

Received 21 Feb 2011 Accepted 27 Apr 2011

Co-Editor: Weicai Yang

Invited Expert Review

**Risk assessment and ecological effects of transgenic *Bt* crops on
non-target organisms**

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This is an Accepted Article that has been peer-reviewed and approved for publication in the *Journal of Integrative Plant Biology*, but has yet to undergo copy-editing and proof correction. Please cite this article as an “Accepted Article”; doi: 10.1111/j.17447909.2011.01047.x

Abstract Application of recombinant DNA technology has resulted in many insect-resistant varieties by genetic engineering (GE), and the crops expressing Cry toxins derived from *Bacillus thuringiensis* (*Bt*) have been planted on a wide scale in the world, and they have been providing an effective tool for pest control. However, one ecological concern regarding the potential effects of insect-resistant GE plants on non-target organisms (NTOs) has been continually debated. We here briefly summarized the data regarding development and commercial use of transgenic *Bt* varieties, elaborated the procedure and methods for assessing non-target effects of insect-resistant GE plants and synthetically analyzed the related research results mostly published within recent 5 year. A mass of laboratory and field studies have shown that the currently available *Bt* crops have no direct detrimental effects on non-target organisms due to their narrow spectrum of activity, and *Bt* crops are increasing the abundance of some beneficial insects and improving the natural control of specific pests. Meanwhile, use of *Bt* crops such as *Bt* maize and *Bt* cotton results in significant reductions of insecticide application, clear benefits on environment and farmer health have been reported. Consequently, *Bt* crops can be a useful component of IPM systems to protect the crop from the targeted pests.

Key words *Bt* crops, non-target organisms, risk assessment, IPM

Introduction

Within the context of integrated pest management (IPM), insect pest resistant cultivars developed through conventional plant breeding methods have been used with great effectiveness against important pests in numerous cropping systems. It was estimated that the economic value of host plant resistance to the major pests of wheat in the USA to be ca. US\$192 million per year (Smith 2005). In addition to high efficiency, using insect-resistant cultivars for pest control is easy to operate and safe to the environment. However, the wide-spread use of host plant resistance had been constrained by the limited availability of elite cultivars possessing high levels of resistance to key pest species before the appearance of recombinant DNA technology (Kennedy 2008). The application of recombinant DNA technology has eliminated the constraint and provided a more efficient tool to develop insect-resistant varieties by genetic engineering (GE). Comparing with conventional plant breeding procedures the new technology has a number of advantages. Firstly because the techniques of genetic engineering allow genes to be inserted directly into advanced crop breeding lines or cultivars, the time required to develop commercially acceptable resistant cultivars is greatly reduced (Smith 2005). Secondly it is greatly increased that the potential array of available resistance traits that can be used to obtain insect-resistant crops; Further, it is possible to identify and use insect-resistant genes from any organism. Finally since the gene products that confer resistance can be well defined, and it is possible to test them directly to address questions regarding health and environmental effects (Kennedy 2008).

The first insect-resistant GE plants were produced in 1987, when genes coding for a Cry toxin derived from a soil bacterium *Bacillus thuringiensis* Berliner were expressed in tobacco (Vaeck et al. 1987). Since then many novel Cry protein and more new type insecticide proteins such as protease inhibitors, lectins and alpha-amylase inhibitors, etc. have been exploited (Malone et al. 2008). In 1995, the transgenic crop cultivars of *Bt* maize and *Bt* cotton were firstly approved for commercial release in the USA and first planted in 1996 (Hellmich et al. 2008; Naranjo et al. 2008; James 2009). Henceforth, the number of countries selecting to grow biotech crops has increased steadily. In 2009 more than 14 million farmers in 25 countries are cultivating biotech

crops, with a global production area of 134 million hectares (James 2009; Marshall 2010).

Bt crops not only provided an effective alternative tool for controlling target insects (Wu et al. 2008), but also provided many social, environmental and economic benefits such as reducing the use of chemical insecticides, benefiting to environment and human health, and increasing farm income (Wang 2007, Brookes and Barfoot 2010; Choudhary and Gaur 2010; Huang et al. 2010; Hutchison et al. 2010; Tabashnik 2010). For example, the direct global farm income benefit from *Bt* cotton was \$ 2.9 billion in 2008. Within this, 65% of the farm income gain has derived from yield gains (less pest damage) and the balance (35%) from reduced expenditure on crop protection (spraying of insecticides) (Brookes and Barfoot 2010). Nevertheless like any technology there have been questions about the potential risks transgenic plants may have on the environment. One of the major ecological concerns regarding the environmental risks of insect-resistant GE plants is their potential effects on non-target organisms (NTOs) (Romeis et al. 2006, 2008, 2009).

In order to safely and sustainably utilize transgenic biotechnology in pest control, the potential impact of *Bt* crops on non-target organisms including pest natural enemies, pollinators, microbes and mammalians *etc.* have been extensively studied worldwide in the last 20 years. A huge body of research data is available so far. To make the data more accessible and easily understood, we here summarized and analyzed the data regarding development and commercial use of transgenic *Bt* insect-resistant varieties, assessment process and procedure for non-target effects of insect-resistant GE plants and the related assessment results mostly published within recent 5 years. It is expected that the current article will provide a general image reflecting the development and use, as well as the risk assessment of *Bt* crops worldwide.

Crop varieties transformed with *Bt* genes

Many plants, such as cotton, maize, potato, tomato, rice, eggplant and crucifer vegetables, have been genetically transformed with genes derived from soil bacteria

Bacillus thuringiensis (*Bt*) coding for proteins that are highly active against many important pests (see details in Table 1). In addition to the δ -exotoxins like vegetative insecticidal protein VIP3A, the well-known insecticidal endotoxins are highly selective, and represent a class of numerous proteins with insecticidal action on larvae from various orders: Cry1 and Cry2 are toxic for lepidopteran pests, Cry2A for lepidopteran and dipteran pests, and Cry3 for coleopteran pests (Malone et al 2008) (Table 1). The first generation of *Bt* crops normally expressed single Cry proteins (Cry1A) with specific activity against lepidopteran pests (Bollgard I expressing Cry1Ac). To broaden the spectrum of protection and to delay the evolution of pest resistance to *Bt*, other insecticidal active *Bt* toxins such as Cry1F and Cry2A, VIP3A, have been added to the list of commercialized traits, and often they are presented as pyramided genes in a single variety (e.g. Dow Agrosciences' Widestrike cotton (Cry1F+Cry1Ac) and Monsanto's Bollgard II (Cry1Ac+Cry2Ab2) (James 2009) (Table 1). The use of non-*Bt* insect resistance traits with totally different modes of action, such as protease inhibitors or lectins, solely or in combination with *Bt*, has long been advocated as a means of delaying selection for resistant pest (Malone et al. 2008), although there are no these kind of transgenic plants commercialized so far (James 2010a). In the long view, new transgenic plants expressing novel Cry or other insecticidal proteins, stacked genes or fusion proteins will increase in importance in the coming years (Ferré et al. 2008).

