

# Insect Resistance to Bt Toxins in GM Insect Resistant Crops

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## Introduction

In March 2010 Monsanto announced its GM Bt cotton Bollgard I had hit significant problems in the Indian State of Gujarat: the insects the GM cotton was supposed to kill had developed resistance to the GM toxin produced by the plants.<sup>i</sup> As a result more insecticides were being applied to control the pink bollworm, which can be a serious pest in cotton.

This was the first major breakdown in Bt resistance anywhere in the world, although not the first case in which the breakdown of other insect resistance had been recorded in the field. Pesticide costs on infested crops have been reported to rise by a nearly a third.<sup>ii</sup>

Reports from the US in 2011 suggest a similar breakdown may be taking place in Bt maize.

This briefing looks at the development of Bt resistance in the field and how these breakdowns have occurred in India and elsewhere. It also looks at some inherent difficulties in getting GM Bt technology to work effectively and suggests alternative to GM and pesticides.

## Bt cotton in India

Bt cotton was approved for cultivation in India from 2002. The Maharashtra Hybrid Seeds Company Ltd (known as MAHYCO and owned by Monsanto) and RASI Seeds Pvt Ltd (under license from Monsanto) sold the GM seed to farmers under the brand name Bollgard. By 2010 industry estimates put the area under Bt cotton in India at 9.4 million hectares.<sup>iii</sup>

The introduction of Bt cotton was highly controversial from the start. MAHYCO was accused of “aggressive and even false marketing of Bt Cotton” to persuade farmers to adopt Bt cotton.<sup>iv</sup> Many farmers persuaded by this high pressure selling found the GM Bt varieties did not perform very well. The poor performance of expensive Bt cotton varieties plunged some poor farmers further into debt. Large debts have led many of the poorest farmers in India to commit suicide, and the failure of Bt cotton crops may have contributed to this serious problem.<sup>v</sup>

## Bt cotton

Bt cotton is genetically modified to produce a protein toxic to caterpillars of cotton pests, such as the moth (Lipidoptera) species pink bollworm (*Pectinophora gossypiella*) and cotton bollworm (*Helicoverpa armigera*). The caterpillars prefer to feed on the ovaries and rinds of the cotton bolls, which means they can have a major impact of yield of cotton if infestation levels are high (ie, above one caterpillar per boll).

Bt toxins attach themselves to the walls of the insect gut, and eventually this causes the gut wall to leak toxins into the rest of the insect, feeding stops and death soon follows.

The first Bt cotton varieties in India were genetically modified to produce a Bt toxin known as Cry1Ac. In early Bt crops the inserted gene from soil bacteria proved unstable and did not work, so researchers had to replace it with a synthetic copy.<sup>vi</sup> Researchers had to produce a synthetic version of the gene to overcome this problem.

## Bt cotton “pro-poor”?

Amid the hyperbole surrounding GM crops is the oft-repeated claim that GM crops, and Bt cotton in particular, are hugely beneficial to poor farmers. Some commentators have referred to the “astonishing success”<sup>vii</sup> of GM crops, and others that GM is a technology from which “Africa could benefit most”.<sup>viii</sup> Academics have also made claims for yield increases and financial benefits for poor farmers from Bt crops.<sup>ix and x</sup>

However portrayals of Bt technology as “pro-poor” have been challenged, including by academic Dominic Glover for being “*selective and misleading*” for ignoring that “*performance and impacts of GM crops have in fact been highly variable, socio-economically differentiated and contingent on a range of agronomic, socio-economic and institutional factors*”. Glover’s review of 46 peer reviewed studies on the performance of Bt cotton in China, India and S Africa<sup>xi</sup> concluded that:

*“Because some academics, and behind them commentators, policy analysts and politicians, have been too eager to draw general conclusions from insufficient evidence, a misleading impression has been created in public and policy discourse that Bt cotton has already proved its value as part of a sustainable, productive agricultural livelihood for poor farmers in China, India and South Africa. That conclusion is not well supported by the available evidence. Bt cotton is evidently a functional technology that can be advantageous for some farmers in certain circumstances, but it is still not conspicuously ‘pro-poor’.*

*“The manner in which the impacts of Bt cotton have been evaluated, interpreted and represented has therefore done a disservice to the public and policy debates that surround the benefits, risks, social purposes, human values and trade-offs involved in pursuing the GM route towards crop improvement and attacking hunger and poverty. There is a risk that bad policy decisions will be made, on the basis of insufficient evidence and inappropriate interpretation of such data as is available.”*

### **Getting Bt plants to work**

To be successful GM insecticide traits must produce lethal levels of the insect toxin throughout the period the plant is vulnerable to pest attack in the parts of the plant where such attacks will do most damage if allowed to progress unhindered.

