Chapter 10
Effects of GE Crops on Non-target Organisms

Steven E. Naranjo

Abstract Genetically engineered (GE) crops have now been part of the agricultural landscape for 25 years and are important tools in crop production and Integrated Pest Management (IPM) in 26 countries. Considerable research has addressed many associated issues including environmental and food safety, as well as economic and social impacts. Non-target effects have been a particularly intensive area of study, and extensive laboratory and field research has been conducted for transgenic Bt crops that produce the insecticidal proteins of a ubiquitous bacterium, *Bacillus thuringiensis*. This body of evidence and the quantitative and qualitative syntheses of the data through meta-analysis and other compilations generally indicate a lack of direct impacts of Bt crops on non-target macro-invertebrates. The data also clearly show that Bt crops are much safer to non-target organisms than the alternative use of traditional insecticides for control of the pests targeted by the Bt proteins. Some indirect effects on arthropod natural enemies associated with reduced abundance or quality of Bt target herbivores have been shown, but the ramifications of these effects remain unclear and would be shared by other pest control technologies. As one tactic in the IPM toolbox, Bt crops have contributed to large reductions in insecticide use. While reduced insecticide use and reduced herbivory may be involved in precipitating new pest problems in Bt crops, it also has broadened opportunities for deployment of another key IPM tactic, biological control.

Keywords Transgenic Bt crops · Risk assessment · Meta-analysis · Ecological guilds · Biological control · Integrated pest management

10.1 Introduction

Genetically engineered (GE) crops have now been part of the agricultural landscape for 25 years and their geographic scope and breadth of traits continues to advance. By 2018, nearly 192 million hectares of GE crops were cultivated in 26 countries, with 21

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of these developing nations. The USA leads the world in adoption of GE crops with Brazil, Argentina, Canada and India among the top five. Spain and Portugal are the only European Union countries growing GE crops, and this is limited to relatively small areas (< 121 K hectares) of insect-resistant maize. Another 40 countries or so allow for the importation of GE crop products for food, animal feed and other processing uses.

The primary GE crops currently under cultivation involve those that have been engineered to either display tolerance to several broad-spectrum herbicides or selective resistance to specific insect pest groups, primarily those belonging to the Orders Lepidoptera and Coleoptera. GE cotton, maize and soybean often include varieties that offer both traits. Major GE crops include soybean, maize, cotton, and canola (oilseed rape), grown in many adopting countries, with much smaller plantings of herbicide-tolerant alfalfa and sugar beets, virus-resistant papaya and squash, insect-resistant eggplant (brinjal), sugarcane and cowpea, and quality enhanced traits in apple, potato and pineapple in a smaller number of countries including the USA, Canada, China, Bangladesh, Costa Rica, Indonesia and Nigeria.

Consistent with this 25-year adoption, there has been considerable research addressing many associated issues including environmental and food safety, economic and social impacts, and effects on crop production and protection. The potential negative effects of GE crop technology have been perhaps most visible and controversial in the area of environmental and food safety. The GE crops that have been scrutinized most in this regard are those with insect-resistance, and that will be the major focus of this chapter.

10.2 Insect-Resistant Crops

At present, all insect resistant crops are based on the production of one or more of the crystal (Cry) and vegetative (VIP) proteins of a ubiquitous gram-positive bacterium, *Bacillus thuringiensis* (Bt). These so called Bt crops comprise about 54% of all GE crops produced globally and are grown in 22 countries. The insecticidal properties of this bacterium have been known for more than 100 years and commercial products based on this organism have been available since the 1940s. Bt spray products occupy > 90% of the bio-pesticide market and are an important tool for pest control in organic farming and stored grain, and for control of larval mosquitos. Presently, Bt cotton and Bt maize are the dominant forms of transgenic, insect-pest control technology globally. Bt soybean has been grown in several South American countries since 2012, Bt sugarcane was approved for production in Brazil in 2017, Bt cowpea was approved for Nigeria in 2019 and several countries are evaluating Bt rice for potential production in the future. Bt eggplant (brinjal) was initially granted approval for cultivation in India in 2009, but a governmental moratorium was imposed shortly thereafter citing the need for more testing and evaluation. In 2014, Bangladesh began to allow cultivation of Bt brinjal and adoption rates have grown quickly, with > 20,000 farmers growing about 1200 ha (2.5% of total crop) in the 2018–19 growing season.

A recent summary of all insect-resistant GE crops, year of approval and adoption rates are provided in Naranjo et al. (2020).

Several other crops producing Bt Cry proteins are under research and development in different parts of the world. One relatively unique crop is a Bt cotton with resistance to plant bugs (Heteroptera) and thrips (Thysanoptera) that is currently under regulatory review and expected to be available to USA farmers in 2021 (Naranjo et al. 2020). Several non Bt approaches are currently under investigation and development. One of these is RNA interference (RNAi), a conserved immune response in eukaryotes whereby double-stranded RNA (dsRNA) produced in the organism itself directs the repression of a specific gene sequence. In crop biotechnology, the approach identifies a gene that controls a vital biological function in the target organism and then produces the associated dsRNA in the plant where it is taken up by the target insect through feeding. This process has the potential to be very selective to the target organism because of the gene specificity. The first commercial event with this technology was approved in 2017 as an additional approach for control of corn rootworm but is not yet being commercially grown due to some lingering trade issues. This RNAi trait will be added to maize already producing multiple Bt and herbicide tolerance traits. Finally, CRISPR-based approaches that allow genome editing have the potential to further revolutionize pest control, but the technology is in the early stages of development for this purpose and there remain many biological and regulatory challenges (Naranjo et al. 2020).