Commercial planting of insect-resistant *Bt* crop

So far, *Bt* maize and *Bt* cotton are the only insect-resistant GE crops for commercially planting (James 2010a). *Bt* genes (*Cry1Ac*, *Cry1Ab*, *Cry2Ab*, and *Cry1F*) of cotton have commercialized in 11 countries in 2009 (Table 2), and the total planting area reached 15 million hectares that comprised about half of all the cotton grown in the world in 2009 (Figure 1) (Naranjo 2010). The area planted to *Bt* cotton globally in 2010 was 19.6 million hectares up by 4.6 million hectares over 2009 (Figure 1) (James 2010b). China and India are the two major cotton growing countries. In 2009, 8.4 million hectares of hybrid *Bt* cotton were planted in India, which made India displace

China to be the largest *Bt* cotton growing country. To delay the development of pest resistance, *Bt* cotton varieties containing two different Cry proteins (Bollgard II and Widestrike) have been gradually adopted by some countries in recent years. Since 2004, growers in Australia have been exclusively using Bollgard II (expressing Cry1Ac and Cry2Ab) instead of Bollgard I (expressing Cry1Ac) (Naranjo et al. 2008). *Bt* cotton varieties with two Cry proteins is becoming common, and most *Bt* cotton is also genetically engineered to be herbicide tolerant (Naranjo 2010). Maize transformed with *Bt* genes (*Cry1Ab*, *Cry1F*, *Cry3Bb1*, *VIP3A*, *Cry34Ab1/Cry35Ab*, *Cry2Ab*) has been commercially planted in 16 countries worldwide in 2009 (Table 2), and the total planting area reached 35.3 million hectares (Figure 1). In 2010, *Bt* maize was grown on 39 million hectares, an increase of 3.7 million hectares, or a year-over-year growth rate of 10% (Figure 1) (James 2010b). After USA, Brazil has become the second largest *Bt* maize growing country with 5.0 million hectares in 2009 (Marshall 2010). There were seven countries (USA, Argentina, Canada, the Philippines, South Africa, Honduras and Chile) planting maize with double stacked traits for herbicide tolerance and insect resistance. USA, Canada are the only two countries to grow triple stack maize with one gene for European corn borer, a second for root worm and a third for herbicide tolerance (James 2009). It seems that the growth of biotech maize stacked with double and triple genes versus single gene is typical of the shift in all countries that deploy stacked genes in maize.

Assessment of IRGE plant effects on NTOs

The assessment of the potential effects of IRGE plants on non-target organisms includes two phases, namely pre-market risk assessment (PMRA) that was carried out prior to commercialization of a GE plant and post-market monitoring (PMM) that was conducted after commercialization of a GE plant (Sanvido et al. 2005; 2009). Approval for commercial cultivation of a specific transformation event is based on the PMRA where potential adverse effects of the GM plant on the environment are assessed on a case-by-case basis. However, PMRA can not absolutely eliminate any uncertainty (Hill and Sendashonga 2003; Levidow 2003; Sanvido et al. 2005). Therefore a GE plant is in

principle only approved for limited release after PMRA. Subsequently PMM should be carried out to cope with the scientific uncertainties inherent to risk analysis in PMRA before large-scale release of the GE plant to the market.

Pre-market risk assessment (PMRA)

Assessment procedure and methods

To assess IRGE plants effects on NTOs, problem formulation should be firstly established by analyzing available information on the GE plants (introduced traits, expression pattern and mode of action of insecticidal proteins), and identifying the potential exposure of any non-target organism to insecticidal proteins. Problem formulation is used to define the scope of the risk assessment and generates testable scientific hypotheses and endpoints to measure that are relevant for decision-making (Wolt et al. 2010).

For practical reasons, only a small fraction of all possible terrestrial organisms can be considered for regulatory testing. Therefore, to assess the effect of IRGE plants on NTOs, appropriate species should be selected (Romeis et al. 2008; Dutton et al. 2003; Garcia-Alonso et al. 2006). Firstly the species should represent different ecological functions such as herbivory, pollination of cultivated and wild plants, predation and parasitism of pest organisms and decomposition in the soil. In addition, the species with special aesthetic or cultural value or species classified as threatened or endangered should also be considered for risk assessment (Romeis et al. 2008). In addition, since risk is a function of hazard and the likelihood that this hazard will be realized, the NTOs will not be affected, if they have not chance to get in contact with the insecticidal protein by the plant (exposure). Thus the species that is highly exposed to the insecticidal protein and is most likely affected by the protein should be chosen. Finally, practical considerations include the ease of working with a species, the potential for unambiguous taxonomic recognition, the ability to rear the species in captivity, the availability of permanent source colonies and validated and accepted test methods should be considered for species selection (USEPA 2007; Romeis et al. 2008).

Once the surrogate test species are selected, they would be evaluated moving through the tiered testing procedure that has been recommended and well accepted by

regulators and risk assessors (Dutton et al. 2003; Garcia-Alonso et al. 2006; Romeis et al. 2006; Romeis et al. 2008; EPA 2002; USEPA 2007). The procedure starts with laboratory tests (lower tier), followed by semi-field (glasshouse) and field (higher tier) tests (Figure 2). Lower tier tests serve to identify potential hazards and are typically conducted in controlled conditions. Lower-tier test are designed to measure a specific endpoint (or set of endpoints) under worst conditions using protein concentrations that are normally 10-100 times higher than those present in plant tissues. In general experiments, typical measurement endpoints are mortality, fecundity, development duration, body mass or the percentage of individuals that reach a certain life-stage (Dutton et al. 2003; Romeis et al. 2011). Under these conditions a lack of adverse effects may provide enough confidence that there is no risk and no further data would be needed (Romeis et al. 2011). However, if potential hazards were detected or if unacceptable uncertainties about possible hazards remain. Higher tier tests should be conducted that include more complex semi-field (that is, under containment using live GE plant material) or open field tests. These tests can serve to confirm whether an effect can still be detected under more realistic rates and routes of exposure to the protein. Field tier tests should provide more ecological information and answer questions related to the effects observed in laboratory and semi-field tests (Dutton et al. 2003). The structure and species-diversity of organism's community in general were investigated as measurement endpoints (Romeis et al. 2008). In cases where uncertainty about the risk remains after higher tier studies, one can always return to lower tiers to conduct additional studies. In exceptional cases, higher-tier studies or studies using alternative designs may be conducted at the initial stage of the risk assessment process when lower tier tests are not possible. Movement between tiers takes place either because the available information is insufficient to accept the risk hypothesis of 'no effect' or because the results of risk hypothesis have the adverse effects. Where no hazard or risk is detected, effective tiered processes prevent costly and unnecessary testing.

