

## COMPATIBILITY OF INSECT-RESISTANT TRANSGENIC PLANTS WITH BIOLOGICAL CONTROL

Jörg ROMEIS<sup>1</sup> and Anthony M. SHELTON<sup>2</sup>

<sup>1</sup> Agroscope FAL Reckenholz  
Swiss Federal Research Station for Agroecology and Agriculture  
Reckenholzstr. 191  
8046 Zurich, Switzerland  
joerg.romeis@fal.admin.ch

<sup>2</sup>Department of Entomology  
Cornell University, NYSAES  
630 W. North St.  
Geneva, NY 14456, U.S.A.  
ams5@cornell.edu

### SESSION 7 INTRODUCTION

Insect resistant transgenic crops that express genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) are grown on a steadily increasing area worldwide since their first introduction in 1996. In 2004, *Bt*-transgenic plants were grown on 22.4 million ha worldwide (James 2004).

*Bt* (Cry) toxins are known to have a very specific mode of action and plants commercialized today target either lepidoteran pests, including stem borers in maize and the budworm-bollworm complex in cotton, or coleopteran pests including the Colorado Potato beetle, *Leptinotarsa decemlineata*, and corn rootworms, *Diabrotica* spp. (James 2004 ; Shelton *et al.* 2002;). *Bt*-crops should not be viewed as silver bullets to solve all insect pest problems but should be regarded as just another tool to help manage certain pest populations in an economically viable and environmentally safe manner.

While in some areas of the world, especially in Europe, the debate is focusing on the potential environmental risks that could come with the large scale deployment of *Bt*-transgenic crops, other countries are investing time and efforts to evaluate how these crops can be implemented in integrated pest management (IPM) programs for sustainable pest control. One factor of particular interest in this respect is the impact of *Bt*-transgenic crops on non-target organisms that fulfil important ecological and economic functions within the agricultural system. This includes pollinators and biological control agents such parasitoids and predators that are of importance for natural pest regulation. Since *Bt*-transgenic plants express proteins with insecticidal properties, their effects on non-target arthropods should be assessed within an ecological risk assessment prior to commercialization of the crop (Conner *et al.* 2003; Dutton *et al.* 2003). Research to date on commercialized *Bt* crops indicates that the expressed Cry toxins do not have any direct effect on species belonging to orders other than the target

insects (Lepidoptera or Coleoptera) (O'Callaghan *et al.* 2005). This is not surprising given the long history of safe and very targeted use of microbial *Bt* products (Glare and O'Callaghan 2000).

Thus, *Bt*-transgenic crops have the potential to be a viable alternative to conventional insecticides. In cotton fields, broad-spectrum insecticides are generally applied for the control of lepidopteran pests, i.e. the bollworm-budworm complex. Around the globe, deployment of *Bt* cotton has consistently resulted in a 60-80% decrease in insecticide applications in this crop (Fitt *et al.* 2004). Similarly, *Bt* sweet-corn has been found to be a suitable alternative for control of lepidopteran pests (Musser and Shelton 2003). In other crops such as maize, the introduction of the *Bt* gene to control the European corn borer, *Ostrinia nubilalis*, has not lead to substantial insecticide decreases simply due to the fact that this pest is generally not controlled by foliar insecticides so many growers simply did not treat and were resigned to the losses (Phipps and Park 2002).

The published information available to date reveals no detrimental impact of *Bt*-transgenic crops on the abundance or efficiency of biological control agents. In cases where *Bt* crops replaced the use of conventional insecticides (e.g., cotton or sweet-corn), substantial positive effects on the biocontrol fauna have been reported, resulting in increased control of potential secondary pests such as aphids (Reed *et al.* 2001; Wu and Guo 2003). Thus *Bt*-transgenic crops should be regarded as a biocontrol friendly technology that can help promote the conservation of biological control agents for key pests in cropping systems that are currently dominated by insecticide use. Furthermore the replacement of broad-spectrum insecticides by *Bt* crops opens up an opportunity for biocontrol of secondary pests, such as plant and stink bugs in cotton, that were controlled by the insecticides applied against the lepidopteran pest complex (Green *et al.* 2001; Wu *et al.* 2002).

The following session will provide information on the non-target risk assessment conducted by biotech companies as part of the regulatory process (Graham Head). This will be followed by examples from *Bt*-maize (Rick Hellmich) in the U.S. and *Bt* cotton in the U.S. (Steven Naranjo), China (Kongming Wu) and Australia (Gary Fitt) on how *Bt* crops can be implemented in IPM systems.

## REFERENCES

- Conner, A. J., Glare, T. R., and Nap, J. P. 2003. The release of genetically modified crops into the environment - Part II. Overview of ecological risk assessment. *Plant Journal* **33**, 19-46.
- Dutton, A., Romeis, J., and Bigler, F. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: *Bt*-maize expressing Cry1Ab as a case study. *Biocontrol* **48**, 611-636.
- Fitt, G. P., Wakelyn, P. J., Stewart, J., James, C., Roupakias, D., Hake, K., Zafar, Y., Pages, J., and Giband, M. 2004. "Global Status and Impacts of Biotech Cotton." Report of the second expert panel on biotechnology of cotton. International Cotton Advisory Committee.

- Glare, T. R., and O'Callaghan, M. 2000. "Bacillus thuringiensis: Biology, Ecology and Safety". John Wiley and Sons Ltd, Chichester, U.K.
- Green, J. K., Turnipseed, S. G., Sullivan, M. J., and May, O. L. 2001. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. *Journal of Economic Entomology* **94**, 403-409.
- James, C. 2004. "Preview: Global Status of Commercialized Biotech/GM Crops: 2004", ISAA Brief No. 32, International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY, U.S.A.
- Musser, F. R., and Shelton, A. M. 2003. Bt sweet corn and selective insecticides: Impacts on pests and predators. *Journal of Economic Entomology* **96**, 71-80.
- O'Callaghan, M., Glare T. R., Burgess, E. P. J., and Malone, L. A. 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology* **50**, 271-292.
- Phipps, R. H., and Park, J. R. 2002. Environmental benefits of genetically modified crops: Global and European perspectives on their ability to reduce pesticide use. *Journal of Animal and Feed Sciences* **11**, 1-18.
- Reed, G. L., Jensen, A. S., Riebe, J., Head, G., and Duan, J. J. 2001. Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. *Entomologia Experimentalis et Applicata* **100**, 89-100.
- 346 Shelton, A. M., Zhao, J. Z., and Roush, R. T. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annual Review of Entomology* **47**, 845-881.
- Wu, K. M., and Guo, Y. Y. 2003. Influences of *Bacillus thuringiensis* Berliner cotton planting on population dynamics of the cotton aphid, *Aphis gossypii* Glover, in northern China. *Environmental Entomology* **32**, 312-318.
- Wu, K., Li, W., Feng, H., and Guo, Y. 2002. Seasonal abundance of the mirids, *Lygus lucorum* and *Adelphocoris* spp. (Hemiptera: Miridae) on Bt cotton in northern China. *Crop Protection* **21**, 997-1002.

## ASSESSING THE INFLUENCE OF *Bt* CROPS ON NATURAL ENEMIES

Graham HEAD

Monsanto LLC, A2NA  
800 North Lindbergh Blvd.  
St. Louis, MO 63167, U.S.A.  
graham.p.head@monsanto.com

### 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.

347

### 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,

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<sup>®</sup> or Ingard<sup>®</sup>) 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.

348

## 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 chan-

nels 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 reasons. 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). Thus predators feeding on these different prey species will be exposed to very little *Bt* protein. 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, *Chrysoperla 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: Apidae) 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.).

351

## 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



(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

- Al-Deeb, M. A., Wilde, G. E., and Higgins, R. A. 2001. No effect of *Bacillus thuringiensis* corn and *Bacillus thuringiensis* on the predator *Orius insidiosus* (Hemiptera : Anthocoridae). *Environmental Entomology* **30**, 625-629.
- Bernal, J. S., Griset, J.G., and Gillogly, P.O. 2002. Impacts of developing on *Bt* maize-intoxicated hosts on fitness parameters of a stem borer parasitoid. *Journal of Entomological Science* **37**, 27-40.
- Betz, F. S., Hammond, B. G., and Fuchs, R. L. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regulatory Toxicology and Pharmacology* **32**, 156-173.
- Candolfi, M., Brown, K., Reber, B., and Schmidli, H. 2004. A faunistic approach to assess potential side-effects of genetically modified *Bt*-corn on non-target arthropods under field conditions. *Biocontrol Science and Technology* **14**, 129-170.
- Dutton, A., Klein, H., Romeis, J., and Bigler, F. 2002. Uptake of *Bt*-toxin by herbivores on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecological Entomology* **27**, 441-447.
- English, L., and Slatin, S. L. 1992. Mode of action of delta-endotoxin from *Bacillus thuringiensis*: a comparison with other bacterial toxins. *Insect Biochemistry and Molecular Biology* **22**, 1-7.
- EPA Biopesticides Registration Action Document (BRAD) *Bacillus thuringiensis* Plant-Incorporated Protectants. October 2001.
- [http://www.epa.gov/pesticides/biopesticides/pips/Bt\\_brad2/5-benefits.pdf](http://www.epa.gov/pesticides/biopesticides/pips/Bt_brad2/5-benefits.pdf) (last accessed April 18, 2005)
- Hagerty, A. M., Kilpatrick, A. L., Turnipseed, S. G., Sullivan, M. J., and Bridges, W. C. 2005. Predaceous arthropods and lepidopteran pests on conventional, Bollgard, and Bollgard II cotton under untreated and disrupted conditions. *Environmental Entomology* **34**, 105-114.

- Head, G. P., Brown, C. R., Groth, M. E., and Duan, J. J. 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. *Entomologia Experimentalis et Applicata* **99**, 37-45.
- Hilbeck, A., Moar, W. J., Pusztai-Carey, M., Filippini, A., and Bigler, F. 1998a. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* **27**, 1255-1263.
- Hilbeck, A., Baumgartner, M., Fried, P. M., and Bigler, F. 1998b. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* **27**, 480-487.
- Hilbeck, A., Moar, W. J., Pusztai-Carey, M., Filippini, A., and Bigler, F. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. *Entomologia Experimentalis et Applicata* **91**, 305-316.
- James, C. 2004. "Preview: Global Status of Commercialized Biotech/GM Crops: 2004", ISAAA Briefs No. 32, ISAAA, Ithaca, NY.
- Lozzia, G. C. 1999. Biodiversity and structure of ground beetle assemblages (Coleoptera Carabidae) in *Bt* corn and its effects on non target insects. *Bollettino di Zoologica Agraria e di Bachicoltura* **31**, 37-58.
- Lundgren, J. G., Razzak, A. A., and Wiedenmann, R. N. 2004. Population responses and food consumption by predators *Coleomegilla maculata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. *Environmental Entomology* **33**, 958-963.
- McClintock, J. T., Schaffer, C. R., and Sjoblad, R. D. 1995. A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. *Pesticide Science* **45**, 95-105.
- Mendelsohn, M., Kough, J., Vaituzis, Z., and Matthews, K. 2003. Are *Bt* crops safe? *Nature Biotechnology* **21**, 1003-1009.
- Nickson, T. E., and Head, G. 2000. Environmental monitoring of genetically modified crops. *Journal of Environmental Monitoring* **1**, 101-105.
- Perlak, F. J., Fuchs, R. L., Dean, D. A., McPherson, S. L., and Fischhoff, D. A. 1991. Modification of the coding sequence enhances plant expression of insect cotton protein genes. *Proceedings of the National Academy of Sciences* **88**, 3324-3328.
- Pilcher, C. D., Obrycki, J. J., Rice, M. E., and Lewis, L. C. 1997. Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. *Environmental Entomology* **26**, 446-454.
- Raps, A., Kehr, J., Gugerli, P., Moar, W. J., Bigler, F., and Hilbeck, A. 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the non-target herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Molecular Ecology* **10**, 525-533.

- Romeis, J., Dutton, A., and Bigler, F. 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *Journal of Insect Physiology* **50**, 175-183.
- Schuler, T. H., Poppy, G. M., Kerry, B. R., and Denholm, I. 1998. Insect-resistant transgenic plants. *Trends in Biotechnology* **16**, 168-175.
- Sharples, F. E. 1991. Ecological Aspects of Hazard Identification for Environmental Uses of Genetically Engineered Organisms. In "Risk Assessment in Genetic Engineering" (M. A. Levin, and H.S. Strauss, Eds.), pp. 18-31. McGraw-Hill, New York.
- Sisterson, M. S., Biggs, R. W., Olson, C., Carriere, Y., Dennehy, T. J., and Tabashnik, B. E. 2004. Arthropod abundance and diversity in *Bt* and non-*Bt* cotton fields. *Environmental Entomology* **33**, 921-929.
- Wu, K. M., and Guo, Y. Y. 2005. The evolution of cotton pest management practices in China. *Annual Review of Entomology* **50**, 31-52.
- Xia, J. Y., Cui-Jin, J., Ma, L. H., Dong, S. L., and Cui, X. F. 1999. The role of transgenic *Bt* cotton in integrated insect pest management. *Acta Gossypii Sinica* **11**, 57-64.

## INTEGRATION OF *B<sub>T</sub>* MAIZE IN IPM SYSTEMS: A U.S. PERSPECTIVE

Richard L. HELLMICH<sup>1</sup>, Dennis D. CALVIN<sup>2</sup>, Joseph M. RUSSO<sup>3</sup>,  
and Leslie C. LEWIS<sup>1</sup>

<sup>1</sup>USDA-ARS

Corn Insects and Crop Genetics Research Unit, and Department of Entomology  
Iowa State University  
Ames, IA, 50011, U.S.A.  
rhellmi@iastate.edu, leslewis@iastate.edu

<sup>2</sup>Department of Entomology, Pennsylvania State University  
University Park, PA 16802, U.S.A.  
dcalvin@psu.edu

<sup>3</sup>ZedX, Inc.  
Bellefonte, PA 16823, U.S.A.  
russo@zedxinc.com

### ABSTRACT

357

*Bt* maize has become increasingly popular with United States (U.S.) growers since it was commercially available in 1996. Yield protection, reduced need for insecticides, improved grain quality, and ease of use are benefits that motivated growers to plant 32 percent of total acres to *Bt* maize in 2004. Rapid adoption of a technology raises many questions concerning product longevity and how the technology will influence the maize agricultural ecosystem. Overuse could result in the development of resistant insects, economic populations of secondary pests, or influence populations of non-target organisms. Grower strategies for using *Bt* maize in the U.S. vary regionally and depend on targeted and secondary pests, cropping practices, and insect resistance management requirements. A challenge for scientists and educators has been to try to keep grower recommendations uniform and grounded in principles of Integrated Pest Management (IPM). The talk will highlight a project funded by United States Department of Agriculture Risk Avoidance and Mitigation Program (USDA-RAMP) that uses site-specific high-resolution information to help merge transgenic technology with traditional insect IPM tools.