10.3 The IPM Context

The breadth and scope of GE crop technology is undeniably large on the world stage as are the potential solutions they contribute to agriculture in the face of a rapidly growing human population. However, it is important to keep their role in focus when thinking about crop productivity and crop protection, especially with Bt crops. Whether one considers Bt crops to be a form of host plant resistance or alternatively a convenient method for the delivery of a selective insecticide, they represent only a single tactic within the integrated pest management (IPM) toolbox. Effective and sustainable crop protection must include multiple tactics that are carefully integrated to manage multiple pests within agricultural landscapes. Nonetheless, some global compilations based on the adoption of Bt cotton and maize suggest that they have contributed significantly to economic and environmental gains. For example, Brookes and Barfoot (2020b) estimate that Bt cotton and Bt maize have increased global farm level incomes by $63.6B and $59.6B, respectively, from the period 1996-2018 with 55% of the benefits derived by farmers in developing countries. The associated environmental gains over this same time period in terms of insecticide use reductions also is large. Brookes and Barfoot (2020a) further estimate that foliar and soil insecticide use in Bt crops has declined by a total of 331 and 112 M kilograms (active ingredient) in Bt cotton and Bt maize, respectively. The associated decline in insecticide use for Bt soybeans over the period 2013–2018 in South America is

14.9 M kg. These insecticide reductions have paid dividends, particularly in cotton by facilitating improved biological control of non-target pests (Naranjo et al. 2020; Romeis et al. 2019).

Though not unique to Bt crops, seeds treated with neonicotinoid insecticides for seedling and early plant stage pest control have become nearly ubiquitous in field crop production, particularly for maize and cotton in the USA. While the non-target impacts of this technology remain unclear, this trend has the potential to partially erase some of the positive gains in reduced foliar and soil insecticides use with adoption of Bt crops. Growing target pest resistance to several Bt Cry proteins also has the potential to erode some of these gains in insecticide reductions in both maize and cotton (see Chapter 9 by Fleischer et al.).

10.4 What Is a Non-target Organism?

The focus of this chapter is to consider what we know about the effects of GE crops on non-target organisms. What is a non-target organism? Very simply, a non-target organism is broadly defined as any organism that the transgenic technology was not intended to control. Given that the intended targets of Bt crops are quite narrow, for example, several species of corn rootworm beetles (*Diabrotica* spp.) for Cry3 Bt maize, and several dozen species of caterpillars (various bollworms, defoliators and stalk borers) for Cry1, Cry2 and VIP Bt maize and cotton (Naranjo et al. 2020), the list of non-targets is potentially quite extensive. In Bt crops, non-targets include other arthropod crop pests that are not susceptible to Bt proteins and a wide range of organisms, many of which provide important ecosystem services such as biological control, pollination and decomposition. Much of the research focus has been placed on arthropods and other invertebrates, but some attention has been placed on vertebrates and it is common for regulatory agencies to require testing on a wide range of organisms including birds, mammals, fish and multiple invertebrate groups as part of the registration process for Bt crops. For instance, the US-Environmental Protection Agency considers Bt engineered into crops to be plant-incorporated-protectants (so-called PIPs), and regulatory oversight includes a process similar to that required for pesticides. This process often involves the use of surrogate species in a tiered testing system (see below) starting with laboratory experiments under extreme-dose, worst-case exposure conditions, but is increasingly emphasizing more extensive evaluations on non-target organisms in crop fields.

This chapter will focus primarily on the effects of Bt crops on non-target arthropods. These organisms are often among the most abundant and important residents of agricultural fields where they serve a wide variety of ecosystem functions and represent a significant portion of agroecosystem biodiversity. The focus here on insect resistant Bt crops stems from the fact that much of the non-target research conducted has focused on these crops. It is recognized that Bt proteins engineered into crops represent a different risk to non-target organisms and overall biodiversity than Bt proteins applied as foliar spray treatments. For example, Bt proteins are continually produced in Bt crop plants and these proteins are protected from the environmental degradation (e.g. rain, UV exposure) common in sprayable products applied to the plant surface. To date, over 700 scientific studies have been completed to assess effects of Bt crops on non-target invertebrates in both the laboratory and in the field. These collective data have been the subject of dozens of review articles. The data also have been used in more quantitative, synthetic studies called meta-analyses, which is simply a way to enhance the rigor and power of testing for non-target effects by statistically combining the results of multiple studies. The most recent synthesis that looked at multiple Bt crops and examined both field and laboratory studies was Naranjo (2009). The study including two dozen individual and pyramided (two proteins) Bt Cry proteins, eight Bt crops in 20 countries, and over 300 species in three Phyla (Arthropoda, Annelida, Mollusca). Other meta-analyses have since been published but with a narrower focus on single Bt crops or restricted regions of the world. A summary of these meta-analyses will be presented and discussed. For coverage of the other environmental risk issues associated with GE crops, including gene flow, invasiveness and soil ecosystem effects, the reader is directed to several recent reviews (Guan et al. 2016; Naranjo et al. 2020; Romeis et al. 2019).

