# 2169 Global Resistance Strategies for Cotton: Biotechnology and Pesticides

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## Abstract

**Transgenic crops offer great promise for improved pest management and the reduction of impacts of cropping on the environment. However, to provide maximum long-term returns from these crops, it is essential that resistance be delayed in the targeted pests. In theory, the most promising ways to manage resistance will be to maintain "refuges" of completely susceptible plants within the cropping system, especially when the transgenic plants express the insecticidal toxin at a very high level (a "high dose") or, better yet, when two insecticidal genes are expressed ("pyramided") in the same plant. Refuges provide susceptible insects to aid in dilution of resistance. In theory, pyramided cultivars will delay resistance longer and with smaller refuges than comparable single toxin cultivars. Across the range of environments where Bt cotton has now been deployed resistance management strategies of varying levels of rigour have been deployed. In some cases structured refuges and other components provide a conservative resistance strategy while in other cases resistance management is reliant on natural refuges and little other explicit management is used. Here I explore the basis of resistance management strategies for Bt cotton, provide and update on results to date and speculate on likely future changes.**

## INTRODUCTION

The evolution of resistance in target pests is clearly one of the major challenges to the long term sustainable use of Bt cottons. In all environments where Bt cotton is now grown it is likely that some level of resistance management will be required on the part of growers (Roush, 1994), although the level of responsibility will depend on the situation. Resistance management has a long tradition with conventional pesticides although in almost all cases management strategies have not been implemented until resistance was easily measured in field populations, but which time the situation was almost lost. Although many of the approaches to resistance management for GM crops follow a broadly similar theoretical basis to those used with pesticides, it has been possible with Bt technology to implement pre- emptive resistance management strategies and so maximise options to achieve sustainable use. The following discussion explores the underlying basis of Bt cotton resistance risks and management strategies targeted mostly at the Helicoverpa/ Heliothis complex.

## RESISTANCE RISKS and OPTIONS

Resistance to conventional Bt sprays has evolved in field populations of the diamond back moth, *Plutella xylostella* through the excessive use of Bt formulations in horticultural crops (Tabashnik, 1994a). There are now numerous cases of laboratory colonies of *Helicoverpa* species selected for resistance to Cry proteins (Akhurst *et al.* 2003; Tabashnik *et al.,* 2003). As these are the most widespread targets for Bt cotton technology they and similar Bt sensitive Lepidopteran pests (e.g. pink bollworm *Pectinophora gossypiella,* rough bollworm *Earias spp.*) represent a significant threat. Strategies for the management of Bt cotton have been exhaustively explored with population genetic models and innovative methods to modify the selection environment imposed by Bt cotton on the pest (Tabashnik, 1994b; Tabashnik *et al.,* 2004; Caprio, 1994; Gould, 1994, 1998; Roush, 1994, 1996, 1997, 1998). Although the debate concerning the appropriateness of certain strategies continue (Vacher *et al.,* 2004; Tabashnik *et al.,* 2004), the overwhelming body of evidence supports the use of a refuge strategy combined with the highest possible efficacy of the Bt plants (Tabashnik *et al.,* 2003). High efficacy can be achieved by high expression of single proteins, use of multiple proteins (much preferred) or through combination with other HPR traits. In terms of requirements for industry or individual farmers the main questions centre on 1) how best to deploy and manage refuge crops (which may not necessarily be cotton) in ways appropriate for the pest complex to be managed, 2) the farming system in which cotton is grown and 3) the economic and educational circumstances of farmers. Strategies appropriate or workable for large capital intensive farmers may not be workable for small-holders. Some attempts are being made to rationalize these constraints (e.g. Fitt *et al.,* 2004).

In contrast to much of the history of pesticide resistance in cotton production systems, transgenic cotton provides a real opportunity to implement pre-emptive management strategies as part of a technology package built around IPM where the risk of resistance is significant. Resistance is not an inevitable consequence of the deployment of Bt cottons, but susceptibility to Bt proteins should be viewed as a valuable natural resource to be managed as carefully as the soil and water upon which production depends directly. Defining the risk of resistance requires a sound understanding of the biology and ecology of the system, while defining the components of a resistance management strategy requires an ordered process that engages all stakeholders to identify a workable response.

