tivation, the textile industry and the mechanism of the futures market. More information on the congress can be obtained from:

IV Brazilian Cotton Congress
Av. 87 No. 662 - Setor Sul
Goiânia, Goiás 74093-300, Brazil
Tel: 55-62-5410163
Email: 4cba@cultura.com.br
Website: http://www.4cba.com.br

The World Cotton Research Conference-3 was held in Cape Town, South Africa, from March 9-13, 2003, hosted by the ARC Institute for Industrial Crops and sponsored by CIRAD, FAO, ICAC, Cotton South Africa, the Agricultural Research Council of South Africa, and many private companies. Over 300 researchers from 38 countries, in addition to representatives of several international organizations attended the conference. Brazil, Turkey and the USA offered to host the World Cotton Research Conference-4 in 2007. The International Committee of the WCRC-3 will consider proposals from the three countries in its meeting in Poland during the ICAC plenary meeting, and will take a decision that will be announced in *THE ICAC RECORDER*.

Bollgard II: A New Generation of Bt Genes Commercialized

The first transgenic cotton resistant to lepidoteran pests was approved for commercial utilization in Australia and the USA in 1996/97. The gene has proved its worth in a number of countries across continents, but controversy still continues regarding its environmental safety. Even though some countries are convinced that the technology is safe, they are not willing to adopt it because of potential trade implications with importing countries that are not yet convinced that the technology is risk-free. Thus, efforts to promote and adopt transgenic cotton varieties are continuing, and much area is already under commercial production. It is expected that the greatest increase in area in 2003/04 will be in China (Mainland) and India. Colombia is expected to become a transgenic cotton-growing country in 2003/04, and a number of other countries will intensify their efforts in the field of biotechnology to get closer to the commercialization stage.

Eight countries have already adopted transgenic varieties resistant to lepidopterans, herbicides or both. The Cry1Ac toxin has eradicated bollworms and budworms from cotton fields. 2003/04 will be the first year that a new protein, Cry2Ab in Bollgard II varieties, will join the fight against bollworms and budworms in Australia and the USA. Monsanto is the sole owner of the Bollgard II technology. On December 23, 2002, the Monsanto Company announced that they had received full U.S. regulatory clearance for its Bollgard II insect-protected cotton technology, clearing the way for large-scale use of the second Bt gene along with the first Bt gene introduced in 1996/97. In Australia, the Office of the Gene Technology Regulator has already given approval for limited commercial release of Bollgard II, after a comprehensive scientific assessment and a public consultation process. While in the USA approval includes the whole country, in Australia it extends only to the established cotton growing areas of New South Wales and Queensland, and a new cotton growing area in the north not to exceed 800 hectares. Other countries are still experimenting but are not expected to adopt Bollgard II in 2003/04. Argentina has proved more willing to adopt biotechnology in the past and could accept Bollgard II in a year or two. No country has adopted the Bollgard II technology without first using Bollgard.

Benefits of Bollgard II

The U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have already confirmed the food, feed and environmental safety of Bollgard II, which uses the same soil bacterium found in Bollgard, but in a different gene. The primary objective remains the same-control of target insects that damage bolls. However, Bollgard II has additional advantages, some of which are long-lasting and some short-term.

- The basic objective of finding the second Bt gene is to delay the development of resistance. Target lepidopterans, if fed on the Cry1Ac Bt toxin for years, will develop resistance. Bollgard II has two Bt genes at the same time, because insects can develop resistance to one gene faster than to two genes working in the same genotype.
- The Cry proteins are not equally effective against all bollworms. The Cry1Ac gene in Bollgard offers maximum resistance to the tobacco budworm *Heliothis virescens* and to the American bollworm *Helicoverpa armigera*, but comparatively less resistance to other lepidoterans. The second objective of inserting the Cry2Ab gene is to extend the spectrum of bollworms and budworms controlled by Cry proteins. The Cry2Ab gene in Bollgard II provides equally good control of fall armyworm *Spodoptera frugiperda*, beet armyworm *Spodoptera exigua*, cabbage looper *Trichoplusia ni*, and soybean looper *Pseudoplusia includens*, in addition to bollworms and budworms already controlled by Bollgard.
- Some bollworms and budworms survive on Bollgard varieties, particularly towards the end of the fruit formation stage. The phenomenon, which occurs due to a low amount of Cry1Ac toxin in the flowering stage, is responsible for some

loss in yield. It is not recommended to use insecticides at this point to control bollworm and budworms surviving on Bollgard varieties because of the cost benefit ratio. Cry1Ac levels are usually expressed 1 to 3 parts per million, while Cry2Ab in Bollgard II varieties is expressed from 7 to 19 parts per million. The higher dose of toxins in the form of Bollgard II will save the plant from late season losses.