10.5 Effects on Non-target Organisms

10.5.1 How to Characterize Risk

Globally, an environmental risk assessment (ERA) is generally conducted before any GE crop can be approved and released for production in the field. While each country has their own set of processes, there are many similarities among them (Schiemann et al. 2019). The approach commonly used involves a problem formulation process to establish protection goals and to assess current knowledge and identify areas of concern or uncertainty. Biodiversity and its associated ecosystem services is a typical protection goal evaluated in the ERA. Through problem formulation, risk hypotheses are developed and subsequently tested. Most regulatory bodies use conventional tier testing that starts with worse-case exposure in the laboratory and escalates through more complex and realistic tiers only if the null hypothesis of no risk is rejected or other uncertainties exist. Several considerations are important in identifying non-target species to assess, including their potential sensitivity to the insecticidal compounds in the GE crop. This is often predicated on phylogenetic considerations, especially for Bt where there is a long history of known effects on specific taxonomic groups (e.g. Lepidoptera). Another consideration is the relevance of the non-target group and this is established from knowing what taxa can be found in and around crop fields and if they are at risk of exposure. Relevance might also be based on consideration of the important ecosystem services provided, for example arthropods involved in biological control, pollination or decomposition. A further, practical consideration is the availability of select taxa and the ease with which high quality organisms can be reared and maintained. This might necessitate the use of representative surrogate species. Finally, it is critical that studies conducted in support of risk assessment are rigorous and can meet minimum quality standards (Romeis et al. 2019; Schiemann et al. 2019). In the end, a tiered approach is a balance between ecological reality and practicality. Regardless of the process it is ultimately up to decision-making bodies of each jurisdiction to determine the balance of risks and benefits to society as a whole.

The approach for assessing the non-target risk of the newer generation of GE crops such as those based on RNAi is still a developing field. Many of the same considerations employed for Bt crops would likely apply but nuances in how the technology works to effect pest control require careful consideration (Schiemann et al. 2019). For the one crop utilizing RNAi technology so far approved in the USA (corn rootworm control), the ERA was similar to the typical tiered system used for other crops with PIPs such as Bt. One additional supporting evaluation that can be added to identify species most likely to be at risk is to use bioinformatics to determine if the non-target organism shares a sufficient genomic match in the affected genomic sequence to that in the target.

10.5.2 General Non-target Effects

Although the topic area of non-target effects of Bt crops has enjoyed its share of controversy and debate, the extant body of research supports the conclusion that these crops have minimal negative effects on non-target organisms, and certainly less impact that the alternative use of insecticides to control the same target pests. Three broad and several more specific meta-analyses have been published in the past 15 years, beginning with Marvier et al. (2007). Based on funding from the US-EPA, this group developed the first database that attempted to compile the global English-language published research on the effects of Bt crops and Bt cry proteins on non-target organisms (primarily Arthropods but also including Annelida and Mollusca) in 2005. The database included studies conducted in both the laboratory and the field, although the Marvier et al. (2007) study examined only field studies. Their analyses showed that the abundance of all non-target invertebrates combined was slightly lower in Bt maize and cotton compared with non-Bt crops, but that abundances were much higher in Bt crops compared with non-Bt crops that had been treated with insecticides to suppress Bt targeted pests. They further concluded that taxonomic affiliation did not alter these general findings and that it was unclear if the observed reductions of abundance in Bt crops were due to direct toxicity or indirect effects causes by lowered target prey/host availability in the case of natural enemies.

Two subsequent and more detailed meta-analyses followed, including Naranjo (2009), who updated the Marvier database and examined both laboratory (discussed below) and field studies from an ecological rather than a taxonomic context. In general, analyses of field studies showed little difference in the abundance of various ecological guilds when insecticides were not applied to either the Bt crop or its non-Bt counterpart (Fig. 10.1a). This comparison tests the hypothesis that the plant itself, either directly or indirectly, affects non-target organism abundance. The one notable

