Bollgard® II versus WideStrike™ Biotech Cotton

Biotech varieties were grown on 95% of the area planted to Upland cotton in the USA in 2008/09. Biotech varieties include single gene and stacked gene insect resistance, in addition to herbicide resistance. The insect resistant events approved in the USA are Bollgard®, Bollgard® II and WideStrike™. Bollgard® carrying the Cry 1Ac gene was commercialized in 1996/97, Bollgard® II, with the Cry 1Ac and Cry 2Ab genes, in 2003/04 and WideStrike™, with Cry 1Ac+Cry 1F, in 2005/06. The purposes of adding a second gene to Bollgard® II and WideStrike™ were to enhance the spectrum of pests controlled and delay the development of resistance, a danger that is more likely when there is a single type of toxin. It is estimated that over 90% of the Upland cotton area in the US in 2008/09 was planted to Bollgard®+ Bollgard® II varieties and only 1-2 % to WideStrike™ varieties, both in the single gene and the stacked gene forms to add the herbicide resistance characteristic.

In the USA, losses due to various pests are estimated every year. The report presented at the 2008 Beltwide Cotton Conferences showed that the bollworm/budworm complex caused more damage to cotton in 2007 than other insects (Williams, 2008). According to Williams (2008) the average cotton yield in 2007/08 in the USA was lower by 0.913% because of Heliothines. The Lygus bug was number two, lowering yield by 0.683%; thrips were third with 0.578%, cotton fleahoppers were forth with 0.477% and aphids were fifth with a yield reduction of 0.320%. All the sucking pests together caused more than double the yield loss caused by the budworm/bollworm complex, but against them farmers have to rely on chemical controls, while biotech cotton is the main defense against budworms and bollworms. Cotton was planted on 4.3 million hectares in the USA in 2007/08, and the average yield was 985 kg/ha. The 0.913% yield loss caused by Heliothines comes to nine kilograms of lint per hectare which is equal to US$61.4 million at the average Cotlook A Index price of US$1.61/kg for 2007/08. It is not possible to eliminate losses as long as budworms and bollworms exist as pests on cotton; it is, however, definitely possible to lower those losses. *Helicoverpa zea* is the most important of the Heliothines affecting cotton in the USA, and WideStrike™ and Bollgard® II insect resistant technologies are supposed to minimize those losses. Yield losses to bud and bollworm attacks were estimated at 3.7% from 1985 to 1995 (Gianessi and Carpenter, 1999). Thence the need to stack another gene or find a stronger one; this and many more aspects of WideStrike™ and Bollgard® II technologies are compared in this article.

Bollgard® II Technology

Monsanto developed Bollgard® II Event 15985 by re-transformation of Bollgard® cotton Event 513. Bollgard® II produces two proteins that provide effective control of the major Lepidopteran pests of cotton, including the cotton bollworm *Helicoverpa zea*, tobacco budworm, *Heliothis virescens*, pink bollworm *Pectinophora gossypiella*, and beet armyworm *Spodoptera exigua*. Bollgard® II also produces the β-D-glucoronidase (GUS) marker protein. The GUS protein has no insecticidal properties and is used as a marker to facilitate the detection of plants capable of producing Cry 2Ab. The GUS produced in Bollgard® II is extremely safe. In fact, GUS is present in intestinal epithelial cells, intestinal microflora bacteria and numerous foods. Cry 2Ab is also designated as Cry 2Ab2, Cry IIB, Cry B2 and Cry IIAb. Bollgard® II provides greater control of tobacco budworm, pink bollworm and cotton bollworm than Bollgard®, plus it provides additional control of secondary lepidopteron insects such as beet armyworm *Spodoptera exigua* and fall armyworm *Spodoptera frugiperda*. The fall armyworm feeds on foliage

and developing fruit forms. In cotton, the damage is severe when the larvae feed on developing bolls. The beet armyworm damages seedlings, growing tips in young plants and small bolls. The young larvae make a loose web over the feeding site for protection. Older larvae chew irregular holes in leaves and also feed on squares, flowers, and bolls. Square damage by the beet armyworm differs from bollworm damage in that the surrounding bracts and foliage are often damaged by the beet armyworm but not by bollworm. However, the beet armyworm primarily feeds on plant leaves. It is known (ICAC, 2008) that there is variability in the expression of Bt toxin in various plant parts and at various stages of plant development. Toxin expression declines in the terminal leaves throughout the season, as well as within individual leaves, as they age. Thus, it was feared that the target insects would encounter progressively lower protein levels as they moved downward on the plant thereby increasing their chances of survival for a little longer or of escaping altogether. During this period, pest damage would continue. Both types of armyworm can cause sever losses in yields, so it became necessary to have more effective control, similar to the control of the tobacco budworm and other insects against which Bollgard® is effective.