Variability in the Cry1Ac Quantity

The amount of Cry1Ac, which is different in different parts of the plant, has much to do with the ability of the plant to resist target pests. Studies have been undertaken by Greenplate et al (2000) and many others to investigate if the amount of Cry1Ac protein remains the same in all plant parts throughout the growing season. They also looked into the effect of location of the quantity of the toxin in the same variety. An average of twelve trials conducted in nine states in the USA revealed that environmental sites, sampling time and tissue type contribute significantly to the variability among Cry1Ac levels in the same variety. Terminal parts were found to have more Cry1Ac compared to squares and bolls.

Cry1Ac in Plant Tissues

Tissue	µ/g dry weight
Terminal	22.3
Square	14.1
Boll	17.1

Plants were found to have a maximum Cry1Ac expression four weeks post-pinhead-square stage. Mean Cry1Ac concentration within specific tissues, although variable from one sampling time to another, showed no specific trend over time to either increase or decrease. The study suggested that similar tissues of same physiological age might express Cry1Ac at levels around tissue/age-specific mean throughout the fruiting cycle. The environmental effect was found to be significant in the expression of Cry1Ac. Variation among site locations was much greater compared to variation among tissues and the age of the tissue. Variation among the twelve trials ranged from 7.4 micrograms per gram (µ/g) dry weight to 31.5 µ/g dry weight.

The Australian Experience with Bollgard II

Studies were conducted on Bollgard II in Australia in 2002/03. The amount of toxin expressed in fruit forms and other parts of the plant was evaluated to assess additional effects of the second Bt gene. Trials revealed that Bollgard II genotypes had a two to three times higher quantity of the toxin in the terminal leaves than Bollgard. Bollgard genotypes on average expressed the Cry1Ac toxin at 27 µ/g of dry weight, compared with 150 µ/g in Bollgard II.

Researchers also evaluated the expression of toxin in the flower bud between the two types of transgenic cottons. It is more important to have a higher protein expression in the flowering parts than in leaves because target insects attack flowering parts. It is known that Bollgard varieties have higher toxin levels in leaves than in squares. The Australian data revealed that on average Bollgard varieties produced half the amount of protein in squares versus leaves (27 versus 50 micrograms). On the contrary, Bollgard II genotypes produced more protein in squares than in leaves. The first position retention in Bollgard II varieties also improved over Bollgard.

No genetically engineered products are approved for sale as food in Australia and New Zealand unless they undergo a safety assessment by Food Standards Australia New Zealand. Approval will only be given if the genetically engineered food is found to be as safe and wholesome for human consumption as its conventionally-produced counterpart. After assessing products derived from Bollgard II, Food Standards Australia New Zealand found them safe for human consumption and approved the sale of oil and linters from Bollgard II cotton containing genes that confer insect protection to the cotton plant. Australia also decided that food products containing oil and linters derived from Bollgard II cotton would be exempt from GM labeling requirements, unless novel DNA and/or protein are found in the final food.

Bollgard II Trials in the USA

Bollgard II has been evaluated extensively in the USA for four years. The comparison included Bollgard II with Bollgard, sprayed non-Bt and unsprayed non-Bt varieties. The trials were conducted at Louisiana State University during 1999/00 and 2000/01. The sprayed plots received weekly applications (early July through mid-August) of an insecticide for worm control; the other plots received no insecticide applications for worm control. All other insect pests were controlled on an as-needed basis and applications were made through the entire trial. Insect damage was assessed weekly from early July through mid-August.

The strongest indication of the Bollgard II effect was seen in the form of significantly less damage to squares. If there is minor damage to squares more bolls are formed resulting in higher yields. Yield data revealed that during both years, Bollgard II gave a higher yield over Bollgard and other entries in the trials. The data below indicates that even if Bollgard or Bollgard II genes are used for protection from fruit loss, some loss in yield still occurs in Bollgard though the loss is much lower. Non-Bt varieties sprayed with insecticides as needed suffered as much as 6.2% square damage. Bollworm damage adds to square damage, as the most damaged squares will not even become bolls.

