ASSESSING THE INFLUENCE OF BT CROPS ON NATURAL ENEMIES

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ABSTRACT

Transgenic Bt crops expressing proteins derived from Bacillus thuringiensis (Bt) currently are grown commercially in ten countries on over 20 million hectares. Assessing their environmental safety is a critical part of the regulatory approval process and product stewardship for Bt crops. The environmental safety testing process for Bt crops follows a standard risk assessment approach, and involves multiple tiers of laboratory and field testing. Lower tier testing is based primarily upon pure protein tests, with testing concentrations keyed off of the maximum possible environmental exposure for a variety of non-target species. Test species are chosen based on considerations of the product and region, and typically include insect predators and parasitoids. These laboratory studies have not found any direct toxic effects of Cry1, Cry2 or Cry3 proteins against any generalist predator or parasitoid. The results of these studies agree with other laboratory and field studies conducted prior to and post-commercialization of Bt crops. Collectively, the non-target studies performed to date demonstrate that Bt crops do not have any unexpected toxic effects on natural enemy species, as would be predicted from knowledge of the mode of action and specificity of Bt proteins.

INTRODUCTION

Two decades of advances in the areas of molecular biology and genetics have led to the creation of exciting new opportunities in agriculture. The use of genetic engineering techniques to transfer traits useful in insect, disease and weed control have provided farmers with a new set of tools to control some old, intransigent problems (James 2004; Schuler et al. 1998). Some of the first genetically engineered crops, and some of the most widely used, have been modified to express insecticidal crystalline (Cry) proteins derived from the common soil bacterium Bacillus thuringiensis (Bt) Berliner (Perlak et al. 1991). These so-called Bt crops are protected from the feeding of various groups of pest insects. They provide pest control solutions that are highly effective and yet very specific, leading to substantial direct benefits for farmers as well as providing greater flexibility in crop management practices.

Since 1995, various biotechnology companies, including Monsanto, Syngenta, Dow and Dupont-Pioneer, have registered varieties of corn, cotton and potatoes that express Bt proteins for commercial use in ten countries. The Bt cotton and Bt corn products, in particular,
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are widely accepted and used, with total global adoption exceeding 20 million hectares (James 2004). In cotton, the proteins expressed (Cry1Ac, Cry1F and Cry2Ab) confer protection from a broad array of lepidopteran herbivores, enabling the use of broad spectrum insecticides to be greatly reduced and, in some cases, eliminated. Bt cotton (particularly varieties expressing Cry1Ac and sold as Bollgard® or Ingard®) has been registered for commercial use in Argentina, Australia, China, Colombia, India, Mexico and South Africa. Some of the most exciting possibilities for such a product exist in tropical systems where substantial broad spectrum insecticides would otherwise be used. In areas of Asia, such as India and China, cotton crops may be sprayed more than ten times in a year in the absence of Bt cotton in an attempt to control severe lepidopteran pest outbreaks (e.g., Wu and Guo 2005). Bt corn, modified to express either Cry1Ab or Cry1F to combat a set of stalk-boring Lepidoptera, or Cry3Bb1 to control feeding by coleopteran Diabrotica spp., has similar potential to Bt cotton. In 2004, about 12 million hectares of Bt corn were planted in the United States, almost 50% of corn acres in Argentina were planted with Bt corn, and smaller amounts were planted commercially in Canada, the Philippines, South Africa and Spain.

A critical part of the introduction of such products is to ensure their safety and safe use. This involves comprehensive laboratory and field testing to ensure that the products’ characteristics are understood and that they are used correctly. In this paper, I describe the environmental safety assessment process used for Bt crops, with particular emphasis on the assessment of impacts of Bt crops on natural enemies. I then summarize the results of regulatory and related testing of the impacts of Bt crops on natural enemies, and compare the results with what would have been predicted from knowledge of the mode of action of Bt proteins.

