Chapter 4
The Effects of Transgenic Crops on Non-target Organisms

Chandrakanth Emani

Abstract A non-monitored cultivation of transgenic crops can potentially have adverse effects on animal biodiversity when the transgenic plants or their expressed products negatively impact the organisms that are not intended to be the targets that need to be controlled. Agro-ecosystems house a diverse array of species above and below the cultivated ground that can come in contact with the cultivated plants and their metabolites. When a transgenic crop intended for pest control is planted in the field, the resulting effect on the agro-ecosystem cannot exclude the rest of the species in the habitat non-intended to be harmed by the transgenics, and these are defined as the non-target species. The present review summarizes the possible effects of transgenic plants on non-target species in agro-ecosystems with a focus on possible strategies to minimize the unintended effects of transgenic crop cultivation on animal biodiversity, while complementing the efforts of integrated pest management.

Keywords Transgenic crops · Non-target species · *Bt* maize · Monarch butterfly · Pollinator

4.1 Introduction

Transgenic crops are the fruits of biotechnological research that enable plant genetic engineers to ensure the stable integration, desired level of expression, and predictable inheritance of numerous agriculturally important genes. The present agricultural revolution sometimes referred to as gene revolution, which continues the green revolution of the 1960s resulted in the cultivation of transgenic crops expressing herbicide tolerant and insect resistant genes. Herbicide resistant transgenics, especially glyphosate-resistant soybean, cotton and corn have contributed to effective weed management strategies in the respective crops (Green and

C. Emani (✉)
Department of Biology, Western Kentucky University-Owensboro,
4821 New Hartford Road, Owensboro, KY 42303, USA
e-mail: chandrakanth.emani@wku.edu

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Owen 2011). Unlike the herbicide-resistant transgenics, the insect-resistant transgenics involve effect of the expressed *cry* genes derived from the soil bacterium *Bacillus thuringenesis* on living organisms in the biosphere such as insects of the species Lepidoptera and Coleoptera (Arpaia 2012). Since the insect species including insect pests make up a significant proportion of an agro-ecosystem, legitimate concerns exist of the commercial transgenics affecting the non-target insect and soil microorganism populations. Monitoring agencies such as the USDA and the European Union provide guidelines for the non-target organism testing prior to transgenic commercialization, but few if any of the guidelines relate to risks at the ecological level (Arpaia 2012). An agro-ecosystem also depends on physiological field functions such as microbial decomposition, nutrient cycling, crop pollination by animals and biological pest control and these in turn involve diverse animal species other than insects (Curtis et al. 2002) and hence, a thorough examination of transgenic cultivation effects should include all these in the non-target populations. Some of the recent reviews on effects of transgenics on non-target organisms dealt with studies focused on arthropods at field and laboratory level (O'Callaghan et al. 2005; Lövei and Arpaia 2005; Romeis et al. 2006), sometimes utilizing meta-analysis (Marvier et al. 2007; Lövei et al. 2009). Reviews also exist of the effects on soil microorganisms (Widmer 2007; Filion 2008) and soil-associated meso and macro-fauna (Icoz and Stotzky 2008). The present review aims to present an overview of various studies on the effect of transgenic crops on non-target organisms focusing on sound environmental assessments with emphasis on agro-ecosystems.

4.2 The Study that Started it All—The *Bt* Maize-Monarch Butterfly Controversy

The much discussed and dissected study that started a serious discussion on the effect of transgenic crops on non-target insect populations was the Losey et al. (1999) report that showed the *Bacillus thuringenesis* (Bt) corn plants' pollen on monarch butterflies. In a laboratory assay, it was demonstrated that larvae of monarch butterfly, *Danaus plexippus*, reared on milkweed leaves, dusted with pollen from *Bt* corn, ate less, had slow growth and high mortality compared to those reared on normal corn pollen (Losey et al. 1999). It was argued that the dispersal of the corn pollen by wind to almost 60 m and its deposition on other plants might affect non-target insect populations. Subsequent studies examined various flaws in the experimental set up (Hodgson 1999) such as non-relatable field conditions in terms of pollen availability and existence of milk weed plants in the real world, inappropriate experimental controls and the absence of stringent quantification of the amount of pollen used in the experiments. However, environmental groups and media seized the opportunity to sensationalize the issue of transgenic crops being a threat to non-target insect populations.