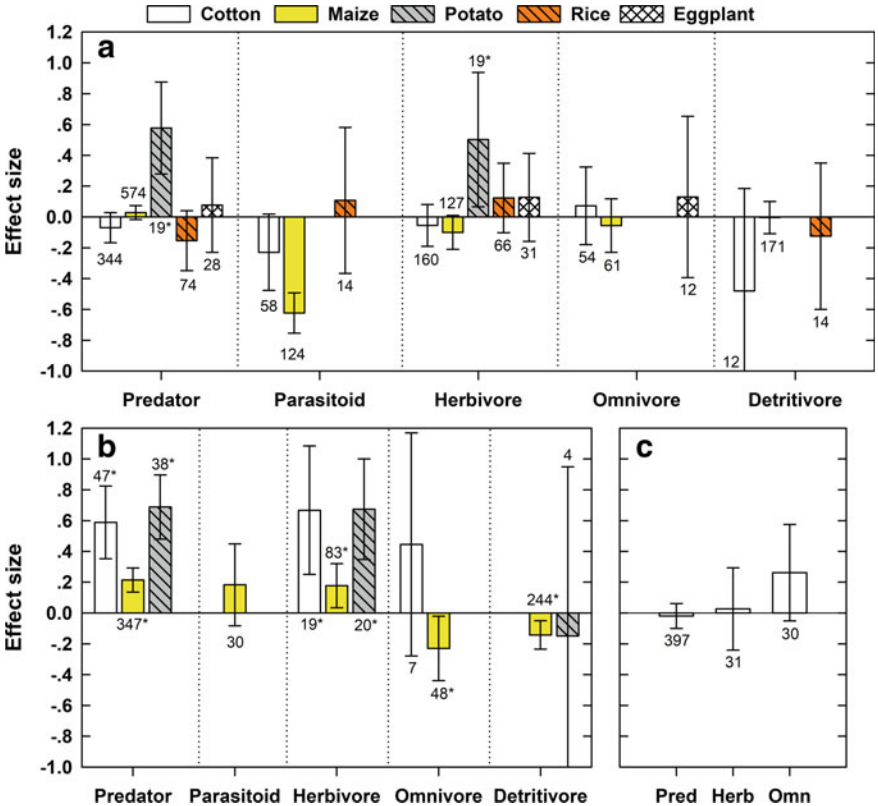

Fig. 10.1 Meta-analyses of field studies that examined the comparative abundance of non-target invertebrates in Bt and non-Bt crops. Meta-analysis quantitatively combines the results of multiple studies using a metric called the effect size that takes into account the variability, sample sizes and the magnitude of differences in individual comparative studies. The data are plotted such that a negative effect size denotes a lower abundance in the Bt crop compared with the non-Bt crop; a positive effect size denotes the opposite. Here the data are parsed into ecological guilds that represent different ecosystem functions. **a** Neither the Bt nor non-Bt crop received any insecticide treatments. These analyses test the hypothesis that the Bt protein or any other differences in the Bt plant affected non-target abundance either directly or indirectly. **b** Here the non-Bt crop was sprayed with insecticide and these analyses test the hypothesis that the method used to control the Bt targeted pest affects non-target abundance. **c** Finally, both the Bt and non-Bt crop are treated with insecticides to control both target and non-target pests and these analyses test the realistic hypothesis that management of pests in both Bt and non-Bt crops affect non-target abundance. The numbers above or below the bars denote sample size and the asterisks denote statistical significance of the effect size, i.e. significantly lower or higher than zero *Data modified* from Naranjo (2009) to include additional studies up to 2013

exception was a large reduction in the abundance of insect parasitoids in Bt maize. This pattern was found to be entirely due to a large number of USA based studies that examined densities of a specialist exotic parasitoid that attacks the European corn borer, a primary target of Bt maize. With effective control of the parasitoid's host there was an expected reduction in abundance in Bt maize fields. This is an example of an indirect ecological effect as parasitoids require their hosts to survive but are not necessarily directly affected by Bt proteins. Such indirect ecological effects would be expected of any tactic that lowers the target pest (the goal of pest management) and is not something unique to the deployment of a Bt crop. Another result of note is the effect of Bt potato on predators and herbivores (Fig. 10.1a). Here, the abundance of both groups was higher in the Bt crop. This is another example of an indirect ecological effect in which higher herbivore populations in Bt potato, primarily sucking insects (insects that feed by inserting their straw-like mouthparts into plant parts) led to a corresponding increase in predators responding to higher prey availability. The reason for increased sucking insect populations has not been studied, but it has been suggested that it is related to the lack of induced plant defenses in Bt potato when its primary targeted defoliator is controlled, and/or the lack of collateral control previously provided by insecticides (see Non-target Pests below). Other functional guilds (herbivores, omnivores and detritivores) were unaffected in Bt maize, cotton or potato in comparison with untreated non-Bt controls. At the time of this early meta-analysis, fewer studies had been conducted in Bt rice and eggplant, but results indicated no effects of Bt crops on any ecological guild (Fig. 10.1a). Most ecological guilds were more abundant in Bt maize, cotton and potato when the comparative non-Bt crop was treated with a variety of insecticides for control of target pests (Fig. 10.1b). The results for detritivores in Bt maize provides another example of indirect ecological effects wherein springtails, the primary detritivores in the system, were released from control by soil dwelling predatory beetles when insecticides were applied. Why omnivores were less abundant in unsprayed Bt maize compared with sprayed non-Bt maize is not completely understood. Heterogeneity analysis indicated that this pattern was due to omnivorous ants. Ants were not affected by Bt maize when either the Bt or non-Bt crop were insecticide-free suggested it is not a direct effect of the Bt proteins. When insecticides are used in both the Bt and non-Bt crops, a common situation in cotton, which harbors multiple pests, the abundance of the ecological guilds available for analyses were the same in both crops. While different pest complexes would have been targeted in Bt and non-Bt cotton, both systems rely on relatively broad-spectrum insecticides for non-target pest control, albeit generally fewer applications are needed for the Bt crop (Fig. 10.1c).

Several other crop and region targeted meta-analyses have subsequently been conducted. Comas et al. (2014) focused on arthropod herbivores, predators and parasitoids common to maize systems in Spain and generally found no effects of Bt maize. Although Bt rice has yet to be commercially approved for widespread cultivation in China, there have been numerous laboratory and field studies to examine potential non-target effects. Using a similar ecological guild approach to Naranjo (2009), Dang et al. (2017) conducted meta-analyses on laboratory and field studies of Bt rice in China. This extended on previous meta-analyses involving rice, adding new studies published in both English and Chinese. Their synthesis found patterns of lower herbivore and parasitoid density, and higher abundance of detritivores in Bt rice in the field but no differences for predators. Aside from parasitoids, these results differed from previous meta-analyses. Unfortunately, this study lacks methodological details; for example, it is unclear if they included studies involving insecticide use and if those were parsed in their analyses. Furthermore, the study authors do not provide any clear discussion on the underlying reasons for such differences. A global meta-analysis for Bt maize generally supported prior analyses in finding a general lack of differences in most non-target groups with the exception of expected declines in parasitoids associated with European corn borers Pellegrino et al. (2018). Krogh et al. (2020) published a systematic review and global meta-analyses focused on soil invertebrates in Bt maize. As in prior studies on maize, they generally found no effects of Bt maize on the non-target fauna and their inclusion of soil invertebrates extends and complements existing syntheses.