### INTRODUCTION

The most damaging pests of maize in the United States Corn Belt are the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) and corn rootworm complex,

*Diabrotica* spp. (Coleoptera: Chrysomelidae). European corn borer is a chronic pest of maize in the eastern two-thirds of North America. In the U.S., it is annually responsible for over one billion dollars in yield and control costs. Before transgenic maize was available, *O. nubilalis* was managed by planting insect resistant maize and by using rescue treatments of chemical insecticides (Mason *et al.* 1996). Western corn rootworm, *Diabrotica virgifera* LeConte, and northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, also are estimated to cost U.S. growers more than a billion dollars in crop losses and control costs annually. Historically, these pests have been controlled by crop rotation and prophylactic use of soil insecticides. Biocontrol of these pests has not been embraced by most U.S. growers because it is usually not cost effective. There have been limited successes with *O. nubilalis*. Biological sprays of *Bacillus thuringiensis* Berliner (*Bt*; e.g., Dipel) are commonly used by seed producers to protect seed corn and by organic growers. Inundative and inoculative releases of the egg parasitoid, *Trichogramma ostrinae* Pang and Chen (Hymenoptera: Trichogrammatidae), can significantly reduce *O. nubilalis* damage in sweet corn (Hoffman *et al.* 2002; Seaman *et al.* 1996; Wright *et al.* 2002) but is not commercially available. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin shows potential for controlling *O. nubilalis* under field conditions (Bing and Lewis 1991). Biocontrol of corn rootworm is nonexistent, although there is some indication that certain species of nematodes could be useful (Journey and Ostlie 2000; Nickle *et al.* 1994). In areas in and near Illinois, crop rotation has failed as a cultural control method for western corn rootworm because some rootworm beetles oviposit in soybean fields (Sammons *et al.* 1997). Also, in some areas of Iowa, Minnesota and South Dakota populations of northern corn rootworm have adapted to crop rotation through a two-year or more extended diapause (Krysan *et al.* 1986).

358

Transgenic maize hybrids with a gene from *B. thuringiensis* are revolutionizing maize insect management. Transgenic maize with resistance to *O. nubilalis* was commercially available in the U.S. in 1996. Use of this maize has steadily increased where in 2004 32% of field maize in the U.S. was *Bt* (USDA–NASS 2004). The perceived value of *Bt* maize tends to increase going from east to west and in areas where univoltine and bivoltine moths overlap (e.g., southern Minnesota and South Dakota). Growers are attracted to *Bt* maize because it protects their yield, simplifies pest management, and in some cases leads to better quality grain (Munkvold *et al.* 1997).

Along with the advancements of molecular biology, there have been improvements in computer processing power. Faster computers allow scientists to evaluate and integrate data sets that were not practical even ten years ago. A collaboration of scientists supported by a grant from USDA–RAMP provides unique approaches to improve field crop IPM by using site-specific high-resolution information to help merge transgenic technology with traditional insect management tools. Maize and *O. nubilalis* development models driven by degree days lay the foundation for these analyses, which allow entomologists to determine when corn borer presence and potential damage are most detrimental to maize development. An economist then uses these data to access where in the Corn Belt *Bt* maize is the most economical. These types of analyses could open doors for improving biocontrol because they also could use degree-day models on large spatial scales to tie together the phenologies of biocontrol agents with those of maize pests.

## METHODS

The *Bt* maize Economic Tool or BET program is composed of several models: pest phenology, maize phenology, site-specific weather data and an economic component. The maize and corn borer phenology models, using site-specific (~ 1 km<sup>2</sup> resolution) historical weather data as input, calculate the weather-driven seasonal synchrony of maize growth stage and the period of European corn borer stalk tunneling for every location east of the Rocky Mountains. An economic analysis model calculates the potential yield losses associated with all possible “seasonal” synchronies. The site-specific weather data, which serve as input into both crop and pest models, are derived from either observations or forecasts. Data from observations are the result of interpolating between weather stations; while, data from forecasts are derived from numerical weather prediction models. Site-specific weather data are increasingly becoming a popular input choice for local crop and pest models (Magarey *et al.* 2001; Russo 2000). These models extend from collaborative efforts of many scientists, but the chief designers of the BET program are Drs. Dennis D. Calvin, Jeffrey Hyde (Penn State University), and Joseph M. Russo (ZedX, Inc.).

The BET model is available at an interactive website ([www.essc.psu.edu/bet/](http://www.essc.psu.edu/bet/)). Grower inputs include seed-maturity class and planting date, and inputs for the economic analysis include first and second generation *O. nubilalis* densities (mean larvae per plant), *Bt*-pest control, seed premium, average yield, planting rate, and maize price. Default values are based on long-term averages. Growers can update these values to reflect local conditions and economics.

359

## RESULTS AND DISCUSSION

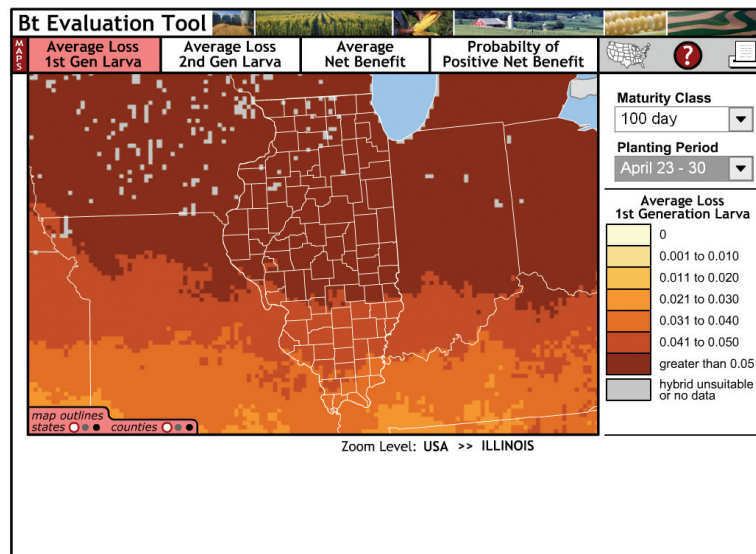
The BET model provides growers with a tool that allows them to make decisions whether to plant *Bt* maize based on the probability of positive net benefits. A grower in central Illinois, for example, who traditionally plants a 105-day hybrid the last week in April, could expect first generation *O. nubilalis* to overlap with an attractive stage of maize (Fig. 1). Similar output for second generation *O. nubilalis* suggests potential problems are less (data not shown), primarily because most maize would be past the vulnerable stage when *O. nubilalis* were present. This grower could expect a positive return on *Bt* maize 31 out of 33 years (Fig. 2). This type of information provides a starting point for growers to assess whether the technology is cost effective in their area, which could minimize overuse of the technology. Overuse of *Bt* technology can result in selection for insects that are resistant. Prescriptive use of a pest control instrument is one form of resistance management, which would apply to transgenic crops and any other pest control technology.

A grower also could evaluate planting date possibilities that reduce the overlap of first or second generation *O. nubilalis*. For example, if a grower is most concerned with first generation *O. nubilalis* he or she may want to plant *Bt* maize early and non-*Bt* maize later; and vice versa if second generation borers are a concern. Such information also could be valuable



for organic growers who might decide to alter planting dates and seed maturity to avoid peak pest densities. An organic organization also potentially could evaluate places to establish farms that would minimize pest problems.

A visual tool for evaluating the overlap of pest and plant phenologies offers possibilities for evaluating biocontrol agents. Scientists have long puzzled over why effectiveness of biocontrol agents varies spatially and temporally. For example, Clark *et al.* (2001) have found very high levels of parasitism in maize in Nebraska, but other researchers (Bruck and Lewis 1999; Lewis 1982) found consistently low levels over several years in Iowa. Granted many factors contribute to the success of parasitoids, but one could start with a specific parasitoid phenology model and overlay it with maize and *O. nubilalis* phenologies. First-level analyses would entail modeling various planting dates and locations to determine if patterns emerge that provide clues to more efficient use of a particular parasitoid. Second-level analyses could follow whereby other factors are incorporated into the model, such as the phenology of plants that provide shelter, nectar, or other benefits to the biocontrol agent. Ultimately this systems approach to pest management could allow investigators to incorporate unique features of landscapes into the analyses. One could be overwhelmed by the near infinite combinations of pests, biocontrol agents, and environments, but the system outlined here could allow scientists to identify patterns of effective combinations. Bottom line, this approach provides a way to visualize complex interactions and provides a valuable tool toward developing more sustainable approaches to pest management.



**Figure 1.** *Bt* Evaluation Tool (BET) model results showing average loss of first generation *O. nubilalis* through Corn Belt states when a 100-day maturity seed is planted the last week in April.

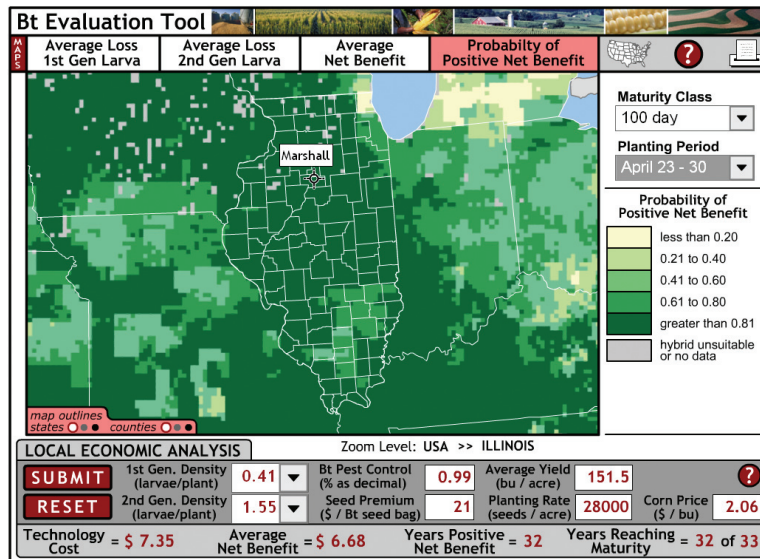


Figure 2. BET model results showing probability of positive net benefits of using *Bt* technology for controlling *O. nubilalis* through Corn Belt states when a 100-day maturity seed is planted the last week in April. Local economic analysis focuses on Marshall County in Illinois.

## ACKNOWLEDGEMENTS

This research was supported by a grant from USDA–CSREES, Risk Avoidance and Mitigation Program (RAMP). Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA, Iowa State University or Pennsylvania State University.

## REFERENCES

- Bing, L. A., and Lewis, L. C. 1991. Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environmental Entomology* **20**, 1207-1211.
- Bruck, D. J., and Lewis, L. C. 1999. *Ostrinia nubilalis* (Lepidoptera: Pyralidae) larval parasitism and infection with entomopathogens on corn fields with deterrent border vegetation. *Journal of Agricultural and Urban Entomology* **16**, 255-272.
- Clark, T. L., Witkowski, J. F., and Foster, J. E. 2001. Parasitism rates in European corn borer (Lepidoptera: Crambidae) larvae collected from six maize hybrids. *Journal Entomological Science* **36**, 342-351.
- Hoffmann, M. P., Wright, M. G., Pitcher, S. A., and Gardner, J. 2002. Inoculative releases of *Trichogramma ostriniae* for suppression of *Ostrinia nubilalis* (European corn borer) in sweet corn: Field biology and population dynamics. *Biological Control* **25**, 249-258.

- Journey, A. M., and Ostlie K. R. 2000. Biological control of the western corn rootworm (Coleoptera: Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae*. *Environmental Entomology* **29**, 822-831.
- Krysan, J. L., Foster, D. E., Branson, T. F., Ostlie, K. R., and Cranshaw, W. S. 1986. Two years before the hatch rootworms adapt to crop rotation. *Bulletin of the Entomological Society of America* **32**, 250-253.
- Lewis, L. C. 1982. Present status of introduced parasitoids of the European corn borer, *Ostrinia nubilalis* (Hübner), in Iowa. *Iowa State Journal of Research* **56**, 429-436.
- Magarey, R. D., Seem, R. C., Russo, J. M., Zack, J. W., Waight, K. T., Travis, J. W., and Oudermans, P. V. 2001. Site-specific weather information without on-site sensors. *Plant Disease* **85**, 1216-1226.
- Mason C. E., Rice, M. E., Calvin, D. D., Van Duyn, J. W., Showers, W. B., Hutchison, W. D., Witkowski, J. F., Higgins, R. A., Onstad, D. W., and Dively, G. P. 1996. European Corn Borer- Ecology and Management. North Central Regional Extension Publication No. 327.
- Munkvold, G. P., Hellmich, R. L., and Showers, W. B. 1997. Reduced *Fusarium* ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. *Phytopathology* **87**,1071-1077.
- Nickle, W. R., Connick, W. J. Jr., and Cantelo, W. W. 1994. Effects of pesta-pelletized *Steinernema carpocapsae* (All) on western corn rootworms and Colorado potato beetles. *Journal of Nematology* **26**,249-250.
- Russo, J. M. 2000. Weather Forecasting for IPM. In "Emerging Technologies for Integrated Pest Management: Concepts, Research, and Implementation" (G. G. Kennedy, and T. Sutton, Eds.), pp. 25-42. APS Press, St. Paul, MN.
- Sammons, A. E., Edwards, C. R., Bledsoe, L. W., Boeve, P. J., and Stuart, J. J. 1997. Behavioral and feeding assays reveal a western corn rootworm (Coleoptera: Chrysomelidae) variant that is attracted to soybean: *Environmental Entomology* **26**, 1336-1342.
- Seaman, A., Hoffmann, M., Gardner, J., and Chenus. S. 1996. Pilot Testing of *Trichogramma ostriniae* Releases in Fresh Market Sweet Corn for Control of European Corn Borer. pp. 149-154. In 1996 New York State Vegetable Project Reports Relating to IPM. NY IPM Publication 121.
- USDA-NASS. 2004. Crop production: acreage supplement, pp.24-25. United States Department of Agriculture, National Agriculture Statistics Service, Washington, D.C. <http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0604.pdf> (last accessed April 2005).
- Wright, M. G., Kuhar, T. K., Hoffmann, M. P., and Chenus, S. A. 2002. Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. *Biological Control* **23**, 149-155.

