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Deployment of Transgenic Crops for Pest Management : Ecological Considerations and Their Biosafety to the Environment

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INTRODUCTION

Significant progress has been made over the past decades in handling and introduction of exotic genes into plants, and has provided opportunities to modify crops to increase yields, impart resistance to biotic and abiotic stress factors, and improve nutrition. Genes from bacteria such as *Bacillus thuringiensis* (Bt) and *B. sphearicus* have been used successfully for pest control through transgenic crops on a commercial scale (Hilder and Boulter, 1999; Sharma *et al.*, 2004). Insecticidal genes such as Bt, trypsin inhibitors, lectins, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins, and small RNA viruses can also be used alone or in combination with Bt genes for pest management (Sharma, 2009). In addition to widening the pool of useful genes, genetic engineering also allows the use of several desirable genes in a single event, and thus reducing the time required to introgress novel genes into the elite background.

The Bt toxin gene was cloned in 1981, and the first transgenic plants were produced by mid-1980s (Barton *et al.*, 1987; Fischoff *et al.*, 1987; Vaeck *et al.*, 1987). Since then, several crop species have been

genetically engineered to produce Bt toxins to control the target insect pests. Genes conferring resistance to insects have been inserted into maize, cotton, potato, tobacco, rice, broccoli, lettuce, walnuts, apples, alfalfa, and soybean (Hilder and Boulter, 1999). The first transgenic crop was grown in 1994, and large-scale cultivation was taken up in 1996 in USA. Since then, there has been a rapid growth in the area under transgenic crops in USA, Australia, and China. The area planted to transgenic crops increased from 1.7 million ha in 1996 to over 148 million ha in 2010 (James, 2010). Transgenic crops were grown in several countries, and most of the area planted to genetically improved crops is in Australia, Canada, Argentina, China, USA, South Africa, and India. Among the crops produced, insect-resistant cotton and maize, herbicide-resistant soybean, cotton and canola, and tomatoes with a long shelf-life are widely grown worldwide. In addition to the reduction in losses due to insect pests, the development and deployment of transgenic plants with insecticidal genes will also lead to a major reduction in insecticide sprays, reduced exposure of farm labor and nontarget organisms to pesticides, increased activity of natural enemies, reduced amounts of pesticide residues in the food and food products, and a safer environment to live.

The benefits to growers have been higher yields, lower costs, and ease of management. Additional impact of these insect-resistant transgenic crops is in terms of reduction in number of pesticide applications. The additional benefits to growers have been the ability to control insect pests that have become resistant to commonly used insecticides, and reduction in crop protection costs. Genetically modified (GM) crops have been used commercially for more than 10 years. Available impact studies of insect-resistant and herbicide-tolerant crops show that these technologies are beneficial to farmers and consumers, producing large aggregate welfare gains as well as positive effects for the environment and human health (Qaim, 2009). The advantages of future applications could even be much bigger. Transgenic crops can contribute significantly to global food security and poverty reduction. Nonetheless, widespread public reservations have led to a complex system of regulations. Overregulation has become a real threat for the further development and use of transgenic crops. The costs in terms of foregone benefits may be large, especially for developing countries.

RISK ASSESSMENT OF INSECT RESISTANT TRANSGENIC CROPS TO THE ENVIRONMENT

The focus of biosafety assessment and regulations need to be on safety, quality, and efficacy. Various approaches addressing the risks focus on establishing good standards of laboratory practice, efficiency and security of the containment facilities, and effects of genetically modified organisms on human health and the environment (Levin and Strauss, 1993; Sharma *et al.*, 2002). The risk is assessed in the form of probability that a modified organism (or the DNA inserted in it) will be able to enter the human body and survive there, and result in expression of anticipated level of the inserted DNA. Risk also measures damage in the form of harm likely to be caused to a person by exposure to the modified organism. A comprehensive risk assessment is necessary once a plant has to be released for small-scale experiments, and commercial production. The scientists concerned, the biosafety committee, and the national/international regulatory authorities should determine whether it is acceptable to release the specific transgenic plants, and if needed, restrictions to be imposed. Field containment should be in place to limit the possible environmental impact of the release experiment. This may include isolation from the sexually compatible species, prevention of flowering, use of male-sterile lines, and subsequent monitoring protocols. Information required for risk assessment includes organization and the people involved, DNA donor, the receiving species, the transgenic plant, target environment, conditions of release, transgenic plant x environment interaction, and monitoring and management of the waste (NAS, 1989).

DNA Donor and Receiving Species

It includes complete information about the donor and the receiving species. The receiving plant species forms the baseline with which the transgenic plant should be compared. Information on the donor species indicates the type of information needed about the transgene. Information should also be provided about the vector used in the transformation, and antibiotic or herbicide resistance genes used as a selection marker. Finally, there should be complete molecular data on the genes inserted, stability of expression, change in allergenicity, toxicity and persistence in particular environmental conditions, and ability to invade new habitats. The changes in the transgenic plant should be measured against the unmodified control genotype.

