Second Generation of Bt Cotton is Coming

Transgenic crops have been cultivated commercially for almost ten years now. China started commercial cultivation of transgenic crops by planting the first virus resistant tobacco and later tomatoes in the early 1990s. Commercial cultivation of genetically engineered crops started in the U.S. in 1994 with delayed ripening tomatoes. Commercial cultivation of transgenic cotton became a reality in 1996, when it was planted in the USA. An estimated 5.3 million hectares in the world were planted to transgenic cotton varieties in 2000/01. The U.S. is the largest producer of transgenic cotton. According to the International Service for the Acquisition of Agri-Biotech Application, 44.2 million hectares were planted to transgenic crops in the world in 2000/01. In 2000/01, transgenic cotton comprised 12% of the total transgenic crops area in the world compared to about 9% in 1998/99 and 1999/00, and 11% in 1997/98. Such a share in the overall transgenic area in the world indicates that transgenic cotton varieties are being adopted at about the same rate as other transgenic crops.

At the time commercial cultivation of genetically engineered cotton began in 1996, six countries had already started commercial production of other transgenic crops. The technology is expensive and many developing countries do not have ready access to it. The limited availability of genetic engineering technology is preventing the easy spread of transgenic cotton varieties, particularly to developing countries, and regulatory processes to start commercial production of transgenic varieties are not in place in many countries. Thus, transgenic crops were grown only in thirteen countries during 2000/01, compared to six in 1996/97, and almost 90% of the total transgenic area of 44.2 million hectares was in developed countries.

Commercial cultivation of transgenic cotton spread to Argentina, Australia, China (Mainland), South Africa and the USA almost three years ago; since then only Mexico has joined these countries. It is estimated that Mexico planted transgenic cotton in 2000/01 on about 25% of its total cotton area. There are many other cotton producing countries, both large and small that could grow transgenic Bt cotton successfully, and it could serve to reduce their heavy use of pesticides.

Transgenic Cotton Area in Various Countries–2000/01

Currently, only two types of transgenes are available for commercial production in cotton. There are two herbicide-tolerant genes and a gene offering resistance to bollworms. The two kinds of herbicide-tolerant genes, called BXN and Roundup Ready, are already being utilized. Each gene has been derived from soil bacterium. A cotton plant having either one of these two genes offers resistance to the broad leaf herbicide bromoxynil or glyphosate. More details about this can be found in the June 1998 issue of *THE ICAC RECORDER*.

The bollworm-resistant gene has also been isolated from a soil bacterium, *Bacillus thuringiensis*, but it only provides resis-

Transgenic Crops World Area

Year	All Crops	Cotton	
	(Ha)	(Ha)	%
1996/97	2.8	0.8	29
1997/98	12.8	1.3	11
1998/99	27.8	2.5	9
1999/00	39.9	3.7	9
2000/01	44.2	5.3	12

Transgenic Cotton Area in Various Countries

Year	Area in 2000/01		Estimate 2001/02
	(Mill. Ha)	(%)	(%)
Argentina	0.03	5	5
Australia	0.15	30	30
China (Mainland)	1.00 *	15-20	25
Mexico	0.02	25	30
South Africa	0.04	40	40
USA	3.93	72	75

* Approximate area.

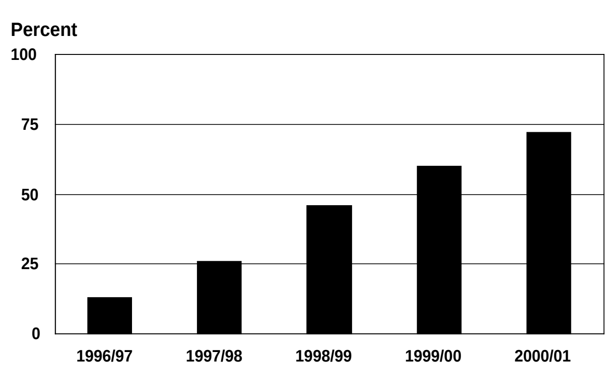
tance to a specific type of bollworms and not to sucking insects. Both herbicide-tolerant genes are compatible with the bollworm-resistant gene and can be put together in one genotype to make higher use of the technology. Such genotypes have been grown commercially in the USA since 1996. According to the Agricultural Marketing Service of the USDA, 72% of the total area grown under cotton in the USA during 2000/01 was planted to transgenic cotton varieties.