Yet several studies have found that the CryA1c toxin is not uniformly expressed at a high level throughout the cotton plant or through the growing season. This leaves the plant more vulnerable to pest attack. Crucially it also means it is more likely pests *will* develop resistance. Since pests are exposed to lower, sub-lethal, levels of the toxin, the less susceptible individuals can survive this dose, go on to breed and eventually become dominant. At this point the pest population would be resistant and effectiveness of GM Bt cotton with Cry A1c toxin would be lost.

#### **Bt toxins**

Bt is a generic name given to a group of toxins produced by the soil bacterium *Bacillus thuringiensis*. Cry1Ac is one such toxin. There are many more. Each toxin is most effective against one group of insects – either *Lepidoptera* (moths and butterflies) or *Colleoptera* (beetles).

Although these toxins are not sufficiently broad spectrum to kill all insects, they may impact on non-target species in the same group that come into contact with the toxin via their food supply<sup>xii and xiii</sup> or, in some cases, unrelated species.<sup>xiv and xv</sup>

Bt toxins sprayed externally on the crop are classed as insecticides, and yet the large amounts constantly produced by GM Bt plants and released into the soil are not.

Researchers in India looked at the expression of Cry1Ac in eight different varieties of GM Bt cotton.<sup>xvi</sup> They found expression, “[I]n the boll-rind, square bud and ovary of flowers was clearly inadequate to confer full protection to the fruiting parts,” and that, “[I]ncreasing levels of *Helicoverpa armigera* survival were correlated with the toxin levels decreasing below 1.8 µg/g in the plant parts.”

They also found that expression of the toxin “decreased consistently as the plant aged”.

The most important parts of the plant in terms of yield were not adequately protected against major pest because insufficient toxin was present to kill the target pest from the start and the levels

reduced as the plant grew. To deal with this problem the research team recommended that biopesticides should be used on cotton 90-100 days after sowing and:

*“Subsequently, any of the conventional insecticides such as endosulfan, thiodicarb, uinalphos and chlorpyrifos, or new molecules such as spinosad, emamectin benzoate, novaluron or Indoxacarb can be used at economic threshold levels of one larva per plant.”*

To maintain control of a major pest cotton farmers are being sold expensive Bt seed (Bollgard 1), which then has to be used in combination with bio and/or chemical insecticides to ensure that control is sufficient and pest do not develop resistance to Bt toxins.

More recently other researchers have investigated the expression of Bt toxin in GM plants and found abnormalities in the plants that expressed Cry1A including<sup>xvii</sup>:

- no flowering.
- flowers dropping off.
- failure to set seed.

The researchers concluded, “[H]igh levels of the Cry1Ac protein are selected against during regeneration of transformed events,” and, “[D]eveloping transgenics that express optimal levels of cry gene is not routine, due to the detrimental effects of high expression of these genes.” They recommended that in future genetic modification should target chloroplast proteins.

These research findings may go a long at to explaining why Indian farmers had problems with Bt cotton when it was first introduced.

### **Bt resistance in Gujarat**

Monsanto’s statement said pink bollworms resistant to Cry1Ac were, “[C]onfirmed in four districts in Gujarat – Amreli, Bhavnagar, Junagarh and Rajkot.”<sup>xviii</sup> The company attempted to blame the emerging resistance on illegal Bt cotton cultivation in Gujarat prior to its approval by the Indian Government.

The statement was tantamount to closing the door after the horse has bolted:

*“The findings in Gujarat are an important reminder to Indian farmers. When using Bt cotton products it is essential to regularly monitor and scout fields throughout the season for insect presence and plant appropriate non-Bt refuge. Furthermore, farmers must adopt measures such as need-based application of insecticide sprays during the crop season, and properly manage crop residues and unopened bolls after harvest. Examples of such practices include tillage and cattle grazing to minimize the survival and spread of pink bollworm.”*

The procedures described above should have been common practice, and farmers should have been given the necessary information and training before they were first sold Bollgard 1 Bt cotton. It appears that sales and profit took precedence over education. The Indian press reports that between 2002 to 2009 yields fell and pesticide costs increased.<sup>xix</sup>

### **Resistance to Bt elsewhere**

Although the development of field resistance in Gujarat is the worst example so far, four other instances confirm different pests developing resistance to Bt toxins in the field in other parts of the world:

- *Helicoverpa zea* (Cotton Bollworm) in Bt cotton in the US.<sup>xx</sup>
- *Busseola fusca* (stem borer) in Bt Maize in South Africa.<sup>xxi</sup>
- *Spodoptera frugiperda* (fall armyworm) in Bt maize in Puerto Rico.<sup>xxii</sup>
- *Diabrotica virgifera virgifera* (western corn rootworm) Bt Maize in the US.<sup>xxiii</sup>

The US breakdown in western corn rootworm began in Iowa and may have spread already to Illinois, Minnesota<sup>xxiv</sup> and beyond to up to six states. It has the potential to become a serious breakdown.