Conditions of Release and the Target Environment

The risk to the target environment requires qualitative judgment, and should be based on a case-by-case study, depending on experience. Information about the purpose of the release and agronomic requirements are important for risk assessment at the national and international level. Ecological information about the release site, survey of plant species growing in the target region, and the nature of pollen dissemination are also important. The anticipated target and nontarget organisms with which the transgenic plant will interact need to be determined. Information should be recorded whether the transgenic plant would become a better or worse host for the target and nontarget pests.

Interaction Between the Transgenic Plant and the Environment

It is important to describe the invasiveness of the transgenic plant in the wild habitat, ability to propagate sexually/asexually, possibility of transferring the transgene to the same or related species, or to microorganisms, and the consequences of gene transfer.

Monitoring and Waste Treatment

Once the transgenic plants are released into the environment, there is a movement of pollen, seed, and the plants outside the immediate environment of release. It is important to monitor the transgenes in the environment after the release, and the efficiency with which it is possible to destroy the plant material, if necessary. Efficient methods of detecting the transgenic plants and the transgene in the nontarget species are necessary. It can be done by visual marker (e.g., β-glucorinidase) or a selectable marker (e.g., antibiotic resistance, or molecular analysis, e.g., PCR and southern hybridization). Methods of destroying the plant material at the end of the experiment should also be described. Commercial releases follow once the results of experimental releases have been found to be satisfactory. The risk assessment should be carried out by the multidisciplinary biosafety committee with expertise in molecular biology, environmental science, entomology, pathology, and any other field as appropriate. The governmental authorities, environmental groups, NGOs, and progressive farmers may be involved during the multilocation testing to make the process transparent, and assure the public that care is being taken to minimize risks to the environment and human health.

Effects on Target and Non-target Pests

Efficacy of transgenic crops for controlling the target and the nontarget pests need to be determined in each region (Sharma and Ortiz, 2000; Sharma, 2009). Some of the pests maintain high pest densities on alternate hosts. The issues that need to be addressed while introducing transgenic crops for pest control include effect on target and non-target insects, performance limitations, secondary pest problems, insect sensitivity, evolution of insect biotypes, and environmental influence on gene expression.

Effect of Transgenic Plants on Population Dynamics

Effects of transgenic crops on the population dynamics of the insects would be similar to the plants with conventional host plant resistance (Luginbill and Knipling, 1969; Sharma, 2004), e.g., continuous planting of the stem fly-resistant (*Cephus cinctus*) wheat cultivars would completely suppress the stem fly populations below the economic threshold levels within six years. The stem flies can also be kept under check by alternate planting of the resistant and susceptible cultivars. Similar models for the effect of insect-resistant cultivars on insect abundance have also been developed for sorghum shoot fly (*Atherigona soccata*), spotted stem borer (*Chilo partellus*), sorghum midge (*Stenodiplosis sorghicola*), and sorghum head bug (*Calocoris angustatus*) (Sharma, 1993; Sharma *et al.*, 1999). In wheat, no direct relationship has been observed between the planting of the resistant cultivars and the population of the Hessian fly (*Mayetiola destructor*) (Foster *et al.*, 1991). Expression of resistance is not the same under different population densities, and under different environmental conditions.

Performance Limitations

The Bt transgenic crops cannot produce the same dramatic effects on insect mortality as the synthetic insecticides. The farmers need to be educated about the efficacy and mode of action of transgenic crops. The expectations have to be real, and remedial measures should be taken as the situation warrants. The effects of the transgenic crops on insects will be relatively slower, but cumulative over time. Transgenic crops may not be able to withstand the pest density in some seasons. Therefore, careful monitoring of pest populations is an essential component of pest management involving the transgenic crops. The value of the transgenic crops can be best realized when deployed as a component of pest management for sustainable crop production

(Sharma *et al.*, 2004). The current CryIAb construct employs PEP-carboxylase promoter, which enables the expression in the green tissue, and as a result, the expression is greater in the young plants. Some insects such as stem borers and shoot fly migrate into the plant whorl or stem tissue with incomplete chlorophyll formation. If the toxin is expressed in insufficient amounts in such tissues, the insects can develop mechanisms to withstand low levels of toxins in the transgenic plants. Behavioral avoidance of the tissue expressing the toxin gene can be another component in insect resistance to the transgenic plants. Therefore, care should be taken to express the toxins in sufficient amounts at the site of damage/feeding by the insects.