Insect resistance to Bt toxins is most often related to recessive genes or accumulation of several minor genes (Gahan *et al.,* 2001; Mahon *et al.* 2007). Some cases of semi-dominant Bt resistance have also been identified. The use of refuges essentially allows part of the pest population to be unexposed to Bt protein by developing in non-Bt cotton, or some other host plant, and for the insects produced in the refuge to mate with any surviving individuals from Bt crops that may carry resistance genes. Prior to selection the vast majority of resistance alleles in an equilibrium population will be carried in the heterozygous condition, and so it is the survival of heterozygotes which essentially drives the rate of evolution of resistance.

Whether heterozygotes are able to survive on Bt crops will depend on the dose of Bt toxin present, and hence the selection pressure imposed. In the case of *H. virescens* and Bollgard cotton in the USA, efficacy of Cry IAc protein truly represents a high dose. Heterozygotes would likely be killed by the plants and, consequently, any survivors in the field would be homozygous for resistance alleles. This is not the case in Australia, China and India, where *H. armigera* is the dominant target and is naturally more tolerant of Cry IAc as is H. zea in the USA. Most of the single gene Cry1Ac expressing varieties show marked declines in efficacy through the growing season (Daly and Fitt 1998, Fitt *et al.,* 1998; Olsen and Daly 2000; Wu and Guo 2005). In this case the survivors are essentially Bt susceptible individuals.

There seems little doubt that pyramided two toxin varieties are an important next step for sustainable resistance management of Bt cottons (Roush 1998). Bollgard II varieties expressing both Cry IAc and Cry 2 Ab have been released in the USA and Australia, although in the US they now coexist alongside single gene Cry1Ac varieties, whereas in Australia, the single gene options were removed within one season of Bollgard II being commercialised. This rapid transition was considered critical to safeguard the longevity of the Cry1Ac/Cry2Ab pyramided product by removing selection on Cry 1Ac alone.

A central question for resistance management is whether a structured refuge is required (i.e. explicit planting of a non-Bt crop) and, if so, how large should it be and how should it be deployed in space or time. In some environments naturally occurring refuges, usually other crops grown in the farming system, provide adequate dilution. The need for structured or unstructured refuges needs to be determined on a case-by-case basis and requires significant biological information about the system. In Australia and the USA a structured refuge strategy has been deployed, whereas in China, India and smallholder systems in Africa resistance management relies on the effectiveness of natural refuges. In China cotton makes up only 10% of the cropping area and maize covers a huge 55% (Wu and Guo 2005). Wu *et al.* (2002, 2004) demonstrated that maize can provide an adequate refuge, but concerns about the risk of resistance remain because of past experience with the rapid emergence of pesticide resistance even though non-cotton crops are largely unsprayed. Similar evidence is available

for India where diverse agricultural systems accompany cotton (Qaim, 2003), although quantitative studies of refuge crop productivity are not yet available. Strategies that rely on natural refuges are easy to deploy for small-holders but suffer from the weakness that other farmers often bear the responsibility for Bt resistance management but do not share in the benefit and may remove that "service" at any time. It is critically important that individual countries research and adopt a management strategy appropriate for their environment and cropping system and not simply adopt strategies applied elsewhere. The specific ecological features and assumptions which dictate the need for those strategies may not apply in all countries, particularly where small-holder production systems result in a diverse mosaic of cropping and alternative hosts for the target pests. Again a "case-by-case" assessment of management needs is critical.

Where structured refuges are required, different methods can be used to deploy the refuge in space through the use of seed mixes, intermingled blocks or strips of non-transgenic and Bt transgenic plants (Roush *et al.,* 1998). Appropriateness of different methods is determined by the biology of the pest involved, particularly the mobility of immatures (which could subvert the value of seed mixtures) and of adults (Dillon *et al.,* 1998). An understanding of adult movement is critical to determine the minimal requirements for area and spatial arrangement of refugia to ensure generation of sufficient unselected moths and the likelihood of random mating and gene flow within the local population.