Transgenic cotton has become popular in the U.S. at a much faster rate than expected and transgenic area in the U.S. is equivalent to almost 15% of world area. Data from the USDA (2000) show that 20% of area was planted to the stacked gene varieties having herbicide-tolerance and bollworm-resistance genes during 2000/01.

Herbicide-resistant BXN and Roundup Ready cottons have been approved for growth on a commercial scale in the USA since the beginning of transgenic production. So far, Australia has grown Bt varieties only but will start growing the Roundup Ready varieties next year. It is assumed that most of the new varieties will be the stacked-gene type because Bt varieties have already met the target area permissible for transgenic varieties. It is estimated that in other countries most area is under Bt varieties, and that herbicide-resistant cotton is grown only on a small scale, if at all.

Although the Bt gene (Cry1Ac) derived from the soil bacterium *Bacillus thuringiensis* offers the greatest resistance to the tobacco budworm *Heliothis virescens*, it is also quite effective against the cotton bollworm *Helicoverpa armigera*. In the Yellow River Valley of China (Mainland), *Helicoverpa armigera* is a major cotton pest and the continued indiscriminate use of insecticides resulted in the development of resistance to a variety of insecticide groups. Not only did yields drop significantly, but cotton area also declined. China (Mainland) had to look for alternate means of controlling the cotton bollworm and found that Bt cotton is a good choice. China also claims to have developed its own transgenic cotton with a gene different from Cry1Ac. Estimates from China (Mainland) suggest that the area planted under transgenic varieties in 2000/01 increased by 200,000 hectares to a total of over one million hectares. The severity of the bollworm problem and its solution in the cultivation of transgenic bollworm-resistant cotton suggests that transgenic cotton might have been grown on a much larger area than reported in 2000/01. Personal communications indicate that most of the area in Hebei and Shandong provinces may already be under transgenic varieties or will be planted to such varieties in 2001/02. Some unconfirmed sources indicate that Bt cotton may have been planted on over one million hectares thus making 5.3 million hectares the world Bt cotton area. However, all countries except the USA will continue to grow bollworm-resistant varieties, and the demand for herbicide-tolerant varieties outside the USA will remain limited. The main reason for the low demand for the herbicide-tolerant gene is the availability of alternate means of controlling weeds and the low use of herbicides in most countries.

Transgenic Cotton Area in the USA
(Percentage of Total Area)



Environmental Safety

The Cry1Ac protein is a member of a large group of insecticidal proteins produced in nature by the bacteria *Bacillus thuringiensis*. Research conducted before the commercial application of the gene, and experience during the last five years, show that Cry1Ac binds to specific receptors in the mid-gut of sensitive insects, but does not affect mammals or insects that do not have the receptors. The presence of the specific receptors makes the Cry1Ac protein specifically toxic to a particular group of insects, affecting mainly the lepidopteran insects, and particularly the cotton bollworm *Heliothis virescens*. All other insects, fish, wildlife and beneficial insects in cotton fields are not affected. The insecticidal protein in the plant begins to break down immediately after the plant dies, thus it does not accumulate in the soil and it does not have the chance of leaching down in the soil and contaminating underground water.

Limitations to Technology Spread

The cotton bollworm is the most damaging cotton pest in the world. There are many countries confronted with the problem of bollworm control that have the potential to use transgenic varieties to reduce the use of pesticides. The increase in transgenic area in the USA from zero to 72% of total area in five years is testimony to the fact that transgenic varieties, including the variety resistant to bollworms, are more profitable than growing normal varieties and controlling bollworms with insecticides. Similarly, Australia reached the cotton area allowed by regulators in less than five years. Under the current resistance management program, Australian growers can plant only 30% of the cotton area to transgenic varieties and this target has already been reached. Transgenic cotton area is going to increase in China (Mainland). India conducted large-scale trials during 2000/01 and is close to commercial adoption of Bt cotton. Among the major cotton producing countries, Pakistan and Turkey are other potential users of Bt cotton but still probably many years away from commercial adoption. Two countries where Bt cotton does not seem to have a future in the short term are Greece and Spain because of public pressure against genetically engineered crops, including cotton.

Most of the countries that grow transgenic crops are industrialized. Adoption of genetic engineering technology outside the U.S. is comparatively slow, even though the benefits are substantial. Among all the crops with transgenic varieties for commercial cultivation, soybean and corn account for the most area, with 25.8 million soybean hectares and over 10 million corn hectares, compared to 5.3 million hectares of GE cotton. Soybean and corn are major crops in industrialized countries, unlike cotton that is produced mostly in developing countries. It is not the lack of desire to grow genetically engineered varieties that is limiting the spread of the technology, but it will be many years before the currently available Bt gene is utilized in most countries. Many other genes with different effects will slowly follow. The following are some of the reasons that hinder the easy spread of the latest production research technology to many countries.