# FIELD ABUNDANCES OF INSECT PREDATORS AND INSECT PESTS ON $\delta$ -ENDOTOXIN-PRODUCING TRANSGENIC COTTON IN NORTHERN CHINA

Kongming WU, Kejian LIN, Jin MIAO, and Yongjun ZHANG

Institute of Plant Protection  
Chinese Academy of Agricultural Sciences  
Beijing 100094, China

wkm@caasocse.net.cn

## ABSTRACT

China is one of the largest producers of cotton in the world. Insect pests such as cotton bollworm, cotton aphid, and mirids are the major factors that contribute to a decrease in cotton production. Transgenic cotton that expresses a gene derived from the bacterium *Bacillus thuringiensis* (*Bt*) has been deployed for combating cotton bollworm since 1997 in China, and expanded rapidly to 3.7 million of the total cotton acreage of 5.3 million ha in 2004. Field monitoring on the change of pest status derived from *Bt* cotton commercialization in a large scale were conducted during 1998-2004 in Hebei Province. The results indicated that *Bt* cotton efficiently controls cotton bollworms, while the decrease of pesticide applications allows the build up of high populations of predators, such as lady beetles *Coccinella septempunctata*, lacewings *Chrysopa sinica*, spiders and others in mid-season. Furthermore, planting *Bt* cotton efficiently prevented the resurgence of cotton aphids caused by insecticide use for control of cotton bollworm. However, the investigation of the seasonal dynamics of mixed populations of mirids showed that mirid density increased drastically, probably due to a reduction in the number of foliar insecticide applications in *Bt* cotton fields.

363

## INTRODUCTION

Cotton bollworm, *Helicoverpa amigera* (Hübner) (Lepidoptera: Noctuidae) is a serious pest of cotton, *Gossypium hirsutum* L. in China. To combat the unprecedented *H. armigera* pest pressure in the early 1990s, cotton farmers in China had to apply synthetic pyrethroids, endosulfan and organophosphate insecticides at 2~3 day intervals during critical periods, resulting in more than 20 sprays during the season (Wu and Guo, 2005). In recent years, control of this pest has relied heavily upon commercial cultivation of *Bt* cotton that expresses a gene encoding an insecticidal protein from the bacterium *Bacillus thuringiensis* Berliner, which has been expanded rapidly to 3.7 million of the total cotton acreage of 5.3 million ha in 2004 (James, 2004).

There are numerous arthropods in cotton fields. While the *Bt* protein is toxic to only a narrow spectrum of lepidopteran species, the dynamics of other species may be indirectly affected. Effects on non-target species may be positive due to the removal of disruptive pesticides, or negative due to the effective removal of prey (Fitt, 1994). Because a total insect control strategy in cotton involves both lepidopterous and non-lepidopterous pests, it is necessary to understand the population dynamics of insect predators and insect pests after *Bt* cotton deployment (Pilcher *et al.*, 1997; Riggin-Bucci and Gould, 1997; Wilson *et al.*, 1992). The influences of *Bt* cotton on cotton bollworm, cotton aphid, and mirids have been evaluated in field trials (Wu and Guo, 2003; Wu *et al.*, 2002; Wu *et al.*, 2003). However, previous studies do not provide an insight in the population changes of predators and secondary pests derived from *Bt* cotton commercialization in the long term or on a large scale. Here we report the monitoring results on the change in abundance of insect predators and insect pests associated with *Bt* cotton planting in northern China.

## MATERIALS AND METHODS

### COTTON VARIETIES

A transgenic cotton variety expressing the Cry1Ac gene (NuCOTN33B), a transgenic cotton variety (SGK321) expressing Cry1Ac and CpTI genes and its parental line (Shiyuan321) supplied by Monsanto Co. (St. Louis, MO) and Shijiazhuang Academy of Agricultural Sciences (Shijiazhuang, Hebei Province), respectively, were used in the experiments.

### EXPERIMENTAL DESIGN

Experiments were conducted from 1998 to 2004 at Langfang Experimental Station of the Chinese Academy of Agricultural Sciences, located in Hebei Province. Experiments consisted of three treatments (two transgenic cotton varieties and one conventional cotton). The field was layed out as a randomized complete block replicated three times. Each plot was about 0.033 hectare and was seeded at the rate expected to produce 45000 plants per planted hectare. No insecticide was sprayed in *Bt* cotton plots and its control plots (normal variety). Cotton was maintained with standard agronomic practices for northern China.

### SAMPLING FOR INSECT PESTS AND PREDATORS

Each treatment was sampled every 3-4 d from middle-June to early-September, and each sample consisted of 5 sites with a total number of 100 cotton plants. Field counts consisted of eggs and larvae of cotton bollworm, immature and adult *Lygus pratensis* Linnaeus, *Lygus lucorum* Meyer-Dür, *Adelphocoris suturalis* Jak., *Adelphocoris fasciaticollis* Reuter and *Adelphocoris lineolatus* (Goeze), and predators, such as lady beetles [*Coccinella septempunctata* Linnaeus, *Leis axyridis* (Pallas), *Propylaea japonica* (Thunberg)], lacewings (*Chrysopa sinica* Tjeder, *Chrysopa septempunctata* Wesmæl, *Chrysopa shansiensis* Kawa and *Chrysopa formosa* Brauer), spiders (*Erigonidium graminicolum* and *Misumenopos tricuspidata*) and *Orius similis*. Three leaves per plant from upper, middle and lower parts of cotton plant were sampled to estimate densities of cotton aphid, *Aphis gossypii* Glover.

## STATISTICAL ANALYSES

Population densities of insects were analyzed using analysis of variance (ANOVA) and means were separated using the protected least significant difference (LSD) test (SAS Institute 1988).

## RESULTS

### PREDATORS

Transgenic *Bt* cotton may affect natural enemies indirectly through the removal of eggs, larvae, and pupae of lepidopteran insects that serve as food sources for predatory arthropods. Considerable reduction in the number of insecticide applications is another important factor that regulates the population dynamics of natural enemies. The field surveys showed that the populations of lacewings (Fig. 1), lady beetles (Fig. 2), spiders and *Orius similis* remained at high densities in *Bt* cotton and conventional cotton through the season. This was likely due to the reduced application of insecticides.

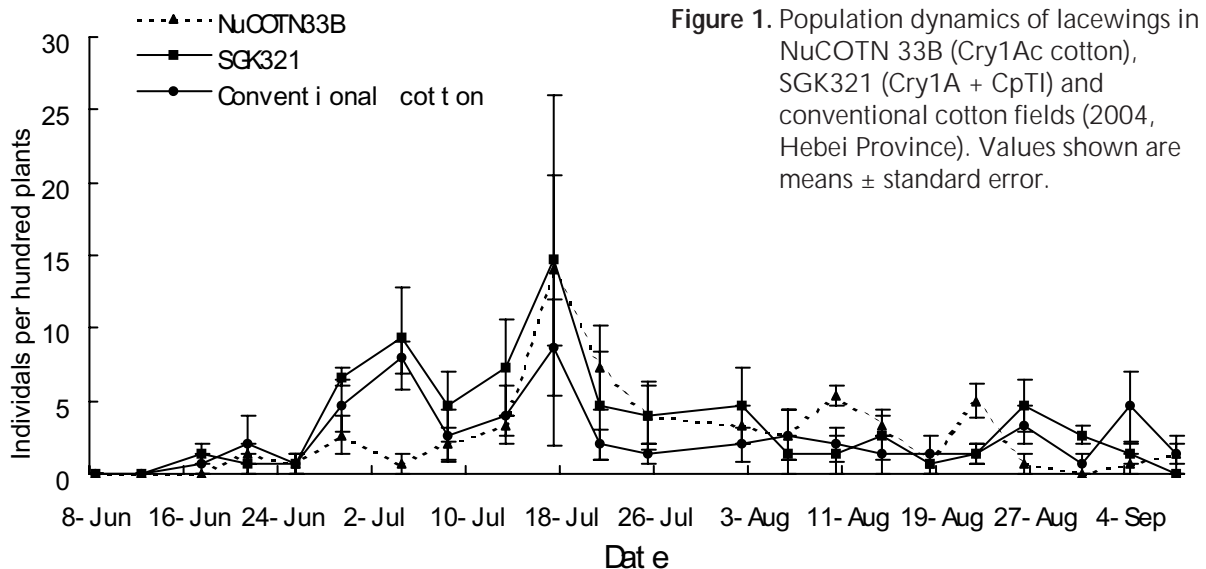


Figure 1. Population dynamics of lacewings in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means  $\pm$  standard error.

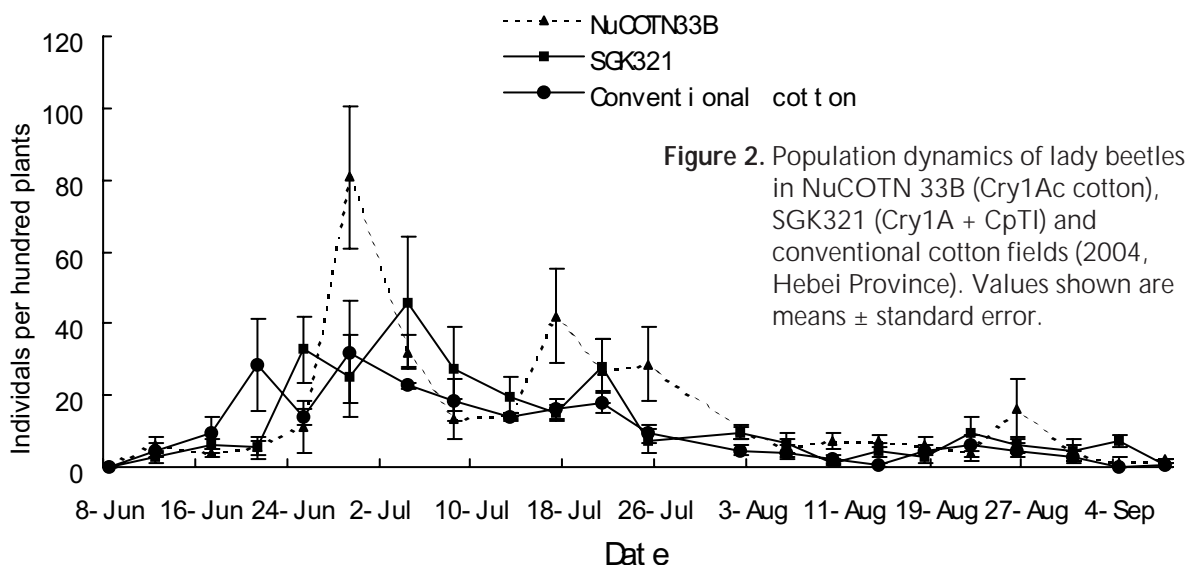
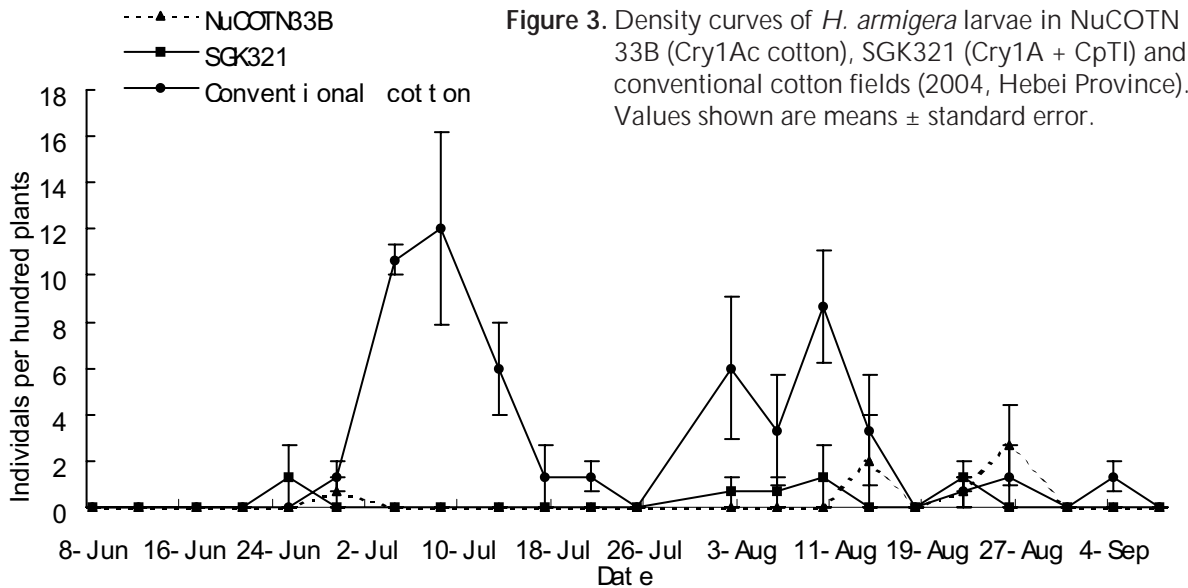


Figure 2. Population dynamics of lady beetles in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means  $\pm$  standard error.