The high dose - refuge strategy makes three key assumptions (Roush 1997, Gould 1998):

- a. Resistance to Bt is likely to be functionally recessive;
- b. Resistance genes are at low frequency in natural populations;
- c. Random mating occurs among individuals from refuges and Bt crops

These assumptions seem reasonable based on current knowledge of Bt resistance in field populations of *Plutella* and laboratory selection of resistance in *Heliothis/ Helicoverpa* populations. Baseline levels of Bt susceptibility have been defined in many countries (Forrester *et al.,* 1993; Stone and Sims, 1993; Sims *et al.,* 1996; Kranthi *et al.,* 1999; Luttrell *et al.,* 7 1999; Wu *et al.,* 1999, Babu *et al.,* 2002; Liao *et al.,* 2002; Jalali *et al.,* 2004;). In most cases there is substantial geographic variation in tolerance with as a much as a ten-fold variation in LC50. Such studies provide baseline information on tolerance and often generate a discriminating dose for use in monitoring studies. Few studies have directly estimated background frequencies of Bt resistance alleles, although some estimates are now emerging. Gould *et al.* (1997) were the first to provide an estimate of background frequencies of Bt resistance in H. zea and indicated that starting frequencies may be higher than the $10^{-4}$ assumed by most simulation modelling studies for natural populations. Li *et al.* (2004) provide estimates for *H. armigera* in China of 0.00107 and 0.00059 – at or below $10^{-3}$.

Using an F2 screen (Andow and Alstad 1998), researchers in Australia (Mahon *et al.* 2007; Downes et al in press) have confirmed that Cry1Ac resistance is at very low frequency [$<10^{-4}$] but have established the background frequency of an allele for Cry2Ab resistance which is present at the surprisingly high frequency of 0.004 in field populations well above the levels normally assumed in simulation models. Despite these caveats and concerns, there is substantial field evidence that resistance to Bt proteins has not evolved in any situation as a result of deployment of a Bt crop (Tabashnik *et al.* 2003, Bates *et al.* 2005). That resistance has not occurred to date is likely due to a range of factors including significant fitness costs associated with most cases of Bt resistance (but see Mahon *et al.* 2007), and the dilution afforded by natural or structured refuges combined with high dose and low initial gene frequencies for Bt resistance alleles. This evidence provides strong support for the real opportunity and robustness provided by pre-emptive management strategies (Fitt 2000, Tabashnik *et al.,* 2003).

Another issue with potential impact on the stability of resistance management strategies for Bt cotton is the possible deployment of the same Bt genes in other crops. Clear mechanisms are required at national levels to address the potential conflicts of industry need and resistance risk posed by such situations. Fitt (1997) provides a protocol for considering such an issue, while Fitt *et al.* (2004) consider similar issues of conflicting use of Cry 1A proteins in maize and cotton in Kenya.

## Current Practice for Bt cotton resistance management.

### Australia

In Australia the principal targets for Bt cotton are *Helicoverpa armigera* and *H. punctigera.* The possibility of these insects developing resistance to Bt is a real concern, particularly for H. armigera, which has consistently developed resistance to synthetic pesticides (Forrester *et al.,* 1993; Fitt, 1989, 1994). The cotton industry in Australia has been proactive in addressing resistance concerns, emphasised by programs since 1984 to manage resistance to pyrethroids and other insecticides (Roush and Daly 1990, Forrester *et al.* 1993). The industry has established a Transgenic and Insecticide Management Strategy (TIMS) committee with representatives of growers, consultants, researchers, seed companies and chemical industries, to devise, endorse and implement strategies. The Australian strategy (Fitt, 2004) targets H. armigera and is based on the use of large refugia combined with a defined planting window, a requirement for mandatory cultivation of Bt crops after harvest, control of volunteer Bt plants in fields, defined spray thresholds for other pests and a monitoring program for Bt resistance levels in field populations (Fitt, 2004).