Square Damage in USA Trials in 2000/01

Bollgard II	0.7%
Bollgard	1.7%
Sprayed non-Bt	6.2%
Unsprayed non-Bt	>15%

Reports indicate that the cotton flower attracts bollworms more than squares, flower buds or bolls. According to Gore et al (2001), during 1996/97, the first year of Bollgard cotton in the

USA, a large number of bollworm *Helicoverpa zea* larvae was found feeding on white flowers in many Bollgard fields across the United States. Little information is available on why the bollworm larvae are more commonly observed on white flowers, but the possible explanation could be related to the amount of toxin in the white flower compared to other parts. The nutritional value of the flower could also attract more bollworm larvae. Gore et al (2001) compared the conventional form with Bollgard and Bollgard II forms of DP 50. The bollworm larvae were reared in the laboratory. Various flower parts were harvested from the field and the bollworm larvae were allowed to feed on flower parts in small petri dishes. Five larvae were released in each 9.0 cm petri dish. Larval mortality was measured after 24, 48 and 72 hours of larvae release.

The table below indicates that bollworm survival varied among floral parts after 24, 48 and 72 hours of larvae release into the petri dishes. Bollworm survival was minimal on bracts, followed by squares and petals. Square anthers and flower anthers showed higher survival after 24, 48 and 72 hours of bollworm larvae release into the petri dishes. The same trend was seen in all three varieties. Comparison among varieties revealed that the Bollgard II gene let the smallest number of bollworm larvae to survive, particularly after 48 and 72 hours of larvae release. After 72 hours of release only 6% of the larvae survived on Bollgard II bracts compared to 63% and 50% survival on flower anthers and squares, respectively, at the same time interval. The survival rate decreased from 79.2% to 63.8% and 32.6% in conventional, Bollgard and Bollgard II, respectively, after 72 hours of the release of bollworm larvae. Whatever the reason, the results clearly showed that bollworm larvae prefer specific feeding sites on the cotton plant, the highest preference being flower anthers followed by square anthers. The biochemical factors associated with flower bracts made them least preferred. When utilizing the Bt gene technology, it is important to enhance the concentration of the toxin in anthers for effective bollworm control. The same approach seems to have been followed in Bollgard II technology.

Bollworm Survival on Conventional, Bollgard and Bollgard II Flower Parts

Hours	Floral Structure	Varieties			Average
		DP 50 (Conventional)	DP 50B (Bollgard)	DP 50 BII (Bollgard II)	
24	Bracts	83	80	89	84.0
	Petals	98	100	99	99.0
	Flower anthers	98	100	99	99.0
	Square anthers	98	100	100	99.3
	Squares	85	96	97	92.7
	Average	92	95	97	
48	Bracts	67	57	29	51.0
	Petals	95	90	81	88.7
	Flower anthers	98	98	88	94.7
	Square anthers	98	97	72	89.0
	Squares	80	77	38	65.0
	Average	88	84	62	
72	Bracts	48	18	6	24.0
	Petals	81	67	36	61.3
	Flower anthers	95	93	63	83.7
	Square anthers	97	92	50	79.7
	Squares	75	49	8	44.0
	Average	79	64	33	

Transgenic cotton can be viewed as useful from different perspectives but its success depends on growing conditions and the benefit could be less environmental pollution. Allen et al (2000) studied the effectiveness of Bollgard II cotton varieties against foliage and fruit feeding caterpillars in Arkansas. They reported that according to a paper presented by Michael R. Williams at the 2000 Beltwide Cotton Conferences of the National Cotton Council of America, cotton losses due to caterpillar pests did not decline in the United States since the release of Bt varieties from 1996 to 1999. On average, losses due to caterpillars remained around 4.5% from 1996 to 1999, almost the same as prior to the introduction of Bt cotton. In their own studies, Allen et al (2000) showed that Bollgard II varieties exhibited a far lower number of beet armyworm, tobacco budworm, cabbage looper and soybean looper than Bollgard and non-Bt varieties. Thus, the indications are that Bollgard II technology could reduce caterpillar losses in the USA.

Bollgard II has been tested not only on experimental farms but also under field conditions to obtain realistic expectations about the potential fitness of this new technology in transgenic cotton. Bacheler and Mott (2003) undertook studies for three years, from 2000 to 2002, concluding that Bollgard II varieties would seldom require insecticide treatments for caterpillar control in North Carolina, and noted that Bollgard II fields had a higher stink bug population because they were sprayed on average less than once a season.