Meta-analyses also have been conducted to examine the relationship between laboratory and field studies. As noted above, many agencies that regulate GE crops used a tiered system to test for safety and to assess risk. Very often, field studies are conducted regardless of the outcome of early tier testing, specifically by academic and other public research organizations. Field studies also are increasingly being requested of industry by regulatory authorities as part of the registration process. Thus, there are robust datasets from both the laboratory and the field that allows a way to test the validity of the tier system. One such study found that "laboratory studies of transgenic insecticidal crops show effects that are either consistent with, or more conservative than, those found in field studies" (Duan et al. 2010). These findings suggest that the tier system can function to identify harm or the lack thereof in the environment.

10.5.3 Non-target Pests

The unique physiological effect of Bt proteins currently found in GE crops, a characteristic governed by the specific receptors and conditions in a caterpillar's or beetle's gut allowing activation of the Bt proteins, limits their activity to relatively few arthropod pests of crops. Thus, there are often a wide range of other insect and mite pests not affected by Bt crops, particularly in long season crops like cotton and soybean grown in lower latitudes. Many of these pest species are managed much as they were before the advent of Bt crops and represent an equal threat to Bt as well as conventional non-Bt crops. It is these pests that force a greater focus on the principles of IPM, which calls for a suite of integrated tactics to provide effective overall crop protection.

In general, meta-analyses of field abundance studies in Bt cotton, maize, rice and eggplant have shown that non-target herbivores, that would include non-target pests, are no more abundant in Bt crops compared with non-Bt crops when no insecticides are used (see Fig. 10.1a). That is, there is nothing specific about Bt crops themselves that would alter herbivore communities. This conclusion is further supported by meta-analyses of laboratory studies, which clearly show a lack of toxicity of Bt crops to non-target pests (Naranjo 2009). Alternatively, when insecticides are used in non-Bt crops and arthropod abundances are compared to untreated Bt crops, then herbivores, again including non-target pests, are considerably more abundant in Bt crops (Fig. 10.1b) (Naranjo 2009). This does not mean that all these pests are necessarily more problematic in Bt crops but that additional management tactics may be required to suppress their numbers as noted above.

However, some non-target pests (in this case secondary or induced pests) have become more problematic in Bt crops in some production systems. Some of the most visible examples have arisen within sucking insects. This includes, for example, plant bugs in China, Australia, and the USA in cotton. The best documented case comes from China, where multiple species of plant bugs have become more pestiferous in cotton but also in a number of other crops cultivated in the same region (see Chapter 9 by Fleischer et al.). Both plant bugs and stink bugs also have risen in importance as pests of cotton in parts of the mid-southern and southeastern USA. The causes for these increases are not completely understood, but in some areas like China and the USA, the problem appears to be ironically associated with the general reduction in broad-spectrum insecticides that were once used to manage caterpillar pests now effectively controlled by Bt cotton. These insecticides would often provide collateral control of these non-target pests. Similarly, in Australia it is thought that reduced insecticide use for bollworms has allowed plant bugs, stink bugs, leafhoppers and thrips to become more prominent. Sprays now applied to these pests have in turn disrupted a complex of natural enemies and lead to secondary outbreaks of pests such as spider mites, aphids and whiteflies. Growers in India are facing similar issues with mealy bugs, thrips and leafhoppers.

The increased emergence of non-target pests in Bt maize has been relatively minor in comparison to the situation with Bt cotton. Western bean cutworm (a caterpillar, but sensitive to only certain Cry proteins in Bt maize) has been less problematic with the introduction of pyramided cultivars producing at least two Bt proteins but it has been used negatively as an example of the side-effects of Bt crop adoption. While the use of Bt maize and the associated reduction in insecticide use has contributed to the range expansion of this pest, there are many other factors to consider. Among those are basic insect biology, pest and maize phenology, increasing use of conservation tillage afforded by herbicide tolerant maize, soil properties in the expanded range, insect genetics, insect pathogens, pest replacement and climate change (Hutchison et al. 2011). Issues with minor pests like wireworms and grubs (beetles) have been addressed mainly through insecticide-treated seed, a now common practice in the USA as noted before.

The rising importance of some non-target pests in Bt crops is likely associated with the large reductions in insecticides previously applied to control Bt targeted pests. The fact that reduced insecticides and associated conservation of natural enemies did not enhance control of these non-target pests suggests that biological control does not strongly operate for these pests. The induction of natural plant defenses is another factor that may play a role. Both maize and cotton are known to produce defensive compounds in response to certain types of herbivory. For instance, it is well known for maize and cotton that caterpillar feeding leads to the release of volatile compounds that act as attractants for natural enemies, thus facilitating biological control. In addition to such volatile signaling, herbivory, particularly by chewing herbivores, also induces plants to produce defensive compounds that can have negative effects on other herbivores feeding on the plant. In Bt crops this induction by chewing herbivores (caterpillars) is lessened significantly. Studies in cotton showed that a group of chemicals called terpenoids have lower levels of induction in Bt cotton compared with non-Bt cotton in the presence of caterpillar feeding. This difference allows better survival and growth in other pests such as aphids and plant bugs in Bt cotton. Reduced competition from target pests also may play a role and allow non-target pests to perform better in Bt crops.