Effects on non-target organisms

Pest predator and parasitoid. The effects of *Bt* cotton and *Bt* maize on predators and parasitoids have been extensively assessed, most of studies were conducted using tritrophic systems including plants, herbivores and natural enemies. Here we summarized the data that were mostly published from 2005 to 2010 (see Table 3). From the previous data, it can be found that adverse effects on predators (larval survival, consumption rate and body mass) were only reported in the studied where *Bt*-susceptible insects were used as prey (Zhang et al. 2006; Chen et al. 2009; Lawo et al. 2010); and no effects were detected when *Bt*-unsusceptible, even sublethally damaged herbivores were used as prey (Davidson et al. 2006; Obrist et al. 2006; Zhang et al. 2006; Álvarez-Alfageme et al. 2008; Lewandowski and Górecka 2008; Meissle and Romeis 2009; Álvarez-Alfageme et al. 2009, 2010; García et al. 2010; Li and Romeis 2010). Likewise, no negative effects were found when predators were directly fed with *Bt* plant tissues (for example maize pollen) (Li et al. 2008; Duan et al. 2008b; Meissle and Romeis 2009; Ferry et al. 2007) (Table 3). In addition, studies feeding predators with high concentrations of purified Cry proteins revealed no direct toxicity to *Chrysoperla carnea* (Li et al. 2008), *Adalia bipunctata* (Álvarez-Alfageme et al. 2010), *Orius insidiosus* (Duan et al. 2008b) with an exceptional study by Schmidt et al. (2009) reported toxicity of *Escherichia coli*-produced recombinant Cry1Ab and Cry3Bb to first-instar *Adalia bipunctata*. However, the results of the study have been questioned due to the methodological shortcomings that undermine the study's conclusion and that also prevent the reconstruction of the study (Álvarez-Alfageme et al. 2010). And the recent study by Álvarez-Alfageme et al (2010) using a tri-trophic and a bi-trophic experimental systems clarified that *A. bipunctata* is not sensitive to Cry1Ab and Cry3Bb1, and the detected harmful effects reported by Schmidt et al (2009) were artifacts of poor study design and procedures. These results together with earlier data demonstrate that the negative effects observed were a consequence of sublethally intoxicated preys due to *Bt* ingestion, apparently being of lower nutritional quality, but not caused by the direct toxicity of *Bt* toxins (Romeis et al. 2006).

Adverse effects of *Bt* crops on survival, development, and reproduction of some parasitoid species were also observed, when *Bt*-susceptible herbivores were used as

host in studies (Liu et al. 2005a; Liu et al. 2005b; Vojtech et al. 2005; reviewed in Romeis et al. 2006; Ramirez-Romero et al. 2007; Sanders et al. 2007; Chen et al. 2008). Likewise, it was subsequently confirmed that the deleterious effects observed on parasitoids were due to the lower quality of hosts caused by *Bt* toxin ingestion, but not the direct toxicity of *Bt* toxins (Davidson et al. 2006; Faria et al. 2007; Kim et al. 2008; Wang et al. 2007; Chen et al. 2008; Liu et al. 2010). Since parasitoids have particularly close relationships with their hosts, and often possess a relatively narrow host range, they are more likely than predators to suffer significant negative impacts from GE crops if their *Bt* susceptible hosts are treated with *Bt* toxin and are weakened or killed (Romeis et al. 2006).

Similar to laboratory or glasshouse studies, field surveys (higher tier tests) did not find convinced and meaningful negative effects on the population density, abundance, species richness and diversity of natural enemies when transgenic *Bt* cotton or maize were cultivated which were assessed using different methods (Lopez et al. 2005; reviewed in Romeis et al. 2006; Chen et al. 2009; Balog et al. 2010). Naranjo et al. (2005) detected minor changes in abundance of a few non-target taxa occurring with the cultivation of *Bt* corn and cotton, while almost all these effects were explained by expected changes in target pest populations. A recent meta-analysis suggested that no uniform effects of *Bt* cotton, maize and potato on the functional guilds of non-target arthropods, but insecticide effects were much larger than those of *Bt* crops (see review by Wolfenbarger et al. 2008).

Pollinators and butterflies. Pollinators play an important functional role in most terrestrial ecosystems. As the world's most abundant and widespread pollinators, honey bees have drawn much attention, and they had been used as a group of indicators for pre-market risk-assessment of *Bt* crops (EPA 2001). Feeding tests with *Bt* plant pollen have been extensively performed on honeybee, and no effect was observed on their longevity, feeding and learning behavior, development of hypopharyngeal glands, superoxide dimutase activity, and intestinal bacterial communities in most of the studies (Bailey et al. 2005; Babendreier et al. 2005; Liu et al. 2005; Babendreier et al. 2007; Rose et al. 2007; Hofs et al. 2008; Liu et al. 2009a; Han et al. 2010b). Contrarily

Han et al. (2010a) showed that honey bee feeding behaviour was disturbed during a 7-day oral exposure to cotton pollen expressed Cry1Ac+CpTI toxin. However lack of long-term exposure did not provide enough evidences to support the results. A recent meta-analysis of 25 independent studies suggested that the *Bt* proteins used in GE crops to control lepidopteran and coleopteran pests do not negatively impact the survival of larvae or adults of honey bee (Duan et al. 2008a). Likewise no effects were detected on abundance, diversity, colony activity and development of honey bee in field surveys (Rose et al. 2007; Hofs et al. 2008).

Butterflies are more than just useful indicator species, they represent some of the most spectacular and visually appealing organisms in the world and play many vital roles in ecosystems (Bonrbrake et al. 2010). The observations that pollen from *Bt* corn line dusted onto milkweed leaves caused mortality of monarch larvae (Losy et al. 1999) prompted much public interest. Later, more thorough research indicated that the likely effect of *Bt* maize on monarch was negligible because of limited exposure and low toxicity of *Bt* maize pollen to monarch larvae (Sears et al. 2001; Stanley-Horn et al. 2001; Hellmich et al. 2008), although some adverse effects were observed on mortality, development, body weight and larval behavior of butterflies in lab or glasshouse experiments where the test insects were artificially exposed to high levels of insecticidal *Bt* proteins (Mattila et al. 2005; Lang and Vojtech 2006; Prasifka et al. 2007; Perry et al. 2010).

Microorganisms and macroorganisms in soil. *Bt* toxins from transgenic plants can enter soil in three different ways: 1) plant pollen deposited in and around *Bt* crop field during anthesis; 2) root exudate; 3) plant residues after harvest (Heckmann et al. 2006; Vaufleury et al. 2007; Zwahlen et al. 2007; Li et al. 2007). The potential impacts of *Bt* plants on soil organisms depend, at least in part, on the persistence of the transgenic-derived protein and its biological activity in soil. Research showed that *Bt* toxins can bind to clay particles and humic substances from soils, which renders the proteins resistant to biodegradation but with retention of larvicidal activity (Zwahlen et al. 2003; Clark et al. 2005; Viktorov 2008; Saxena et al. 2010). While most studies suggested *Bt* proteins from transgenic plants break down relatively rapidly at early

stage after entering soil and only small amount of them can remain for long, so that *Bt* proteins do not bioaccumulate in soil (Ahmad et al. 2005; Li et al. 2007; Margarit et al. 2008; Shan et al. 2008; Rauschen et al. 2008; Daudu et al. 2009; Icoz and Stotzky 2008a,b; Zurbrügg et al. 2010).