Many studies have shown that the quantity of toxin from the Bollgard Cry1Ac gene declines toward the end of plant maturity and the end of the growing season. At these stages, if a large number of bolls are yet vulnerable to caterpillar attack, insecticide applications may be required, according to work done by Gore et al (2001) and mentioned above. The same conclusion has been made by Akin et al (2003) in their studies undertaken in Mississippi. Akin and his colleagues collected bolls from the first and second positions starting from the seventh node to the nineteenth node in Bollgard and Bollgard II varieties. Using the enzyme-linked immunosorbent assay (ELISA) technique, they measured the Bt toxin separately as Cry1Ac and Cry2Ab. The data showed that CryA1c

protein was highest in the first and second position bolls on the ninth node. The first and second position bolls on the fifth node contained almost 5 ppm of Cry1Ac compared to around 7 ppm at the ninth node. The concentration of Cry1Ac protein started declining after the ninth node and came down to about 4 ppm on the seventeenth node. The concentration was slightly higher on all nodes in the first position bolls compared to the second position bolls on the same nodes. A similar trend was noted for Cry2Ac on nodes from seventh to seventeenth. The ninth node had the maximum concentration, over 7 ppm, which dropped to 5 ppm on the first position bolls and 4 ppm in the second position bolls on the seventeenth node. The work indicated that the individual concentration of Cry1Ac and Cry2Ab would decline in the dual toxin Bollgard II varieties. However, the concentration of Bt toxins together in Bollgard II varieties would be double the concentration in Bollgard varieties. It was concluded that bolls of the same phenological age would have the same concentration of toxins in Bollgard II varieties. Such a conclusion confirms that environmental factors will continue to be important in the expression of toxins in cotton. But the effect of the decline in toxin expression on the survival of target pests needs to be seen.

Effect of Bt Genes Under Sprayed and Unsprayed Conditions

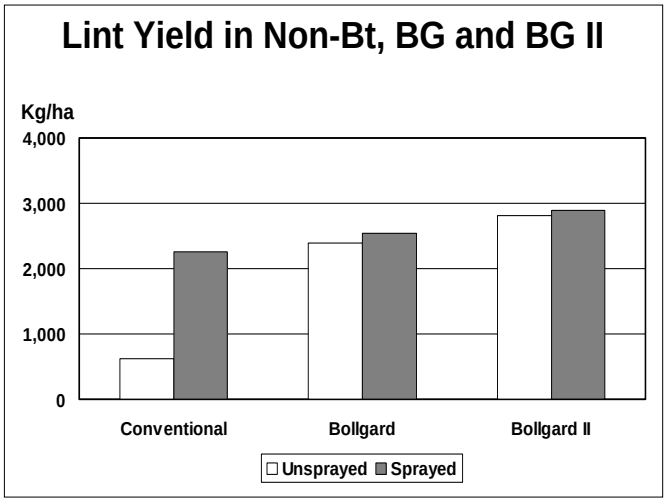
Insecticide Regime	Genotype	No. of Bollworm Adults/ha (%)	No. of Bolls Damaged/ha (%)	Lint Yield (kg/ha)
Unsprayed trials	Non-Bt	11.24	8.66	902
	Bollgard	2.47	2.00	1,137
	Bollgard II	0.52	0.15	1,185
System trials	Non-Bt	7.31	8.16	833
	Bollgard	1.55	3.25	941
	Bollgard II	0.79	0.60	1,001
Average	Non-Bt	9.37	7.93	870
	Bollgard	1.95	2.40	1,047
	Bollgard II	0.53	0.33	1,100

The additive action of the two Bt genes has been shown by Catchot and Mullins (2003) in terms of damage to squares and bolls and lint yields in system trials. They tested a Bollgard II variety against its isogenic Bollgard line and against an isogenic conventional variety at many locations under different treatments, which they called system treatments. The unsprayed trials were not treated at all for lepidopteran insects throughout the growing season. However, when non-lepidopteran insects reached threshold levels, the entire experimental area was sprayed with an insecticidal product that had no or very little lepidopteran activity. In the system trials, each variety was managed independently according to the threshold of the lepidopterans at various stages of development. But, as in the case of unsprayed trials, the entire area was sprayed with the appropriate insecticides with minimum or no activity toward lepidopterans. The data for seasonal mean damage to squares and bolls and lint yield is shown in the table.

According to Catchot and Mullins (2003), the average of all unsprayed trials (not sprayed against lepidopterans) they conducted in the mid-south and east Texas in the USA showed 0.52% and 0.15% damage to squares and bolls in Bollgard II compared to 2.47% and 2.0% in the case of Bollgard, and 11.24% and 8.66% in the case of non-Bt isogenic lines. Bollgard II protection against lepidopterans gave a higher yield by 317 kg/ha over non-Bt, and 53 kg/ha over the Bollgard line. A similar trend was seen in the system trials, but the margin in yield over non-Bt cotton declined due to non-Bt protection against lepidopterans. Bollgard II exhibited a higher yield over Bollgard and non-Bt recurrent parents.