Secondary Pest Problems

Most crops are not attacked by a single pest species, but a number of insect pests. In the absence of competition from the major pests, secondary pests may assume a major pest status (Hilder and Boulter, 1999). The Bt toxins may be ineffective against such pests, e.g., leaf hoppers, mirid bugs, root feeders, mites, etc. (Sharma and Pampapathy, 2006). This will offset some of the advantages expected of the cultivation of transgenic crops. Management of phytophagous stink bugs is necessary in transgenic Bt cotton (Greene *et al.*, 1997; Sharma and Papmpapathy, 2006). Insecticide application for the control of stink bugs is necessary if more than 20% of the bolls are damaged in mid- to late-season. There are no differences between transgenic and nontransgenic cultivars in boll weevil or aphid damage, beneficial arthropods or fiber characteristics (Parker and Huffman, 1997). Effective and timely control measures should be adopted for the control of secondary pests on transgenic crops. There is a need to deploy protease inhibitor and lectin genes that are effective against sucking pests, along with the Bt genes, to make genetically modified plants to be more effective against insect pests for effective crop protection (Sharma, 2009). While there is a trend to develop target specific compounds for chemical control, it will be desirable to have genes with broad-spectrum activity for use in genetic transformation of crops, provided this does not influence the beneficial organisms.

Insect Sensitivity

There are many species of insects that are not susceptible to the currently available Bt proteins. There is a need to broaden the pool of genes, which can be effective against insects that are not sensitive to the currently available genes. *Heliothis virescens* is less sensitive to

Cry1Aa, Cry1C and Cry1E, while *Spodoptera littoralis* is insensitive to most of the Bt toxins (Gill *et al.*, 1992). *Spodoptera litura* is less sensitive to toxins from *B. thuringiensis* var *kurstaki* than *H. armigera, Achaea janata, Plutella xylostella,* and *Spilosoma obliqua* (Meenakshisundram and Gujar, 1998). Bt toxins CryIC and Cry1E, which are active against *H. virescens* (MacIntosh *et al.*, 1991), are ineffective against *H. armigera* (Chakrabarti *et al.*, 1998). Cry1B is slightly active against *H. armigera*, while it has been reported to be inactive against *H. virescens* (Hofte and Whiteley, 1989). No significant differences in leaf miner, *Liriomyza trifolii* damage were observed between Bt and non-Bt cotton hybrids (Dhillon and Sharma, 2010a). Thus, there are considerable differences in the sensitivity of different insect species to various Bt toxins, and due care has to be taken to deploy Bt toxins in different crops/cropping systems. The first generation transgenics had only one Bt toxin gene, and were less effective for the control of less sensitive species. However, the second generation transgenic plants have more than one toxin gene and are more effective against the sensitive target and non-target insect pests.

Evolution of New Insect Biotypes

Experience from the conventional breeding has shown that there is a direct relationship between planting of insect resistant cultivars and emergence of new biotypes, e.g., Hessian fly, *Mayetiola destructor* in wheat, green bug, *Schizaphis graminum* in sorghum, and rice gall midge, *Orseolia oryzae* in rice, etc. (Smith, 2005). The time needed for adaptation to antibiosis resistant genes has been predicted to be 3 to 8 years. In case of green bug, *S. graminum*, the breeding programs continue to struggle to keep pace with the evolution of new biotypes (Wood, 1971; Daniels, 1981). However, there is no relationship between the deployment of green bug-resistant wheat cultivars and the development of new biotypes (Porter *et al.*, 1997). For sorghum, only 3 of the 11 biotypes of green bug have shown a correlation between the use of resistant hybrids and the development of new biotypes. Based on the analysis of specific insect-plant interactions, future plant resistance efforts should focus on the use of most effective resistance genes, despite the past predictions, of what effect these genes may have on the target insects.

Environmental Influence on Gene Expression

Variations in efficacy within the growing season and between seasons also may be influenced by environmental factors (Olsen *et al.*,

2005). There have been some failures in insect control through the transgenic crops. Cotton bollworm, *Helicoverpa zea* destroyed Bt cottons due to high tolerance to Bt toxin, CryIAc in Texas, USA (Kaiser, 1996). Similarly, *H. armigera* and *H. punctigera* destroyed the cotton crop in the second half of the growing season in Australia because of reduced production of Bt toxins in the transgenic crops (Hilder and Boulter, 1999). Possible causes for the failure of insect control may be because of inadequate production of the Bt toxin, effect of environment on expression of transgene, locally resistant insect populations, and development of resistance due to inadequate management. Cotton crop flooded with 3 to 4 cm deep water for 12 days lost resistance to insects as compared with the control plants irrigated normally (Wu *et al.*, 1997). Similar reaction has been observed in Bt cotton grown under overcast and rainy weather continuously for 21 days. Epistatic and environmental effects on foreign gene expression also influence the stability, efficacy, and durability of the foreign genes (Sachs *et al.*, 1998). Expression of transgene is also influenced by site of gene insertion, gene construct, epistasis, somaclonal mutations, and the physical environment. Appropriate evaluation and selection procedures should be used in a breeding program to develop crop varieties with pest-resistant traits conferred by the foreign genes.