Researchers in Iowa found field evolved resistance in western corn rootworm in maize. This the first instance of a beetle (*Coleopteran*) pest evolving resistance to a Bt toxin, as opposed to previous cases involving moth species (*Lepidopteran*).

The Iowa researchers reported, “[S]evere rootworm feeding injury to Bt maize contained populations of western corn rootworm that displayed significantly higher survival on Cry3Bb1 maize.”<sup>xxv</sup> Growing the same GM maize variety on the same field for at least three years consecutively was a contributing factor, and the authors concluded:

*“Even with resistance management plans in place, sole reliance on Bt crops for management of agriculture pests will likely hasten the evolution of resistance in some cases, thereby diminishing the benefits that these crops provide.”*

Other contributing factors are the low level of compliance to provide non-GM refuges, which research suggested ranged between 66% and 78% in 2008,<sup>xxvi</sup> and that the Cry3Bb1 toxin is not produced at a high enough concentrations to cause high pest mortality.<sup>xxvii</sup>

In the neighbouring state of Illinois, similar patterns of resistance breakdown haven been reported, although so far not confirmed by the laboratory testing of insects collected in the field. The University of Illinois Extension service report “severe” root damage caused by the corn rootworm in maize crops genetically modified with the same Cry3Bb1 Bt protein which has lost its potency in Iowa.<sup>xxviii</sup> Damage has even occurred when the Bt proteins Cry34Ab1/Cry35Ab1 are “pyramided” in plants (see below).

Tabashnik et al<sup>xxix</sup> reported the first case of field evolved resistance to Bt in the major cotton pest *Helicoverpa zea* (cotton bollworm), although other cotton pests monitored did not exhibit any significant changes in the presence of resistance genes. The research team found the presence of resistance genes in wild populations increased significantly after the introduction of Bt cotton with Cry1Ac present, clearly suggesting that there was selection pressure for that gene.

However the resistance has not so far caused any control breakdowns. The authors put this down to:

- Resistance in Bollworm still being relatively rare in the pest population.
- Insecticides are used as a back up to Bt to augment bollworm control.
- Pest mortality remains at 48-60% despite the high level of resistance.
- Biotech companies have received approval to “pyramid” several Bt genes into the same variety, each aimed at killing the same pest.
- Refuges have been effective at preventing resistance genes becoming established.

### **Fighting the tide**

The biotech corporations selling Bt crops (cotton and maize) require farmers growing their seed to adopt a high-dose refuge (HDR) strategy, which aims to prevent individuals with resistance to the Bt toxins becoming dominant in pest population. This is a legal obligation for farmers insisted upon by the US Environmental Protection Agency.

The strategy has two prongs:

- Ensuring insects receive lethal doses of Bt toxin (at levels up to 25 times the lethal dose).
- Providing 20% of the crop areas as non-GM refuges in order to ensure non-resistant insects are available to breed with any individuals who survive this dose. As the resistance gene is not dominant, mating with a non-resistant adult means the recessive resistance gene is not expressed in the offspring, so they gain no advantage from carrying it. Non-GM

crops grown nearby may also serve as a refuge and help prevent resistance developing.

However there is evidence that there is a significant level of non-compliance with refuge provision.<sup>xxx</sup> In some US states or counties GM Bt crops are dominating production to such an extent that non-GM crops are less than 20% of the area sown, so insufficient to act as a refuge. For instance in 2010 Bt cotton was 90% of crops grown in Tennessee. Bt maize crops are less dominant. Iowa, at 76% BT maize, was the only state close to 80% of planting in 2010,<sup>xxxi</sup> although locally the percentage may be much higher. Bt maize uptake is predicted to rise in the US,<sup>xxxii</sup> and there is concern that non-compliance with refuge rules will lead to more resistance developing.

The strategy of pyramiding Bt genes to increase the chance of killing the target pest may also fail in the longer term. One laboratory-based research project looking at levels of resistance to Cry1Ac and Cry2Ab Bt toxins in cotton reported, “[L]aboratory selection of pink bollworm with Cry2Ab caused up to 420-fold cross resistance to Cry1Ac as well as 240-fold resistance to Cry2Ab.” However they concluded:

*“[T]he asymmetrical cross-resistance seen here does not threaten the efficacy of pyramided Bt cotton against pink bollworm,”* and recommended that, *“(I)ncorporating the potential effects of such cross-resistance in resistance management plans may help to sustain the efficacy of pyramided Bt crops.”*<sup>xxxiii</sup>

#### **Pyramided and stacked GM traits**

These two terms refer to crop varieties with more than one GM trait present:

- Pyramiding is when two or more Bt genes aimed at the same pest are present in order to slow pest resistance developing. Each trait will produce a Bt toxin acting on different parts of the insect gut, which increases the chances of the pest being killed.
- Stacking usually refers to multiple GM traits aimed at different pests or different herbicide tolerances or combinations thereof.