A report presented at the 2002 Beltwide Cotton Conferences by D. S. Brickle and A. L. Catchot of Monsanto, about a similar trial conducted in 2001, also showed that Bollgard II gave higher yields and provided more effective control against bollworms, beet armyworm and soybean loopers than Bollgard. Bollgard II varieties left untreated for lepidopteran pests averaged 103 kg/ha lint more than Bollgard varieties left untreated for lepidopterans. The data collected on Bollgard II, Bollgard and conventional genotypes under unsprayed conditions for beet armyworm larvae showed 0.1, 9.6 and 10.2 larvae per meter of row. Similar data for soybean loopers showed 0.4, 8.0 and 10.7 larvae per meter of row respectively on Bollgard II, Bollgard and conventional varieties.

Trial data on yield comparisons among non-Bt, Bollgard and Bollgard II varieties is always variable, mainly because of the pest complex. Assuming that there was absolutely no pest attack and the three types of varieties, non-Bt, Bollgard and Bollgard II, were grown under similar agronomic conditions, there would be no difference in yield. But the higher the pressure from target pests, the more significant the difference in yield. The difference in yield could also be reflected in terms



of insecticide sprays required to control the target boll and budworms based on their thresholds. Under such a situation, the yield difference between the unsprayed conventional and sprayed conventional varieties is supposed to be very high. A similar trend could be found between a sprayed conventional variety, unsprayed Bollgard and unsprayed Bollgard II variety. The data given in the chart (Sherrick et al 2003) is limited to conditions in the U.S. southeast region, but it clearly indicates the impact of a single Bt gene toxin and the combined effect of two proteins under sprayed and unsprayed conditions versus a conventional variety under sprayed and unsprayed conditions.

Beet Armyworm

One of the advantages of the Bollgard II gene is resistance to beet armyworm *Spodoptera exigua* that primarily feeds on plant leaves. Previous studies on Bt varieties have shown variability in the expression of toxin in various plant parts. The same plant parts contained variable quantities of the toxin at various stages of development. The toxin expression declines in the terminal leaves throughout the season as well as within individual leaves as they age. So, the fear is that target insects will encounter low protein levels as they move downward on the plant and increase their chances of survival for a little longer, if not escape all together. During this period, damage will continue. Sparks and Norman (2002) studied the survival of beet armyworm larvae on young and old leaves containing the Bt toxin. They planted three varieties in Texas—DPL 5415 (no Bt gene), NuCotton 33B (Bollgard gene) and NuCotton 33BII (Bollgard II gene). The studies were conducted in the laboratory on leaf samples collected in the field on the 86-day old crop from four different places on the plant, the third, sixth, ninth and twelfth leaf from the main terminal. Bioassay studies were done using one-day-old larvae. Nineteen days later, similar samples were collected and bioassays were done using five-day-old larvae. Leaf samples were collected from five different plants in each of the four replications per variety using lab-reared colonies of beet armyworm. Leaves were cut in 7/8 inch leaf disks and placed in plastic cups. One beet armyworm larva was placed on the leaf disk and mortality was checked every two days. At each check, the surviving larvae were provided with a fresh leaf sample. Data were recorded for ten days on the one-day old larvae and for eight days on the five-day old larvae. The surviving larvae were weighed for their ability to tolerate the toxin doses.

The results revealed that two days after the larvae were released on the leaves, mortality was low in each test (one-day and five-day-old larvae) and generally remained so for the younger leaves in DPL 5415. It confirmed that the mortality at later stages is the result of the Bt toxin rather than the handling and disease effects on the colonies. The data revealed that the presence or absence and type of Cry protein and leaf age had a significant impact on mortality and weight of the larvae after ten days of feeding. The average data across leaf ages showed that 88.3% of the larvae died after ten days when the one-day-old larvae were released on Bollgard II leaves compared to 20.4% on Bollgard leaves, and only 12.9% on non-Bt variety. The five-day-old larvae could tolerate a higher dose of Cry2Ab toxin, and only 46.3% of the larvae died after eight days on Bollgard II leaves compared to less than 1% mortality on the Bollgard and non-Bt varieties. The Bollgard II gene not only killed a higher percentage of larvae but also the weight of the surviving larvae was much lower compared to the other varieties. The average data for the three varieties showed that larvae surviving on the non-Bt variety had the greatest weight. The one-day-old larvae showed minimum mortality on the third position leaves and a linear mortality increase with the increase in the age of the leaves. Similar results were achieved on the five-day-old larvae where mortality increased from 3.3% on the third position leaves to 26.7% on the twelfth position leaves after eight days of feeding. The weight of the larvae surviving after ten days and eight days decreased with the increase in the age of the leaf.