TESTING AND REGULATION OF BT CROPS

The environmental safety of Bt crops has been addressed throughout their development process and has involved review by regulatory agencies and scientific experts from the government, academia, and industry. In particular, environmental safety is a criterion in the initial product design, and then is the focus of substantial laboratory and field testing. Regulatory review typically occurs through Ministries of Agriculture and/or the Environment. For example, in the U.S., this primarily involves the Environmental Protection Agency Office of Pesticide Programs (EPA OPP) and, secondarily, the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS).

TRANSGENIC PRODUCT DESIGN

Proteins being considered for use in insect-protected transgenic crops are screened based on effectiveness and specificity. The aim is to find proteins with high activity against the target pest insects and little or no activity against other taxa. As a consequence of this selection process, proteins that might cause adverse environmental impacts because of either broad toxicity or activity against key non-target groups are eliminated early in the development process. The choice of Bt crystalline (Cry) proteins for currently commercialized insect-protected transgenic crops are an illustration of this approach. These proteins must be ingested to be insecticidal. Once ingested, the mode of action of Bt proteins is complex and involves:
solubilization, proteolytic stability, binding to the midgut epithelium, formation of ion channels in the midgut cells, and finally lysis of these cells (English and Slatin 1992). These proteins are highly specific in their effects because of this mode of action, particularly compared to other proteins that have insecticidal properties such as lectins and protease inhibitors. Only a few insect groups have the appropriate mid-gut characteristics and binding sites for a particular *Bt* Cry protein to be active. For example, Cry1-type proteins control various Lepidoptera, Cry2-type proteins affect certain Lepidoptera and Diptera, and Cry3 proteins control certain Coleoptera. Unrelated non-target species are unaffected.

Apart from selecting insecticidal proteins based upon the mode of action, efforts also are made to choose proteins with a history of safe use. Where possible, proteins that have been previously used in comparable ways without environmental problems are preferred. This was another reason for the choice of *Bt* Cry proteins. These proteins have been used extensively in foliar sprays for over 30 years. In that form, they also have been scrutinized by regulatory agencies. They have proven to be extremely safe with respect to both human safety and environmental impacts (EPA 2001; McClintock *et al.* 1995).

**SAFETY TESTING**

The environmental safety testing process for *Bt* crops follows a standard risk assessment approach, and involves multiple tiers of laboratory and field testing (Sharples 1991). The assessment is specific to the product and region, and considers the nature of the trait, crop plant biology, local farming practices, and the local ecological community. The tests used are shaped by the requirements of regulatory agencies (such as the EPA and the USDA-APHIS in the U.S.), as well as by product stewardship considerations (Nickson and Head 2000). The overall environmental risk assessment can be thought of as addressing two basic areas: first, whether the transgenic crop is biologically equivalent to comparable untransformed varieties other than the presence of the *Bt* protein, and second, whether the *Bt* protein has any direct or indirect effects on the ecological community (through toxicity, gene flow, or selection for pest resistance). Potential non-target impacts of *Bt* crops primarily fall into the latter category.

Because *Bt* proteins are chosen for their insecticidal properties, possible impacts on non-target insect species are a particular source of concern. Lower tier (early) testing for such impacts is based primarily upon pure protein tests, with testing concentrations keyed off of the maximum possible environmental exposure for a variety of non-target species. Where appropriate, testing uses relevant plant tissues. Test species are chosen based on considerations of the product and region, and typically include insect predators, parasitoids and pollinators, as well as soil-dwelling and aquatic invertebrates. These species are selected to be representative of different taxa and ecological guilds, and often are economically important species. The results of these tests can be compared to the known properties of the protein (mode of action). Different routes of exposure to the insecticidal protein are assessed, including direct consumption of leaf tissue by herbivores, deliberate or incidental feeding on pollen, and ingestion of plant material that has become incorporated into the soil. Where some hazard is indicated in lower tier testing, or significant uncertainty remains in the hazard and/or exposure assessment, higher tier studies may be initiated. Higher tier tests are more field-
based and may be carried out both prior to and after commercialization. In these studies, the product is compared with reasonable agronomic alternatives. After commercialization, work can take place in commercial-sized fields managed with standard grower practices.