DEVELOPMENT OF RESISTANCE

Development of Resistance in Insect Populations

Insect pest populations have shown a remarkable capacity to develop resistance to chemical pesticides. Over 500 species of insects have developed resistance to insecticides (Moberg, 1990). Development of resistance to Bt toxins will diminish the value of transgenic crops for pest management. Most of the transgenic plants produced so far have Bt genes under the control of cauliflower mosaic virus (CaMV35S) constitutive promoter, and this system may lead to development of resistance in the target insects as the toxins are expressed in all parts of the plant (Harris, 1991). However, several site or tissue specific promoters have been developed in the recent past to overcome this problem. Toxin production may also decrease over the crop-growing season. Decreasing levels of toxin may lead to development of resistance to the toxin used, and to other related Bt toxins to which the insect populations may initially be quite sensitive. Low doses of the toxins eliminate the most sensitive individuals of a population, leaving a population, in which resistance can develop

much faster. Since some Bt toxins have a similar mode of action, resistance developed against one toxin may lead to development of cross-resistance to other toxins. However, there are reports that insects selected for resistance to one Bt toxin may not be resistant to other Bt toxins (Sharma and Ortiz, 2000). In some insect species, the probability of development of resistance may be very low, e.g., *Ostrinia nubilalis* has been observed to develop some tolerance to low levels of CryIA(b) in the diet, but it has not been possible to initiate or sustain the insect colonies at concentrations in the diet closer to the actual levels expressed in the transgenic maize plants (Lang *et al.*, 1996). Development of resistance to Bt may not be a serious issue since the Bt and the pests have co-evolved for million of years (Tabashnik, 1994). Resistance to Cry1Ac-producing Bt cotton is associated with recessive "r" alleles at the BtR locus in the strains of pink bollworm, *Pectinophora gossypiella*, and identify the cadherin locus as a candidate for molecular monitoring of pink bollworm resistance to Bt cotton (Tabashnik *et al.*, 2005). Using simulation modeling, Gustafson *et al.* (2006) estimated that *Helicoverpa zea* will take >30 years to develop resistance to Bt cotton in USA. An analysis of resistance monitoring data from five continents, Tabashnik *et al.* (2009) observed that in 41 studies that evaluated responses of field populations of 11 lepidopteran pests to four Bt toxins produced by Bt corn and cotton, most target pest populations remain susceptible, whereas field-evolved resistance has been documented in some populations of *Spodoptera frugiperda* (J.E. Smith) to Cry1F in Bt corn in Puerto Rico, *Busseola fusca* to Cry1Ab in Bt corn in South Africa, *H. zea* (Boddie) to Cry1Ac and Cry2Ab in Bt cotton in the southeastern United States, and *P. gossypiella* to Cry1Ac in Bt cotton in Gujarat, India. Field outcomes are consistent with predictions from theory, suggesting that factors delaying resistance include recessive inheritance of resistance, abundant refuges of non-Bt host plants, and two-toxin Bt crops deployed separately from one-toxin Bt crops. However, transgenic crops that target haplodiploid or parth-enogenetic pests will require careful consideration of the effects of reproductive mode, fitness costs, and incomplete resistance for development of resistance to transgenic crops (Crowder and Carrière, 2009).

Development of Resistance to Herbicides

Herbicide genes have been inserted into several crops to provide selectivity for herbicides that are degraded in the environment quickly. There is a need to know whether the herbicide-resistant plants can establish as a weed, and the possibility of gene transfer

into the wild relatives of the crop plant. Genes from plants engineered for herbicide resistance could cross over to other plants, creating super weeds. Genes introduced into genetically transformed crops can spread into closely related native species (Chevre *et al.*, 1997). Studies in Norway and the United States have shown that the gene for herbicide resistance can move from cultivated canola to wild relatives. Genes from the conventionally bred *Brassica napus* have been moving to the wild turnip, *B. rapa* (Raybould and Gray, 1993). Genes from unrelated sources may change the fitness and population dynamics of the hybrids between native plants and the wild species.