The table reveals that variety and leaf age interaction effects are significant whether the larva is a day old or five days old. The one-day-old larvae presented some mortality even in the absence of the toxin, but all the five-day-old beet armyworm larvae survived when they were fed for eight days on a non-Bt variety as well as on a Bt variety carrying only the Cry1Ac gene. 76.6% of beet armyworm larvae died after eight days on Bollgard II leaves. Only 10% of the five-day-old larvae feeding on the third position leaves was killed by Cry2Ab (Bollgard II), indicating that terminal growth is the most likely location where beet armyworm larvae could survive.

Effect of Leaf Age and Variety on Mortality of Beet Armyworm

Variety	Leaves from Terminal	Mortality (%) One-day Old Larvae (10 days feeding)	Mortality (%) Five-day Old Larvae (8 days feeding)
DPL 5415			
	3	1.7	0.0
	6	13.3	0.0
	9	1.7	0.0
	12	35.0	1.7
NuCotton 33B			
	3	18.3	0.0
	6	0.0	0.0
	9	35.0	0.0
	12	28.3	1.7
NuCotton 33B II			
	3	65.0	10.0
	6	88.3	26.7
	9	100.0	71.7
	12	100.0	76.7

Harris et al (2002) conducted field studies for three years, starting in 1999, on Bollgard and Bollgard II against bollworms and other insects under sprayed and unsprayed conditions in the state of Mississippi, and measured lint damage caused by various pests. Sprayed plots received two caterpillar treatments while unsprayed fields received five treatments for the damaged-plant bug. Data on feeding damage by beet armyworm larvae on leaves per 9.14 meters were recoded at three stages. At each stage the non-Bt variety and the Bt variety showed damage ranging from 0.8-5.8 under sprayed conditions and 1.0-4.5 under unsprayed conditions. The extent of the damage was almost the same on the Bollgard variety under sprayed and unsprayed conditions. The Bollgard II variety did not show any damage due to beet armyworm at any stage when fields were checked three times during the season. The feeding damage by beet armyworm larvae on leaves per 9.14 meters was zero.

Sivasupramaniam et al (2003) conducted similar studies and compared the effect of feeding on various parts of the plant on bollworm weight. Vegetative and flower parts were included as feeding material and the varieties used were three isolines, DP 50, DP 50B and DP 50BII. Various plant tissues as given in the table below were freeze-dried, finely powdered and utilized in all assays. ELISA and tobacco budworm quantitative bioassays were conducted to study the expression profile. Activity against bollworm larvae was ascertained using a diet-based assay, where the tissue in agar was overlaid on diet (2% tissue in 0.2% agar), and infested with the first instar larvae. Data were recorded seven days after infestation.

The ELISA and quantitative bioassay data showed that all tissues under investigation expressed Cry1Ac alone and in combination with Cry2Ab in all parts of the plant. However, as discussed earlier in this paper, the protein quantity was different in different parts of the plant. The results from the quantitative bioassay analysis showed that expression profile of proteins in nine different parts of the plant was similar in both types of transgenics. Bollgard had the highest amount of Cry1Ac in the terminal leaf followed by petals and anthers. Bollgard II also had the highest quantity of Cry2Ab in the terminal leaves. But squares were also found to have a high quantity of Cry2Ab.

Mean Weight of the Surviving Bollworm Larvae (g)

Plant Part	DP 50	DP 50B	DP 50BII
Large leaf	77.3	27.8	6.1
Terminal leaf	63.5	19.0	5.7
Square	48.8	12.6	3.4
Bract	80.0	28.8	10.5
Calyx	83.8	25.5	12.5
Petal	56.2	16.7	5.4
Anthers	39.3	14.7	5.6
Ovule	25.0	8.9	3.2
Small boll	26.5	11.1	3.6
Average	55.6	18.3	6.2

The ELISA analysis showed the highest quantity of Cry1Ac in the terminal leaves. However, the ELISA test showed the highest quantity of Cry2Ab in ovules, at least double that in many other parts and ten times more than in the calyx. The weight of bollworms surviving on these plant parts varied. In general, DP 50 produced the most healthy bollworm larvae followed by Bollgard. Bollworm larvae surviving on the Bollgard II variety had the least weight. None of the larvae surviving on Bollgard II lived beyond the second larval stage or seven days post infestation.