### COTTON BOLLWORM

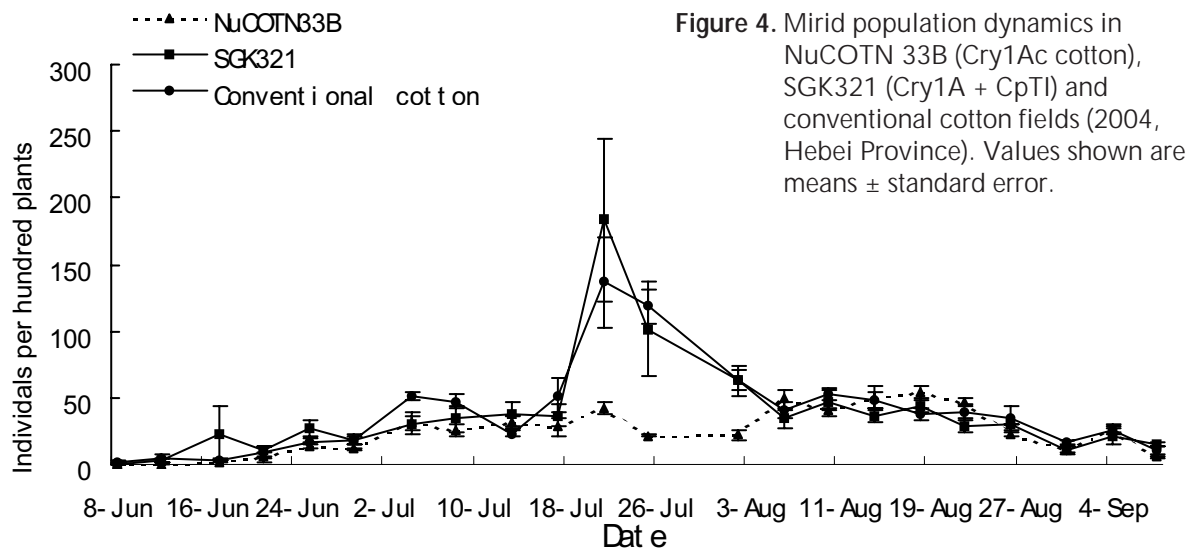
The field evaluations from 1998 to 2004 indicated that *Bt* cotton provided good control of the cotton bollworm. Data for 2004 illustrate this trend (Fig. 3). In this year, the bollworm occurred slightly, and the larval densities on July 8 and August 10 in conventional cotton fields were significantly higher than those on *Bt* cotton ( $P < 0.05$ ). Historically, potentially damaging bollworm larval densities have developed in transgenic cotton under severe egg densities in some years. However, in recent years, the regional occurrence of cotton bollworm in northern China has decreased drastically and this is likely due to the large-scale deployment of *Bt* cotton



366

### MIRIDS

Investigations on the seasonal population dynamics of a species-complex of mirids in *Bt* cotton fields indicated that mirid density on *Bt* cotton increased drastically, probably due to the reduced number of insecticide sprays (Fig. 4). This suggests that mirids have become key insect pests in *Bt* cotton fields, and their damage to cotton could increase further with the expansion of the *Bt* cotton growing area if no additional control measures are adopted.



## APHIDS

Cotton aphid is one of the most important insect pests after cotton bollworm in cotton production in China. Historically, the period during which the cotton aphid caused yield loss was restricted to the seedling stage of cotton plants. Before the 1970s, aphids could easily be controlled by seed treatment with insecticide. In the mid 1970s, aphids became an important insect pest of cotton due to insecticide-induced resurgence in mid and late season. Since the 1980s, its damage to cotton has become more serious and frequent because insecticide sprays directed against *H. armigera* killed most natural enemies, such as ladybeetle and lacewing that are major predators of cotton aphids. Field experiments on the population dynamics of cotton aphids in Bt cotton fields indicated that cotton aphid populations were effectively controlled at a low level, probably due to high densities of ladybeetle and lacewing populations (Fig. 5). This suggests that Bt cotton planting could effectively prevent resurgence of cotton aphids caused by insecticide use for control of cotton bollworm.

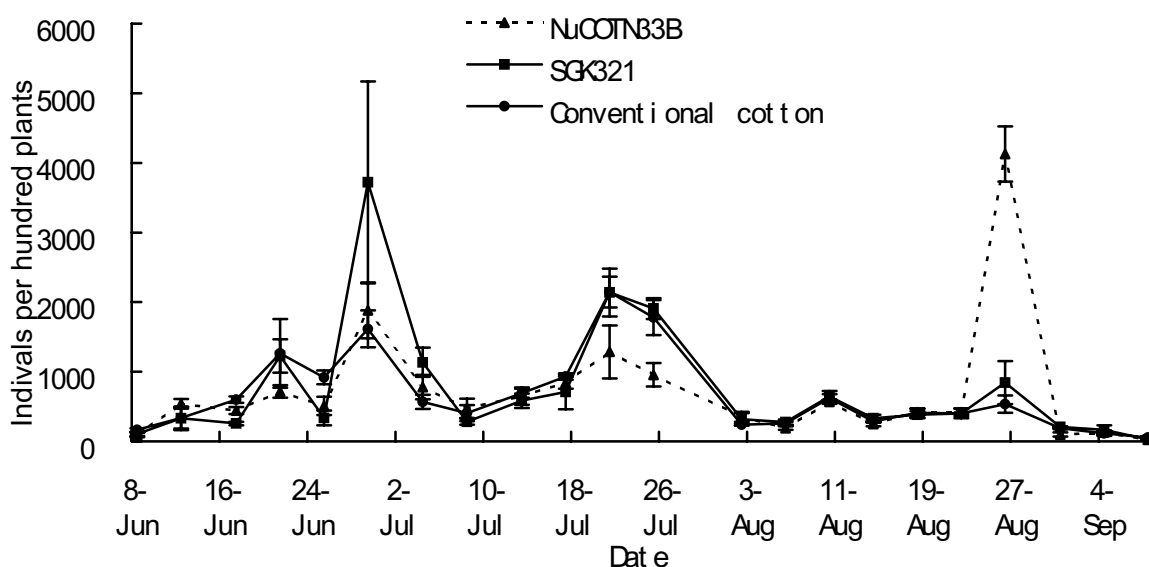


Figure 5. Population dynamics of cotton aphid in NuCOTN 33B (Cry1Ac cotton), SGK321 (Cry1A + CpTI) and conventional cotton fields (2004, Hebei Province). Values shown are means  $\pm$  standard error.

## DISCUSSION

Transgenic cotton, containing *Bt* genes, offers great potential to dramatically reduce pesticide use for control of major lepidopteran pests (Wu and Guo 2005). The greatest threat to the continued efficacy of *Bt* cotton against *H. armigera* is the evolution of resistance (Gould 1998). In addition to current resistance management theory of non-transgenic refuges for the preservation of susceptible alleles, it is also important to consider the prudent use of insecticides, especially late in the season, to reduce overall larval densities in transgenic fields. If late-season survivors in *Bt* fields (under direct selection by the toxin) are reduced by foliar insecticides, the total number of resistance alleles in a region could be reduced. Therefore, it is important that late-season larval density on *Bt* cotton plants be carefully monitored and controlled with effective insecticides.



In addition to the control of Lepidoptera, a control strategy for mirids needs to be developed. In contrast to the greater dispersal ability of the cotton aphid and cotton bollworm, mirids can only fly short distances to adjacent fields. Weeds near cotton fields are their major host plants before moving to cotton, and any action to destroy the weed may decrease the likelihood that the pests will occur and damage the crop. In addition, an alfalfa / cotton system can result in major outbreaks of the mirids in cotton fields. It is thus important to avoid *Bt* cotton planting in fields adjacent to alfalfa and other host plants that mirids prefer (Zhang et al. 1986).

## REFERENCES

- Fitt, G. P. 1994. Field Evaluation of Transgenic Cottons in Australia: Environmental Considerations and Consequences of Expanding Trial Size. In "Proceedings of the 3rd International Symposium on Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms", November 1994 Monterey, California, pp. 37-48.
- Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* **43**, 701-726.
- James, C. 2004. International service for the acquisition of agri-biotech applications (ISAAA). Brief No. 32.
- Pilcher, C. D., Obrycki, J. J., Rice, M. E., and Lewis, L. C. 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. *Environmental Entomology* **26**, 446-454.
- Riggin-Bucci, T. M., and Gould, F. 1997. Impact of intraplot mixtures of toxic and non-toxic plants on population dynamics of diamondback moth (Lepidoptera: Plutellidae) and its natural enemies. *Journal of Economic Entomology* **90**, 241-251.
- SAS Institute. 1988. SAS/STAT user's guide, release 6.03 ed. SAS Institute, Cary, NC.
- Wilson, F. D., Flint, H. M., Deaton, W. R., Fischhoff, D. A., Perlak, F. J., Armstrong, T. A., Fuchs, R. L., Berberich, S. A., Parks, N. J., and Stapp, B. R. 1992. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects. *Journal of Economic Entomology* **85**, 1516-1521.
- Wu, K., and Guo, Y. 2003. Influences of *Bt* cotton planting on population dynamics of the cotton aphid, *Aphis gossypii* Glover, in northern China. *Environmental Entomology* **32**, 312-318.
- Wu, K., and Guo, Y. 2005. The evolution of cotton pest management practices in China. *Annual Review of Entomology* **50**, 31-52.
- Wu, K., Li, W., Feng, H., and Guo, Y. 2002. Seasonal abundance of the mirids, *Lygus lucorum* and *Adelphocoris* spp. (Hemiptera: Miridae) on *Bt* cotton in northern China. *Crop Protection* **21**, 997-1002.

- Wu, K., Guo, Y., Nan, L. V., Greenplate, J. T., and Deaton, R. 2003. Efficacy of transgenic cotton containing a cry1Ac gene from *Bacillus thuringiensis* against *Helicoverpa armigera* (Lepidoptera: Noctuidae) in northern China. *Journal of Economic Entomology* **96**, 1322-1328.
- Zhang, Y., Cao, Y., Bai, L., and Cao, C. 1986. Plant bug damage on cotton in different growing stages and the threshold for control. *Acta Phytophylacica Sinica* **13**, 73-78.

# FUNCTIONAL NON-TARGET DIFFERENCES BETWEEN *Bt* AND CONVENTIONAL COTTON

Steven E. NARANJO

USDA-ARS, Western Cotton Research Laboratory  
4135 East Broadway Road  
Phoenix, AZ 85040, U.S.A.

*snaranjo@wcr.l.ars.usda.gov*

## ABSTRACT

A five-year field study was conducted in Arizona to assess the long term impact of transgenic cotton expressing the Cry1Ac  $\delta\delta$ -endotoxin of *Bacillus thuringiensis* (*Bt*) on population densities of 22 taxa of foliar-dwelling arthropod natural enemies and on the effect of the natural enemy community on key pests in the system. Multi-year analyses of arthropod abundance revealed small, but statistically significant, reductions in five common arthropod predator taxa in unsprayed *Bt* compared with unsprayed non-*Bt* cotton. In contrast, the use of conventional insecticides led to large reductions in 13 predator taxa. Furthermore, functional studies conducted over a three-year period indicated that the small reductions in abundance observed in *Bt* cotton may have little ecological meaning. Sentinel eggs and pupae of *P. gossypiella* experienced the same rates of mortality, primarily from predation, in both *Bt* and non-*Bt* cotton and cohort-based life tables for *B. tabaci* demonstrated that rates of sucking predation, parasitism and dislodgement (chewing predation in part) were unchanged between *Bt* and non-*Bt* cotton. Results demonstrate that long-term and multi-factor studies are required to examine meaningful non-target effects in the field.

## INTRODUCTION

Transgenic crops expressing the insecticidal proteins of *Bacillus thuringiensis* (*Bt*) have been commercially available in the U.S. since 1996 and their adoption continues to expand rapidly in the U.S. and other parts of the developed and developing world (James 2004). In 2004 it was estimated that *Bt* cotton represented about 46% of all upland cotton production in the U.S. (USDA 2004). Use rates are much higher in Arizona where *Bt* cotton was grown on 81% of the upland cotton acreage in 2003, most of it (74%) in a stacked configuration with transgenes conferring glyphosate resistance (Tronstad *et al.* 2004). The primary target of *Bt* cotton in Arizona and southern California is the pink bollworm, *Pectinophora gossypiella* (Saunders), a caterpillar that feeds within the cotton fruit and is difficult to control with conventional insecticides (Henneberry and Naranjo 1998). *Bt* cotton is extremely effective in controlling this pest (Flint and Parks 1999).

As with any new technology, both benefits and risks are associated with transgenic crops in agricultural production systems. The use of transgenic crops have led to significant reductions in conventional, broad-spectrum insecticides, improved suppression of target pests, improved yields, reductions in production costs leading to increased profitability, and increased opportunities for biological control (Cannon 2000; Edge *et al.* 2001; Federici 2003; Shelton *et al.* 2002). Some of the potential risks include outcrossing through pollen drift, horizontal transfer of transgenes to other organisms, food safety, loss of susceptibility to *Bt* toxins in target pests, and effects on non-target organisms and biodiversity (Cannon 2000; Conner *et al.* 2003; Marvier 2001; Shelton *et al.* 2002; Wolfenbarger and Phifer 2000). Despite the long history of safety associated with the topical use of *Bt* endotoxins (Federici 2003; Glare and O'Callaghan 2000) the season-long expression of these toxins in crop plants has prompted research to address potential ecological concerns.

A growing number of studies have examined non-target effects in both the laboratory and field (see reviews by Glare *et al.* 2001, Lovei and Arpaia 2005; Pilson and Prendeville 2004; O'Callaghan *et al.* 2005; Schuler *et al.* 1999) with most concluding that *Bt* crops are highly selective. Laboratory studies have tended to focus on defining the effects of direct exposure or indirect exposure via trophic interactions to *Bt* toxins on the biology of non-target species while most field studies have focused primarily on changes in abundance and diversity of non-target taxa. Relatively few studies have examined predator/prey or host/parasitoid interactions, especially in the field (Bourguet *et al.* 2002; Orr and Landis 1997; Sisterson *et al.* 2004) and all have been relatively short-term in duration and have examined only a few of the potential interactions that may occur between natural enemies and their prey or hosts in transgenic crops.