- There are two ways to acquire GE technology. Interested countries can either buy the technology from the existing owners or develop their own systems to convert their own varieties into transgenic varieties. Both options are expensive.
- There are scientific limitations to the utilization of this technology because only a specific gene expresses a particular toxin. Under currently applicable international patent laws, the gene cannot be inserted into local varieties without the permission of a company that owns this gene.
- It is critical to grow transgenic varieties on a large scale, particularly the Bt varieties. Large-scale production is impossible under the small scale farming systems unless every producer in the area commits to grow Bt varieties.
- Bollworm pressure varies from country to country and among regions within countries. The Bt gene varieties are not intended for all kinds of bollworms.
- There are countries that can grow, or have grown, only varieties of local origin. The Bt gene is available primarily in varieties of U.S. origin, and the U.S. varieties may not be suitable for all production conditions throughout the world. The Bt gene has to be transferred into local varieties, an additional step for most countries other than the U.S. There are other situations like India's, where millions of hectares are grown from the hybrid seed in the F_1 generation or, rarely, in the F_2 generation. Such conditions may also limit the use of transgenic varieties.
- Genetic engineering is a new technology still not completely understood. It is the responsibility of researchers to educate the public about benefits and potential risks of the technology. Any new developments have to be thoroughly tested before they are adopted in commercial production. Because the technology is expensive, countries are hesitant to develop something that may not be acceptable at the end use level.
- Some recent developments indicate that, unlike conventional breeding, the genetic engineering technology has the potential to be misused. Thus, some countries are highly cautious, probably more than required, to welcome the technology. Both developed and developing countries need assistance in the identification of biotech needs and priorities in addition to assessing potential socio-economic impacts.
- Experience from countries that have adopted this technology shows that there is a need to regulate its use. Many countries do not yet have such regulatory systems in place to make use of GE technology.
- Although some decisions remain with local governments, many countries need guidance to develop systems for genetically engineered crops. Transgenic cotton produced using recombinant DNA techniques has been available for five years, and new genes and new approaches are already being developed to make use of genetic engineering techniques. While the benefits of transgenics have been realized and no long-term effects have been detected to date, no doubt some possible environmental effects remain as areas of concern and there is a need to regulate biotechnology as a science. The technology is progressing and the sooner growers feel confident about adopting it, the better it will be for cotton.

Bollgard® II Gene

Biotechnology can be employed in many ways to improve plants and their products. Identifying not only the suitable genes, but also their functions and how they work, provides researchers with crucial knowledge to improve crop plants. One of the avenues could be "wide crosses" hybridization, in which genes are moved from one species or one genus to another to create a plant variety that does not and cannot exist in nature. But, because cotton is considered to be the highest insecticide-consuming crop, the major emphasis in cotton has been on agronomic traits particularly for savings on insecticide use. Communications from China (Mainland) show that they have developed a transgenic cotton having a different gene than Monsanto's Cry1Ac, but reports on the performance of this gene are not available because it has not been tried outside China (Mainland). However, it is expected to be as good as Cry1Ac, otherwise it will not gain acceptance against Bt. Egypt is also said to have developed a drought resistant transgenic cotton of its own. Many other countries are in the process of developing the capability to produce their own transgenics having a variety of characteristics.

Most researchers recognize that insects will develop resistance to the Bt toxin, and efforts are underway to avoid its development. Using refuge was always considered an interim solution. One of the strongest solutions proposed by supporters of the technology is to identify a second gene and insert it into cotton along with the Cry1Ac gene. With the advancement of knowledge about the technology during the last decade, researchers are convinced that such an option is possible and could be available soon.

Researchers have met the expectations of industry, and a stacked gene variety called Bollgard® II, with a new gene, Cry2Ab, is available. Cry2Ab has been added to the DP 50 variety, which already had the gene Cry1Ac. Bollgard® II has been tested in the field for two years against non-engineered DP 50 and the Cry1Ac variety DP50B. According to Voth (2001), a combination of the Cry2Ab and Cry1Ac genes shows promise for improved insect efficacy and an increased spectrum of control. Tobacco budworm, cotton bollworm and pink bollworm are more susceptible to the Cry1Ac protein than to Cry2Ab, whereas fall armyworm, beet armyworm, cabbage looper and soybean looper are more susceptible to Cry2Ab than to Cry1Ac. The level of the Cry2Ab expression measured in the ELISA is > 10 times the level of the Cry1Ac expression seen in Bollgard® II plants. This relationship is consistent and was seen for all sites, sampling times, and tissue types. The high plant expression of Cry2Ab contributed to higher efficacy against important lepidopteran insects in cotton.