Effects of *Bt* crops on soil macroorganisms have been studied including mites, collembola, earthworm, snails *etc.* In general, no toxic effects of Cry proteins on macroorganisms have been reported in lab and field experiments (Ahmad et al. 2005; Heckmann et al. 2006; Vercesi et al. 2006; Vaufleury et al. 2007; Zwahlen et al. 2007; Hönemann et al. 2008; Hönemann and nentwig 2009; Liu et al. 2009b; Bai et al. 2010). To our knowledge, only one laboratory study reported that *Bt* maize have negative impact on growth and egg hatchability of snail (Kramarz et al. 2009). However, the risk was not well established due to the lack of certain important information, and additional tests should be supplemented. Field investigations suggested that crop management practices and/or environmental conditions (e.g. heavy rainfall during the growing season), pesticide use had the greatest impact on these species diversity and evenness, rather than the crop itself (*Bt* or isoline) (Birch et al. 2007; Cortet et al. 2007; Griffiths et al. 2007a).

The effects of *Bt* crops on microbes remains a concern in recent years. A number of studies on the effects of *Bt* crops on soil microorganisms have failed to find any significant effects in lab experiment, in microcosm and under field condition (Baumgarte and Tebbe 2005; Griffiths et al. 2005; Shen et al. 2006; Devare et al. 2007; Griffiths et al. 2007b, Knox et al. 2008; Miethling-Graff et al. 2010; Oliveira et al. 2008; Tan et al. 2010). In contrast, a study from Castaldini et al. (2005) reported differences between *Bt*176 maize and non-*Bt* maize in rhizospheric eubacterial communities, inmycorrhizal colonization, soil respiration, bacterial communities, and mycorrhizal establishment, while the risk was not well established. In general, a conclusion has been drawn that *Bt* toxins were not the direct factors causing negative effects on microbes, but other factors, such as plant growth stage, duration of plant straw decomposition, plant hybrid and variety *etc.*, may have stronger effects on the microorganisms than the presence of Cry protein.

Aquatic organisms. Although aquatic organisms are not likely to be exposed to the insecticidal *Bt* proteins through their expressions in crop plants with exception of aerial deposition of pollen or runoff transport of soil-bound *Bt* residue and crop material, several studies have been conducted to evaluate the potential effects of *Bt* plants on aquatic organisms. Most studies showed no effects of *Bt* maize byproducts on aquatic insects (add what kinds of aquatic organisms) (Pokelsek et al. 2007; Swan et al. 2009; Jensen et al. 2010). Contrarily Bøhn et al. (2008, 2010) showed that the Cry1Ab toxin expressed in maize reduced fitness performance of *Daphnia magna*, which is a crustacean (phylum: Arthropoda) invertebrate that inhabits ponds and lakes in most regions of the world. However, given the poor experimental design, the physiological relevance of the findings is questionable; conclusions of adverse effects cannot be drawn from these studies (Monsanto 2010). Rosi-Marshall et al. (2007) reported that consumption of Cry1Ab expressed in plant parts such as corn pollen, stalks and cobs increased mortality and reduced growth in caddisflies, which were related to lepidopteran insects that are the targeted pests of Cry1Ab protein expressed in *Bt* corn. However, the study was questioned to miss important background information on methodology and plant material used in the study by Beachy et al. (2008), so that the conclusions drawn in the paper seemed pre-mature for eco-system effects speculative. Chambers et al. (2010) assessed the influence of *Bt* maize detritus on benthic macroinvertebrate abundance, diversity, biomass, and functional structure in situ in 12 streams adjacent to *Bt* maize or non-*Bt* maize fields using combined laboratory and field approaches. There were no significant differences in total abundance or biomass of benthic macroinvertebrate and trichopterans between *Bt* and non-*Bt* streams. These studies demonstrate that further studies with better experimental design are needed for assessment of the potential effects of *Bt* crops on aquatic organisms.

Birds and mammals. There have been several articles published since 2005 that described research reports of the impacts of *Bt* maize on the mammalian and birds. Studies were carried out to compare the performances, such as growth rate, weight gain, food intake, feed efficiency, fecundity and broilers *etc.* of the animals feeding transgenic plant tissues with those feeding control plant tissues (Aeschbacher et al.

2005; Flachowsky et al. 2005; Rossi et al. 2005; Hammond et al. 2006; MacKenzie et al. 2007; Finamore et al. 2008; Wiedemann et al. 2007; Trabalza-Marinucci et al. 2008). The possible health effects of *Bt* crops through multigeneration in mammals and poultry were also measured, which was designed to clarify and enlighten the safety of long-term *Bt* crop consumption (Flachowsky et al. 2005; Halle et al. 2005; Kilic and Akay 2008). All the current datasets show *Bt* plants has no toxicity effects on mammalian development and health due to the fact that the normal mode of toxic action for *Bt* proteins is very unlikely to occur in the vertebrate digestive systems (Siegel et al. 1987; McClintock et al. 1995; Broderick et al. 2006).

Post-market monitoring

Assessment procedure and methods

PMM ensures the detection and prevention of adverse effects on the environment possibly deriving from commercial cultivation of GM crops, which divided into case-specific monitoring (CSM) and general surveillance (GS) (European Community 2001; European Council 2002; European Union 2003). CSM aims to assess the anticipated adverse effects that can not be identified with certainty in PMRA, but may occur during commercial cultivation; GS, in contrast, aims at detecting adverse effects on the environment that were not anticipated during PMRA. Therefore, GS has to be performed in all cases, while CSM may not be required when the conclusions of PMRA identify an absence of risk or negligible risk (European Council 2002). Although there have been not wide-accepted PMM strategies for GM plant cultivation established so far, some articles have described the conceptual frameworks for the design of environmental PMM programmes for GM plant cultivation based on current governmental legislation and common risk analysis procedures (Sanvido et al. 2005; Sanvido et al. 2007; Sanvido et al. 2008, 2009). Because GS has to be done in PMM programmes, here we introduce a general procedure for GS. The procedures of GS included defining safeguard subjects, collecting reports on adverse incidents via existing surveillance programmes and reporting system on adverse environmental effects, analysis of reports on adverse incidents, valuating of reports on adverse

incidents from general surveillance and determining the likelihood with GM plants cultivation, finally determining possible causalities with GM plants and taking a final decision (Sanvido et al. 2005). For instance, to monitor the non-target effects of GE plants, the general protection goal in monitoring of *Bt* crops is the biodiversity by using GS (Sanvido et al. 2005). The GS plan may comprise the following elements: 1 □ farm questionnaires and/or other surveillance approaches; 2 □ literature review; 3 □ information for operators and farmers; 4 □ alert “hot line”; and 5 □ integration of information from surveillance programs by third parties (Wilhelm 2010).