10.5.4 Valued Non-target Organisms

While all non-target organisms could be considered valuable for multiple reasons, there are several groups that hold special significance because of the way they are valued by both agriculture and the public. Such groups include pollinators (e.g. honeybees), charismatic butterflies (e.g. Monarchs) and moths of special economic value (e.g. silk moths). Natural enemies that provide biological control services also would fall into this group, but they will be discussed separately below due to the key roles they play in crop protection. One charismatic insect came to represent the debate about the safety of GE crops more than all others, the Monarch butterfly, a well-known resident of North America. In 1999, a laboratory study presented in the prominent science journal Nature suggested that pollen from a Bt11 event of Bt maize in the USA could cause larval mortality when applied in large quantities to the surface of the butterfly's milkweed host plant. Interestingly, the pollen of Bt11 contains very low levels of Bt proteins but the anthers contain high levels. It is speculated by some that there was anther contamination of the pollen during the study. In addition to lots of negative popular press coverage, this study also precipitated a large research effort by multiple scientific groups in the mid-western USA to examine many aspects of this issue in both laboratory and field studies. Data from these studies and others was then used to construct a robust risk assessment that took into account many variables including factors relative to hazard (toxicity) and exposure. Ultimately, hazard was found to be low, especially with the primary Bt maize events under production that contained very little Bt in their pollen. This coupled with the very low potential for exposure (timing and extent of pollen dispersal from corn, proportion of Bt maize in the butterfly's breeding habitat, etc.) to the toxin in the butterfly's habitat ultimately led to a conclusion of negligible risk in the field (Sears et al. 2001). This was the same conclusion reached by the US-EPA for valued non-target butterflies during the registration process prior to 1996. The susceptibility of the Monarch to Bt proteins was never doubted given its taxonomic affinity with target caterpillars and a meta-analysis of laboratory studies on Monarch and other valued Lepidoptera showed this to be true (Naranjo 2009). Some recent work suggests that drift from commonly used insecticides for soybean pest management may be more toxic to monarch larvae that pollen drift from Bt maize. The larger focus currently is on the widespread use of glyphosate and other herbicides on herbicide-tolerant maize leading to a significant reduction in the abundance of the butterfly's host plant (milkweed) within and bordering maize fields.

Pollinators are another important non-target group, and awareness has been heightened even more with the current issues surrounding declining honeybee health and colony collapse. A meta-analysis based on 25 laboratory studies showed that survival of neither adult or larval stage honeybees was affected by Bt proteins targeting either caterpillar or beetle pests (Duan et al. 2008). An independent meta-analysis that included honeybees as well as bumble bees reached the same conclusion based on both survival and development in the laboratory (Naranjo 2009). Relatively few field studies have examined pollinators in general, but laboratory studies on a few species of bees indicate a lack of hazard from Bt proteins.

10.5.5 Non-target Effects on Arthropod Natural Enemies

Arthropod natural enemies represent another valuable group of organisms that require consideration in assessing risks from GE crops. They can potentially provide biological control services critical to controlling target and non-target pests, may help to ameliorate the evolution of resistance to Bt crops, and represent important members of communities in natural and managed habitats overall. Due to their importance there has been considerable research in assessing the impact of Bt crops on biological traits (e.g. survival, development, reproduction), abundance, and to a more limited degree, biological control function. There are multiple pathways by which natural enemies can be potentially exposed to Bt proteins (Fig. 10.2). First, most predators and parasitoids directly feed on vegetative and reproductive plant tissue or plant products such as nectar and pollen; some species such as hover flies and certain lacewings feed exclusively on nectar and pollen as adults. Such an exposure route is frequently called bi-trophic—plant to natural enemy. Secondly, predators and parasitoids can be exposed to Bt proteins through their prey or host, which have fed directly on the plant. This route is referred to as tri-trophic—plant to prey to natural enemy. A third pathway is related to tri-trophic exposure, but involves natural enemies feeding on honeydew produced by certain plant-sucking insects. Soil dwelling natural enemies can potentially be exposed to Bt proteins entering the soil through root exudates, decaying plant material or dead arthropods—a combination of bi- and tri-trophic exposure. Natural enemies on the borders of a crop or in adjacent habitats may be exposed to Bt proteins that have left the field through various mechanisms in the soil or air such as transport by ground water or dispersion of pollen and plant debris—again, bi-trophic exposure. Finally, predators, parasitoids and herbivores from the

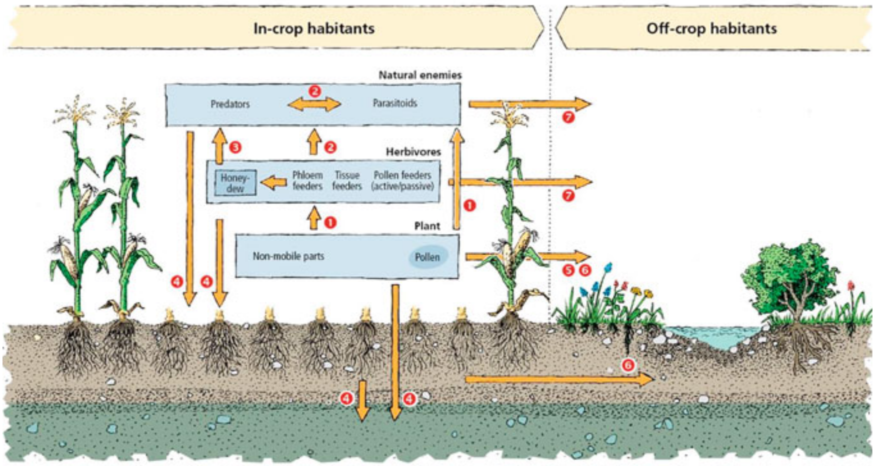

Fig. 10.2 Conceptual diagram showing the potential exposure pathways for natural enemies in Bt crop fields: (1) herbivores and natural enemies can feed directly on pollen and other plant parts; (2) predators and parasitoids can be exposed to Bt proteins in plants by consuming herbivores that have fed on the plant; (3) natural enemies can feed on honeydew secreted by various heteropteran insects that have feed on Bt plants; (4) soil dwelling natural enemies may be exposed to Bt proteins entering the soil through root exudates, decaying plant material or dead arthropods; (5,6) natural enemies outside the crop may be exposed to Bt proteins that have left the field through various mechanisms in the soil or air; (7) trophic interactions may occur outside the crop field via organisms originating in the crop. From Romeis et al. (2019), drawing by Ursus Kaufmann, Agroscope, Switzerland

crop can engage in trophic interactions outside the crop field as they move within the agroecosystem.