Bollgard® II has been developed using the biolistic transformation technology. Trials conducted for two years in the USA during 1998/99 and 1999/00 show that the two genes in the Bollgard® II genotypes segregate independent of each other (Penn et al 2001). The two years of data also show that the expression of Cry2Ab did not comprise the expression of Cry1Ac in Bollgard® varieties. There were no differences in the lepidopteran activity level between the terminal shoot and squares. However, the lepidopteran activity level decreased in older leaves, which is also true in the case of Bollgard®. Analysis of samples at 2, 4, 6 and 8 week intervals shows a sharp decline in the lepidopteran activity in Bollgard® II compared to Bollgard® but even the lower activity in the larger leaves was still 2-3.5 times higher in Bollgard® II compared to Bollgard®. Cry1Ac expression in Bollgard® II by site, time and tissue type are all similar to Bollgard® Cry1Ac expression and there was no difference in the levels of activity of Bollgard® II at each individual field site.

According to a paper presented at the 2001 Beltwide Cotton Conferences (Voth, 2001), Monsanto is licensing the gene to seed companies liberally. At least six companies already have a license and thus the new gene is expected to be available in a number of new varieties developed by various companies at the same time. An efficacy profile is being defined and all regulatory requirements are being completed. It is expected that Bollgard® II varieties will be available for at least small commercial cultivation in 2002/03. In the meantime, studies will continue to redefine threshold for the stacked gene varieties, develop new scouting methods, and study economic benefits and any undesirable effects on the plant. Work will also continue to perfect refuge requirements for the stacked gene varieties. It is anticipated that once commercial production starts, Bollgard varieties will be replaced by Bollgard® II in about five years. Bollgard® II cotton varieties will also be available containing the Roundup Ready® gene.

New Refuge Requirements for 2001/02

Transgenic cotton has been strictly regulated in the USA. Concerns were expressed since the beginning of commercial cultivation of Bt cotton varieties, that if Bt cotton is planted year after year on a large area bollworms will develop resistance to the Bt toxin. The concern was taken seriously by the U.S. and Australian cotton industries. Australia put a ceiling on the maximum area to be planted to Bt cotton at each farm, while in the USA, a refuge crop system was adopted. Farmers were required to plant ten hectares of conventional cotton varieties for every 40 hectares of Bollgard cotton, and to treat conventional varieties with insecticides other than foliar Bt products. Farmers also had the option to plant 4% of their area to unsprayed normal varieties (4 hectares for every 100 hectares). The objective was to produce a hybrid generation of bollworms affected by the Bt toxin and delay the development of resistance for as long as possible. The approach seems to have worked well as no significant resistance has been reported so far.

Bollgard® II varieties will be planted during 2002/03, and refuge requirements have been revised for 2001/02. According to Mullin (2001), the new refuge requirements are as follows:

20% Sprayed Option

This is an amendment to the existing 20% (or 10 hectares of non-Bollgard area for every 40 hectares of Bollgard area) option with the additional requirement that all Bollgard fields must be within 1.6 kilometers (preferably within 0.8 kilometers) of the associated refuge.

5% Unsprayed Option

The requirement for the unsprayed option has been increased from approximately 4% (or 4 hectares of non-Bollgard for every 100 hectares of Bollgard) to a true 5%, or 5 hectares of unsprayed non-Bollgard refuge for every 95 hectares of Bollgard. Additionally, the unsprayed refuge must be at least 45.75 meters wide (150 feet or approximately 48 rows in conventional row-width cotton) and all associated Bollgard fields must be within 0.8 kilometers of the unsprayed refuge. These requirements apply to all users of the 5% unsprayed option regardless of the percent of cotton area planted to Bollgard in a particular area/region. The treatment restrictions for the unsprayed option remain the same as those in place for the 4% unsprayed option in 2000.

5% Embedded Option

A third option has been added for 2001/02, which is the "embedded" option. Unlike the 5% Unsprayed Option, this option allows the refuge to be treated with any insecticide at the same time the Bollgard is treated, as long as the refuge is "embedded" in the field or the "field unit."

