazards. *Proc. R. Soc. B* 284, 20170440.
- Svobodová, Z., Skoková Habuštová, O., Holec, J., Holec, M., Boháč, J., Jursík, M., Soukup, J., Sehnael, F., 2018. Split application of glyphosate in herbicide-tolerant maize provides efficient weed control and favors beneficial epigeic arthropods. *Agric. Ecosyst. Environ.* 251, 171–179.
- Tabashnik, B.E., Carrière, Y., 2017. Surge in insect resistance to transgenic crops and prospects for sustainability. *Nat. Biotechnol.* 35, 926–935.
- Tabashnik, B.E., Breéault, T., Carrière, Y., 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nat. Biotechnol.* 31, 510–521.
- Tamburini, G., De Simone, S., Sigura, M., Boscutti, F., Marini, L., 2016. Conservation tillage mitigates the negative effect of landscape simplification on biological control. *J. Appl. Ecol.* 53, 233–241.
- Tank, J.L., Rosi-Marshall, E.J., Royer, T.V., Whiles, M.R., Griffiths, N.A., Frauendorf, T.C., Treering, D.J., 2010. Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17645–17650.
- Thomazoni, D., Degrande, P.E., Silvie, P.J., Faccenda, O., 2010. Impact of Bollgard genetically modified cotton on the biodiversity of arthropods under practical field conditions in Brazil. *Afr. J. Biotechnol.* 9, 6167–6176.
- Tian, J.-C., Collins, H.L., Romeis, J., Naranjo, S.E., Hellmich, R.L., Shelton, A.M., 2012. Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one of its major predators. *Transgenic Res.* 21, 1303–1310.
- Tian, J.-C., Wang, X.-P., Long, L.-P., Romeis, J., Naranjo, S.E., Hellmich, R.L., Wang, P., Earle, E.D., Shelton, A.M., 2013. Bt crops producing Cry1Ac, Cry2Ab and Cry1F do not harm the green lacewing, *Chrysoperla rufilabris*. *PLoS One* 8, e60125.
- Tian, J., Long, L., Wang, X., Naranjo, S., Romeis, J., Hellmich, R., Wang, P., Shelton, A., 2014. Using resistant prey demonstrates that Bt plants producing Cry1Ac, Cry2Ab, and Cry1F have no negative effects on *Geocoris punctipes* and *Orius insidiosus*. *Environ. Entomol.* 43, 242–251.
- Tian, J.-C., Yao, J., Long, L.-P., Romeis, J., Shelton, A.M., 2015. Bt crops benefit natural enemies to control non-target pests. *Sci. Rep.* 5, 16636.
- Tian, J.-C., Romeis, J., Liu, K., Zhang, F.-C., Zheng, X.-S., Xu, H.-X., Lu, Z.-X., 2017. Assessing the effects of Cry1C rice and Cry2A rice to *Pseudogonatopus flavifemur*, a parasitoid of rice planthoppers. *Sci. Rep.* 7, 7838.
- Tian, J.-C., Wang, G.-W., Han, H.-L., Romeis, J., Zhang, F.-C., Ye, G.-Y., Xie, M.-C., Wang, G.-Y., Lu, Z.-X., 2018a. The rice planthopper parasitoid *Anagrus nilaparvatae* is not at risk when feeding on honeydew derived from *Bacillus thuringiensis* (Bt) rice. *Pest Manage. Sci.* 74, 1854–1860.
- Tian, J.-C., Wang, X.-P., Chen, Y., Romeis, J., Naranjo, S.E., Hellmich, R.L., Wang, P., Shelton, A.M., 2018b. Bt cotton producing Cry1Ac and Cry2Ab does not harm two parasitoids, *Cotesia marginiventris* and *Copidosoma floridanum*. *Sci. Rep.* 8, 307.
- Teague, W.M., Steffens, J.C., 1991. The environmental control of insects using plant resistance. In: Pimentel, D. (Ed.), *CRC Handbook of Pest Management in Agriculture*, 2nd Edition. CRC Press, Boca Raton, FL, pp. 131–156.