Interaction Between Two Toxins

Cry1Ac and Cry2Ab are protein toxins that can interact and affect the performance of one or both toxins. Monsanto has already undertaken studies on this subject and it was reported by Greenplate et al (2002) that there is no interaction between the two Cry proteins. They designed a study to quantify the bio-efficacy of Cry1Ac/Cry2Ab (Bollgard II) cotton and compared it with Cry1Ac (Bollgard) in the tobacco budworm *Heliothis virescens* bioassay. Three isolines of a variety having Cry1Ac only, Cry2Ab only and Cry1Ac+Cry2Ab were used to examine the relative contribution of each toxin to the total efficacy of Bollgard II, in addition to studying the nature of the interaction (synergistic/antagonistic or additive) of the individual toxins in the 2-gene cotton. Purified Cry1Ac was used as a standard for comparison.

Cry1Ac and Cry2Ab Proteins in Two Isolines (µ/g dry weight)

Plant Part	ELISA Analysis		Quantitative Bioassay	
	Bollgard	Bollgard II	Bollgard	Bollgard II
Large leaf	0.9	419	21.3	200
Terminal leaf	8.3	372	36.1	263
Square	4.8	642	21.6	221
Bract	0.6	302	10.4	110
Calyx	1.3	137	8.9	43
Petal	5.6	380	34.5	90
Anthers	5.8	583	24.5	68
Ovule	4.5	1243	22.3	170
Small boll	5.0	792	22.6	198
Average	4.1	541.1	22.5	151

The data for the quantity of Cry proteins in three different isolines is given in the table. The Bollgard II line contained a simple additive quantity of Cry1Ac and Cry2Ab as measured individually in two different isolines. The results showed that the addition of Cry2Ab to Cry1Ac in cotton provided a highly significant and uniform increase in lepidopteran bioactivity. The lepidopteran activity in the *H. virescens*

quantitative assay was 3-6 times higher in the 2-gene cotton. Using the enzyme-linked immunosorbent assay test on every lyophilized plant sample in the study, it was confirmed that neither toxin was influenced by the presence of the other. ELISA results confirmed that the level of each toxin in the 2-gene isoline is identical to the level found in its single-gene isoline.

Quantity of Cry Proteins in Three Isolines (mg/g of dry weight)

Isolines	Cry1Ac	Cry2Ab
Cry1Ac	7.2	0.0
2-gene	7.0	412.0
Cry2Ab	0.0	417.7

Jackson et al (2003) studied the *Helicoverpa zea* bollworm population production and associated damage to bolls in Bollgard and Bollgard II cotton versus the conventional variety under sprayed and unsprayed conditions. Trials were conducted at three locations in North Carolina and the results were presented at the 2003 Beltwide Cotton Conferences. DP50 was grown in pure form, in Bollgard and Bollgard II forms at all locations. Insecticide treatments included in-furrow applications of aldicarb (Temik) for the control of early season sucking insects, a mid-season application of an insecticide for the control of plant bugs and stink bugs, and two applications of a suitable insecticide for supplemental bollworm control. Other agronomic operations were carried out as recommended in the area.

According to the authors, the 2002/03 season in North Carolina was characterized by high bollworm pressure. Data were recorded in smaller plots for bollworm larvae, bollworm pupae, bollworm adult and damaged bolls; the data were converted into a per-hectare basis. They found that, on average, 400,000 bolls per hectare were affected by bollworm under sprayed DP50 compared to 190,650 bolls in Bollgard DP50 and only 23,315 bolls in Bollgard II DP50. Bollworm damage under unsprayed conditions was reduced in Bollgard II by 172 times over the non-Bt gene of the same variety, and by 45 times over the Bollgard variety. Insecticide treatments drastically reduced the number of bolls damaged by bollworm to 142,814; 35,530; and 2,464 bolls/ha in DP50, Bollgard DP50 and Bollgard II DP50 respectively.

Prospects for Bollgard II

In the USA, growers have already reached the potential area for transgenic cotton by planting 77% of the total cotton area under transgenic varieties in 2002/03. However, only 40% of the total area under transgenic varieties had the Cry1Ac gene, 3% in pure form and 37% in stacked form with the Roundup ready herbicide resistant gene. The recommended adherence to the planting of a refuge crop will continue for Bollgard II and for Bollgard. The main purpose of the refuge crop is to delay the development of resistance to the Bt toxin. It is still feared that target insects could develop resistance to both toxins at the same time. Thus, in the USA, it is anticipated that the approval of Bollgard II technology could bring some increase in area planted to Bt genes. The main reason for the increase in area could be the increase in the spectrum of insects controlled by Bollgard II over Bollgard. Because a larger area will attract a larger number of Bollgard plus Bollgard II target pests, more growers will be interested to grow Bollgard II. The cotton area that will benefit the most in the USA will be the one affected by loopers and fall armyworms, such as south Georgia and Mississippi.