Thus far, no unintended adverse ecological impacts have been identified for any commercialized Bt crop, despite the comprehensive regulatory assessment in multiple countries, extensive commercial use, and post-commercial monitoring (e.g., Betz et al. 2000; EPA 2001; Mendelsohn et al. 2003).

REGULATORY TESTS OF NATURAL ENEMIES

Important criteria in choosing suitable natural enemies for testing are comparable to the criteria used for selecting any non-target species: they should adequately represent organisms relevant to the cropping system where the Bt crop will be used; they potentially should be exposed to the Bt proteins expressed in Bt crops; they should be relatively easy to work with in the laboratory; and suitable laboratory colonies must be available.

Potential routes of natural enemy exposure to Bt proteins include direct feeding on pollen, nectar or other plant tissues of Bt crops, or secondary exposure through feeding on prey species that have themselves fed upon Bt plants. Bt protein expression in Bt crops is highest in actively growing green tissues, lower in older vegetative tissues and reproductive tissues, and lowest or absent in the phloem (Head et al. 2001; Raps et al. 2001). This suggests that regulatory testing should focus upon those natural enemies that opportunistically feed on pollen or vegetative tissues of crops. Furthermore, direct routes of exposure generally lead to much greater exposure to the Bt proteins in Bt crops than secondary exposure for several reason. First, the level of Bt protein that is present in herbivores that have fed on Bt plants is far lower than the level of Bt protein present in the plant tissues, presumably because of dilution effects (Dutton et al. 2002; Head et al. 2001). Second, some insects, particularly phloem feeders like aphids, ingest only minimal amounts of Bt protein because little or no Bt protein is present in the parts of the plant where they are feeding (Head et al. 2001; Raps et al. 2001). Third, arthropod predators usually prey upon a variety of species, some or all of which may not be feeding on the Bt crop at all. Therefore regulatory testing logically focuses on direct exposure to Bt proteins through ingestion of pollen or green tissues.

As described above, several representative natural enemies typically have been included among the lower tier regulatory tests. Indicator organisms tested for currently registered lepidopteran-active Bt proteins (e.g., Cry1Ab, Cry1F, Cry1Ac and Cry2Ab) have included lady beetles, the green lacewing, Chrosoperla carnea Stephens (Neuroptera: Chrysopidae) and a parasitic Hymenoptera such as Nasonia vitripennis Walker (Hymenoptera: Pteromalidae). An additional reason for the choice of these species was their history of testing with microbial pesticides which provides useful comparative data.

Among generalist predators, lady beetles are a logical choice for testing because of their abundance and importance within cropping systems, and particularly corn and cotton agroecosystems. Studies have been conducted with the convergent lady beetle, Hippodamia convergens Guerin-Meneville (Coleoptera: Coccinellidae) and the pink-spotted lady beetle,
Coleomegilla maculata De Geer (Coleoptera: Coccinellidae). Of these two species, C. maculata is the preferred species for testing because it is more of a generalist predator and more readily feeds on pollen than H. convergens (Lundgren et al. 2004).

Other coleopteran generalist predators also may be suitable for regulatory testing. In particular, ground beetles (Carabidae) and rove beetles (Staphylinidae) are logical candidates for lower tier tests, and have been used in assessing the impact of conventional insecticides and Bt crops expressing coleopteran-active proteins. These taxa are ecologically and economically important within agro-ecosystems, and fill diverse niches. Many are capable of feeding on pollen. For example, Pterostichus spp. and Amara spp. are abundant carabids within corn fields, and could be adapted for laboratory testing.

Green lacewings are important generalist predators in many crops, but typically are less abundant and influential in corn and cotton cropping systems than coccinellids and heteropteran predators such as Geocoris spp. and Orius spp. (e.g., Candolfi et al. 2004; Hagerty et al. 2005). Furthermore, green lacewings consume little pollen in the field and primarily feed upon on phloem-feeding aphids, and thus their exposure to the Bt proteins in Bt crops will be limited (Head et al. 2001; Raps et al. 2001). For these reasons, green lacewings are being used less as test species for Bt crops, and instead are being replaced with species like the insidious flower bug, Orius insidiosus Say (Heteroptera: Anthocoridae). This species is highly abundant in crop systems, readily feeds on pollen, and also feeds on leaves and other green tissues under certain conditions.