4.3 Effects of Transgenic Crops on Natural Pests

The negative effects of transgenic crops on non-target organisms can be functionally categorized at the levels of ecological, agricultural and other anthropocentric values (Arpaia 2012). If looked at under these broad perspectives, a more realistic picture about the effect of transgenics on the non-target organisms can be elucidated. A starting point for this kind of examination would be with natural pests and the natural pest control due to predators and parasitoids in agriculture that does account for about 95% control of potential pests (Debach and Rosen 1991). Transgenic plants with insect resistant genes potentially introduce novel metabolites into an agro-ecosystem such as *cry* toxins (Naranjo 2009), proteinase inhibitors (Malone et al. 2000) and lectins. The expressed insecticidal toxins are selectively and specifically toxic to various insect orders, and this specificity by itself can be considered as an important characteristic that limits any effects on non-target insect populations (Arpaia 2012). Nevertheless, after examining many lab studies related to effects of the transgenic insecticidal toxins using meta-analysis, it was concluded that the few studies that showed significant negative effects of transgenics on non-target species were replete with limitations in terms of sample size, statistical insufficiency and the duration of toxicity tests (Lövei et al. 2009). An extensive meta-analysis of extant literature on the invertebrate non-target effects that revealed hazards identified in laboratory tests may not always manifest in field, and the minor negative effects exhibited by transgenic *Bt* plants were insignificant when compared to insecticide-based pest suppression (Marvier et al. 2007; Naranjo 2009). Studies with transgenic *Bt* potatoes also examined the natural pests (Wolfenbarger et al. 2008) as well as the tangential effects on sucking herbivores that maintain the toxic products upon ingestion (Obrist et al. 2006) showed no adverse effects of the expressed Bt toxin on non-target species such as the lacewings (Andow et al. 2006), ladybirds (Dhillon and Sharma 2009), ground beetles (Haughton et al. 2003; De la Poza et al. 2005) and honeybees (Duan et al. 2008). Varying to insignificant effects were seen on parasitoids, but these indirect effects need to be considered in an ecological contexts such as abundance and diversity of the parasitoids for both pest and non-pest species (Arpaia 2012).

4.4 Effects of Transgenic Crops on Pollinators

Since transgenic plants, just like the regular crop species depend on pollinators for their optimal reproduction, it is imperative to consider the effects of the expressed transgenic products on the various pollinator insect species that are non-target population. Recent studies showed no deleterious effects of transgenic herbicide-tolerant or insect-tolerant on pollinators (Malone and Burgess 2009). The one *Bt* toxin that was shown to have a potent effect on Hymenopteran insects was Cry5 (Garcia-Robles et al. 2001), but no Cry5-exprssing plants have been approved for commercial cultivation. The more popular Cry1 toxin expressing plants have no effect on pollinators such as honeybees (Ramirez-Romero et al. 2005; Rose et al.

2007), a fact confirmed by extensive meta-analysis of laboratory tests assessing honeybee survival on commercial *Bt* crops (Duan et al. 2008). Other transgenes such as serine protease inhibitors were shown to affect honeybees and bumblebees at very high concentrations (Malone and Burgess 2009). Herbicide tolerant transgenic crops may have an indirect effect on pollinators (Haughton et al. 2003) in an agro-ecosystem due to reduced flowering.