Cotton hosts a rich diversity of parasitoid and arthropod predator species (van den Bosch and Hagen 1966; Whitcomb and Bell 1964), and these natural enemies are known play an important role in regulating pest herbivore populations (e.g., Eveleens *et al.* 1973; Naranjo and Ellsworth 2005; Stoltz and Stern 1978). Conventional cotton production relies heavily on the input of insecticides which typically have broad toxicity to both pests and their natural enemies. The pattern of insecticide use in cotton is one of the most severe constraints to realizing the potential of natural biological control in this system. Improving the compatibility between chemical and biological control depends on minimizing the effects of insecticides on natural enemies through reductions in use of broader-spectrum materials and adoption of more selective compounds (Hull and Beers 1985; Newsom *et al.* 1976). Transgenic *Bt* crops have the potential to contribute to natural enemy conservation through both their selective activity and associated reductions in the broad-spectrum insecticides they replace. However, longer-term and more inclusive studies are needed to define any potential unintended effects of transgenic crop production.

A five-year field study was conducted in Arizona to assess the long term effects of *Bt* cotton expressing the Cry1Ac d $\delta$ -endotoxin on natural enemy abundance and on the potential impact of the natural enemy community on pest populations. The objectives were to compare: 1) populations of a large group of common natural enemy taxa and several key target and non-target pests between *Bt* and non-*Bt* cottons and to contrast any potential effects relative to conventional production practices using an array of selective and broad-

spectrum insecticides, and 2) rates of natural enemy-induced mortality on two key pests between unsprayed *Bt* and non-*Bt* cotton using sentinel prey and field life table studies.

## MATERIALS AND METHODS

### STUDY SITE AND EXPERIMENTAL DESIGN

Cotton plots were established at the University of Arizona, Maricopa Agricultural Center, Maricopa, Arizona between 1999-2003. All plots were planted in early April of each year and grown according to standard agronomic practices for the area. Each year included a contrast between Deltapine NuCOTN 33B, a transgenic cultivar expressing the Cry1Ac insecticidal protein of *B. thuringiensis*, and its non-transgenic parent cultivar Deltapine 5415. A randomized complete block design with four replications was used in all years; plot size varied from 0.12-0.17 ha. Studies in 2001 and 2002 included positive control treatments which consisted of split plots of *Bt* and non-*Bt* main plots that were sprayed for *P. gossypiella*, other lepidopteran pests, *Bemisia tabaci* (Gennadius) and *Lygus hesperus* Knight based on established action thresholds (Ellsworth and Barkley 2001; Ellsworth *et al.* 1996; University of California 1996). Applications were made on 12 and 20 July and 2 August in 2001, and 12 and 25 July and 16 and 28 August in 2002 consisting of organophosphates, pyrethroids, carbamates and insect growth regulators.

### ARTHROPOD NATURAL ENEMY AND PEST DENSITY

Studies in all years tracked the density of a consistent, selected complex of 22 taxa of foliage-dwelling arthropod natural enemies, primarily predators, along with densities of various key pests including *P. gossypiella*, *B. tabaci* and *L. hesperus*. Most arthropods were sampled using a standard sweep net (38-cm diameter). Two sets of 25 sweeps were collected weekly in each plot between early June and mid-September each year. Densities of immature aphelinid parasitoids attacking *B. tabaci* (*Eretmocerus* spp. and *Encarsia* spp.) were estimated by weekly leaf samples (20-30 per plot) from the seventh mainstem node below the terminal. Densities of *B. tabaci* nymphs and adults were estimated weekly from early July through mid September each year using standard methods (Naranjo and Flint 1994; 1995). Densities of *P. gossypiella* larvae were estimated by counting all larvae inside 100 hostable green bolls per plot every two weeks from early July onward. Adult moth density was monitored weekly with beginning in June with pheromone traps. The abundance of other larval lepidopterans as well as *L. hesperus* were estimated from sweep net samples.

### PEST MORTALITY STUDIES

Mortality of *P. gossypiella* and *B. tabaci* was examined in unsprayed plots of *Bt* and non-*Bt* cotton from 2001-2003. To examine mortality of *P. gossypiella* eggs, small cards containing 20 eggs (1 d old) obtained from the USDA-ARS rearing facility in Phoenix, AZ were pinned under the bracts of cotton bolls that were approximately 20 day old to simulate oviposition by female moths. Twenty cards (one per plant) were placed in each plot and left exposed for 24 hours after which they were examined under magnification in the laboratory for evidence of predation. Intact eggs that remained were then held at 27°C for an additional 6 d to evalu-

ate parasitism. The experiment was repeated 3-4 in 2001-2003. The mortality of *P. gossypiella* pupae (USDA-ARS rearing facility in Phoenix, AZ) were studied on four dates each in 2002-2003. Individual pupae were placed at the base of 20 cotton plants in each plot and left exposed for 24 hours. This simulated a type of pupation site used by larvae exiting bolls. Pupae that remained were returned to the laboratory and examined under magnification for evidence of predation. Intact pupae were held for three weeks to evaluate parasitism. Appropriate controls were run for both sentinel egg and pupae. Mortality of *B. tabaci* nymphs was examined using an *in situ* life table approach (Naranjo and Ellsworth 2005). Briefly, the method involves marking the location of individual settled 1<sup>st</sup> instar nymphs ( $\geq 50$  per plot) with a non-toxic felt pen on the underside of leaves and then repeatedly observing these nymphs every 2-3 d until death or adult emergence. Mortality due to dislodgment (from wind, rain and chewing predators), sucking predation, parasitism, and unknown causes was recorded for each of the four nymphal instars. Life table studies were repeated twice each year between mid-July and early September in 2001-2003.

## ANALYSES

Yearly analyses were conducted for all arthropod taxa (Naranjo 2005a) but only multiyear analyses will be highlighted here. These analyses were conducted by calculating seasonal mean densities for all taxa for each replicate plot in each year and entering block and year as random effects. Arthropod counts were transformed by  $(x+0.5)^{0.5}$  or  $\ln(x+1)$  throughout as necessary to achieve normality and homoscedasticity before analyses; untransformed means are presented. The response variable for egg and pupal mortality of *P. gossypiella* was the proportion missing, eaten or parasitized. Marginal mortality rates were calculated from *B. tabaci* life table data based on apparent mortality using the methods outlined by Elkinton *et al.* (1992) (see Naranjo and Ellsworth 2005 for details) to correct for mortality due to contemporaneous agents. Mixed model ANOVA was used to test for treatment effects in each year where block and trial were entered as random effects. Mixed model ANOVA was also used for multi-year analyses with block, year and trial within year entered as random effects. Proportional and marginal mortality values were transformed by  $\arcsin$  prior to analyses as needed.

373

## RESULTS

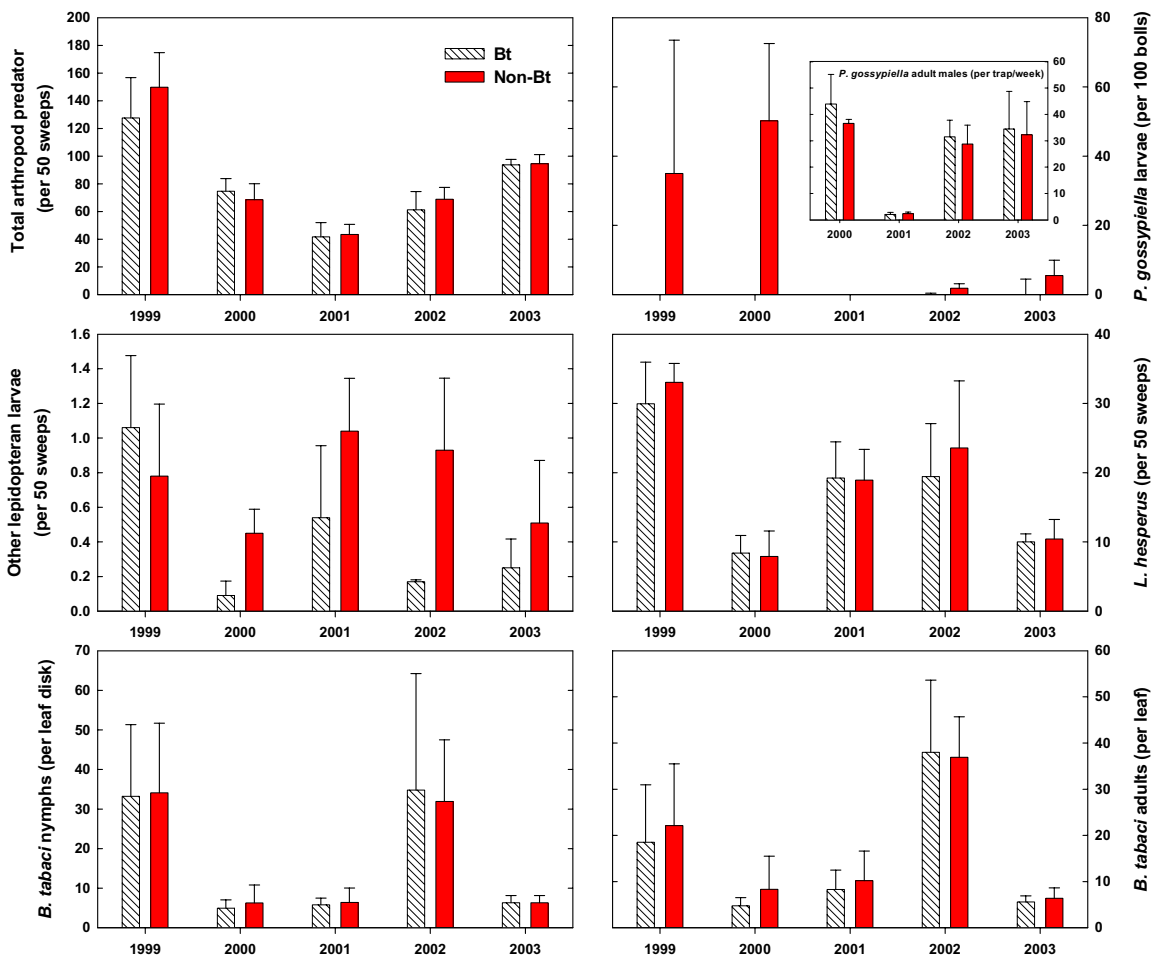
### ARTHROPOD ABUNDANCE

Yearly analyses revealed few differences between *Bt* and non-*Bt* cotton in any natural enemy taxa and no differences for the natural enemy community as a whole (Fig. 1). However, multiyear analyses revealed significant ( $P < 0.05$ ) declines in seasonal densities of five predator taxa in *Bt* compared with non-*Bt* cotton including a group of miscellaneous spiders, *Hippodamia convergens* Guérin-Méneville, *Geocoris punctipes* (Say), *Nabis alternatus* Parshley and *Drapetis nr. divergens* (Table 1). In general, the changes in density were smaller than those observed in individual years; however, the increased sample size of the analyses improved power considerably allowing smaller changes to be detected. Overall, the mean decline in these five taxa was around 19%. In contrast, the use of insecticides resulted in signifi-

cant ( $P < 0.05$ ), and larger declines in 13 individual taxa averaging nearly 48% (Table 1). As expected, there were essentially no *P. gossypiella* larvae in *Bt* cotton and densities of other lepidopterans were reduced in most years (Fig. 1). However, populations of two other key pests, *B. tabaci* and *L. hesperus* were similar in *Bt* and non-*Bt* cotton (Fig. 1).

## MORTALITY OF PEST INSECTS

Eggs of *P. gossypiella* were readily preyed upon in both *Bt* and non-*Bt* cottons with no significant differences ( $P > 0.05$ ) in rates of predation in each of three individual years or all years combined (Fig. 2). No parasitism was detected in eggs that survived predation in any year. Pupae of *P. gossypiella* placed on the soil surface beneath plants also experienced high rates of mortality and there was no significant difference ( $P > 0.05$ ) in rates of pupal mortality between *Bt* and non-*Bt* cottons in either year or both years combined (Fig. 2). Most pupae disappeared presumably from the action of chewing predators and no parasitism was observed in intact pupae held in the laboratory after field exposure.



**Figure 1.** Seasonal mean density of arthropod natural enemies and pests over all sample dates. Error bars are 95% confidence intervals. From Naranjo (2005a).

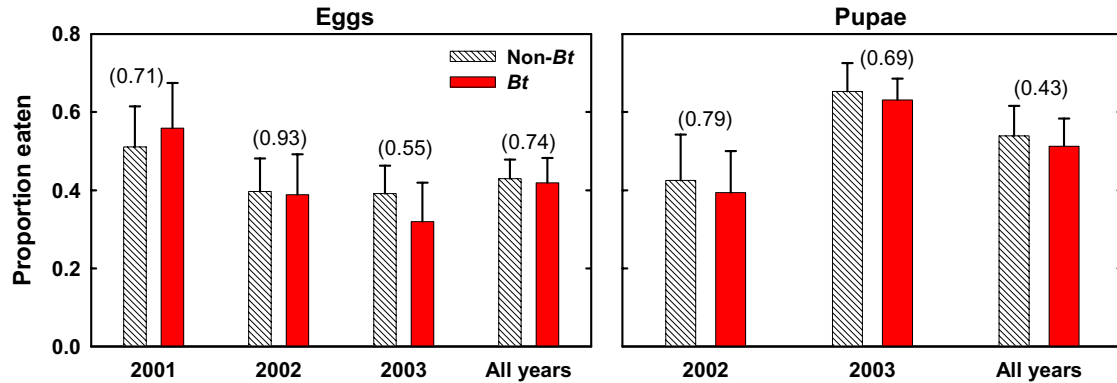
**Table 1.** Overall change in mean densities of arthropods (per 50 sweeps) in *Bt* and non-*Bt* cottons (5 years) and in sprayed and unsprayed cottons (2 years), Maricopa, AZ, 1999-2003. Data from Naranjo (2005a).

