Resistance to Bt Toxin in Cotton

The 1996/97 cotton crop year made history in cotton production with the commercial planting of Bt cotton resistant to lepidopteran insects. Cotton farmers in the USA and cotton researchers all over the world have been hearing about Bt cotton for over ten years. The cotton industry was impatient to see Bt cotton growing in the field making use of genetic engineering technology on a large scale. Since the acceptance of chromosomes as carriers of hereditary material, developments have been made step by step to understand the mechanism of genetic control and the utilization of smaller units than a whole chromosome. Biochemical genetics led to the concept that a gene is the smallest unit of heredity and that one gene is responsible for the production of one enzyme. The next significant development was the recognition and understanding of the Watson and Crick model of the double helix DNA (Deoxyribose Nucleic Acid) structure. Once the DNA structure became known to researchers, they started exploring possibilities to transform and regenerate plants. Regeneration of a plant from a single cell was long established in other crops before it was successfully tried in cotton. Once a plant could be regenerated from cellular plant tissues, the next attempt was to induce genetic material in somatic tissues and obtain genetic expression of a trait through somatic culture, the real lead to the development of transgenic plants in crops and especially in cotton.

First Insect Resistant Bt Cotton

Researchers identified a gene in the soil bacteria *Bacillus thuringiensis* (Bt) which codes for an insecticidal protein. They were able to isolate and transfer that gene into cotton so that a toxin could be produced inside the plant. The transformed cotton with a Bt gene resistant to lepidopteran insects is called Bollgard™ in the USA and Ingard™ in Australia. Both Bt cottons are capable of producing CryIA, a protein which is toxic to most bud/bollworms. In October 1995, the Monsanto Company received final regulatory approval from the US Environmental Protection Agency for planting Bt cotton on a commercial scale in the USA. Only two cotton varieties resistant to budworms and bollworms, including *H.armigera*, tobacco budworm and pink bollworm, were available for commercial cultivation in 1996/97. The Bt varieties are named NuCOTN 33[B] and NuCOTN 35[B] and have been developed from their isogenic commercially grown Delta and Pine Land varieties DPL 5415 and DPL 5690, respectively. Many other varieties with a Bt gene are close to being released but only NuCOTN 33 and NuCOTN 35 were offered for sale during 1996/97. It is estimated that in 1996/97 Bollgard Bt cotton was grown on about 700,000 hectares in the USA. According to the ICAC, cotton was planted on about 5.53 million hectares in the USA during 1996/97. NuCOTN 33 alone was grown on about 10% of the total area and emerged as the largest single variety in the USA. NuCOTN 35 covered about 2% of the total US area. It is assumed that similar Bt cotton varieties have prospects for over 2 million hectares in areas affected by tobacco budworm and cotton bollworm.

Some Future Bt Cottons

1996/97 will prove to be a crucial year for Bt cotton. The technology has proved its worth in small scale trials, but 1996/97 will show the reaction of farmers. Early reports during the 1996/97 season in the USA showed that Bt cotton was not completely safe from bollworms and farmers started doubting the technology. Either claims made by Bt cotton promoters were not true or farmers did not fully understand that the Bt gene would not provide 100 percent protection against all types of bollworms and it would not eliminate the use of insecticides. However, the technology will certainly reduce significantly the use of insecticides and any misunderstandings will not affect the research being done in this field.

After the release of Bt cotton resistant to lepidopteran insects and BXN cotton resistant to a herbicide, transgenic cotton resistant to other herbicides and insects is expected. Insect and herbicide resistance is just one aspect of transgenic cotton. Foreign genes can bring in any imaginable feature and characteristic to cotton. Reports are already pouring in about the very recent pigment alteration patent granted to the American company Calgene Inc. by the US Patent Office. The company owns Stoneville Pedigree Seed Company and claims that its scientists have developed blue and red fibers and are now focussing on enhancing the shades. Pigment alteration of genetically-modified cotton plants has enhanced the prospects of producing naturally colored blue jeans. The fiber quality of presently available naturally colored green and brown fibers is not equivalent to white cotton fiber. Development of pigment alteration in the genetically-modified cottons will not require additional work to improve fiber quality. Pigment alteration will be an addition to the existing qualities of white cottons with no other effects on the plant. Tomorrow you may also find black, yellow and other naturally existing colors in cotton. Similarly, many other developments now seem to be within the reach of researchers although it might take quite some time before they become available to the public. A lot of work is also being conducted to improve fiber quality parameters through genetic engineering. In the near future the following developments are expected:

Year	**Development**
1996	Commercial scale production of Bt cotton resistant to lepidopteran insects and Buctril herbicide.
1997	Bt cotton resistant to Glyphosate herbicide will become available. It will be called "Roundup Ready®."
1997-98	Cotton resistant to Sulfonylurea herbicide will become available to farmers.
1999	Multiple Bt genes resistant at the same time to a group of insects or separately to a group of herbicides will become available.
2000	It is hoped that Bt cotton resistant to insects and herbicides at the same time will become available.
2000+	Within the first decade of the 21st Century, cotton resistant to boll weevil will become available for commercial cultivation.
2000+	It is assumed that genes responsible for fiber characteristics are already known. Large scale utilization/adoption is expected in the first decade of the 21st Century.
2000+	Biopolyester cotton, colored cotton, etc.

Because most of the work in genetic engineering is done in the private sector, the full status of genetic engineering research is not known. The above list is just an indication of a few developments and much more may be underway using this very expensive technology.

Impact of Bt Cotton

Bt toxin is specific to a narrow range of insects and quickly breaks down to non-toxic compounds when exposed to ultraviolet light and other natural environmental factors. Its quick breakdown makes it unharmful to other species of living organisms. On the target insect, the toxin attacks the digestive system and the insect stops eating as soon as it feeds on the Bt protein. Commercial scale production of Bt cotton will have the following effects:

- At the end of 1996, the global market for pesticides is expected to be over US$29,000 million. Though most pesticides go to vegetables and cereal crops, cotton receives about 10% of total pesticides used in the world. Among the major field crops, rice accounts for about 13% of total pesticides while—because of larger area—maize uses 11%. Pesticide applications to rice and maize and most vegetable and cereal crops are in the form of herbicides rather that insecticides. Pesticide use on cotton is mostly in the form of insecticides. According to a study conducted by the ICAC during 1995, with the exception of a few countries, insecticides represent 75% or higher of the total pesticides used on cotton. Among exceptions, Syria and the USA are significant because the minimum need for insecticides in Syria and the extensive use of herbicides in the USA keep the share of insecticides low. Introduction of Bt cotton on a larger area ultimately will change cotton's reputation as a heavy user of insecticides. It may be possible to eliminate insecticides in areas where *Helicoverpa* or *Heliothis* are the only pests at boll formation stage. If not eliminated, insecticide use will definitely be reduced.
- The insect pattern is going to change. If one species of insects is suppressed strongly and continuously—the result of Bt cotton—some major insects will become minor pests. Similarly, some minor insects may become major insects.

Also new insects may adapt to cotton more quickly due to less use of insecticides.

- One of the major concerns of the cotton industry is the development of insect resistance to the endotoxin produced by the Bt gene. Insects, like all other living organisms, have a tendency to become used to lighter doses of chemicals and slowly become used to lethal doses. As insects acquire the ability to withstand normal lethal doses, the quantity of insecticides applied has to be increased. In the case of Bt cotton, because the endotoxin is in the plant all the time, the possibility of developing resistance and developing it more quickly is very high.
- Large scale utilization of Bt genes or any sort of chemical resistance from within the plant (even transformed cotton not having the Bt gene), is going to affect the economics of cotton production. If transgenic cotton resistant to a specific insect becomes available, cotton production may be revived in countries or areas where it was stopped because of this particular insect's attack.
- Seed is a genetic carrier of all phenotypic expressions. It is already a very important input in cotton but the importance of seed is going to increase in the changed scenario. Adoption of Bt cotton seed purchased at a higher price will ensure the supply of pure seed and careful planting for better establishment.
- Biological control of insect pests will become more popular.

Why Insects Will Develop Resistance

Insect resistance to Bt endotoxin was not known prior to 1985. In the last ten years, a number of studies have proved that insects can develop resistance to a Bt endotoxin. Laboratory studies conducted by Monsanto researchers confirmed the genetic capability of the tobacco budworm to adapt to the Bt toxin (Stone et al, 1989). Gould et al (1995) isolated a population of the tobacco budworm which showed high levels of resistance to Bt toxin in the laboratory and fed them on Bt cotton. They noted that some of these resistant colonies were able to survive on Bt cotton. Survival of low population levels indicated that the gene determining resistance may be present in a population at a proportion of 1 in 1,000 and most probably it is in a recessive position. In addition to the Monsanto researchers, many other groups will be monitoring the resistance of worms in 1996/97. In the USA, *H.virescens* and in Australia *H.armigera* will probably be the first to express resistance to Bt endotoxin under field conditions. Budworms and bollworms will develop resistance at least due to the following reasons:

- The endotoxin will be present in all parts of the plant from the very beginning to the end of the season. As soon as the seed germinates, the endotoxin will be there in the same toxic amount. One after the other, all generations of insects feeding on cotton as a primary host will face the endotoxin at all times during the growing season and thus will have an ample chance to develop resistance.
- The currently available Bt gene has the ability to produce only one endotoxin, ''CryIA,'' which is protein in nature. In conventional farming where cotton is sprayed more than once, farmers are advised not to repeat products with the same chemistry. In Bt cotton, there will be only one chemical to which the bollworms will be exposed. The chemistry of the presently available endotoxin produced by the Bt gene cannot be changed unless a new gene with a similar toxic effect but with a different chemistry is identified and induced in the cotton plant.
- The number of available insecticides for control against cotton bollworm, *H.armigera*, tobacco budworm or pink bollworm is very high and cotton growers have plenty of choice. Depending upon insect pressure, price and pest complex, growers make different selections and delay development of resistance to various groups of insecticides. Such changes will not be available if there is only one type of Bt cotton planted over a large area by all farmers in one region.
- Most cotton insects and particularly bollworms are highly adaptable species. They can develop resistance through resistant genes and in other ways. However, some insects have the capability to alter the receptor binding in the gut which affects proteolytic activity. Because Bt protoxins are solubilized and activated in part by gut proteinases, lower proteolytic activity could result in less toxin in the insect gut and reduced toxicity. (Views circulated by Brenda Oppert, Karl J. Kramer and William H. McGaughey at the National Forum on Insect Resistance to *Bacillus thuringiensis* organized by the USDA from April 15-16, 1996.)

How to Delay Resistance

There are no reports based on field observations showing any resistance to Bt CryIA endotoxin in cotton. However, there is a strong feeling that target insects will develop resistance in five to seven years. Development of resistance could happen earlier, even in three years, if appropriate steps are not taken. The mechanism of resistance could be such that it may result in broad resistance to many diverse Bt toxins. Researchers can find new toxins and develop a new line of toxicity but chances are that some insects like the tobacco budworm may develop a resistance which may not be able to be substituted. Alternatively, resistance could also be very specific to a particular toxin and could easily be managed through substitution of a new Bt gene. According to Gould (1995) there are a number of strategies to delay development of resistance to the available endotoxin in cotton. They are

- High levels of a single toxin**—**Currently there is only one toxin which is responsible for resistance to lepidopteran insects. In order for the bud/bollworms to delay expression of resistance to a single toxin, it is recommended that Bt genes capable of producing a high level of toxin in the plant be identified. Like high doses of insecticides, it will take a longer time for the insect to develop resistance to higher concentrations of a particular toxin.

- High levels of more than one toxin—Development of resistance to higher levels of more than one toxin will take many years, thus providing enough opportunities to identify and induce new Bt genes.
- Mixture of resistant and susceptible populations (a modified form of refuge population)—Bt cotton with one or more highly effective toxins if grown in mixture with isogenic non-Bt cotton will permit the production of insect hybrids through mating between resistant and susceptible populations. If resistance is recessive, it will take a few years to develop a truly homozygous resistant population.
- Low level of expression—A low level of expression could help to encourage biological control. Low levels of the toxin or toxins will only slow down insect growth/activities. Predators and parasites could survive and multiply and consequently produce a strong natural defense against an insect. There are advantages for this approach but, because there is no support from biotech companies, almost no work is being conducted on these lines.
- Target specific gene expression—A very specialized approach is to produce an endotoxin in a particular part of the plant and at a specific stage of crop development. It is not clear how this approach will work in cotton but it seems that production of a toxin could be restricted to only bolls or leaves in the case of bollworms and sucking insects, respectively.

There is no way that resistance can be avoided in Bt cotton if the genetic base for production of toxic compounds is so limited. However, development of resistance could be delayed. The Monsanto Company, who is the sole promotor of the Bollgard gene in cotton, recommends planting a refuge crop but it is hard to say that large scale trials have been completed. Under circumstances when other choices are not available and development of resistance is contemplated at a faster rate compared with insecticides, dilution of the population exposed to the Bt gene seems to be a viable solution. The Monsanto Company recommends to its Bt cotton growers to set aside a ‘‘refuge’’ area where cotton containing Bollgard gene is not planted. According to the company, to ensure that the Bollgard gene remains effective for years to come, growers are required to plant the refuge area in direct proportion to the Bollgard cotton they have on their farms. The following two types of refuges are recommended in the USA and NuCOTN cotton growers must opt for one of these options:

- For every 100 hectares of Bollgard cotton planted on the farm, 25 hectares of conventional cotton varieties (not containing the Bollgard gene) must be planted and treated with insecticide (excluding foliar Bt products).
- For every 100 hectares of Bollgard cotton planted on the farm, 4 hectares of conventional cotton varieties (not containing the Bollgard Bt gene) must be planted and treated with any insecticide except those used for worm control. The company provides a list of excluded products.