4.5 Effects of Transgenic Crops on Soil Fertility Inducers

The availability of beneficial nutrient-rich fertile soils is dependent on effective microbial functioning in the soil and the presence of soil-dwelling invertebrates involved in nutrient recycling and decomposition of organic matter is a significant parameter of an efficient agro-ecosystem (Moore et al. 1988). In an agro-ecosystem, plants themselves contribute to the thriving of beneficial soil fertility inducers through the release of useful soil exudates (Brussaard et al. 2007; Arpaia 2012). Cry1 toxins expressed in *Bt* corn did not show any adverse effects on one of the most efficient soil fertility inducer, earthworm (Saxena and Stotzky 2001; Schrader et al. 2008). Though some laboratory studies showed effect of Bt corn on mean fresh weight of earthworms after a 160 day exposure, the same was not observed in the field (Zwahlen et al. 2003). Cry toxins were proposed to be hazardous to the nitrogen fixing nematode population of *C. elegans* (Höss et al. 2008), but this was later dubbed an indirect effect as the Cry toxin in *Bt* maize samples was not sufficiently high to produce the same toxic effects in growth chamber studies (Saxena and Stotzky 2001), a fact further corroborated by both glasshouse studies (Griffiths et al. 2007b) and field studies (Griffiths et al. 2007a). Apart from *Bt* maize, nematodes being effected was studied with *Bt* oilseed rape and a direct correlation was observed between transgenic oilseed rape and the abundance of fungal feeding nematodes (Manachini and Lozzia 2002). Studies on a model decomposer, the woodlouse with *Bt* maize and purified cry toxins did not show any adverse effects (Escher et al. 2000; Clark et al. 2006). Another important group of soil fertility inducers and indicators of soil health that live in root zones of plants, the collembolan were exposed to purified cry toxins and did not show any adverse survival, growth or reproduction impacts (Sims and Martin 1997). Other decomposers such as diplopods that regularly occur in corn fields showed no adverse effects when fed with *Bt* maize (Weber and Nentwig 2006).

4.6 Effects of Transgenic Crops on Soil Microorganisms

Soil microorganisms are involved in fundamental processes such as decomposition of organic matter, mineralization, chemical decomposition and improvement of soil structure (Gupta and Yeates 1997) and root exudates released by field crops selectively regulate these organisms in an agro-ecosystem (Lynch 1994). A majority of recent studies (Icoz and Stotzky 2008) indicate *Bt* transgenics having no adverse effects on soil microorganism populations. Xue et al. (2005) found a lower ratio between gram-positive and gram-negative bacteria in fields of *Bt* maize, but a reverse of the same in a *Bt* potato field. Root exudates of *Bt* corn were shown to result in genetic modification specific to a changed physiology and composition of root exudates that in turn affect symbiotic and rhizosphere microorganisms (Castaldini et al. 2005), but this might be disadvantageous to the individual crop and would not affect the agro-ecosystem as a whole (Widmer 2007).

4.7 Revisiting the Monarch Butterfly Controversy—Assessing Risks in Field

The study of Losey et al. (1999) combined with the charismatic and iconic christening by over enthusiastic environmentalists and media of the lepidopteran monarch butterfly triggered numerous studies on the effect of transgenic corn on monarch butterfly populations. Later field studies showed that the risk to monarch butterfly in terms of toxic levels of transgenic pollen is minimal simply due to the limited spatial distribution of pollen (Pleasants et al. 2001) and the insignificant exposure of larva during the pollen shed (Oberhauser et al. 2001). Studies in USA transgenic *Bt* corn fields specific to effects on monarch butterfly larvae continuously exposed to the transgenic crop during anthesis showed insignificant effects on mortality (Dively et al. 2004), though laboratory studies continued to show reduction in feeding and weight gain (Anderson et al. 2004; Prasifka et al. 2007). The differences in field results was later attributed to the fact that early larval instars are less exposed to *Bt* pollen drift as they feed on the upper third of milkweed plants that have lesser densities of anthers and the larva tending to move on the underside of leaves avoiding contact with anthers (Pleasants et al. 2001; Anderson et al. 2004; Jesse and Obrycki 2003).

4.8 Conclusion

After the rich dividends reaped by the agricultural community through green revolution, the commercial cultivation of transgenic crops is being seen as its successor and this important scientific event is being christened as the gene revolution (Birch and Wheatley 2005). Though the adoption of transgenic technology was one of the fastest across the world (James 2009), the backlash in Europe as compared to USA can be attributed to societal and political differences (Marshall 2009). An important factor when examining issues such as the subject of this review puts the onus on the scientific community to properly educate the general public and the political decision makers, and when disseminating their findings take into consideration the larger perspective of the agro-ecosystem instead of jumping to unwarranted conclusions based on individual laboratory studies.

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