Taxa	Order: Family	1999-2003		2001-2002	
		Non-Bt density <sup>a</sup>	Prop. $\Delta$ (P) <sup>b</sup>	Unsprayed density <sup>a</sup>	Prop. $\Delta$ (P) <sup>b</sup>
<i>Dictyna reticulata</i> Gertsch and Ivie	Araneida: Dictynidae	0.62±0.05	0.121 (0.56)	0.62±0.07	-0.443 (0.02)
<i>Misumenops celer</i> (Hentz)	Araneida: Thomisidae	2.59±0.28	-0.038 (0.42)	1.30±0.13	-0.410 (<0.01)
Salticidae	Araneida: Salticidae	0.33±0.07	-0.268 (0.07)	0.11±0.02	-0.143 (0.71)
Other Araneida	Araneida	0.63±0.14	-0.233 (0.02)	0.10±0.04	0.154 (0.73)
<i>Collops vittatus</i> (Say)	Coleoptera: Melyridae	1.65±0.29	-0.062 (0.51)	0.67±0.09	-0.349 (0.02)
<i>Hippodamia convergens</i> Guérin-Meneville	Coleoptera: Coccinellidae	1.20±0.13	-0.189 (0.04)	0.48±0.12	-0.613 (0.03)
Anthicidae	Coleoptera	1.48±0.23	-0.095 (0.33)	0.32±0.10	-0.439 (0.21)
Other Coccinellidae	Coleoptera	0.59±0.18	-0.132 (0.56)	0.20±0.04	-0.538 (0.05)
<i>Geocoris punctipes</i> (Say)	Heteroptera: Lygaeidae	7.30±1.69	-0.176 (0.01)	6.22±0.37	-0.781 (<0.01)
<i>Geocoris pallens</i> (Stål)	Heteroptera: Lygaeidae	4.30±0.79	0.058 (0.38)	2.08±0.25	-0.677 (<0.01)
<i>Orius tristicolor</i> (White)	Heteroptera: Anthocoridae	4.89±0.67	0.054 (0.21)	5.39±0.26	0.270 (0.01)
<i>Nabis alternatus</i> Parshley	Heteroptera: Nabidae	2.53±0.25	-0.238 (<0.01)	1.01±0.17	-0.837 (<0.01)
<i>Zelus renardii</i> Kolenati	Heteroptera: Reduviidae	0.71±0.20	-0.011 (0.77)	0.05±0.02	-0.714 (0.05)
<i>Sinea</i> spp.	Heteroptera: Reduviidae	0.01±0.01	0.370 (0.74)	-	-
<i>Lygus hesperus</i> Knight	Heteroptera: Miridae	18.8±2.23	-0.073 (0.35)	34.5±2.28	-0.557 (<0.01)
<i>Pseudatomoscelis</i> <i>seriatus</i> (Reuter)	Heteroptera: Miridae	10.3±2.33	0.044 (0.98)	1.80±0.20	-0.398 (0.02)
<i>Spanogonicus</i> <i>albofasciatus</i> (Reuter)	Heteroptera: Miridae	2.99±0.53	0.052 (0.38)	0.73±0.10	0.234 (0.65)
<i>Rhinacloa forticornis</i> Reuter	Heteroptera: Miridae	0.26±0.07	-0.160 (0.31)	0.03±0.02	0.000 (0.96)
<i>Chrysoperla carnea</i> s.l. Stephens	Neuroptera: Chrysopidae	2.27±0.21	-0.042 (0.56)	4.08±0.24	-0.105 (0.26)
<i>Drapetis</i> nr. <i>divergens</i>	Diptera: Empididae	19.3±4.31	-0.118 (0.02)	17.8±2.35	-0.387 (<0.01)
Aphelinid parasitoids	Hymenoptera: Aphelinidae	6.18±1.26	-0.273 (0.21)	3.64±0.63	-0.317 (0.08)
Other Hymenoptera	Hymenoptera	1.72±0.20	0.039 (0.59)	1.31±0.15	-0.208 (0.13)

<sup>a</sup> Overall means ( $\pm$ SE) based on seasonal means in four replicate main plots in each of five years for Bt and non-Bt contrasts (n=20) and two years for unsprayed and sprayed contrasts (n=8).

<sup>b</sup> Prop.  $\Delta$  is the proportional change in density in Bt cotton relative to non-Bt cotton or sprayed cotton relative to unsprayed cotton. Numbers in parentheses following prop.  $\Delta$  are P-values; values < 0.05 are bolded.

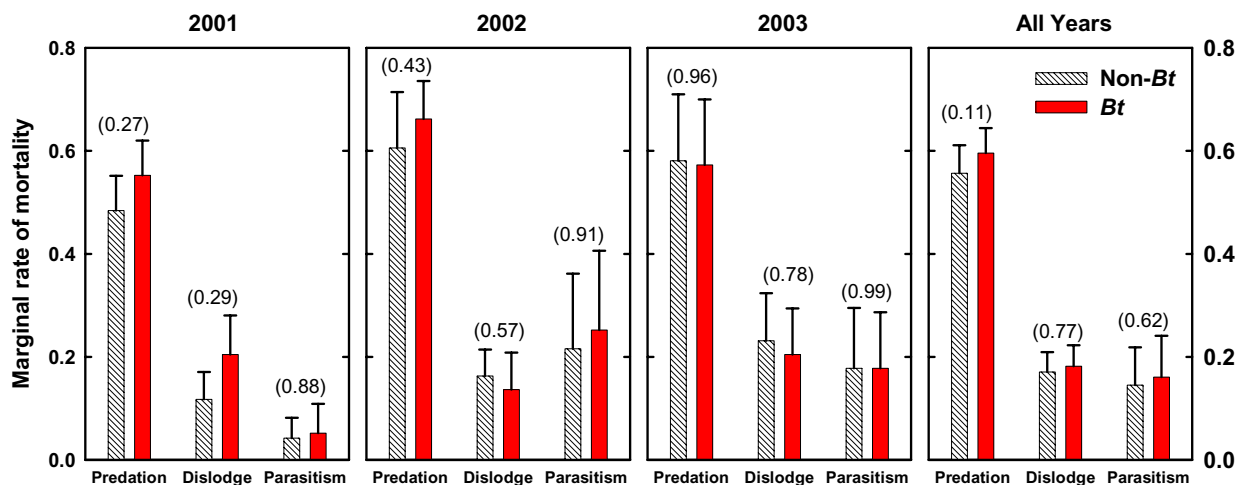




**Figure 2.** Comparison of natural enemy induced mortality of sentinel *P. gossypiella* eggs and pupae between unsprayed *Bt* and non-*Bt* cottons over a three-year period. Numbers above paired bars are *P*-values for ANOVAs of each individual year or all years combined. Error bars represent 95% confidence intervals. Results for each individual year based on 3-4 separate experiments. From Naranjo (2005b).

Nymphs of *B. tabaci* were subject to high rates of predation, moderate rates of dislodgement, and low to moderate rates of parasitism over the three years of study (Fig. 3). Marginal rates of predation did not differ significantly ( $P > 0.05$ ) between *Bt* and non-*Bt* cotton in any year or all years combined. Rates of dislodgement, which included the effects of chewing predation and weather (Naranjo and Ellsworth 2005) varied over years but not as a result of the use of *Bt* cotton. Parasitism was generally low but there was no difference ( $P > 0.05$ ) in rates of parasitism between the two cottons.

376



**Figure 3.** Comparison of natural enemy induced mortality of natural cohorts of *B. tabaci* nymphs between unsprayed *Bt* and non-*Bt* cottons over a three year period. Numbers above paired bars are *P*-values for ANOVAs of each individual year or all years combined. Error bars represent 95% confidence intervals. Results for each individual year based on two separate experiments. From Naranjo (2005b).

## DISCUSSION

Although separate analyses each year generally indicated no negative effects, combined analyses across the five years revealed a significant average decline of about 19% in five predator taxa representing four orders including *H. convergens*, *G. punctipes*, *N. alternatus*, *D. nr divergens* and a group of miscellaneous spiders. With very few exceptions there was a numerical decline in seasonal population density of all these taxa in *Bt* compared with non-*Bt* cotton in all five years. Combining the data sets simply allowed for a larger sample size and correspondingly greater statistical power to discern smaller changes in density.

The causes for these declines are uncertain but could be associated with sampling error, declines in target or non-target prey abundance, or sublethal effects resulting from exposure to *Bt* toxins. Sampling error seems to be an unlikely cause because populations were consistently lower in *Bt* cotton for the five taxa in the majority of years. There was also no obvious difference in the canopy structure between *Bt* and non-*Bt* cotton that could have affected sampling efficiency. Many stages of *P. gossypiella* are relatively invulnerable to natural enemies (Henneberry and Naranjo 1998) and so it is unlikely that the absence of this prey in *Bt* cotton would have measurable effects on generalist predator populations. *B. tabaci* was the most abundant prey for the affected predators but densities of immature and adult stages were similar in both *Bt* and non-*Bt* cotton. Other potential caterpillar prey occurred at low densities and differed relatively little between *Bt* and Non-*Bt* cotton, however, because all are foliage feeders they are susceptible to predation and reductions in their density may have influenced predator populations. Direct feeding on the plant by *G. punctipes* and *N. alternatus* could expose these predator to *Bt* toxins, however, Armer *et al.* (2000) found no negative effects for *Geocoris* and *Nabis* spp. feeding directly on *Bt* potato foliage. Pollen feeding may be an avenue of exposure to the predaceous bugs as well as *H. convergens* but this has not been examined in these species. *D. nr. divergens* largely specialize on adult *B. tabaci* which are phloem feeders and unlikely to possess *Bt* toxins in their bodies. However, Ponsard *et al.* (2002) observed modest declines (H<sup>27</sup>%) in longevity of adult *G. punctipes* and *O. tristicolor* (but not *Nabis* spp.) feeding strictly on *Bt* intoxicated *S. exigua* compared with larvae feeding on non-*Bt* cotton in the laboratory. Although, these predators would not feed exclusively on such caterpillars in the field it does suggest a potential explanation that merits further study.

The biological relevance of these declines in *Bt* cotton is also uncertain. Clearly, conventional alternatives to the use of *Bt* cotton, as represented by the positive controls in this study, are many times more damaging to the natural enemy community, causing much large reductions in density and affecting a broader range of taxa. Such disruptions by broad-spectrum insecticides have been shown repeatedly to compromise the natural biological control of cotton pests (e.g., Eveleens *et al.* 1973; Stoltz and Stern 1978). On the contrary, the results of functional studies here demonstrated that the overall contribution of the natural enemy community to mortality of *P. gossypiella* eggs and pupae and *B. tabaci* nymphs was equal in *Bt* and non-*Bt* cotton that received no additional insecticide applications. In addition there was no indication of resurgence by key pests in the system (*B. tabaci* or *L. hesperus*) that might indicate a reduction in natural control. Thus, the small declines in several taxa of natural enemies in *Bt* cotton observed here may not be ecologically meaningful in terms of at least some trophic interactions.

The lack of association of reductions in density of some predator taxa and rates of natural enemy mortality on two key pests in the system may be explained by the general feeding behavior of most of these predators. Reductions in the density and associated activity of any one species in the complex is offset or replaced by the activity of other members of the community. Life table studies with *B. tabaci* revealed that most of the mortality from any one source is replaceable (Naranjo and Ellsworth 2005). Thus, a reduction in predation *G. punctipes* or *N. alternatus*, for example, could be easily replaced by *Orius tristicolor* White or *Zelus renardii* Kolenti. However, there are limits to the amount of mortality that can be replaced by the natural enemy community. Life table studies with *B. tabaci* also have shown that the use of broad-spectrum insecticides which cause large reductions in natural enemy density can significantly reduce the contribution of these natural enemies to pest mortality leading to a situation where the continued use of insecticides are required for pest suppression (Naranjo 2001; Naranjo and Ellsworth unpublished data). In contrast, the use of selective insecticides for *B. tabaci* only slightly reduces the abundance of various natural enemies (Naranjo *et al.* 2004) but allows the complex to continue contributing significant mortality that enables long-term pest suppression in the absence of additional insecticides (Naranjo 2001; Naranjo and Ellsworth unpublished data).

Transgenic *Bt* cotton appears to represent a highly effective and selective technology for lepidopteran pest control. The long-term studies described here suggest that negative effects of *Bt* cotton on non-target arthropods, particularly natural enemies, are minimal and that even small declines in density of some taxa do not appear to be associated with any meaningful changes in the function of the overall natural enemy community. The use of *Bt* cotton and other selective methods of pest control will continue to advance the important role of biological control in cotton IPM in the western U.S. and elsewhere.

## ACKNOWLEDGMENTS

I thank Kim Beimfohr, Rebecca Burke, Luis Cañas, Melanie Charney, Scott Davis, Greg Owens and Jeffrey Rivas for expert technical assistance and Peter Ellsworth (Univ. Arizona) for assistance with insecticide applications. This study was funded entirely by public funds appropriated to the USDA-ARS through CRIS project 5344-22620-015-00D.

## REFERENCES

- Armer, C. A., Berry, R. E., and Kogan, M. 2000. Longevity of phytophagous heteropteran predators feeding on transgenic *Bt*-potato plants. *Entomologia Experimentalis et Applicata* **95**, 329-333.
- Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N., and Pagliari, C. 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). *Environmental Biosafety Research* **1**, 49-60.
- Cannon, R. J. C. 2000. *Bt* transgenic crops: risks and benefits. *Integrated Pest Management Reviews* **5**, 151-173.