For large fields, 5% of the field would be planted to a non-Bollgard variety, the rest with Bollgard. If the Bollgard field needed treatment for bollworms (or any other pest), the entire field, including the refuge, could be sprayed with the same insecticide at the same time (i.e., within the same 24-hr. period). The refuge could not be treated with any insecticide labeled for lepidopteran control independently of the associated Bollgard field(s). For very large fields (more than 1.6 kilometers long or wide), multiple refuge blocks across the field should be used.

For smaller field situations, fields could be grouped into "field units" so that one of the smaller fields or a portion of one of the fields would serve as the "embedded" non-Bollgard refuge. Likewise, this embedded refuge could be treated with the same insecticide at the same time that all of the associated Bollgard fields were sprayed, but could not be treated with any insecticide labeled for lepidopteran control independently of the associated Bollgard fields. Any fields contained within a 1.6-kilometer square area (one mile by one mile) can be considered a "field unit."

As required for the 5% untreated option, the embedded refuge within a field or "field unit" must be at least 45.75 meters wide in all areas where the cotton bollworm or the tobacco budworm is a potential pest.

For areas where pink bollworm is the only pest of concern, growers are allowed to mix individual rows of non-Bollgard with Bollgard rows to embed their refuge, as long as the non-Bollgard rows represent at least 5% of the total Bollgard cotton. An example of how this "embedded" option would be planted is placing a non-Bollgard variety in one seed hopper and putting Bollgard seed in the remaining seed hoppers, resulting in interspersed rows of a non-Bollgard variety across a Bollgard field. Interspersing rows of non-Bollgard varieties and Bollgard varieties within a field is not allowed where cotton bollworms or tobacco budworm can be significant pests.

Community Refuge Plan

If farmers are small growers and are unable to meet the 45.75 meters requirement, multiple growers in an area can work together to ensure that the Bollgard cotton and refuge fields are appropriately sized and placed to provide optimum insect resistance management (IRM) value. The Community IRM plan must meet the requirements of either the 5% unsprayed option or the 20% sprayed option, or an appropriate combination of the two options. For 2001/02, growers will not be allowed to use the 5% embedded option within a community. The larger area bounding the entire group of farms would form a geographic "community" and the refuge requirements would apply to the community of growers and the geographic community exactly as they apply to a single grower. The community refuge agreement among growers must require that an appropriate amount of refuge (depending on the option chosen) is associated with the total amount of Bollgard grown by the community and all distance requirements are met for all Bollgard fields included in the community. Each community must designate a coordinator for the total community refuge plan. This coordinator should be knowledgeable about all requirements of the community plan and agree to represent the group to explain the plan. The coordinator will act as a facilitator and/or spokesperson for the community refuge group.

Need for Education on Genetically Engineered Cotton

More than 40 transgenic crop varieties are available for cultivation with enhanced agronomic and/or nutritional characteristics or one or more features of pest protection (insect and viruses) and tolerance to herbicides. The most widely used transgenic pest-protected plants express insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt). The genetic engineering technology and its applications are spreading. The rate of acceptance is the highest among all research developments in the history of agriculture. But still, there is reluctance on the part of some researchers and countries to accept this technology. Rather, genetic engineering is seen as a short lived and disposable technology. It is assumed that this technology is highly risky and available for only a short period of time. While many concerns are valid, there are others that can be satisfied through scientific education. Genetic engineering technology is not completely understood yet, and the public needs to be educated about the usefulness as well as potential risks of this technology.

The International Cotton Advisory Committee realized the importance of public education on the subject and in January 2000 formed an expert panel comprised of nine international experts from eight countries. The expert panel noted that six national academies of science—Brazil, China (Mainland), Great Britain, India, Mexico and the USA—and the Third World Academy of Sciences issued a joint statement not only endorsing biotechnology but urging companies, governments and charities to extend it to the developing world. Most biotechnology research is in the private sector, and there is a need to ensure that the benefits of biotechnology are extended to developing countries at a reasonable cost. The seven academies say private companies must "share with the public sector more of their capacity for innovation" and that "care should be taken so that research is not inhibited by over-protection of intellectual property" (patents on genetic discoveries). The ICAC Expert Panel on Biotechnology in Cotton concluded that the technology can bring much good to cotton but should be used carefully. The Report of the Expert Panel is available online free of charge at:

<http://www.icac.org/icac/meetings/plenary/59cairns/documents/e_biotech.pdf>.

Hard copies can be requested from the ICAC Secretariat at publications@icac.org.

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