Effects of *Bt* crop on arthropod population dynamics

***Bt* cotton.** A relatively large number of pest species that are not susceptible to the *Bt* toxins expressed in transgenic cottons affect cotton production worldwide. The sucking pests including cotton aphid, thrip, whitefly, leafhopper, *etc* and the spider mites are the major non-target pests in *Bt* cotton fields, which are not susceptible to *Bt* proteins used currently (Wu and Guo 2005; Arshad and Suhail 2010; Manna et al. 2010). In general, most of these species exhibit the same pest status and continue to be managed identically in *Bt* and conventional cotton system. However, due to the reduced use of insecticides for bollworms and the change of pest management regimes in *Bt* cotton fields, these secondary pest populations have increased and gradually evolving into key pests in USA, India, China, Australia and other countries (Gouse et al. 2004; Sharma et al. 2005; Williams 2006; Wilson et al. 2006; Ho et al. 2008; Lu et al. 2008; Li et al. 2010; Zhao et al. 2011). For example, in Australia, the green mirid (*Creontiades dilutus*), green vegetable bug (*Nezara viridula*), leaf hoppers (*Austroasca viridigrisea* and *Amrasca terraereginae*), and thrips (*Thrips tabaci*, *Frankliniella schultzei* and *F. occidentalis*), have become more prominent (Lei et al. 2003; Wilson et al. 2006). In India, the reduction in insecticide use increased incidence of sucking and other pests such as mired bugs, mealy bugs, thrips and leaf eating caterpillar (Karihaloo and Kumar 2009; Nagrare et al. 2009). Field surveys conducted over 10 years in six major cotton growing provinces (i.e., Henan, Hebei, Jiangsu, Anhui, Shangdong, and Shanxi) of northern China showed that mirid bugs (Heteroptera: Miridae) have progressively increased population levels and acquired pest status in *Bt* cotton fields (Lu et al. 2010).

In addition, it was also found that spider mites have been observed to occur at higher levels in *Bt* cotton during drought season (Wu and Guo 2005). These emergent pests have forced Chinese farmers to continue the use of chemical pesticides, however, the increase in insecticide use for the control of these secondary insects was smaller than the reduction in total insecticide use due to *Bt* cotton adoption (Wang et al. 2009).

To date, there have been no confirmed monitoring results of negative effects of *Bt* cotton on insect pest predators. Based on field investigations, populations density of the major species of predators such as predatory spiders, coccinellids, chrysopids and small flower bug in the transgenic *Bt* cotton fields were not different from in the conventional *Bt* cotton field, but significantly increased over that in the conventional cotton fields applied with pesticides (Sisterson et al. 2007; Sharma et al. 2007; Dhillon and Sharma 2009). In India, it was found that the predator populations (*Chrysoperla* spp., *Orius* spp., *Coccinella* spp., *Brumus* spp., *Vespa* spp., *Lycosa* spp., and *Aranews* spp.) were similar on Bollgard I, BollgardII and conventional cotton (Manna et al. 2010). As expected, the population densities of parasitic wasps (*Trichogramma confusum*, *Microplitis* spp., *Camponotus chlorideae*, and *Meteorus pulchricornis*) decrease significantly owing to poor quality and lower density of *H. armigera* in *Bt* cotton fields due to the close relationship between parasitoids and their hosts (Wu and Guo 2005; Yang et al. 2005; Xia et al. 2007). A three-year field surveys showed that planting of *Bt* cotton increased the diversity of arthropod community (Men et al. 2003) and the results were confirmed by the study from Cui et al. (2005) with natural enemy sub-community. Thus the biological control function of natural enemies in *Bt* cotton field did no change comparing with conventional *Bt* cotton (Naranjo 2005; Wolfenbarger et al. 2008; viewd in Naranjo 2009).

Bt maize. Similar to *Bt* cotton, *Bt* maize did not affect the non-target arthropods at population levels in *Bt* maize in long-term or short-term monitoring by sampling methods used including visual surveillance, sticky cards, pitfall traps, and litterbags (Pons et al. 2005; Eizaguirre et al. 2006; Higgins et al. 2009; Virla et al. 2010). Serious problems with secondary pests stemmed from experiences with *Bt* cotton were not found in *Bt* maize due to declining insecticide use against target lepidopteran pests,

although minor pests species increased in *Bt* maize in some countries for commercial planting (Hellmich et al. 2008). For example, in Germany, six-year monitoring of non-target arthropods in *Bt* maize (expressing Cry 1Ab toxin), there were no different in population density of aphids, thrips, heteropterans, aphid specific predators, spiders and carabids (Schorling and Freier 2006). Abundance and species richness of foliage-dwelling spiders (Araneae) were equal or higher in *Bt* maize fields and adjacent field margins than the non-transgenic maize field (Ludy and Lang 2006). In USA transgenic *Bt* maize also did not affect the community abundance of non-target arthropod based on 3-year field investigations (Higgins et al. 2009). Predaceous arthropods were equal or more abundant on *Bt* than non-*Bt* maize (Daly and Buntin 2005; Eizaguirre et al. 2006). Overall, the studies indicated no major effects against natural enemies in *Bt* maize fields compared with non-*Bt* maize fields, with the occasional exception of taxa that were dependent on *Bt*-susceptible pests as hosts (Eizaguirre et al. 2006; Rose and Dively 2007). Likewise, no effects were found of *Cry1Ab* and *Cry3Bb1* maize on the diversities of macroorganisms and microorganisms in long-term and short-term field study in USA (Icoz et al. 2008; Priestley and Brownbirdge 2009; Zeilinger et al. 2010). A recent report from Monsanto (2010) confirms that there is a negligible impact from the cultivation of MON 810 expressing Cry1Ab on biodiversity, abundance or survival of non-target species via the analysis of 240 questionnaires from a survey of farmers cultivating MON 810 in six European countries in 2009 and through a detailed analysis of more than 30 publications. It may be concluded that the biodiversity of non-target arthropods was seemingly easy to be effected by the agro-ecological system rather than the Cry toxin (De la Poza et al. 2005; Farinós et al. 2008).