- Conner, A. J., Glare, T. R., and Nap, J. P. 2003. The release of genetically modified crops into the environment. Part II Overview of ecological risk assessment. *Plant Journal* **33**, 19-46.
- Edge, J. M., Benedict, J. H., Carroll, J. P., and Reding, H. K. 2001. Bollgard cotton: an assessment of global economic, environmental and social benefits. *Journal of Cotton Science* **5**, 121-136.
- Elkinton, J. S., Buonaccorsi, J. P., Bellows, T. S., and Van Driesche, R. G. 1992. Marginal attack rate, K-values and density dependence in the analysis of contemporaneous mortality factors. *Researches on Population Ecology* **34**, 29-44.
- Ellsworth, P. C. and Barkley, V. 2001. Cost-Effective *Lygus* Management in Arizona Cotton. In "Cotton, a College of Agriculture Report, Series P-125", pp. 299-307. University of Arizona, Tucson, AZ.
- Ellsworth, P. C., Diehl, J. W., and Naranjo, S. E. 1996. Sampling sweetpotato whitefly nymphs in cotton. *Cooperative Extension, IPM Series No. 6*, University of Arizona, Tucson, AZ.
- Eveleens, K. G., van den Bosch, R., and Ehler, L. E. 1973. Secondary outbreak induction of beet armyworm by experimental insecticide application in cotton in California. *Environmental Entomology* **2**, 497-503.
- Federici, B. A. 2003. Effects of *Bt* on non-target organisms. *Journal of New Seeds* **5**, 11-30.
- Flint, H. M., and Parks, N. J. 1999. Seasonal infestation by pink bollworm, *Pectinophora gossypiella* (Saunders), of transgenic and non-transgenic cultivars of cotton, *Gossypium hirsutum* L., in central Arizona. *Southwestern Entomologist* **24**, 13-20.
- Glare, T. R., and O'Callaghan, M. 2000. "*Bacillus thuringiensis*: Biology, Ecology and Safety", John Wiley and Sons, NY.
- Glare, T. R., O'Callaghan, M., Malone, L. A., and Burgess, E. P. J. 2001. Summary of current scientific awareness of the effect of genetically modified organisms on the natural environment. *Report to the Ministry of the Environment [New Zealand.]* <http://www.mfe.govt.nz/publications/organisms/scientific-awareness-jun01.html> (last accessed March 18, 2005)
- Henneberry, T. J., and Naranjo, S. E. 1998. Integrated management approaches for pink bollworm in the southwestern United States. *Integrated Pest Management Reviews* **3**, 31-52.
- Hull, L. A., and Beers, E. H. 1985. Ecological Selectivity: Modifying Chemical Control Practices to Preserve Natural Enemies. In "Biological Control in Agricultural IPM Systems" (M. A. Hoy, and D. C. Herzog, Eds.), pp. 103-122. Academic Press, New York.
- James, C. 2004. "Preview: Global Status of Commercialized Biotech/GM Crops: 2004". ISAAA Briefs No. 32. ISAAA, Ithaca, NY. <http://www.isaaa.org> (last accessed March 14, 2005)

- Lovei, G. L., and Arpaia, S. 2005. The impact of transgenic plants on natural enemies: a critical review of laboratory studies. *Entomologia Experimentalis et Applicata* **114**, 1-14.
- Marvier, M. 2001. Ecology of transgenic crops. *American Scientist* **89**, 160-167.
- Naranjo, S. E. 2001. Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Protection* **20**, 835-852.
- Naranjo, S. E. 2005a. Non-target effects of *Bt* transgenic cotton: natural enemy abundance. *Environmental Entomology* (submitted)
- Naranjo, S. E. 2005b. Non-target effects of *Bt* transgenic cotton: function of the natural enemy community. *Environmental Entomology* (submitted)
- Naranjo, S. E., and Ellsworth, P. C. 2005. Mortality dynamics and population regulation in *Bemisia tabaci*. *Entomologia Experimentalis et Applicata* (in press)
- Naranjo, S. E., Ellsworth, P. C., and Hagler, J. R. 2004. Conservation of natural enemies in cotton: role of insect growth regulators in management of *Bemisia tabaci*. *Biological Control* **30**, 52-72.
- Naranjo, S. E., and Flint, H. M. 1994. Spatial distribution of preimaginal *Bemisia tabaci* (Homoptera, Aleyrodidae) in cotton and development of fixed-precision sequential sampling plans. *Environmental Entomology* **23**, 254-266.
- Naranjo, S. E., and Flint, H. M. 1995. Spatial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development and validation of fixed-precision sampling plans for estimating population density. *Environmental Entomology* **24**, 261-270.
- Newsom, L. D., Smith, R. F., and Whitcomb, W. H. 1976. Selective Pesticides and Selective Use of Pesticides. In "Theory and Practice of Biological Control" (C. Huffaker, and P. Messenger, Eds.), pp. 565-591. Academic Press, New York.
- O'Callaghan, M., Glare, T. R., Burgess, E. P. J., and Malone, L. A. 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology* **50**, 271-292.
- Orr, D. B., and Landis, D. A. 1997. Oviposition of European corn borer and impact of natural enemy populations in transgenic versus isogenic corn. *Journal of Economic Entomology* **90**, 905-909.
- Pilson, D., and Prendeville, H. R. 2004. Ecological effects of transgenic crops and the escape of transgenes into wild populations. *Annual Review of Ecology, Evolution and Systematics* **35**, 149-174.
- Ponsard, S., Gutierrez, A. P., and Mills, N. J. 2002. Effect of *Bt*-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators. *Environmental Entomology* **31**, 1197-1205.
- Schuler, T. H., Poppy, G. M., Kerry, B. R., and Denholm, I. 1999. Potential side effects of insect resistant transgenic plants on arthropod natural enemies. *Trends in Biotechnology* **17**, 210-216.

- Shelton, A. M., Zhao, J. Z., and Roush, R. T. 2002. Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Annual Review of Entomology* **47**, 845-881.
- Sisterson, M. S., Biggs, R. W., Olson, C., Carriere, Y., Dennehy, T. J., and Tabashnik, B. E. 2004. Arthropod abundance and diversity in *Bt* and non-*Bt* cotton fields. *Environmental Entomology* **33**, 921-929.
- Stoltz, R. L. and Stern, V. M. 1978. Cotton arthropod food chain disruption by pesticides in the San Joaquin Valley, California. *Environmental Entomology* **7**, 703-707.
- Tronstad, R., Husman, S., Norton, R., Norton, E., Clay, P., and Zerkoune, M. 2004. Review of the 2003 Arizona Cotton Season. In "Cotton, a College of Agriculture and Life Sciences Report, Series P-138" pp. 1-4. University of Arizona, College of Agriculture and Life Sciences, Tucson, AZ. <http://cals.arizona.edu/pubs/crops/az1335> (last accessed March 1, 2005).
- University of California 1996. "Integrated Pest Management for Cotton in the Western Region of the United States, 2<sup>nd</sup> Ed", University of California, Division of Agriculture and Natural Resources, Publication 3305.
- USDA. 2004. Acreage. National Agricultural Statistic Service, Agricultural Statistics Board, U.S. Department of Agriculture, Washington, D. C. <http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bba/acrg0604.pdf> (last accessed March 21, 2005)
- Van den Bosch, R., and Hagen, K. S. 1966. Predaceous and parasitic arthropods in California cotton fields. *California Agricultural Experiment Station Publication* 820.
- Whitcomb, W. H., and Bell, K. 1964. Predaceous insects, spiders and mites of Arkansas cotton fields. *Arkansas Agricultural Experiment Station Bulletin* **690**, 1-84.
- Wolfenbarger, L. L., and Phifer, P. R. 2000. The ecological risks and benefits of genetically engineered plants. *Science* **290**, 2088-2093.

## INTEGRATION OF *Bt* COTTON IN IPM SYSTEMS: AN AUSTRALIAN PERSPECTIVE

Gary P. FITT<sup>1</sup> and Lewis WILSON<sup>2</sup>

<sup>1</sup> CSIRO Entomology, 120 Meiers Rd.  
Indooroopilly, Qld, Australia, 4068  
Gary.Fitt@csiro.au

<sup>2</sup> CSIRO Plant Industry  
Narrabri, NSW, Australia 2390  
Lewis.Wilson@csiro.au

<sup>1,2</sup> Australian Cotton Cooperative Research Centre  
Narrabri, NSW, Australia

### ABSTRACT

Insect pests may have a severe impact on cotton production in Australia. Key pests are *Helicoverpa* spp which are well adapted to exploit cropping systems and often evolve resistance to pesticides. Until recently adoption of IPM has been restricted by a lack of non-disruptive tools.

IPM must be founded on a thorough understanding of the ecology of pest and beneficial species, their interaction with the crop and surrounding non-crop environments. Insect resistant transgenic cottons have proved successful in providing a foundation for more sustainable, economically acceptable IPM with the integration of a range of other non-chemical tactics.

In Australia, *Bt* cottons (tradename INGARD®) expressing the CryIAc endotoxin from *Bacillus thuringiensis* subsp. *kurstaki*, were commercialised in 1996/97 and gradually increased in area under an industry agreed deployment strategy which limited use to 30% of the cotton area. Two gene (Cry IAc/Cry 2Ab) varieties (Bollgard II) have been commercialised from 2004/05 and have now completely replaced Ingard varieties. All *Bt* varieties are grown under a comprehensive management strategy designed to minimise the risk of resistance evolving in *Helicoverpa armigera*, the main target pest.

Commercial use of Ingard cotton varieties has reduced pesticide applications for *Helicoverpa* spp by 60%, providing major environmental benefits. Even greater pesticide reductions now occur with Bollgard II varieties. Pre-release environmental impact assessments demonstrated no significant effect of these *Bt* cottons on natural enemies. Commercial experience with *Bt* cotton crops has now shown a 3-4 fold increases in beneficial insect abundance compared to conventional crops. Co-incidentally several selective insecticides (indoxacarb, spinosad, and emamectin) became available for *Helicoverpa* control on conventional cotton,

which further assisted in conserving beneficials. While resistance is the greatest risk for *Bt* cottons their sustained value in IPM systems also requires focus on the management of secondary pests, which are suppressed in conventional cotton by *Helicoverpa* sprays. Enhanced levels of beneficial species help to partially suppress secondary pests.

Maintaining an appropriate balance and retaining the benefits of *Bt* cottons requires vigilant resistance management, sustained efforts to enhance beneficial species and non-disruptive, short residual pesticides for key sucking pests. Overall the stability of these systems will require mobilization of the whole farm environment and greater understanding of the flows of impacts and services between intensive cropping systems and the surrounding landscape.

## INTRODUCTION

Insect pests represent a significant threat to Australian cotton production (Fitt 1994). Key pests include the noctuid moths *Helicoverpa armigera* and *H. punctigera*, spider mites (*Tetranychus urticae*), aphids (*Aphis gossypii*) and mirids (*Creontiades dilutus*), while insecticide resistance in several pests further complicates management.

Pest management using conventional pesticides can be effective but imposes significant economic and environmental costs including disruption of natural biological control agents. Integrated pest management has long been proposed as a more sustainable approach in many situations, however, the adoption of a truly integrated pest management approach has been extremely patchy.

Broadly IPM can be defined as “the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.” (FAO 2002). In this paper we will illustrate how the adoption of *Bt* cotton varieties has assisted the implementation of IPM in the Australian cotton industry.

Many aspects of IPM have been applied in the Australian cotton industry since the late 1970s when the computer based decision support system, SIRATAC, was released to industry (Hearn and Bange 2002). The minimal IPM approach involved the use of sampling systems and thresholds to better time the use of pesticides. However, today IPM represents a more expansive approach which seeks to minimise pesticide use and include a broader range of tactics such as pest resistant varieties, conservation and augmentation of beneficial insect populations, use of selective and short residual insecticides, recognition of the compensatory capacity of the plant and various cultural control practices which have long been associated with the IPM concept.

These broad principles are captured in the “Integrated Pest Management Guidelines for Cotton Production Systems in Australia” produced for the Australian industry (Deutscher, Wilson, and Mensah, 2004 - <http://cotton.crc.org.au/Assets/PDFFiles/IPMGL05/IPMGLFor.pdf>). The Guidelines emphasise four principles:



1. conservation and utilization of beneficial insects;
2. preferential use of selective insecticides;
3. an emphasis on both profitability and sustainability, ensuring that both input costs and yield are considered, rather than the traditional emphasis on maximizing yield;
4. integration of all farm management activities, throughout the annual cycle of production, not just during the cotton season.

It is into this milieu that *Bt* cotton varieties contribute significantly by providing effective control of the key pests without disruption of the system so providing opportunities for the enhanced role of naturally occurring biocontrol agents to be recognised, manipulated and managed to achieve more sustainable systems.

### GENETICALLY MODIFIED COTTONS IN IPM

*Bt* cotton varieties expressing the Cry 1Ac protein from *Bacillus thuringiensis* subsp. *kurstaki* were first registered in Australia in 1996 (INGARD<sup>®</sup>) and gradually increased in area under an industry agreed deployment strategy which limited use to 30% of the cotton area. Two gene (Cry IAc/Cry 2Ab) varieties (Bollgard II) have been commercialised from 2004/05 and have now completely replaced Ingard varieties. All *Bt* varieties are grown under a comprehensive management strategy designed to minimise the risk of resistance evolving in *Helicoverpa armigera*, the main target pest. Fitt (2003; 2004) provides an assessment of the impact of *Bt* cotton in Australia over the first six years of commercial use. While efficacy of INGARD cottons is not consistent through the growing season and can be highly variable (Fitt *et al.* 1994; Fitt *et al.* 1998), growers have learned to manage INGARD varieties and substantial reductions in pesticide use on *Bt* cotton have occurred.

Potential non-target impacts of *Bt* cotton were one of the environmental impacts which required pre-release assessment. Cry proteins from *Bacillus thuringiensis* (*Bt*) introduced have been deployed as safe and effective pest control agents in microbial *Bt* formulations for almost 40 years in many developed and developing countries (Glare and O'Callaghan 2000). Potential impacts of *Bt* cotton on non-target species may involve direct or indirect effects and a range of assessment protocols have been proposed. Schuler *et al.* (2001; 2004) and Poppy (2000) outline a comprehensive, hierarchical protocol for assessing non-target effects commencing with laboratory studies to assess direct or indirect impacts on non-targets or their predators and parasitoids – a worst case scenario, through a second tier of semi-field contained population experiments and finally a third tier of field experiments.