WideStrike™ Technology

WideStrike™® has two genes, Cry 1F and Cry 1Ac, also derived from *Bacillus thuringiensis (Bt)*. Dow AgroSciences developed the technology to control early and late season insects mostly controlled by Bollgard® II as well. To be effective, the Cry proteins must be ingested by the target lepidoteran insects affecting cotton . The target pests have a high pH in the midgut and the protein is dissolved triggering a chain of reactions that ultimately results in the death of a target pest. The Cry 1Ac and Cry 1F genes bind to specific receptor molecules on the midgut epithelial cells of the target pests. Once bound, the receptor produces in the midgut cells, leading to lysis, cessation of feeding and death. The overlap among receptors is incomplete. Cry 1Ac binds to at least three receptors while Cry 1F binds to at least two receptors in the tobacco budworm. In the cotton bollworm, Cry 1Ac and Cry 1F each bind to at least four receptors, of which two are shared. Data submitted by Dow AgroSciences to the US Environmental Protection Agency for approval of WideStrike™ reported that in cotton bollworm approximately 60% of Cry 1Ac binding is to receptors that also bind Cry 1F, and the remaining 40% of Cry 1Ac binding is to receptors that do not bind Cry 1F. Incomplete shared binding is expected to delay cross-resistance when resistance is mediated by receptor changes.

Bollgard® II was deregulated by the U.S. Environmental Protection Agency in December 2002 while WideStrike™ was approved for commercial production in September 2004, almost two years later. WideStrike™ technology is available only through PhytoGen Cottonseed varieties. PhytoGen Cottonseed was established in 1980, but only 3.3% of the cotton area was planted to Phytogen varieties in the USA in 2007/08, compared to 42.9% to Deltapine 29.3% to Bayer CropScience Fibermax varieties and 15.4% to varieties developed by Stoneville Pedigree Seed Company (Anonymous, 2007). The Delta and Pine Land Company, Bayer CropScience and Stoneville Pedigree Seed Company were using Monsanto's Bollgard® technology and only PhytoGen Cottonseed was using WideStrike™ technology. This is also one of the reasons that WideStrike™ is approved only in the USA. In June 2007 when Monsanto bought Delta and Pine Land Company, Monsanto had to sell the Stoneville Pedigree Seed Company. Bayer CropScience now owns Stoneville Pedigree Seed Company and there is a possibility that the area currently planted to WideStrike™ varieties may increase within the next few years. In January 2006, the Dow AgroSciences and Monsanto Company made an agreement on cross-licensed intellectual property rights. The impact with respect to the WideStrike™ character is yet to be seen.

Interaction Between Two Toxins

The Cry protein (Anonymous, 2003) names are assigned according to the similarity in amino acid sequences. In this nomenclature, Cry proteins with the same Arabic numeral i.e. Cry 2, share at least a 45% of amino acid sequence identity. The Cry proteins with same Arabic numeral and uppercase letter, such as Cry 2A share at least a 75% sequence identity. And, the Cry proteins with the same number, uppercase letter and a lowercase letter (for example Cry 2Ab in the case of Bollgard® II) share more than a 95% amino acid sequence identity.

Cry 1Ac and Cry 2Ab are protein toxins that can interact and affect the performance of one or both toxins. Monsanto has already undertaken studies on this subject, and Greenplate *et al* (2002) reported that there is no interaction between the two Cry proteins. The researchers designed a study to quantify the bio-efficacy of Cry 1Ac/Cry 2Ab (Bollgard® II) cotton and compared it with Cry 1Ac (Bollgard®) in the tobacco budworm *Heliothis virescens* bioassay. Three isolines of a variety having Cry 1Ac only, Cry 2Ab only and Cry 1Ac+Cry 2Ab 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 Cry 1Ac was used as a standard for comparison. The studies proved that both genes work independently of each other and that there is no interaction between them.