Development of Resistance to Antibiotic Genes

The antibiotic genes used as a marker to select for gene transfer may lead to development of resistance in pathogens infecting human beings. However, general scientific view is that the risk of compromising the therapeutic value of antibiotics is almost negligible. Most genetically engineered plants contain a gene for antibiotic resistance as an easily identifiable marker. Hypothetically, antibiotic resistance genes may move from a crop into bacteria in the environment. Since bacteria readily exchange antibiotic resistance genes, the antibiotic resistance genes may move into disease-causing bacteria. Gene transfer from plants to microorganisms is possible in laboratory studies (Gebhard and Samalla, 1998), and possibly has happened during evolution (Doolittle, 1999). The probability of movement of genes from plants to human pathogens (antibiotics) is negligible. Several studies have established that there is little chance that such a transfer would occur (Calgene, 1990), but there is a continuing debate whether such a gene should be present in the commercial varieties. Methods have been developed for removing selectable marker genes after selection of the transgenics (Yoder and Goldsbrough, 1994; Ebinuma *et al.*, 1997). There are alternatives to the antibiotic markers, and systems are also available to carry out the transformation without involving any markers. The marker gene can also be excised after two lines are crossed (Dale and Ow, 1991).

GENE ESCAPE INTO THE ENVIRONMENT

Natural transformation is assumed to be the most likely mechanism by which DNA from transgenic plants could be horizontally transferred to bacteria. Under laboratory conditions, plasmid transfer between *B. thuringiensis* subsp. *tenebrionis* and *B. thuringiensis* subsp. *kurstaki* HD 1 (resistant to streptomycin) strains occurs at 10^{-2}

(Thomas *et al.*, 1997). However, no plasmid transfer has been observed in soil release experiments, and in insects on leaf discs. The Bt toxins were detectable on the clay-particle-size fraction of nonsterile soil after 40 days. When the toxins bind on clay minerals, they become resistant to utilization by microorganisms. Binding of the Bt toxins to humic acids reduces their potential for microbial biodegradation (Crecchio and Stotzky, 1998). These results indicate that Bt toxins in transgenic plants and microbes could persist, accumulate, and remain insecticidal in the soil as a result of binding to humic acids, where they might pose an environmental hazard to non-target organisms. To determine the occurrence of naturally transformable bacteria amongst bulk and rhizosphere soil bacteria, different transformation strategies were employed by Richter and Smalla (2007) using either plasmid DNA (IncQ plasmids pSM1890 and pSM1885, conferring GFP, Smr, Gmr and GFP, Smr, Tcr, respectively) or genomic DNA from rhizosphere isolates, which were chromosomally tagged with mini-Tn5 (GFP, Tcr), as transforming DNA. With a single exception, transformants were neither detected in the collection of isolates nor in the rhizosphere bacterial community. *Acinetobacter baylyi* BD413 used as a positive control showed drastically reduced transformation frequencies with plasmid pSM1890 as transforming DNA when mixed with the rhizosphere pellet. Transformation assays indicated that the proportion of rhizosphere or bulk soil bacteria which are naturally transformable is negligibly low.

The greatest risk of a transgenic plant released into the environment is its potential spread beyond the plant area to become a weed. However, there are no records of a plant becoming a weed as a result of plant breeding (Cook, 2000). This may be because of low risk of crop plants to the environment, extensive testing of the crop varieties before release and adequate management practices to mitigate any risks inherent in the crop plants. Plant breeding efforts have been tended to decrease rather than increase the toxic substances, as a result, making the improved varieties more susceptible to insect pests. However, there is a feeling that genes introduced from outside the range of sexual compatibility might present new risks to the environment and humans. However, many of such apprehensions are not supported by data. A study conducted by the National Academy of Sciences, USA (NAS, 1987), has concluded that there is no evidence of hazards associated with DNA techniques, the risks, if any, are similar to those with conventional breeding techniques, the risks involved are related to nature of the organism rather than the process,

and there is a need for a planned introduction of the modified organisms into the environment.

The introgression of transgenes into the wild relatives is of potential concern (Gregorius and Steiner, 1993; Serratos *et al.*, 1997). Pollen dispersal from transgenic cotton is low, but increases with an increase in the size of the source plot (Llewellyn and Fitt, 1996). Interspecific hybridization is a common process, but hybrids are rare, and most are sterile, and there is a rare chance of gene introgression into the wild relatives (Fitter *et al.*, 1990). Transgenic plants may become weeds, except in the context of their normal agricultural environment. Gene escape may occur when the plant invades a semi-natural habitat or transferred into the wild relative, and persist in the uncultivated land. Its' spread can be checked by methods similar to any other single trait. There are differences among plant species to disperse from the environment other than the one in which they are released, and their ability to establish feral populations. Such an event has to be compared with that of the original plant. One of the hazards in gene transfer from the transgenic plants to the wild relatives is of concern if the wild relatives are under selection pressure (biological control) from the pest. If the target pest does not play any role in population regulation of the wild hosts, the gene transfer will not constitute any hazard. The build up of resistance in the wild relatives can also act as a component of pest management to the target pest.