Transgenic *Bt* crops and IPM

In summary, recombinant DNA technology provides an efficient tool to develop insect-resistance breeding for certain crops. The technology has allowed to directly insert foreign genes that may derive from any kind of living organism into crop plants allowing plants to express completely new pest resistance properties. The use of this

approach has resulted in many insect-resistant varieties, and the crops expressing Cry toxins derived from *Bt* have been planted on a wide scale in the world. Thus far, laboratory and field studies conducted have shown that the currently used *Bt* crops generally do not cause apparent unexpected detrimental effects on non-target organisms or their ecological functions they provided and *Bt* crops are increasing the abundance of some beneficial insects and improving the natural control of specific pests due to the reduction of pesticide use. Meanwhile, the use of *Bt* crops such as *Bt* maize and *Bt* cotton results in significant reductions of insecticide application, clear benefits on environment and farmer health have been reported. Consequently, *Bt* crops can be a useful component of IPM systems to protect the crop from the targeted pests. In fact, *Bt* cotton and *Bt* maize have revolutionized pest control strategy in a number of countries and have changed the conventional IPM practices. Certainly, sole use of *Bt* crops can not solve all the problems related to pest regulation, it has to be used with other IPM tactics including chemical pesticides for controlling pests. For example, to control secondary pests such as mirids and spider mites in *Bt* cotton, chemical control especially the use of more specific, less disruptive compounds, remains important together with the use of other IPM tactics such as crop rotation, intercropping. In a word, as an important component of IMP, ideal use of *Bt* crops would include reduction of insecticide use with maintenance of other traditional IPM practices.

Acknowledgements

We would like to thank Jorg Romeis for providing us some valuable literatures and to thank the anonymous reviewers for valuable comments on this paper.

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Table 1. Crop varieties transformed with *Bt* genes for resistance to target pests

| Crop | Insect toxin genes | Target pest order | Major target pests |
|--------------------------------------|-------------------------|--------------------------|---|
| Cotton | <i>CryIAc</i> | Lepidoptera | Tobacco budworm (<i>Heliothis virescens</i>), pink bollworm (<i>Pectinophora gossypiella</i>), cotton bollworm (<i>Helicoverpa zea</i> , <i>Helicoverpa armigera</i>) |
| | <i>CryIAb/c</i> | Lepidoptera | <i>Heliothis virescens</i> , <i>Pectinophora gossypiella</i> , <i>Helicoverpa armigera</i> , <i>Helicoverpa zea</i> |
| | <i>CryIAc + CpTI</i> | Lepidoptera | <i>Pectinophora gossypiella</i> , <i>Helicoverpa armigera</i> |
| | <i>CryIAc + Cry 2Ab</i> | Lepidoptera | <i>Heliothis virescens</i> , <i>Pectinophora gossypiella</i> , <i>Helicoverpa armigera</i> , <i>Helicoverpa zea</i> |
| | <i>CryIA + CryIF</i> | Lepidoptera | Spodoptera spp. |
| | <i>CryIF</i> | Lepidoptera | <i>Heliothis virescens</i> , <i>Helicoverpa zea</i> , beet armyworm (<i>Spodoptera exigua</i>), and soybean looper (<i>Pseudoplusia includens</i>) |
| | <i>Vip3A</i> | Lepidoptera | <i>Helicoverpa zea</i> , <i>Heliothis virescens</i> , <i>Pectinophora gossypiella</i> , <i>Spodoptera exigua</i> , <i>Pseudoplusia includens</i> , cabbage looper |
| | | | <i>Spodoptera exigua</i> , fall armyworm (<i>Spodoptera frugiperda</i>), and cotton leaf perforator (<i>Bucculatrix thurberiella</i>). |
| | | | <i>Heliothis virescens</i> , <i>Pectinophora gossypiella</i> , <i>Helicoverpa armigera</i> , <i>Helicoverpa zea</i> |
| | Maize | <i>CryIAb</i> | Lepidoptera |
| <i>CryIAb + mCry3A</i> | | Lepidoptera, coleopteran | <i>Ostrinia nubilalis</i> , corn rootworm (<i>Diabrotica</i> spp.) |
| <i>CryIF</i> | | Lepidoptera | <i>Ostrinia nubilalis</i> , <i>Spodoptera frugiperda</i> , southwestern corn borer (<i>Diatraea grandiosella</i>), western bean cutworm (<i>Striacosta albicosta</i>), black cutworm (<i>Agrotis ipsilon</i>), <i>Helicoverpa zea</i> |
| <i>Cry34Ab1 + Cry35Ab1</i> | | Coleoptera | <i>Diabrotica</i> spp. |
| <i>Cry34Ab1 + Cry35Ab1 + CryIFa2</i> | | Lepidoptera, Coleoptera, | <i>Ostrinia nubilalis</i> , <i>Diabrotica</i> spp. |
| <i>mcry3A</i> | | Coleoptera | Western corn rootworm (<i>Diabrotica vigifera vigifera</i>), northern corn rootworm (<i>Diabrotica barberi</i>), and Mexican corn rootworm (<i>Diabrotica vigifera zea</i>) |
| | | | <i>Diabrotica</i> spp. |

| | | | |
|------------|--|----------------------------|---|
| | CryIA.105+ Cry2Ab2 | Lepidoptera | <i>Ostrinia</i> spp., <i>Spodoptera frugiperda</i> , |
| | CryIA.105+Cry2Ab+Cry3Bb1 +Cry34Ab1+ | Lepidoptera, Coleoptera | Above-ground insect: <i>Helicoverpa zea</i> , <i>Ostrinia nubilalis</i> , <i>Spodoptera frugiperda</i> , <i>Diatraea grandiosella</i> , sugarcane borer (<i>Diatraea saccharalis</i>), <i>Striacosta albicosta</i> and <i>Agrotis ipsilon</i> ; |
| | Cry35Ab1+Cry1Fa2 | | Below-ground insect: <i>Diabrotica virgifera virgifera</i> , <i>Diabrotica barberi</i> and <i>Diabrotica virgifera zea</i> |
| | Vip3Aa20+mCry3A+Cry1Ab | Lepidoptera, Coleoptera | Above-ground insect: <i>Ostrinia nubilalis</i> , <i>Diatraea grandiosella</i> , <i>Helicoverpa zea</i> , <i>Spodoptera frugiperda</i> , <i>Spodoptera exigua</i> , <i>Agrotis ipsilon</i> , <i>Striacosta albicosta</i> , <i>Diatraea saccharalis</i> , armyworm (<i>Pseudaletia unipunctata</i>), southern cornstalk borer (<i>Diatraea cramboides</i>), common stalk borer (<i>Papaipema nebris</i>); |
| Potato | Cry3A | Coleoptera | Below-ground insect: <i>Diabrotica virgifera virgifera</i> , <i>Diabrotica barberi</i> , and <i>Diabrotica virgifera zea</i> |
| Tomato | Cry1Ac | Lepidoptera | Colorado potato beetle (<i>Leptinotarsa decemlineata</i>) |
| Rice | Cry1Ab Cry1Ab/c | Lepidoptera Lepidoptera | <i>Heliothis virescens</i> , <i>Pectinophora gossypiella</i> , <i>Helicoverpa armigera</i> , <i>Helicoverpa zea</i> |
| Eggplant | Cry1Ac | Lepidoptera | Rice stem borers(<i>Scirpophaga incertulas</i> , <i>Chilo suppressalis</i>) |
| Crucifer | Cry1 | Lepidoptera | <i>Scirpophaga incertulas</i> , <i>Chilo suppressalis</i> |
| vegetables | | | Fruit and shoot borer (<i>Leucinodes orbonalis</i>) Diamondback moth (<i>Plutella xylostella</i>) |