Efficacy of WideStrike™ against other Technologies

There is no doubt that in terms of insect control, both Bollgard® II and WideStrike™ are superior to Bollgard®. Differences, if any, are all due to the 2nd Bt gene and the host genotypes. The two-gene biotech cottons have a broader spectrum of activity and increased efficacy. However, the potential for caterpillar damage remains, and both technologies may require treatment against target insects. Adamczyk *et al.* (2008) compared WideStrike™ with Bollgard® and Bollgard® II to assess

their ability kill the beet armyworm and fall armyworm. Experiments were conducted in 2005/06 and 2007/08 in the field and lab. The field sites varied in 2005/06 and 2007/08. All Lepidopteran insects used in the experiments were lab-reared colonies. In 2005, bioassays were conducted using only fall armyworm larvae. For undertaking bioassay studies using larvae, a single larva was placed in individual petri dishes containing a moistened filter paper and a single lower leaf obtained from all plots for a total of 32 larvae/variety. Leaves were collected when the crop was at peak bloom. The petri dishes were covered and after five days surviving larvae were carefully transferred to new petri dishes containing fresh filter paper and new leaf. This procedure continued until pupation. Live larvae were counted at seven and 10 days. Petri dishes were checked daily for presence of pupae starting from 15 days. In 2007, the beet armyworm larvae were placed in a dish containing a terminal (upper canopy) leaf or a mid-canopy leaf (10 dishes/variety) for a total of 50 larvae/variety. Fall armyworm bioassays were conducted identically, except that only mid-canopy leaves were used. Leaves were also collected at various stages during the growing season. Percent mortality was counted after five days.

Bioassay studies on egg masses were undertaken in the field in 2007/08. Inoculations with beet and fall armyworm egg masses were done using various sections of the plant. Eggs were spread on a piece of nylon cloth and same-size samples were pinned to the underside of a leaf for all traits and covered with a cage that consisted of a condiment cup covered with a hard plastic lid. Five days after inoculation, the infested leaves and the corresponding cages were harvested and transported to the laboratory. Leaf damage was classified on a scale of 0-5, 0 being no damage and 5 being 100% damage.

The results showed that in 2005/06 and 2007/08, WideStrike™ and Bollgard® II performed significantly better than Bollgard® against fall armyworm larvae. Adamczyk *et al.* (2008) observed that in both 2005 and 2007, WideStrike™ had typically higher efficacy than Bollgard® II. They also noted that fall armyworm larvae developed successfully to pupation when fed Bollgard® or Bollgard® II, but not WideStrike™. They related WideStrike™'s greater efficacy against the fall armyworm to the Cry 1F protein. The beet armyworm survived equally well on Bollgard® II and WideStrike™ when mid-canopy leaves were fed to larvae. Late in the season, however, when beet armyworms were fed leaves located in the upper part of the plant (i.e. upper-canopy leaves), larval survival on WideStrike™ was very high (>60%). This means that when WideStrike™ cotton is close to maturity, Cry 1F expression is low in young terminal leaves. Furthermore, beet armyworm mortality on WideStrike™ terminal leaves at over 109 days after planting was similar to that observed on Bollgard®. This means that WideStrike™ may require supplemental insecticide applications to control beet armyworms feeding on younger leaves late in the season. Results of tests using egg masses and cages support the observations and conclusions above.

VipCot™ (Vegetable Insecticidal Protein) lines utilize a single protein (Vip3A). Syngenta discovered this protein 1994. The Syngenta technology has been extensively tested in the USA but not commercialized yet. Although derived from the bacteria *Bacillus thuringiensis* (Bt), Vip is structurally and functionally different from the d-endotoxins employed in current traits. Vip is expressed throughout the entire plant and provides good protection against the cotton bollworm, American bollworm, native bollworm, tobacco budworm, pink bollworm, beet armyworm, fall armyworm, cabbage looper and soybean looper.

Bacheler and Mott (2004) tested WideStrike™, Bollgard® and Vip lines for their efficacy against the cotton bollworm, *Helicoverpa zea*. However, in 2004, Monsanto regulations prohibited direct comparison of these lines using the same test in adjacent fields. So, Bacheler and Mott (2004) evaluated each technology in separate, but adjacent tests within the same field border. The location of the trials normally has high bollworm pressure. The results indicated that under adverse conditions, each of the technologies evaluated may, at times, require protection from bollworms. Additionally, the VipCot™ cotton line sustained European corn borer damage to bolls. Although low (2%) European corn borer may indicate a certain susceptibility in the Vip3A gene. The WideStrike™, VipCot™, and the Bollgard® II lines showed bollworm damage to bolls of 15, 14, and 6%, respectively, at the peak boll damage scouting assessment. Yield differences appeared to correlate with these boll damage trends. The parathyroid-protected counterparts of these same lines showed yield increases of 158, 327, and 207 kg lint/hectare for the WideStrike™, VipCot™ and Bollgard® II lines, respectively. Stink bug levels were extremely low, thus supporting the inference that these yield differences appeared to have been caused by bollworms.