The Monsanto Company signed contracts with farmers for one year. Other requirements of the Monsanto agreement are

- Growers had to pay US$79 by June 25, 1996, for every hectare of cotton they intended to plant with Bt cotton. This is the cost of Bt gene technology in lieu of the insecticide savings. The cost of seed was separate at about US$15 per bag, making the total per hectare cost US$94/ha.
- Growers could replant as often as they needed but they had to make only a one-time technology payment. In the event of a complete crop wipe-out by June 25, growers did not have to pay the fee to Monsanto.
- Growers will allow field inspectors from Monsanto to visit their fields for three years.
- Growers will not transfer seed to any other grower nor will they retain unused seed for next year or plant next year’s crop from seed from their Bt crop.
- Growers who do not comply with the terms of the contract are liable to a substantial non-compliance penalty.

Australian Approach

Australia will be the second country to grow Bt cotton on a commercial scale during 1996/97. In Australia, under the Limited Commercial Trial Program, 30,000 hectares of Ingard cotton will be grown during 1996/97. The Trial Program is part of an effort to give farmers an opportunity to learn how to grow Ingard cotton. About half of the 30,000 hectares, will be included in an extensive research program to collect more data. 320 cotton growers, about 1/4 of the total cotton growers in Australia, will plant Bt cotton as a trial on an area not exceeding 80 ha per grower. Cotton in Australia has been seriously affected by *Helicoverpa armigera*; thus Bt cotton could really be of great significance. In Australia, a resistance management strategy has been successful and the total number of sprays has been reduced to nine in the past few years. This number is still higher than in the USA and Bt cotton could show its impact better under high insect pressure. In Australia, *H. virescens* does not exist on cotton and most sprays are directed to control *H.armigera* which has already developed resistance to many insecticides in that country.

The participating cotton growers will sign an agreement with Monsanto. The agreement signed between the grower and Monsanto is legally binding, spelling out responsibilities of both parties for the successful growing of an Ingard cotton crop and the protection of Monsanto rights. In Australia, the National Registration Authority for Agriculture and Veterinary Chemicals registered six Bt cotton varieties on August 6, 1996, for planting during 1996/97. The work done at the Australian Cotton Research Institute in Narrabri has shown that currently *H. armigera* has no resistance to the Bt toxin. However, a preemptive strategy has been developed to delay resistance to Bt cotton. As in the USA, the approach is to develop a population not exposed to the Bt gene endotoxin. The main recommendations are as follows:

- For every 100 hectares of Ingard cotton, a grower has to plant 10 ha of irrigated non-Bt cotton which will not be treated with insecticides used to control *H. armigera* or, for every 100 ha of Ingard cotton, plant 50 ha of irrigated conventional cotton, which can be treated with insecticides to control *H.armigera* and *H.punctigera*.
- The refuge crop must be planted by November 15 close to the Bt cotton. The refuge crop will be grown like a normal crop and will not be treated with Bt insecticides.
- Twenty hectares of irrigated sorghum or corn will be grown in every season and managed to flower between January 15 to February 28. Sorghum or corn will not be treated with products normally used to control worms.

Plans for Resistance Management

In April 1996, the USDA organized a two day National Forum on Insect Resistance to *Bacillus thuringiensis* which was attended by about 120 cotton researchers, consultants and various other components of the cotton industry. Discussions were concentrated around the following four questions.

1. What are the key components of a resistance management plan for the targeted crop?
2. What are the obstacles in implementing this plan?
3. How can these obstacles be overcome?
4. What follow-up actions need to be conducted at the regional and/or national levels?

There is little doubt among researchers that insects will develop resistance to Bt toxin. The occurrence of resistance will depend on the population biology of the affected pest species, the agroecosystem in which Bt endotoxin is deployed and the pest management strategies followed to control insects including bud and bollworms. The forum realized the need to monitor the situation carefully during the current season and review results at the end of the season. The importance of transparent results and conclusions was stressed. Participants in the forum expressed a need to review and evaluate the refuge population recommendations of the Monsanto Company and to refine next year's plans based on the 1996/97 experience.

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