Yet monitoring of resistance genes in Bt cotton in Australia found:

*“[T]he frequency of cry2Ab resistance alleles in populations from cropping areas is 8-fold higher than that found for populations from non-cropping regions. This report of field evolved resistance to a protein in a dual-toxin Bt-crop has precisely fulfilled the intended function of monitoring for resistance; namely, to provide an early warning of increases in frequencies that may lead to potential failures of the transgenic technology. Furthermore, it demonstrates that pyramids are not ‘bullet proof’ and that rapid evolution to Bt toxins in the Cry2 class is possible.”*<sup>xxxiv</sup>

#### **Other GM approaches and their limitations**

Monsanto and Dow AgroSciences have been granted US regulatory approval for SMARTSTAX™ maize, which contains six Bt toxin genes aiming to deal with several maize pests and as a safeguard against resistance developing. This maize also contains two GM herbicide tolerance traits, (for glyphosate/Roundup and glufosinate ammonium/Liberty), again to provide insurance against resistance in weeds.

The impact of directing so much of the plants’ resources into producing six insect toxins when there may be no pest present could have an impact on yield (known as “yield dag”<sup>xxxv</sup>). Results from Iowa State University maize performance trials showed Smartstax performed less well than both conventional and other GM varieties – the mean yield was 5.75% less than the district average.<sup>xxxvi</sup>

The US policy on the provision of non-GM refuges in GM crops to prevent resistance developing is changing with the introduction of Optimum® AcreMax™ by Pioneer Hybrid, a mixture of seeds in

which 10% do not have the Bt trait for controlling rootworm. This 10% aims, in theory, “[T]o serve as a rootworm refuge.”<sup>xxxvii</sup> The company says this allows refuges for other pests to be further from the crop to make operations for farmers simpler. However some entomologists are not sure the “refuge in a bag” approach will work.<sup>xxxviii</sup>

*“Our results also indicate that deployment of a seed mix refuge with the currently available toxins may present some risks for Diabrotica spp. resistance management... The advantages of a seed mix refuge are convenience to growers (ease of planting, compliance) and increased adult proximity of adults upon emergence. These are offset by the two main disadvantages of a seed mix refuge, namely the potential for larval movement between refuge and Bt-RW plants that can reduce the number of susceptible beetles while increasing the number of potential heterozygotes, and exposure of later-instar larvae to sublethal doses of Bt toxin. Future research is needed to quantify the sublethal dose received by larvae that move between refuge and Bt plants and to determine when and where during the larval life stages sublethal exposures are likely to occur.”*

Purdue University puts it this way:

*“The concern with refuge-in-a-bag, or seed mixes, has always been sub-lethal exposure with toxic plants and non-toxic plants standing side-by-side... You could have a young corn rootworm beetle larva emerge, feed on a toxic plant but not die, and then move over to a non-toxic plant and feed until reaching adulthood. The larva now has sub-lethal exposure to Bt. That's one of the ways that resistance can develop in an insect population more rapidly.*

*“It's that old adage that whatever doesn't kill you makes you stronger. We could be giving those larvae selective advantage in the long term. That was one of the reasons this technology wasn't embraced initially.”<sup>xxxix</sup>*

## Conclusion

Other major outbreaks of resistance in pests of cotton and maize have so far been avoided, but the experiences in Gujarat and the US show this cannot be guaranteed to continue. There is now growing evidence that resistance genes are increasing in wild populations of cotton pests even when two Bt genes are used (for example in Bollgard 2 cotton). The situation in the Bt maize crops in Iowa has the potential to develop along similar lines.

Bt toxin is an insecticide (albeit less broad spectrum than most chemical products), and Bt plants should be licensed as such, as it is when Bt is applied in a spray. GM Bt technology is a continuation of the pesticides arms race farmers have been persuaded to join since the 1950s.

Experience of Bt crops shows that chemical insecticides are still be required to protect the crop against the target pest or secondary pests which develop in Bt crops. There is now real concern that even this is becoming less effective, possibly due to poor compliance with refuges combined with pests receiving sub-lethal doses of the toxin. The effectiveness of the easy option “refuge in the bag” is already being questioned by entomologists and may turn out to be a short-term stop-gap in the evolution of Bt resistance among insect pests.

In the long-term agroecological approaches to pest control should provide a adequate level of control based on long rotations, diversity of crops, diversity of crop varieties, crops breaks, healthy soil, and the presence of a healthy predator, parasite and pest pathogen populations.

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