Cook *et al.* (2008) compared two WideStrike™ varieties with one Bollgard® II variety and two non-Bt varieties without supplemental insecticide applications. Treatment efficacy was determined by examining 25 squares per plot for evidence of heliothine feeding on both the biotech and non biotech varieties at 67, 75, 80, 91 and 97 days of planting. Similarly, boll damage was assessed by examining 25 bolls per plot on the same dates. In another trial , two WideStrike™ varieties were compared with one Bollgard® II variety stacked with Roundup Ready Flex, with and without supplemental insecticide applications against heliothine insects. To check for the presence of heliothines, 25 squares were examined at 75, 80, 91 and 98 days after planting. Bollworm damage was also assessed in the 2nd experiment in the same way as in the first trial. The results showed that both the WideStrike™ and Bollgard® II technologies had significantly fewer damaged squares and bolls compared to Roundup Ready Flex non-insect resistant biotech cotton varieties.

Hardke *et al*. (2008) conducted laboratory studies to test the efficacy of various types of insect resistant technologies against fall armyworm. They selected one conventional/

non-biotech variety each of Bollgard® cotton, Bollgard® II, WideStrike™ and VipCot. Freshly harvested flower buds from all varieties were placed in petri dishes and lab-reared L3 larvae of the fall armyworm were released in the petri dishes to feed exclusively on the flower buds. The supply of flower buds was replenished every 2-3 days or whenever necessary. A minimum of two replicates, each with a total of 30 larvae, produced a total sample size of 60 larvae per variety/line. Dead larvae were counted every 2-3 days. The results showed that the fall armyworm larvae continued feeding on non-biotech squares and by the cut-off point, which was 12 days after initiating the experiment, 100% mortality had not been achieved. Larval mortality on conventional variety flower buds ranged from 1.7% at two days after treatment to 41.6% at 12 days after treatment. On Bollgard® cotton, the mortality rate ranged from 0% at two days after treatment to 65% at 12 days after treatment. Thus, there was not 100% mortality on Bollgard® cotton at the end of the treatment, i.e., 12 days. While only 1. 7% of the larvae had died on Bollgard® II cotton at two days after treatment, the mortality rate had increased to 85% on the 7th day and 88.3% at 12 days after treatment. All the fall armyworm larvae feeding on WideStrike™ variety flower buds were dead at seven days after treatment. VipCot squares produced results similar to that of WideStrike™ technology, and complete mortality was observed at the end of the treatment. Hardke *et al*. (2008) intend to repeat the experiment in the 2008/09.

Many factors determine the survival of lepidopteran pests on biotech cottons. Toxin expression is influenced by genotypes, growing conditions, stage of crop development, plants parts, etc., so all the impact seen on a particular variety/line cannot be directly attributed to the Cry gene or any other biotech gene. Interestingly, larvae of the target pests also behave differently depending on the previous host. Jackson *et al.* (2007) compared two colonies of the fall armyworm on the same variety. They collected late instars of fall armyworm from Bt and non-Bt sweet corn to establish two separate colonies. Two-day old and 5-day old F_1 larvae from each colony were confined to white flowers of two non-Bt cotton varieties, a WideStrike™ variety, and a Bollgard® II variety with cloth cages to evaluate damage potential. The results showed that the Bt corn strain of fall armyworm damaged significantly fewer bolls, because of the previous host crop, compared to the non-Bt corn strain. No differences were detected between strains with respect to boll damage levels caused by 2-day old larvae in WideStrike™ or Bollgard® II cottons. Assessement of 5-day old larvae of the fall armyworm showed that in one out of three replications the damage caused by the Bt corn strain to non-Bt cotton was significantly less than that of the non-Bt strain. Among strains of Bollgard® II and WideStrike™ cottons, no differences were detected in damage levels caused by 5-day old larvae. Leaf tissue bioassays were also conducted to compare survival of two fall armyworm strains that originated from either non-Bt or Bollgard® II cotton. No differences were detected in the survival rates of 3-day old larvae feeding on either non-Bt or Bollgard® II cotton. Results from these studies suggest that there may be some fitness cost associated with fall armyworm development on Bt sweet corn. Because this same phenomenon was not associated with development on Bt cotton, further studies should be conducted to examine the impact of Bt crops on fall armyworm populations.

The above results conflict with some earlier work which showed that F_1 progeny from a fall armyworm strain collected from a Bt corn field were more vigorous in the presence of non-Bt cotton than those from a strain from a non-Bt corn field . This incidence may be explained by a higher expression of the Cry 1Ab protein in Bt sweet corn compared to a strain from a Bt corn field. However, it is clear that the combination of two proteins (Cry 1Ac and Cry 1F) in WideStrike™ is highly effective against fall armyworm in field and laboratory tests. The Cr 1Ac and Cry 2Ab genes together also provide good control of fall armyworm but not as good as WideStrike™.

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