All elements of the management plan are included in a technology contract and are part of the seed label (**http://www.cotton.crc.org.au/Assets/PDFFiles/IMPInga.pdf** ). Growers have five refuge options (sprayed and unsprayed conventional cotton, unsprayed, corn, sorghum or pigeon pea) which have been defined on the basis of ongoing research that quantifies the value of different options in generating moths (e.g. Fitt and Tann, 1996).

Refuge crops must not be treated with Bt sprays and must be no more than 2 km from the Bt crop. An additional element of conservatism was applied in Australian by the imposition of a phased introduction of single gene (Cry IAc) Bt varieties (marketed as INGARD), and a cap on the area of Ingard varieties at 30% of total cotton (Fitt, 2004). In their first year INGARD® varieties were grown on 30,000 ha representing about 8% of the total cotton area in that year. After that the area increased in 5% increments each year up to the 30% cap, which was reached in the 2000/2001 season. BOLLGARD II™ varieties with two Bt genes (Cry1Ac, Cry2Ab) were first approved for commercial use in 2002/03 when they occupied only 5,000 ha. The two gene varieties provide much better efficacy and hence even greater reduction in pesticide requirement, but their main purpose is to provide much greater resilience against the risk of resistance (Roush, 1998). From the start the Australian industry aimed for a rapid transition to BOLLGARD II™, with INGARD® varieties withdrawn after the 2003/04 season. From 2004/05 onwards only BOLLGARD II™ varieties are available, so removing the risk of mixed deployment of single gene and two gene varieties. From 2004/05 onwards the cap of Bt cotton has also been lifted and in the most recent 2006/07 season they occupied about 86% of the total cotton area. It is noteworthy that **all other components** of the resistance management strategy remain unchanged, so maintaining a conservative resistance management approach.

Minor Lepidopterous pests may also pose some risk of evolving resistance to the Bt toxin in transgenic cotton. In Australia, these species include *Earias huegeli, Crocidosema plebiana, Pectinophora scutigera* and *Buccalatrix gossypii*. All four are specialists on Malvaceous plants and occur regularly in commercial cotton. The potential for alternative non-transgenic hosts for these insects is still under investigation, but non-transgenic cotton is currently being used to avoid strong selection.

### USA/ Mexico/ Argentina

In the US a refuge strategy was also deployed for single gene Bt varieties. Growers had four options to choose from to manage resistance (U.S. EPA, 2001):

1. A 20% external sprayed cotton refuge. The 20% conventional cotton acres can be treated with any registered cotton crop protection products, excluding foliar B.t.k. products. Growers are required to plant their 20% refuge within 1 linear mile (preferably 1/2 mile) of Bt cotton.

2. A 5% external structured unsprayed refuge, which requires 5 acres of conventional cotton for every 95 acres of Bt cotton be planted within 1/2 linear mile of any Bt cotton varieties. This block of cotton must be at least 150 feet wide and must be managed (fertility, weed control, and insect management) in a similar fashion to the Bt cotton. This option is an important part of seed production for Bt crops.

3. A 5% unsprayed embedded refuge, where the refuge occurs as a contiguous block within the Bt field. For large blocks, the 5% embedded refuge can occur in multiple blocks. However, for irregular shaped or smaller fields, neighboring fields and farms can combine their refuge provided that it is at least 150 feet wide, and occurs within 1 square mile of the Bt cotton. A grower can treat his refuge with any approved lepidopteran control products (excluding Foliar B.t.k. sprays) provided he treats the Bt cotton as well. The refuge cannot be treated independently of the Bt cotton. For areas that are affected only by the Pink Bollworm, the embedded refuge can be planted in the form of single rows, provided it meets the 5% requirement.