Many studies have examined both bi- and tri-trophic exposure pathways in a number of species in the laboratory. Meta-analyses and other data reviews have shown that bi-trophic exposure, direct feeding on either the plant or artificial diets containing Bt, has no effect on important biological parameters such as development/growth, survival or reproduction (Naranjo 2009; Romeis et al. 2019).

Interpreting the results from exposure studies examining tri-trophic interactions, or feeding on prey that have ingested Bt proteins, has been more problematic. An issue that has not consistently been factored into the interpretation of study results is that prey that are susceptible to Bt proteins (e.g. target caterpillars or beetles) are frequently affected by this feeding even if they do not die from the exposure. Those that survive are typically smaller and grow slower, a sign of sublethal effects from the Bt protein. Natural enemies that in turn use these compromised prey often suffer as well. The question of whether this is a direct or an indirect effect of the Bt protein is important but sometimes muddled. In order to establish that effects are direct, i.e. toxicological, it is necessary to control for prey quality effects. Two approaches have been employed, including the use of prey that are not susceptible to Bt proteins because they are unrelated taxonomically to the target insects, or the use of target insects that have been selected to be resistant to Bt proteins. Both of these strategies

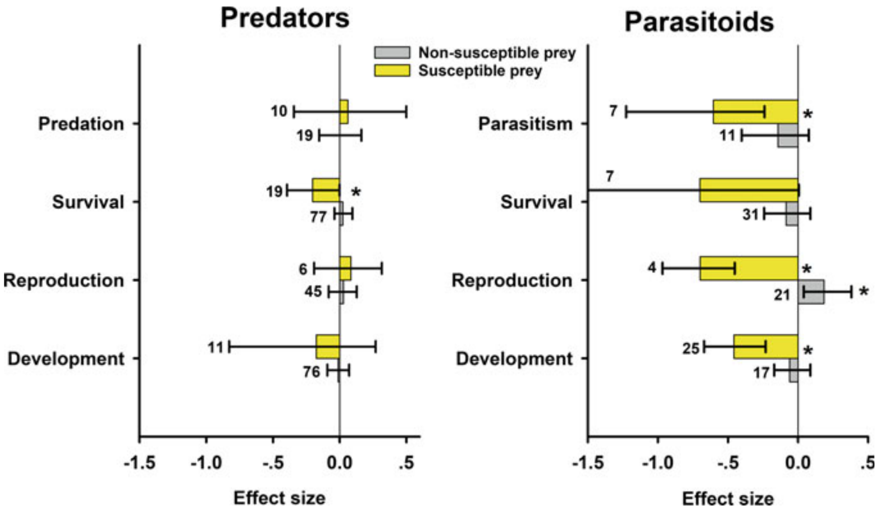

Fig. 10.3 Meta-analyses of laboratory studies that examined the non-target effects of Bt crops on arthropod natural enemies exposed to Bt proteins via their prey or host that had fed on Bt plants (tri-trophic exposure). Bt susceptible prey often suffer sub-lethal effects that degrade their quality as food for natural enemies while non-susceptible or resistant prey are normal. The data are plotted such that a negative effect size would denote a negative impact on performance in the Bt crop compared with the non-Bt crop; a positive effect size denotes the opposite. The numbers next to the bars denote sample size and the asterisks denote statistical significance of the effect size, i.e. significantly lower or higher than zero. Figure modified from Naranjo (2009) and Romeis et al. (2019)

have been used effectively to eliminate prey quality effects and enable a clear testing of direct effects of Bt proteins. Meta-analyses have compared studies where prey quality effects were apparent or were eliminated using non-susceptible or resistant prey (Fig. 10.3) (Naranjo 2009; Romeis et al. 2019). Analyses show that the use of susceptible prey results in slower development and reduced reproduction and parasitism in parasitoids and lower survival in predators. If the effects of prey quality are removed by using non-susceptible or Bt resistant prey then these parameters were either not affected or were affected positively in the case of parasitoid reproduction (i.e. better performance on prey containing Bt proteins). These results demonstrate that Bt proteins do not by themselves have any toxicological effects on the arthropod natural enemies. However, much like the field-based results discussed above there are indirect effects because biological attributes can be negatively affected if natural enemies use compromised prey. The impacts of these indirect effects in the environment are not clear and they are not limited to cases in which Bt crops are being deployed. Any tactic that affects the target prey (previous parasitism, insecticides, other host plant resistance factors, etc.) would likely yield the same indirect effect on the associated natural enemy. It also is unclear if such effects would have any ramification for the biological control services provided by natural enemy populations (discussed below). A key question is if there are enough of these compromised prey or hosts after the action of a control tactic (Bt or otherwise) to materially affect natural enemy dynamics in the field.