In the case of direct effects of Cry IAc and Cry 2Ab proteins on non-target species, the well established specificity of these proteins provides a clear safeguard that greatly reduces risks of direct effects on non-lepidopteran species. Non-targets such as predators which do not feed on the plant are not directly exposed and no evidence exists for a secondary impact through consumption of intoxicated prey. The reported effects of Cry IAb expressed in maize on the survival of lacewing larvae (Hilbeck *et al.* 1998a,b; 1999) were recently shown to be mediated by reduced prey-quality rather than any direct effect of the protein toxin (Romeis *et al.* 2004).

Research in Australia examined the diversity and species richness of invertebrate communities present in unsprayed *Bt* and conventional crops. Across a number of specific invertebrate categories, Fitt and Wilson (2002) found no significant impacts of *Bt* cotton. When analysed as a whole community (Whitehouse *et al.* in press) there were small differences between the communities in *Bt* cotton and conventional cotton, but these differences peaked at different times during the season, and the timing of differences was not consistent between years. Again for the majority of functional groups there was no impact of *Bt* cotton.

Indirect effects on non-target species may be mediated through changes in abundance and diversity of prey. The significance of a reduced density of Noctuid larvae and pupae as food sources for predators or as hosts for parasitoids depends on the importance of *Helicoverpa* life stages in cotton in maintaining local populations of these beneficials. Clearly within transgenic cotton fields, the abundance of some predators and parasitoids may be reduced, particularly those whose survival is closely tied to the abundance of *Helicoverpa*, but this is unlikely to threaten their regional persistence since in the cropping systems where cotton is usually grown a significant proportion of the *Helicoverpa* population is also present on other crops and uncultivated hosts (Fitt 1989; Hearn and Fitt 1992) where parasitoids are also active. None of the known predators that attack Lepidoptera in cotton are specialists; *Helicoverpa* may be only incidental prey items for some key predators whose within-field abundance is maintained by other prey. Other studies have sought further indirect effects of transgenic on parasitoids and non-target herbivores but have generally found no effect of the *Bt* plant itself (e.g., Schuler *et al.* 2001; 2004).

Within-field impacts on non-target insects, even if they do occur, are unlikely to be significant compared to the undoubted impacts from broad-spectrum pesticides. The appropriate control treatment for any experimental comparison of the impact of transgenic cotton must include not only unsprayed non-transgenic conventional cotton, but also conventional cotton with its required management which will usually involve synthetic pesticides (Fitt and Wilson 2002). A similar sentiment has been expressed by EU researchers (<http://europa.eu.int/comm/research/fp5/pdf/eag-gmo.pdf>). Observations over the past 6 years confirm that the abundance of beneficial invertebrates in commercial *Bt* cotton fields is markedly greater than in conventional fields.

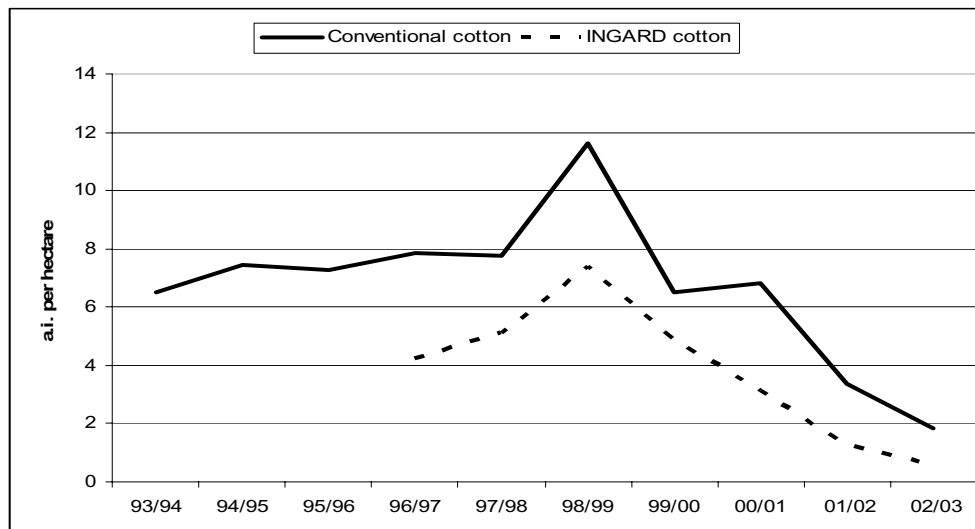
An additional concern with *Bt* cottons is that secondary pests, once suppressed by insecticides applied for *Helicoverpa*, may become significant pests in their own right. The suite of sucking pests (mirids, aphids, stink bugs, cotton stainers etc.) are the group most likely to show such effects. While it is true that sucking pests have become a more significant part of the pest complex in *Bt* crops in some countries (Wilson *et al.* 2004; Wu *et al.* 2002) they have not required additional spraying at levels where the advantage of the *Bt* crop has been significantly eroded. In Australia, an average 60% reduction in sprays applied for *Helicoverpa* was accompanied by no change in sprays for mirids, aphids, mites and thrips (Fitt 2004). Likewise Wu and Guo (2003) report that *Bt* cotton in China help to prevent resurgence of aphid populations. By contrast in the south-eastern USA stink bugs have assumed significant pest status in *Bt* cotton crops (Greene *et al.* 2001).

## IMPACTS OF *Bt* COTTON IN IPM

Commercial use of Ingard cotton varieties in Australia generated an average reduction in pesticide applications for *Helicoverpa* of 56%, with no significant change in pesticide applications for minor pests (Fitt 2004). With Bollgard II varieties now commercialised in the last two years and completely replacing Ingard varieties in the current (2004/05) cotton season, significantly greater reductions compared to conventional cotton have been achieved. Indeed during the 2004/05 season many Bollgard II cotton crops were not sprayed for pests. Anecdotal evidence also shows that researchers now find difficulty in establishing pest populations in field plots for experimental purposes because of the widespread high abundance of predators and parasitoids. As discussed later there have also been simultaneous reductions in pesticide use on conventional cotton over the last 5-6 years, although to a lesser extent.

Ingard and Bollgard II cotton varieties are not perceived as “magic bullets” for pest control in Australia. Instead they are viewed broadly as an opportunity to address environmental concerns about cotton production and more specifically as a foundation to build IPM systems which incorporate a broad range of biological and cultural tactics (Fitt 2000; Wilson *et al.* 2004). The most consistent “winner” from INGARD® technology has been the environment, with reduced pesticide loads.

Coincident with the adoption of *Bt* cotton varieties has been widespread adoption of an IPM approach, supported by a strong extension campaign (Christiansen and Dalton 2002). This is revealed by a significant change in grower attitudes in addition to significant reductions in pesticide use (expressed as active ingredient) on both conventional and transgenic crops (Fig. 1), achieving environmental gains and enhancing future sustainability of the industry.



**Figure 1.** Change in Pesticide Use (active ingredient per hectare) on Australia cotton crops. Significant reductions have been achieved on both conventional and INGARD® cottons over the last 5 years. Source: Cotton Consultants Association Market Audit Survey 2003.

Care must be taken in interpretation of Figure 1 since insecticide use is linked to pest abundance and *Helicoverpa* spp. have been at relatively low densities during the prolonged drought in many cotton areas. Likewise several newer pesticides are active at much lower concentrations than the pesticides they replaced. It also seems unlikely that the reduction in pesticide use on conventional cotton results from the regional impact of *Bt* cotton on *Helicoverpa* abundance since over the period shown the area of *Bt* cotton was limited to a maximum of 30% of the cotton in a region. A number of factors are likely involved here, but one important possibility is that the coincident release of *Bt* cotton and the industry wide extension effort on IPM, allowed many growers to build confidence in the potential for IPM by managing their *Bt* cotton crops. They were able to become more comfortable with seeing a “living” crop, filled with numerous and mostly innocuous or beneficial insects, more attuned the critical importance of managing agronomic inputs, and more willing to work cooperatively with neighbours through the IPM and area wide groups. It will be instructive to view the ongoing management of conventional cotton and the performance and adoption of Bollgard II cotton varieties now that the 30% cap on *Bt* varieties has been lifted. In the 2004/05 season Bollgard II varieties accounted for 70% of the Australian cotton area.

## CONCLUSIONS

IPM systems for future production of many broad acre and horticultural crops will, of necessity, be more complex than the pesticide based systems currently in place. In essence IPM reflects a sound interaction of science and pragmatism to achieve productive, viable and sustainable production systems.

As farming systems change the pest complex will also change. The fundamental role of IPM in reducing pest pressure and insecticide use means that it must continue to evolve. This is particularly so in high value, high input production systems such as cotton. For Australian cotton there seem little doubt that *Bt* cotton varieties have brought considerable gains in management of key pests but more importantly have facilitated a broader recognition and adoption of IPM principles, particularly the importance of natural biological control.

## REFERENCES

- Christiansen, I., and Dalton, B. 2002. Understanding IPM - Industry Attitudes, Practices and Education. In “Proceedings 11<sup>th</sup> Australian Cotton Conference” (D. Swallow, Ed.), pp. 189-196.
- FAO Code of Conduct on Pesticides Definition. 2002. Integrated Pest Management (IPM). FAO, Rome.
- Fitt, G. P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* **34**, 17-52.
- Fitt, G. P. 1994. Cotton pest management: Part 3. An Australian perspective. *Annual Review of Entomology* **39**, 543-562.

- Fitt, G. P. 2000. An Australian approach to IPM in cotton: integrating new technologies to minimise insecticide dependence. *Crop Protection* **19**, 793-800.
- Fitt, G. P. 2003. Deployment and Impact of Transgenic *Bt* cottons in Australia. In "The Economic and Environmental Impacts of Agbiotech: A Global Perspective", (N. G. Kalaitzandonakes, Ed.), pp. 141-164. Kluwer, New York.
- Fitt, G. P. 2004. Implementation and Impact of Transgenic *Bt* cottons in Australia. In "Cotton Production for the New Millennium. Proceedings of the third World Cotton Research Conference, 9-13 March, 2003, Cape Town, South Africa", pp. 371-381. Agricultural Research Council - Institute for Industrial Crops, Pretoria, South Africa.
- Fitt, G. P., Mares, C. L., and Llewellyn, D. J. 1994. Field evaluation and potential impact of transgenic cottons (*Gossypium hirsutum*) in Australia. *Biocontrol Science and Technology* **4**, 535-548.
- Fitt, G. P., Daly, J. C., Mares, C. L., and Olsen, K. 1998. Changing Efficacy of Transgenic *Bt* Cotton - Patterns and Consequences. In "Pest Management - Future Challenges" (M. P. Zalucki, R. A. I. Drew, and G. G. White, Eds.), pp.189-196. University of Qld Press, Brisbane.
- Fitt, G. P., and Wilson, L. J. 2002. Non-Target Effects of *Bt*-cotton: A Case Study from Australia. In "Biotechnology of *Bacillus thuringiensis* and Its Environmental Impact." (R. J. Akhurst, C. E. Beard, and P. A. Hughes Eds.), pp. 175-182. Proceedings of the 4<sup>th</sup> Pacific Rim Conference, CSIRO, Canberra, Australia.
- Glare, T. R., and O'Callaghan, M. 2000. "*Bacillus thuringiensis*: Biology, Ecology and Safety." John Wiley and Sons, Chichester.
- Greene, J. K., Turnipseed, S. G., Sullivan, M. J., and May, O. L. 2001. Treatment thresholds for stink bugs in cotton. *Journal of Economic Entomology* **94**, 403-409.
- Hearn, A., and Bange, M. 2002. SIRATAC and CottonLOGIC: persevering with DSSs in the Australian cotton industry. *Agricultural Systems* **74**, 27-56
- Hearn, A. B., and Fitt, G. P. 1992. Cotton Cropping Systems. In "Field Crop Ecosystems of the World" (C. Pearson, Ed.), pp. 85-142. Elsevier Press, Amsterdam.
- Hilbeck, A., Baumgartner, M., Fried, M. P., and Bigler, F. 1998a. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* **27**, 480-487.
- Hilbeck, A., Moar, W. J., Pusztai-Carey, M., Filippini, A., and Bigler, F. 1998b. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* **27**, 1255-1263.
- Hilbeck, A., Moar, W. J., Pusztai-Carey, M., Filippini, A., and Bigler, F. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. *Entomologia Experimentalis et Applicata* **91**, 305-316.
- Poppy, G. M. 2000. GM crops: environmental risks and non-target effects. *Trends in Plant Sciences* **5**, 4-6.

- Romeis, J., Dutton, A., and Bigler, F. 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *Journal of Insect Physiology* **50**,175-183.
- Schuler, T. H., Denholm, I., Jouanin, L., Clark, S. J., Clark, A. J., and Poppy, G. M. 2001. Population-scale laboratory studies of the effect of transgenic plants on non-target insects *Molecular Ecology* **10**, 1845-1853.
- Schuler, T. H., Denholm, I., Clark, S. J., Neal, S. C., and Poppy, G. M. 2004. Effects of *Bt* plants on the development and survival of the parasitoid *Cotesia plutellae* (Hymenoptera: Braconidae) in susceptible and *Bt*-resistant larvae of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), *Journal of Insect Physiology* **50**, 435-443
- Whitehouse, M. E. A., Wilson, L. J., and Fitt, G. P. 2005. A comparison of arthropod communities in transgenic *Bt* and conventional cotton in Australia. *Environmental Entomology* (in press).
- Wilson, L. J., Mensah, R. K., and Fitt, G. P. 2004. Implementing Integrated Pest Management in Australian Cotton. In "Insect Pest Management: Field and Protected Crops" (A. R. Horowitz, and I. Ishaaya, Eds.), pp. 97-118. Springer, Berlin, Heidelberg, New York.
- Wu, K., Li, W., Feng, H., and Guo, Y. 2002. Seasonal abundance of the mirids, *Lybux lucorum* and *Adelphocoris* spp. (Hemiptera: Miridae) on *Bt* cotton in northern China. *Crop Protection* **21**, 997-1002.
- Wu, K., and Guo, Y. 2003. Influences of *Bacillus thuringiensis* cotton planting on population dynamics of the cotton aphid, *Aphis gossypii* Glover, in northern China. *Environmental Entomology* **32**, 312-318.