4. A community refuge program. A group of growers can opt to use a community refuge option for the 20% sprayed or 5% unsprayed refuge options. This option allows multiple growers in larger cotton producing regions to meet their refuge requirements collectively. This is an attempt to offer the growers more flexibility in their production systems, while still providing protection against resistance development.

A similar approach has been adopted in Mexico and Argentina where a 20% sprayed non-Bt cotton refuge crop is required. With both Bollgard (Cry 1Ac) and Bollgard II (Cry 1Ac/Cry 2Ab) varieties now available in the US the EPA recently approved the removal of a structured refuge requirement only for Bollgard II in the cotton belt east of Texas. This change was based on a compelling data set which clearly showed the value of natural refuges in the reliable multi- cropping regions of the eastern USA. Such approaches may not be applicable in more climatically variable environments where the presence of natural refuge and other crop hosts can vary enormously.

## China

Bt cotton was first grown in China (Mainland) in 1998, with the main target pest being *H. armigera*. Since then areas have increased dramatically to some 2.5 million ha across several provinces of north-western China (Mainland). Cotton is grown largely by small-holders and for that reason no explicit resistance management strategy has been implemented. Chinese researchers from CAAS (Chinese Academy of Agricultural Sciences) and several other institutes have accumulated a considerable database of information on the population dynamics of *H. armigera*. It is argued that the small plot size and diversity of cropping with other H. armigera hosts associated with cotton provides sufficient natural refuge that selection for BT resistance is diluted. Wu *et al.* (2002) show that in the small-scale mixed farming regions of northern China (Mainland), H. armigera develop on cotton, maize, soybean and peanut and these will all contribute to the natural refuge. There was no attempt to deploy other management components and the argument for the effectiveness of natural refuges begs the question of why pesticide resistance has been such a difficult issue for Chinese cotton production.

Recently Wu *et al.* (2004) produced a quantitative study to evaluate the value of refuge crops in China. They show that maize, which is historically the most abundant crop and grown over 70% of agricultural lands, provides an adequate refuge for the third and fourth generations of *H. armigera* in that system. Whether this is related to selective mortality on Chinese Bt cotton is unknown and the long-term sustainability of Bt cotton in China is unclear, although Li *et al.* (2004b) provide sound evidence that the planting of Bt cotton over large areas in China for 5 years has not resulted in a shift in the frequency of Bt resistance alleles in the *H. armigera* population. Field trials indicate that both soybean and peanut can supply refuges for the second and third generations of cotton bollworm. As the most abundant crop, maize is planted widely but has a long sowing date, from April to June in much of the eastern cotton growing regions (Wu et al 2004). The cropping system consisting of wheat, soybean, peanut, maize and Bt cotton appears to provide adequate refuge for *H. armigera* throughout the cotton season. Consequently a strategy reliant on natural refuge has been recommended for much of the cotton region of China (Wu *et al.* 2002, Wu and Guo 2005). Ongoing studies by Wu et al (2006) show that background frequencies of Cry1Ac resistance have not changed in China in the last ten years.

### South Africa

Bt cotton was first introduced in South Africa in 1997/98, with adoption by large-scale farmers growing irrigated and dryland cotton and by small-holder growing only dryland crops. In both cases, Monsanto and government agencies urge the use of a 20% non-Bt cotton refuge with Bt cotton. Adherence to this strategy seems to be poor among small-holders, many of whom may have difficulty grasping the concept (Bennett *et al.,* 2003).

## CONCLUSIONS

The fundamentals of resistance management have been learned through bitter experience with chemical insecticides. One of the most important of these lessons is that implementation of a resistance management program simultaneously with commercial release is essential for success. Although there are many unknown variables, the opportunities for widespread deployment and environmental benefits from Bt cottons are now a reality. Across all growing regions pre- emptive resistance management strategies of some form have been implemented, although there is considerable variation in their rigour. Nonetheless there is no evidence of significant change in Bt resistance gene frequencies and certainly no evidence of field resistance in any country after up to 10 years of deployment. This fact is likely due to the strategies in place, although in some countries there are legitimate ongoing concerns that resistance may well emerge.

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