Issues with differing interpretations of data from tri-trophic studies have created debates in the scientific community. One of the most widely known cases involves the green lacewing, a common and important predator found in many cropping systems. In the late 1990's a group showed that certain biological attributes of green lacewing larvae were negatively affected when feeding on caterpillar prey that have been exposed to certain Bt proteins. They also showed that bi-trophic exposure routes resulted in negative biological effects. Numerous issues with experimental design were identified in these studies, but work conducted in the same laboratory and many others since this initial report have failed to duplicate any of these direct negative findings for several Bt proteins (Romeis et al. 2014). Another debate involved a laboratory-based meta-analysis that reported direct negative effects on arthropod predators and parasitoid by various Bt proteins. This result was surprising and not consistent with many other reviews and meta-analyses, including those discussed here. A rebuttal identified a number of statistical and logical issues with the study but one of the overriding factors was that the study authors failed to account for prey quality issues when examining tri-trophic studies (Romeis et al. 2019). The data presented in Fig. 10.3 shows how different the results can be when prey-mediated effects are not taken into account. Most laboratory studies being done today are cognizant of prey/host quality issue and use proper controls to eliminate their spurious effects.

10.5.6 Effects on Biological Control Function

The impacts of Bt crops on arthropod natural enemies have already been discussed (see Figs. 10.1 and 10.3). Cases where abundance was reduced were associated with indirect ecological effects such as prey scarcity, or possibly with the indirect effects resulting from preying on compromised, Bt susceptible prey. While measures of abundance and general biodiversity are a simple means to gauge non-target effects in the field, the more critical question for natural enemies is whether or not the biological control services they provide have been compromised. Compared with abundance studies relatively few studies have examined some measure of function. Such studies have used a variety of techniques including simple measures of parasitism from field samples, and measurement of predation or parasitism rates on prey artificially placed in the field, to more comprehensive life tables quantifying predation and parasitism rates on natural prey populations. Except for a few cases in which parasitism by specialist parasitoids attacking target pests have been reduced, there is no evidence that biological control capacity differs between Bt and non-Bt crop fields. Even in cases were natural enemies might be less abundant in the Bt crop there is no evidence that biological control services are reduced. For example, a long-term study in Bt cotton showed that a group of five common predators were reduced by about 20% in the Bt crop, but rates of natural enemy induced mortality on two key pests

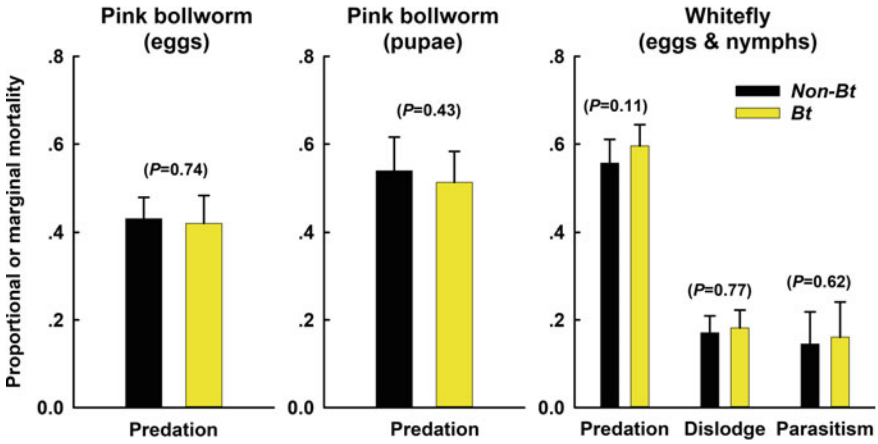

Fig. 10.4 Comparative measurement of biological control function in cotton fields using sentinel pink bollworm eggs and pupae or natural infestations of whitefly nymphs. Both the Bt cotton and the non-Bt cotton were unsprayed. Results for pink bollworm are summarized over four trials in each of two years; results for whitefly are based on two trials in each of three years. Statistical p-values are provided, and error bars denote 95% CIs. Mortality of pink bollworm represented both disappearance and chewing predation; no parasitism was observed for either life stage. For whitefly, dislodged nymphs disappeared from the leaf surface as a result of weather or chewing predation, predation indicates mortality by predators with sucking mouthparts, and parasitism is by several native and exotic aphelinid parasitoids. Data compiled from Naranjo (2005)

of cotton, pink bollworm and whitefly, remained unchanged compared to the non-Bt crop (Fig. 10.4). Overall, opportunities for enhanced biological control in Bt crops have been demonstrated in several systems (see Chapter 9 by Fleischer et al.). These benefits are derived not from anything special about Bt crops, but through the selective control of key pests afforded by the GE technology that allows natural enemy populations to flourish and provide critical ecosystem services within an overall IPM strategy.

10.6 Conclusion

GE crops have become important tools in crop production and protection in many countries and contribute significantly to overall IPM programs. Extensive laboratory and field data have been generated relative to the assessment of ecological risk in these crops, particularly for non-target organisms in Bt crops. This body of evidence and the quantitative and qualitative syntheses of the data through meta-analysis and other compilations generally indicate a lack of direct impacts of Bt crops and the insecticidal proteins they produce on non-target invertebrates. The data also clearly show that Bt crops are much better than the alternative use of traditional insecticides for control of the pests targeted by Bt crops. Some indirect effects on natural enemies associated with reduced abundance or quality of Bt target herbivores have been shown, but the ramifications of these effects are unclear. Crops developed with new technologies based on RNAi gene silencing and CRISPR gene editing have the potential to further revolutionize pest control, but the technologies, particularly CRISPR, are in the early stages of development for crop improvement and there remain many biological and regulatory challenges. As one tactic in the IPM toolbox, Bt crops have had a profound effect on insecticide use patterns. While reduced insecticide use may be involved in precipitating new pest problems in Bt crops it also has broadened opportunities for deployment of biological control.

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