- Todd, J.H., Ramankutty, P., Barraclough, E.I., Malone, L.A., 2008. A screening method for prioritizing non-target invertebrates for improved biosafety testing of transgenic crops. *Environ. Biosaf. Res.* 7, 35–56.
- Toews, M.D., Tubbs, R.S., Wann, D.Q., Sullivan, D., 2010. Thrips (Thysanoptera: Thripidae) mitigation in seedling cotton using strip tillage and winter cover crops. *Pest Manage. Sci.* 66, 1089–1095.
- Torres, J.B., Ruberson, J.R., 2008. Interactions of *Bacillus thuringiensis* Cry1Ac toxin in

- genetically engineered cotton with predatory heteropterans. *Transgenic Res.* 17, 345–354.
- Torres, J.B., Ruberson, J.R., Adang, M.J., 2006. Expression of *Bacillus thuringiensis* Cry1Ac protein in cotton plants, acquisition by pests and predators: a tritrophic analysis. *Agric. Forest Entomol.* 8, 191–202.
- Trichilo, P.J., Leigh, T.F., 1986. Predation on spider mite eggs by the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), an opportunist in a cotton agroecosystem. *Environ. Entomol.* 15, 821–825.
- Triplett, G.B.J., Dick, W.A., 2008. No-tillage crop production: a revolution in agriculture!. *Agron. J.* 100, S153–S165.
- Turlings, T.C.J., Wäckers, F.L., 2004. Recruitment of predators and parasitoids by herbivore-injured plants. In: Cardé, R.T., Millar, J.G. (Eds.), *Advances in Insect Chemical Ecology*. Cambridge University Press, Cambridge, pp. 21–75.
- Vandervoet, T., Ellsworth, P.C., Carrière, Y., Naranjo, S.E., 2018. Quantifying conservation biological control for management of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in cotton. *J. Econ. Entomol.* 111, 1056–1068.
- Van Rijn, P.C.J., van Houten, Y.M., Sabelis, M.W., 2002. How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology* 83, 2664–2679.
- Veres, A., Petit, S., Conord, C., Lavigne, C., 2013. Does landscape composition affect pest abundance and their control by natural enemies? A review. *Agric. Ecosyst. Environ.* 166, 110–117.
- Vojtech, E., Meissle, M., Poppy, G.M., 2005. Effects of Bt maize on the herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) and the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *Transgenic Res.* 14, 133–144.
- Wach, M., Hellmich, R.L., Layton, R., Romeis, J., Gadaleta, P.G., 2016. Dynamic role and importance of surrogate species for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. *Transgenic Res.* 25, 499–505.
- Wäckers, F.L., 2005. Suitability of (extra-)floral nectar, pollen, and honeydew as insect food sources. In: Wäckers, F.L., van Rijn, P.C.J., Bruin, J. (Eds.), *Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and its Applications*. Cambridge University Press, Cambridge, pp. 17–74.
- Walters, F.S., 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, 484–497.
- Wan, P., Huang, Y., Tabashnik, B.E., Huang, M., Wu, K., 2012. The halo effect: Suppression of pink bollworm on non-Bt cotton by Bt cotton in China. *PLoS One* 7, e42004.
- Wang, Y., Li, Y., Romeis, J., Zhang, J., Chen, H., Peng, Y., 2012. Consumption of Bt rice pollen expressing Cry2Aa does not cause adverse effects on adult *Chrysoperla sinica* Tjeder (Neuroptera: Chrysopidae). *Biol. Control* 61, 246–251.
- Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., Qiu, J.-L., 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* 32, 947–952.
- Wang, Z.-X., Li, Y.-H., He, K.-L., Bai, S.-X., Zhang, T.-T., Cai, W.-Z., Wang, Z.-Y., 2017. Does Bt maize expressing Cry1Ac protein have adverse effects on the parasitoid *Macrocentrus cingulum* (Hymenoptera: Braconidae)? *Insect Sci.* 24, 599–612.