Parasitoids typically will only see limited exposure to the Bt proteins in Bt crops because their main route of exposure will be through secondary pathways. Egg parasitoids such as Trichogramma spp. will not be exposed at all. In addition, the Cry1, Cry2 and Cry3 proteins currently expressed in commercial Bt crop varieties are not expected to be directly toxic to Hymenoptera, and the honey bee, Apis mellifera L. (Hymenoptera: Apiidae) routinely is used in non-target testing for Bt crops in any case. Thus, testing of generalist predators usually has taken precedence over testing of hymenopteran parasitoids. As noted earlier, regulatory testing for Bt crops often has included the dipteran pupal parasitoid N. vitripennis. However, other species with greater relevance to corn and cotton cropping systems also are being considered (for example, braconids such as Cotesia spp.).

RESULTS OF REGULATORY AND POST-COMMERCIAL TESTING

TESTING OF PREDATORS

Natural enemies, and particularly generalist arthropod predators, have been the focus of many studies because of their role in the biological control of various agricultural pests. Based on what is known about the limited spectrum of activity of the Bt Cry proteins expressed in currently commercialized Bt crops, no direct toxic effects from Bt crops would be expected for any of these species. As predicted, the Tier 1 (early tier) laboratory studies that have been conducted by companies as part of the regulatory packages for Bt crops have not found any direct toxic effects of Cry1, Cry2 or Cry3 proteins against insect predators for Bt protein concentrations at or much greater than maximum possible exposure under natural conditions.
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(for example, see reviews in Betz et al. 2000; EPA 2001). Obviously these tests are not meant to mimic natural exposure nor do they test all possible species that could be exposed but they do represent highly conservative tests of possible hazard using carefully chosen surrogate species.

Researchers interested in the fate of particular predatory species have carried out additional laboratory and semi-field tests of potential non-target impacts. These tests have used a variety of designs, with differing degrees of realism in terms of the route and level of Bt exposure. Given that many predators feed on some amount of pollen at some point in their life cycle, many of these studies have involved feeding predatory insect species pollen from Bt crops and comparable control lines. None of these studies have found any adverse impacts of Bt pollen on the survival or development of various insect predators (e.g., Pilcher et al. 1997). Comparable studies using Bt corn silks with a heteropteran predator also found no effect (Al-Deeb et al. 2001).

Obviously the above studies involved direct exposure and, under field conditions, exposure also can occur through secondary pathways with predators feeding upon herbivores that had fed on a Bt crop plant. However, secondary exposure of this sort should have relatively little impact on arthropod predators for the reasons outlined above. However, one set of studies has been presented as a possible example of adverse impacts through secondary exposure. Hilbeck et al. (1998a, b; 1999) performed a number of laboratory studies with the predatory lacewing C. carnea, feeding the larvae on lepidopteran larvae that had fed on Bt corn. They found higher mortality and slower development of lacewings exposed to Bt-intoxicated insects than for lacewings fed on comparable controls. Subsequent studies by other researchers indicate that these results actually reflected feeding on nutritionally poorer prey rather than any toxic effect of the Bt protein (Dutton et al. 2002; Romeis et al. 2004). Such a situation should have little relevance to the field because other prey sources that are not affected by Bt crops will be more available and probably preferred under natural conditions. Furthermore, other tritrophic studies by Al-Deeb et al. (2001) with O. insidiosus saw no effect when feeding on Bt-intoxicated prey. In this case, the results were confirmed with direct feeding studies on Bt corn silks and field observations.