Source: James 2009, U.S.EPA, GM crop database, ISAAA.org

Table 2. Global status of commercialized *Bt* cotton and *Bt* maize

| Crop | Countries | Year for commercialization | Insect toxin genes | Total hectares in 2009 (Hectare) |
|---------|-----------------------|----------------------------|-------------------------------|----------------------------------|
| Cotton | Argentina | 1998 | <i>CryIAc</i> | 245,000 |
| | Australia | 1996 | <i>CryIAc</i> | |
| | | 2002 | <i>CryIAc +Cry 2Ab</i> | 180,000 |
| | Brazil | 2005 | <i>CryIAc</i> | |
| | | 2009 | <i>CryIAc +Cry 2Ab</i> | |
| | | 2009 | <i>CryIAc+CryIF</i> | 116,000 |
| | Burkina Faso | 2008 | <i>CryIAc + Cry 2Ab</i> | 15,000 |
| | China | 1997 | <i>CryIAc</i> | |
| | | 1999 | <i>CryIA^b+CpTI</i> | 3,400,000 |
| | Colombia | 2002 | <i>CryIAc</i> | 23,000 |
| | Costa Rica | 2009 | <i>CryIAc</i> | |
| | | 2009 | <i>CryIAc+Cry2Ab</i> | |
| | | 2009 | <i>CryIAc+CryIF</i> | □1,500 |
| | India | 2002 | <i>CryIAc</i> | |
| | | 2006 | <i>CryIAc + Cry 2Ab</i> | 8,400,000 |
| | | 2006 | <i>CryIAc + Cry IAb</i> | |
| | Mexico | 1997 | <i>CryIAc</i> | 46,000 |
| | South Africa | 1997 | <i>CryIAc</i> | |
| | | 2005 | <i>CryIAc + Cry 2Ab</i> | 7,300 |
| | USA | 1996 | <i>CryIAc</i> | |
| | 2002 | <i>CryIAc+ Cry 2Ab</i> | | |
| | 2004 | <i>CryIAc+CryIF</i> | | |
| | 2004 | <i>CryIF</i> | 1,491,000 | |
| Maize | Argentina | 1998 | <i>CryIAb</i> | |
| | | 2005 | <i>CryIF</i> | 1,960,000 |
| | Brazil | 2008 | <i>CryIAb</i> | |
| | | 2009 | <i>CryIF</i> | |
| | | 2009 | <i>VIP3Aa20</i> | |
| | | 2009 | <i>CryIA.105+Cry2Ab</i> | 5,000,000 |
| | Canada | 1997 | <i>CryIAb</i> | |
| | | 2002 | <i>CryIF</i> | |
| | | 2003 | <i>Cry3Bb1</i> | |
| | | 2005 | <i>Cry34Ab1+Cry35Ab1</i> | |
| | | 2007 | <i>mCry3A</i> | □1,300,000 |
| | | 2008 | <i>CryIA.105+ Cry2Ab2</i> | |
| | Chile | 2007 | <i>CryIAb</i> | 28,000 |
| | Czech Republic | 2005 | <i>CryIAb</i> | 6,480 |
| | Egypt | 2008 | <i>CryIAb</i> | 1,000 |
| | Honduras | 2002 | <i>CryIAb</i> | |
| | | 2009 | <i>CryIF</i> | 12,000 |
| | Philippine | 2003 | <i>CryIAb</i> | 392,000 |
| | Portugal ^a | 1999 | <i>CryIAb</i> | 5,094 |
| | Poland | 2007 | <i>CryIAb</i> | 3,000 |
| Romania | 2007 | <i>CryIAb</i> | 3,244 | |

| | | | |
|--------------|------|---------------------------|-------------|
| Slovakia | 2006 | <i>CryIAb</i> | 875 |
| Spain | 1998 | <i>CryIAb</i> | 76,057 |
| South Africa | 1997 | <i>CryIAb</i> | 1,600,000 |
| Uruguay | 2003 | <i>CryIAb</i> | |
| | 2006 | <i>CryIF</i> | 90,000 |
| USA | 1996 | <i>CryIAb</i> | |
| | 1996 | <i>Cry3Bb1</i> | |
| | 2001 | <i>CryIF</i> | |
| | 2005 | <i>Cry34Ab1+Cry35Ab1</i> | |
| | 2007 | <i>mCry3A</i> | |
| | 2008 | <i>CryIA.105+ Cry2Ab2</i> | □17,000,000 |

^ano planting from 2000-2004.

^b*CryIA* represents a fusion gene of *CryIAc* and *CryIAb*

Sources: Clive James 2009 and GM crop database.

Table 3. Studies (published from 2005-2010) under confined conditions assessing effects of *Bt* plants on insect pest predators and parasitoids

| Crop | toxin | Predator/parasitoid Species | Prey/host order/plant tissue | Is the host/prey susceptible to the toxin? | Reported effects | Reference |
|--|---------|---|------------------------------|--|------------------|------------------------------|
| Predators feeding on prey reared on <i>Bt</i> plants | | | | | | |
| Cotton | Cry1Ac | <i>Propylaea japonicav</i> (Coleoptera: Coccinellidae) | Lepidoptera | Y | Y(-) | Zhang et al. 2006 |
| | | <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae) | Lepidoptera | Y | Y(-) | Lawo et al. 2010 |
| | | <i>Propylaea japonic</i> (Coleoptera: Coccinellidae) | Lepidoptera | N | N | Zhang et al.2006 |
| Maize | Cry1Ab | <i>Neoseiulus cucumeris</i> (Acari: Phytoseiidae) | Acari | N | N | Obrist et al. 2006 |
| | | <i>Stethorus punctillum</i> (Coleoptera: Coccinellidae) | Acari | N | N | Álvarez-Alfageme et al. 2008 |
| | Cry3Bb1 | <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae) | Homoptera | N | N | Lewandowski and Górecka 2008 |
| | | <i>Poecilus cupreus</i> (Coleoptera: Carabidae) | Lepidoptera | Y | N | Álvarez-Alfageme et al. 2009 |
| | | <i>Adalia bipunctata</i> (Coleoptera: Coccinellidae) | Acari | N | N | Álvarez-Alfageme et al. 2010 |
| | | <i>Atheta cortaria</i> (Coleoptera: Staphylinidae) | Acari | N | N | García et al. 2010 |
| Potato | Cry1Ac9 | <i>Theridion impressum</i> (Araneae: Theridiidae) | Lepidoptera | Y | N | Meissle and Romeis 2009 |
| | | <i>Adalia bipunctata</i> (Coleoptera: Coccinellidae) | Acari | N | N | Álvarez-Alfageme et al. 2010 |
| | | <i>Stethorus punctillum</i> (Coleoptera: Coccinellidae) | Acari | N | N | Li and Romeis 2010 |
| | | <i>Micromus tasmaniae</i> (Neuroptera: Micromidae) | Hemiptera | N | N | Davidson et al. 2006 |