Bollgard II will be introduced to farmers through Delta and Pine Land Company and Stoneville Pedigree Seed Company in the USA and will be in the commercial scale stage, called the seed multiplication stage, in 2003/04. The seed produced will allow large-scale adoption in 2004/05. At this stage, it seems that only two Delta and Pine Land varieties, DP 424 BGII/RR and DP 468BGII/RR, will be planted on about 5,000-6,000 hectares each in 2003/04. Both varieties have been upgraded from Bollgard/Roundup Ready to Bollgard II/Roundup Ready. Both will have the Bollgard gene too. The Stoneville variety ST 5222B2, made available for planting in 2003/04, will not be in stacked gene form. However, Stoneville plans to introduce Bollgard II stacked gene varieties in the Roundup Ready gene in 2004/05. In the meantime, field testing of other varieties will continue and Stoneville may bring forward more varieties on a commercial scale in 2004/05.

Australia has capped the area devoted to Bollgard cotton (called Ingard in Australia) to 30% of the total area since the adoption of transgenic varieties in 1996/97. Australia planned to plant 5,000 hectares of Bollgard II in 2002/03 mainly for seed increase. During 2003/04, Bollgard II will be planted on about 50,000 hectares while the area under Bollgard will go down accordingly. Australia plans to replace all Bollgard cotton area with Bollgard II varieties in 2004/05.

Bollgard and Bollgard II technologies will both continue to be available for many years. Countries may have other considerations but cost is one of the limitations for the easy spread of genetic engineering technology. It is hoped that, as more insect resistant genes are identified and adopted, other countries may have easier access to the older genes.

Technology Fee for Bollgard II

Bollgard II varieties have the ability to control a broader spectrum of lepidopterans, thus the potential to save more on insecticides. The increase in savings will depend on the population of the target pests controlled. Bollgard II provides better control of fall armyworm *Spodoptera frugiperda*, beet armyworm *Spodoptera exigua*, cabbage looper *Trichoplusia ni*, soybean looper *Pseudoplusia includens* and if these pests are not a serious threat, Bollgard II may not bring any savings in insecticide use as the additional Bt gene will be more costly to farmers.

Monsanto has fixed the technology fee for Bollgard II at US$99/ha, which again will depend on the variable seed drop rate in the USA, as was the case for Bt cotton. Growers who decide to

go for Bollgard II cotton will pay the same technology fee irrespective of seed size/rate used to plant a particular area. The technology fee for Bollgard cotton in the USA is US$79/ha and less in states that have lower insecticide savings. It is assumed that the technology fee for Bollgard II will also be different among states in the USA.

In the past, Monsanto has charged Australian cotton growers more for the same technology offered to U.S. cotton growers because in Australia farmers have higher savings in insecticides. It is assumed that the same philosophy will continue with Bollgard II.

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Limitations on Organic Cotton Production

Organic cotton has been produced for centuries, but it was first officially certified in 1989/90 by Turkey, followed by the USA. Other common names used for organic cotton, particularly at the beginning of production, are green cotton, biological cotton and environment-friendly cotton. There are countries where no insecticides or synthetic fertilizers are used to grow cotton, but production is not sold as organic because it lacks certification. In order to claim that cotton is organic and receive a premium price, cotton production must be recognized as organic by a certifying organization.

Certifying companies, which are well-known among producers, buyers and processors of organic cotton, have established their own organic cotton production standards. The number of certifying companies is small, and standards may vary among them. The Technical Information Section of the International Cotton Advisory Committee has kept track of organic cotton production in the world for many years and has published many articles on the subject. However, in some cases, data from some countries has been unavailable, and the Section has been unable to update the information. It is assumed that production has not increased beyond the experimental stage in countries other than India, Turkey and the USA.

Organic Cotton Production in the USA

The Organic Trade Association, a membership-based business association representing the organic industry in North America through its Organic Fiber Council, has been able to keep a record of organic cotton production in the USA. In the USA, organic production prohibits the use of genetically engineered varieties, irradiation or sewage sludge, as well as toxic and persistent pesticides and synthetic fertilizers.