Numerous field studies also have focused on generalist predators, particularly C. maculata, C. carnea, O. insidiosus, and guilds of carabids because of their abundance in cornfields and their perceived importance. No adverse effects have been seen for any of these species in these studies or in the broader, community-level studies of Bt corn (e.g., Candolfi et al., 2004; Lozzia, 1999; Pilcher et al. 1997) and Bt cotton (Hagerty et al. 2005; Xia et al. 1999). The absence of even indirect trophic effects of Bt corn and Bt cotton in these studies is not surprising because most of these predatory species feed on many different prey species, the vast majority of which are not directly impacted by Bt corn e.g., sucking insects like aphids and whiteflies. In contrast, the insecticidal sprays used in conventional corn had clear adverse impacts, at least transiently, on almost all common predators, and particularly those species foraging above ground (Candolfi et al. 2004). Similarly, the insecticidal sprays used in conventional cotton also had clear adverse impacts on almost all of the important arthropod predators (Hagerty et al. 2005; Wu and Guo 2005; Xia et al. 1999).
TESTING OF INSECT PARASITOIDS

As with arthropod predators, no direct toxic effects from Bt crops would be expected for any of parasitoid species given what is known about the spectrum of activity of the Bt proteins expressed in currently commercialized Bt crops. Furthermore, because the larvae of these groups feed solely on other arthropods, larval parasitoids will not face any direct exposure. Adult exposure also will be very limited because of their occasional feeding on pollen or nectar. However, secondary exposure to Bt proteins may occur if the parasitoids feed on herbivore larvae that have fed upon a Bt crop plant. In addition, indirect effects may occur at the population level if the host species of the natural enemies are a target of the Bt crop and are depressed in numbers.

As with predatory species, the Tier 1 laboratory studies have not found any direct toxic effects of Cry1, Cry2 or Cry3 proteins against parasitoids for Bt protein concentrations at or much greater than maximum possible exposure under natural conditions (see reviews in Betz et al. 2000; EPA 2001). On the other hand, secondary exposure studies indicate that parasitoids that develop on hosts exposed to Bt may be adversely impacted. When reared on Bt-susceptible insects that had fed on Bt corn, the larval development and mortality of the parasitoid Parallorhogas pyralophagus Marsh (Hymenoptera: Braconidae) was adversely affected, but the fitness of emerging adults was not impacted (Bernal et al. 2002).

It should also be remembered that fundamental differences in how Bt plants act relative to conventional insecticides will be a major determinant of the relative impact that these products have on non-target species. With Bt plants, having expression of the insecticidal protein only within the plant and preferentially within certain tissues means that many parasitoids will never be exposed to any Bt protein.

A number of field studies have looked at impacts on parasitoids or the level of parasitism in Bt cornfields. Because of their specificity, species that parasitize the larval stages of target pests of Bt crops would be expected to be rarer in fields of Bt crops than in comparable fields of conventional crops. As expected, the few specialist parasitoids that parasitize Ostrinia nubilalis Hübner (Lepidoptera: Crambidae) and certain other stalk boring Lepidoptera in corn have been found to be rarer in Bt corn than in conventional corn, e.g. Macrocentrus cingulum Brischke (Hymenoptera: Braconidae) (Candolfi et al. 2004). Similarly, the few specialist parasitoids that parasitize foliage-feeding Lepidoptera like Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) in cotton have been found to be rarer in Bt cotton than in non-Bt cotton (e.g., Xia et al. 1999). Of course, it is important to consider these results in the context of alternative practices. As mentioned earlier, the insecticidal sprays used in conventional corn (Candolfi et al. 2004) and cotton (Hagerty et al. 2005; Wu and Guo 2005; Xia et al. 1999) have clear adverse impacts, at least transiently, on these same parasitoid species. Furthermore, any effective pest control practice that decreases the abundance of the host species will have comparable effects.
CONCLUSIONS

Collectively, the non-target studies performed to date demonstrate that Bt crops do not have any unexpected toxic effects on natural enemy species, as would be predicted from knowledge of the mode of action and specificity of Bt proteins. Because of this specificity, Bt crops effectively preserve local populations of various economically important biological control organisms that can be adversely impacted, at least transiently, by broad-spectrum chemical insecticides. The only indirect effects on non-target organisms that have been observed with Bt crops are local reductions in numbers of certain specialist parasitoids whose hosts are the primary targets of Bt crops. Such trophic effects will be associated with any effective pest control technology, whether it be transgenic, chemical, or cultural, as well as with natural fluctuations in host populations.

REFERENCES


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