INTERACTION OF INSECT RESISTANT TRANSGENIC PLANTS WITH NON-TARGET ORGANISMS

One of the major concerns of transgenic crops is their effects on the non-target organisms, about which little is known at the moment. The Bt proteins are rapidly degraded by the stomach juices of the vertebrates. Most Bt toxins are specific to insects as they are activated in the alkaline medium of the insect gut. Bt proteins can have harmful effects on the beneficial insects, although such affects are much less severe than those of the broad-spectrum insecticides. The information that use of genetically modified corn may have toxic effects on the larvae of the monarch butterfly, *Papilio demoleus* has generated a huge amount of publicity, and almost as much misinformation. A review of current research indicates that there may be little risk to monarch butterfly caterpillars from Bt corn pollen. Insect-resistant crops can affect the quantity and quality of non-prey foods for natural enemies, as well as the availability and quality of both target and non-target pests that serve as prey/hosts (Lundgren *et al.*, 2009).

Some inherent qualities of both biological control and transgenic crops provide opportunities to improve upon sustainable IPM systems. For example, biological control agents may delay the evolution of pest resistance to transgenic crops, and suppress outbreaks of secondary pests, while herbicide-tolerant crops facilitate within-field management of vegetational diversity can enhance the efficacy of biological control agents. In a meta-analysis of the information published on the effect of transgenic crops on nontarget insects, Wolfenbarger *et al.* (2008) observed that predators were less abundant in Bt cotton compared to unsprayed non-Bt controls. As expected, fewer specialist parasitoids of the target pest occurred in Bt maize fields compared to unsprayed non-Bt controls. Numbers of predators and herbivores were higher in Bt crops compared to sprayed non-Bt controls, and type of insecticide influenced the magnitude of the difference. No differences in abundance were found when both Bt and non-Bt crops were sprayed. There are no uniform effects of Bt cotton, maize and potato on the functional guilds of non-target arthropods. Use of and type of insecticides influenced the magnitude and direction of effects and the effects of insecticides were much larger than those of Bt crops.

Interactions with Honeybees and Other Pollinators

There are no significant effects of transgenic crops on the honeybees. Transgenic rape does not appear to have harmful effects on the lifespan and behavior of honeybees, but further tests may be necessary (Pham Delegue and Jouanin, 1997). Chitinase in genetically modified oil seed rape did not affect learning performance of honeybees; beta-1,3 glucanase affected the level of conditioned responses, while cowpea trypsin inhibitor (CpTI) induced marked effects in both conditioning and testing phases, especially at high concentrations (Picard-Nizou *et al.,* 1997). The decrease in learning performance induced by CpTI at the individual level has been confirmed at the colony level. Trypsin inhibitor and wheat germ agglutinin (WGA) did not show acute toxicity in honeybees. Consumption of high doses of protease inhibitors induces proteinase over production (Jouanin *et al.*, 1998). Trypsin endopeptidase inhibitor, bovine pancreatic trypsin inhibitor (BPTI), and soybean trypsin inhibitor (SBTI), have been found to be toxic to adult honeybees at 1% weight: volume in sugar solution (Malone *et al.*, 1995). Serine proteinase inhibitor (PI) (from soybean), cysteine PI (OCI from rice), chicken egg white cystatin, and Bowman-Birk soybean inhibitor do not produce harmful effects on honeybees at the concentrations expressed in transgenic plants (Bottino *et al.*, 1988; Girard *et al.*, 1988; Pham

Delegue and Jouanin, 1997). Liu *et al.* (2005) observed no oral acute toxic effects on worker bees. There were no significant differences in superoxide dismutases activity and the longevity of worker bees fed with diets containing the transgenic cotton pollen or non-transgenic parental cotton pollen.

Interactions with Insect Predators

No major differences have been observed in the abundance of predators between the transgenic and non-transgenic crops (Hoffman *et al.*, 1992; Sims, 1995; Wang and Xia, 1997). Nontarget effects of Bt crops on natural enemies have been observed only when Bt-susceptible, sub-lethally damaged herbivores were used as prey or host, with no indication of direct toxic effects (Romeis *et al.*, 2006). Field studies have confirmed that the abundance and activity of parasitoids and predators are similar in Bt and non-Bt crops (maize, cotton, potato, rice and rape). There may be a reduction in the fitness of the predatory chrysopid larvae directly attributable to caterpillars fed on Bt-maize (Hoffmann *et al.*, 1992; Hilbeck *et al.*, 1998). There are no adverse effects of the Bt on the Colorado potato beetle predator, *Coleomegilla maculata* (Giroux *et al.*, 1994). Cry3A-intoxicated *L. decemlineata* can be eaten by *C. maculata* without any observable adverse effects on their survival or predation potential (Riddick and Barbosa, 1998). Its predatory activity can also decrease the rate at which *L. decemlineata* adapts to the Bt toxins if mixed plantings are used (Arpaia *et al.*, 1997). Under choice conditions, the predator showed a distinct preference for the untreated eggs than those treated with Bt (Girard *et al.*, 1994). The predator activity was not affected by pure transgenic and mixed seed potato fields (Riddick *et al.*, 1998).