- Wang, G., Dong, Y., Liu, X., Yao, G., Yang, M., 2018. The current status and development of insect-resistant genetically engineered poplar in China. *Front. Plant Sci.* 9, 1408.
- Weber, N., Halpin, C., Hannah, L.C., Jez, J.M., Kough, J., Parrott, W., 2012. Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. *Plant Physiol.* 160, 1842–1853.
- Wei, W., Schuler, T.H., Clark, S.J., Stewart Jr, C.N., Poppy, G.M., 2008. Movement of transgenic plant-expressed Bt Cry1Ac proteins through high trophic levels. *J. Appl. Entomol.* 132, 1–11.
- Whitehouse, M.E.A., Wilson, L.J., Davies, A.P., Cross, D., Goldsmith, P., Thompson, A., Harden, S., Baker, G., 2014. Target and nontarget effects of novel “triple-stacked” Bt-transgenic cotton 1: canopy arthropod communities. *Environ. Entomol.* 43, 218–241.
- Wilson, L.J., Whitehouse, M.E.A., Herron, G.A., 2018. The management of insect pests in Australian cotton: an evolving story. *Annu. Rev. Entomol.* 63, 215–237.
- Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Watrud, L.S., 2008. Bt crops effects on functional guilds of non-target arthropods: a meta-analysis. *PLoS One* 3, e2118.
- Wu, K.-M., Lu, Y.-H., Feng, H.-Q., Jiang, Y.-Y., Zhao, J.-Z., 2008. Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. *Science* 321, 1676–1678.
- Yu, H., Romeis, J., Li, Y., Li, X., Wu, K., 2014. Acquisition of Cry1Ac protein by non-target arthropods in Bt soybean fields. *PLoS One* 9, e103973.
- Zhang, J., Khan, S.A., Hasse, C., Ruf, S., Heckel, D.G., Bock, R., 2015. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. *Science* 347, 991–994.
- Zhang, J., Khan, S.A., Heckel, D.G., Bock, R., 2017. Next-generation insect-resistant plants: RNAi-mediated crop protection. *Trends Biotechnol.* 35, 871–882.
- Zhang, W., Lu, Y., van der Werf, W., Huang, J., Wu, F., Zhou, K., Deng, X., Jiang, Y., Wu, K., Rosegrant, M.W., 2018. Multidecadal, county-level analysis of the effects of land use, Bt cotton, and weather on cotton pests in China. *Proc. Natl. Acad. Sci. U.S.A.* 115, E7700–E7709.
- Zhao, J.-Z., Cao, J., Collins, H.C., Bates, S.L., Roush, R.T., Earle, E.D., Shelton, A.M., 2005. Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. *Proc. Natl. Acad. Sci. U.S.A.* 102, 8426–8430.
- Zhao, Z., Li, Y., Xiao, Y., Ali, A., Dhilloo, K.H., Chen, W., Wu, K., 2016. Distribution and metabolism of Bt-Cry1Ac toxin in tissues and organs of the cotton bollworm, *Helicoverpa armigera*. *Toxins* 8, 212.
- Zhou, X., Guo, Y., Kong, H., Zuo, J., Huang, Q., Jia, R., Guo, A., Xu, L., 2016. A comprehensive assessment of the effects of transgenic Cry1Ac/Cry1Ab rice Huahui 1 on adult *Micraspis discolor* (Fabricius) (Coleoptera: Coccinellidae). *PLoS One* 11, e0142714.
- Zurbrugg, C., Höhnemann, L., Meissle, M., Romeis, J., Nentwig, W., 2010. Decomposition dynamics and structural plant components of genetically modified Bt maize leaves do not differ from conventional hybrids. *Transgenic Res.* 19, 257–267.
- Zwahlen, C., Andow, D.A., 2005. Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. *Environ. Biosaf. Res.* 4, 113–117.