| | Cry9Aa2 | Hemerobiidae) | | | | | Chen et al. 2009 |
|---|-----------------------------------|--|-------------------|---|-------------------|--|---|
| Rice | Cry1Ab | <i>Pirata subpiraticus</i> (Araneae: Lycosidae) | Lepidoptera | Y | Y(-) | | |
| Predators directly feeding on <i>Bt</i> plant tissue | | | | | | | |
| Maize | Cry1Ab | <i>Chrysoperla carnea</i> (Neuroptera: Chrysopidae) | Pollen | | N | | Li et al. 2008 |
| | Cry3Bb1 | <i>Orius insidiosus</i> (Heteroptera: Anthocoridae) | Pollen | | N | | Duan et al. 2008b |
| | | <i>Theridion impressum</i> (Araneae: Theridiidae) | pollen | | N | | Meissle and Romeis 2009 |
| Potato | Cry3A | <i>Harmonia axyridis</i> (Coleoptera: Coccinellidae), <i>Nebria brevicollis</i> (Coleoptera: Carabidae) | Pollen and flower | | N | | Ferry et al. 2007 |
| Parastoids developing in hosts reared on <i>Bt</i> plants | | | | | | | |
| cotton | Cry1A+CpTI, Cry1Ac | <i>Campoketis chlorideae</i> (Hymenoptera: Ichneumonidae) | Lepidoptera | Y | Y(-) | | Liu et al. 2005a |
| | Cry1A, Cry1Ac+CpTI | <i>Microplitis mediator</i> (Hymenoptera: Braconidae) | Lepidoptera | Y | Y(-) | | Liu et al. 2005b |
| Maize | Cry1Ab | <i>Cotesia marginiventris</i> (Hymenoptera) | Lepidoptera | Y | Y(-) | | Vojtech et al. 2005 Ramirez-Romero et al. 2007 |
| | | <i>Cotesia marginiventris</i> (Hymenoptera) | Lepidoptera | Y | Y(+) ^a | | Faria et al. 2007 |
| | | <i>Campolepis sonorensis</i> (Hymenoptera: Ichneumonidae) | Lepidoptera | Y | Y(-) | | Sanders et al. 2007 |
| Potato | Cry1Ac9, Cry9Aa2 | <i>Apanteles subandinus</i> (Hymenoptera: Braconidae) | Lepidoptera | Y | N | | Davidson et al. 2006 |
| Broccoli | Cry1Ac, Cry1C, Cry1Ac+Cry1C | <i>Pteromalus puparum</i> (Hymenoptera: Pteromalidae) | Lepidoptera | Y | Y(-) | | Chen et al. 2008 |
| | Cry1Ac | <i>Diadegma insulare</i> (Hymenoptera: Ichneumonidae) | Lepidoptera | N | N | | Liu et al. 2010 |
| Chinese | Cry1Ac | <i>Microplitis mediator</i> (Hymenoptera) | Lepidoptera | N | N | | Kim et al. 2008 |

| | | | | |
|---|--|--------------|---|------------------|
| cabbage | Braconidae) | | | |
| Parastoids directly feeding on <i>Bt</i> plant tissue | | | | |
| Cry1Ab | <i>Trichogramma ostriniae</i> (Hymenoptera: Trichogrammatidae) | Maize pollen | N | Wang et al. 2007 |

a: in the presence of the corn leaf aphid
 Y represents Yes, N represents No; (-) represents negative effect, (+) represents positive effect.

Legends

Figure 1. Global planting area of *Bt* cotton and *Bt* maize and both, 1996-2009 (Million hectare). Source from: James, 2002-2010.

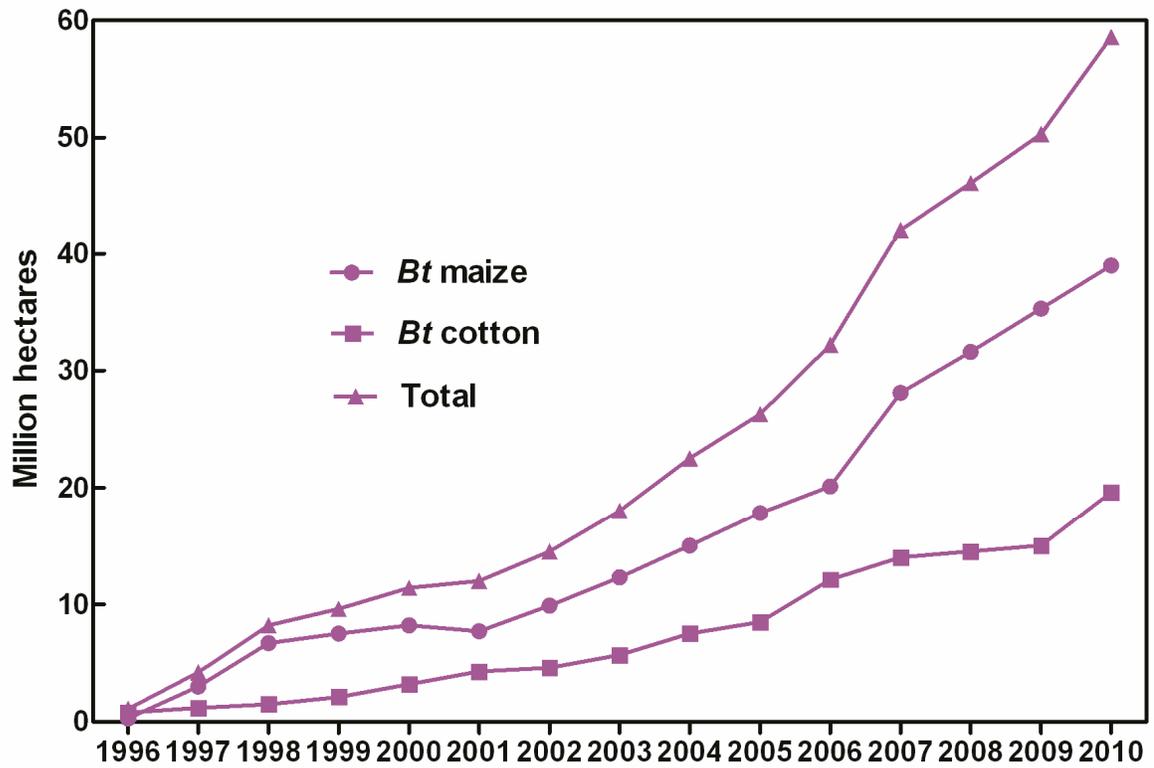


Figure 2. Sequential test procedure for assessing the effects of genetically modified plants on non-target organisms using a tiered scheme.