No significant effects on survival, aphid consumption, development, or reproduction have been observed in *Hippodamia convergens* fed on *Myzus persicae*, reared on potatoes expressing δ-endotoxin of *Bacillus thuringiensis* subsp. *tenebrionis* (Dogan *et al.*, 1996). Two spotted ladybirds, *Adalia bipunctata* fed for 12 days on peach-potato aphids, *M. persicae* on transgenic potatoes expressing lectin from *Galanthus nivalis* have shown a decrease in fecundity, egg viability, and longevity (Birch *et al.*, 1999). Adverse effects on ladybird reproduction were reversed after switching the ladybirds to pea aphids from non-transgenic bean plants. Lozzia *et al.* (1998) did not observe any adverse effect on pre-imaginal development or mortality of *Chrysoperla carnea* when reared on *Rhopalosiphum padi* that had fed on Bt-maize. However, abundance of *Lebia grandis* was lower in pure

and mixed plants of transgenic potatoes than in pure non-transgenic potato plants (Riddick *et al.*, 1998). There was no apparent effect of transgenic cotton on the relative abundance of predatory spiders (*Clubiona* sp. and *Neoscona* sp.), coccinellid (*Cheilomenes sexmaculatus*), and the chrysopid (*Chrysoperla carnea*) (Sharma *et al.*, 2007). However, the abundance of spiders, coccinellids, and chrysopids was quite low in insecticide protected plots towards end of the cropping season. Direct exposure of the coccinelllid predator, *C. sexmaculatus* larvae to Bt toxins resulted in reduced larval survival and adult emergence as compared to the controls (Dhillon and Sharma, 2009). However, there were no adverse effects of the Bt toxins on *C. sexmaculatus* when the larvae were reared on *Aphis craccivora* fed on different concentrations of Cry1Ab or Cry1Ac in the artificial diet.

Thus, the effects of transgenic plants on the activity of predators vary across crops and the insect species involved. Because Bt-transgenic varieties can lead to substantial reductions in insecticide use in some crops, they can contribute to integrated pest management systems with a strong biological control component.

Interactions with Insect Parasitoids

Nontarget effects of Bt crops on natural enemies have been observed only when Bt-susceptible, sub-lethally damaged herbivores were used as prey or host, with no indication of direct toxic effects (Romeis *et al.*, 2006). Increased levels of parasitism by *Campoletis sonorensis* have been observed on transgenic plants compared to the nontransgenic plants, which may be due to fewer larvae on the transgenic plants. *C. sonorensis* and transgenic plants act synergistically, decreasing the larval survival beyond the level expected for an additive interaction (Johnson and Gould, 1992). The parasitoid, *Cardiochiles nigriceps* does not reduce the survival of the host larvae significantly, and its' activity is not influenced by the transgenic plants (Johnson, 1997; Johnson *et al.*, 1997). Egg parasitism of third-generation noctuids on Bt-transgenic cotton has been observed to be lower than in the conventional cottons (Wang and Xia, 1997). Percentage of parasitism by *Diadegma insulare* was not significantly different between the mixed and non-mixed plots of transgenic crop (Riggin Bucci and Gould, 1997). There is no effect of transgenic corn on the parasitization of *O. nubilalis* by *Eriborus terebrans* and *Macrocentrus grandii* (Orr and Landis, 1997).

Intra-field mixtures could serve to decrease density of a target pest such as the diamondback moth, while not adversely affecting the

activity of natural enemies. *Diadegma insulare* was not harmed by exposure to Cry1C, while similar studies involving insecticides significantly reduced parasitism rates on strains of *P. xylostella* resistant to these insecticides (Chen *et al.*, 2008). In another study, Cry1Ac was not detected in newly emerged parasitoid, *Cotesia vestalis*, but detected in *Chrysoperla carnea* larvae fed on Bt-resistant *P. xylostella* larvae reared on Bt transgenic oilseed rape (Wei *et al.*, 2008). However, no Cry1Ac could be detected in *C. carnea* larvae when the lacewings were transferred to *P. xylostella* larvae reared on conventional oilseed rape.

There was a significant reduction in cocoon formation and adult emergence of the ichneumonid parasitoid, *Campoletis chlorideae* reared on *H. armigera* larvae fed on the leaves of transgenic cottons before and after parasitization (Sharma *et al.*, 2007). However, no Bt toxins were detected in *H. armigera* larvae and the parasitoid cocoons with enzyme linked immunosorbent assay. Reduction in cocoon formation was because of early mortality of the *H. armigera* larvae due to Bt toxins in the leaves of transgenic cotton. Survival and development of *C. chlorideae* was also poor when *H. armigera* larvae were fed on the leaves of cotton hybrid Mech 184. The adverse effects of transgenic cotton on survival and development of *C. chlorideae* were largely due to early mortality and possibly poor nutritional quality of *H. armigera* larvae due to toxic effects of the transgene (Sharma *et al.*, 2007, 2008). Bt sprays on chickpea prolonged the larval period, and reduced pupation and adult emergence of the *H. armigera* parasitoid, *C. chlorideae* (Dhillon and Sharma, 2010b). The Bt-intoxicated *H. armigera* larvae also resulted in reduced weight of the cocoons and adults of *C. chlorideae*. Bt toxins were detected in *H. armigera* larvae fed on Bt-sprayed chickpeas, but not in *C. chlorideae*. The adverse effects of Bt on the parasitoid were largely through early mortality of *H. armigera* larvae or poor quality of the insect host.

The effects of transgenic crops on the natural enemies vary across crops and the cropping systems. Some of the variation may be due to differences in pest abundance between the transgenic and the non-transgenic crops. Wherever the transgenic crops have shown adverse effects on the natural enemies, these effects may still be far lower than those of the broad-spectrum pesticides.

Interactions with Microflora in the Rhizosphere

Under field conditions, the microflora of Bt transgenic potato plants has been observed to be minimally different from that of chemically and microbially treated commercial potato plants (Donegan

et al., 1996). It is unlikely that expression of Bt and any other genes in transgenic plants would have an adverse effect on the soil microflora. Decomposition dynamics and bacterial and fungal communities associated with decomposition were strongly affected by surface and incorporated placements, and by temporal factors (Lu *et al.*, 2010). However, no significant differences were observed between Bt and non-Bt rice variety in either decomposition dynamics or in the soil microbial communities associated with residue decay. Mocali *et al.* (2009) observed a significant difference in microbial respiration and diversity among with Bt and control egg plants, but no such effects were observed after 6 and 12 months, suggesting a strong correlation between plants and microorganisms, as well as a short-term impact. The antimicrobial peptide magainin II has activity against a range of micro-organisms. Tubers harvested from potatoes genetically modified (GM) to express a synthetic magainin gene show improved resistance to the bacterial pathogen, *Erwinia carotovora*. There is little likelihood of any major sustained non-target effect of genetic modification using a magainin II transgene on plant-associated and soil microflora and function (O'Callaghan *et al.*, 2008). Transplastomic modification of tobacco with antibiotic resistance marker-gene *aadA* caused a relative decline of a specific *Flavobacterium* population, but not of other bacteria (O'Callaghan *et al.*, 2008). Transgenic cotton leaves have no significant acute toxicity on the earthworm, *Eisenia fetida* from oral exposure to the transgenic cotton line, GK19 (Liu *et al.*, 2009). The average weight, numbers of cocoons and new offsprings of *E. fetida* in the GK19 was lower than in the Simian 3, but the differences were not significant.

CONCLUSIONS

The use of crop protection traits through transgenics will continue to expand in future, and gene pyramiding will become very common. This approach of controlling insects would offer the advantage of allowing some degree of selection for specificity effects, so that pests, but not the beneficial organisms are targeted. Despite numerous future promises, there is a multitude of concerns about the impact of transgenic crops on the environment. The major issues in the environmental assessment of transgenic crops are putative invasiveness, vertical or horizontal gene flow, other ecological impacts, and effects on biodiversity. These are all highly interdisciplinary and complex issues. A crucial component for a proper assessment is defining the appropriate baseline for comparison and decision. The most appro-

priate reference point is the impact of plants developed by traditional breeding as the latter is an integral and accepted part of agriculture. In general, there are no major adverse effects of genetically modified insect-resistant crops on the generalist predators, while some adverse effects have been observed on the host specific parasitoids, which are largely due to early mortality of the host larvae or poor nutritional quality of the inset host, rather than direct toxicity of the insecticidal proteins. Such effects are common for all pest control interventions, including synthetic insecticides, and are not regarded as a risk.

It is equally important to ensure the safety of food derived from transgenic crops based on the principle of nutritional equivalence. Insect-resistant transgenic crops will play a significant role in integrated pest management in the future, reducing the number of insecticide sprays and pesticide residues in food. Concerted efforts are required involving international and advanced research institutes, and the national research organizations to harmonize the regulatory requirements to assess the bio-safety of the food derived from genetically engineered crops and their effects on the non-target organisms for sustainable crop production and food security.

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