UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

February 17, 2004

MEMORANDUM

SUBJECT: EPA Review of DowAgroSciences' Product Durability (Insect Resistance Management) Plan in Support of the Section 3 Application for the Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton, Submitted by Dow AgroSciences [Reg. No. 068467-G; Decision Number 214150; DP Barcode: D290936; Case: 071326; Includes 5 Studies with MRID#'s: 45808415, 45808407, 45808417, 45808418, 460719901]

TO: Leonard Cole, Regulatory Action Leader
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FROM: Sharlene R. Matten, Ph.D., Biologist
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ACTION REQUESTED: To review the product durability plan/insect resistance management (IRM) plan, high dose studies, and field efficacy studies for tobacco budworm, (*Heliothis virescens*, TBW), cotton bollworm (*Helicoverpa zea*, CBW), and pink bollworm (*Gossypiella pectinophora*, PBW) submitted by Dow AgroSciences to support their application for a Section 3 Registration for Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton. Note: the high dose studies and field efficacy studies are reviewed separately, but the summaries of these reviews are contained within this full review of the IRM/product durability plan.

CONCLUSIONS

Dow AgroSciences' (DAS) proposed product durability/insect resistance management (IRM) plan for WideStrike is acceptable. The same refuge options currently mandated by EPA for Bollgard® and Bollgard II®[1] cotton (EPA 2001, 2003) should be appropriate for insect resistance management to WideStrike (MXB-13). This refuge strategy will afford clarity and consistency of the IRM to growers, consultants, extension entomologists, seed dealers, and others that need to understand and implement it.

WideStrike cotton, expressing both the Cry1F and Cry1Ac insecticidal control proteins, is intended to protect cotton from feeding by three key lepidopteran pests of cotton in their respective geographies: tobacco budworm (*Heliothis virescens,* TBW, Lepidoptera: Noctuidae)), pink bollworm (*Pectinophora gossypiella*, PBW, Lepidoptera: Gelechiidae), and cotton bollworm (*Helicoverpa zea*, CBW, Lepidoptera: Noctuidae). In addition, several other lepidopteran pests are controlled by this product: cabbage looper (*Trichoplusia ni*, CL), soybean looper (*Pseudoplusia includens,* SL), beet armyworm (*Spodoptera exigua,* BAW), fall armyworm (*Spodoptera frugiperda*, FAW) and southern armyworm (*Spodoptera eridania*, SAW) (all Lepidoptera: Noctuidae).

The binding patterns of the two proteins in CBW and TBW indicate there are shared and unique binding sites. In TBW, Cry1Ac binds to at least three receptors, while Cry1F binds to at least two, only one of which binds Cry1Ac. In CBW, Cry1Ac and Cry1F each bind to at least four receptors, of which two are shared. For CBW, approximately 60% of Cry1Ac binding is to receptors that also bind Cry1F, and the remaining 40% of Cry1Ac binding is to receptors that do not bind Cry1F. Incomplete shared binding is expected to lead to incomplete cross-resistance when resistance is mediated by receptor changes. Thus, a mutation in a gene that codes for a receptor that bind both ICPs will not prevent all binding of either ICP and thus alone will not allow high survival of the insect bearing even two copies of it, on Cry1F/Cry1Ac stack cotton plants.

The DAS CBW modeling efforts show that we can have high confidence that there will not be a significant change in population fitness of CBW on WideStrike in a 15-year time horizon even without a high dose for either Cry1Ac or Cry1F and incomplete cross-resistance (20 to 60% maximum shared binding)**.** Market share analysis of WideStrike versus Bollgard or Bollgard II had little effect on the rate at which CBW may adapt in either the North Carolina or Mississippi Delta agroecosystem**..** Refuge size, whether sprayed or unsprayed, had no significant impact on CBW population fitness on WideStrike (MXB-13) after 15 years. In the Delta the immigrating non-selected population from alternate hosts further reduces the local rate of adaptation. The local structured refuge only supplies a small proportion of the non-selected insects in the Delta. The availability of CBW alternate hosts, coupled with a non- *Bt* cotton refuge are additional levels of assurance for WideStrike product

[1]Bollgard® and Bollgard II® are trademarks of Monsanto Company.

durability. Additional empirical information is needed on the function and effectiveness of alternate hosts on the rate of CBW adaption.

For TBW, durability is expected to be greater than that predicted using the TBW model by Peck et al. (1999) where the worst case (structured refuge is moved each year) is 17 years. TBW exhibits similar patterns in binding studies as does CBW and Widestrike expresses a high dose against TBW. The Cry1Ac component alone is a high dose, the Cry1F component alone is not quite a high dose. For PBW, WideStrike expresses a high dose of Cry1Ac, just as does Bollgard (Cry1Ac) cotton. Cry1F is not effective against PBW. Current refuge options mandated for management of PBW resistance to Bollgard cotton should be appropriate for WideStrike. Any plan that focuses on TBW, CBW, and PBW should be adequate, to maintain susceptibility in secondary pests, such as FAW, BAW, SAW, SL, and CL. The market mix of different *Bt* cottons (at present, Bollgard and Bollgard II), as well as other control technologies, further reduces the expected selection pressure for resistance against WideStrike.

SPECIFIC RECOMMENDATIONS:

1. *Research Data: Pest Biology and Ecology*. EPA recommends that DAS provide the Agency with relevant IRM research applicable to WideStrike IRM such as that described above: north-south migration of CBW and its impact on both *Bt* corn and *Bt* cotton resistance management and development of Bt-resistant colonies to better understand cross-resistance patterns. Other IRM research is desirable to refine tobacco budworm TBW and CBW resistance models and to develop PBW resistance models. To support alternate hosts as effective refuges of CBW (TBW, PBW), DAS would need to supply published information or data regarding the timing and production of larvae and adults on each alternate host, mating behavior, origin of moth production (i.e., which alternate hosts) both locally and regionally, proximity of alternate host production to *Bt* cotton, survival and fecundity of each host, and fitness of adults coming of alternate hosts. Similarly, DAS should provide appropriate data regarding the effectiveness of supplemental insecticide treatment of *Bt* cotton fields to control putative resistant CBW. This research will improve the strength and reliability of an IRM plan to effectively reduce the likelihood that TBW, CBW, or PBW will become resistant to the Cry1Ac and Cry1F ICPs.

2. *PBW Model*. It is recommended that DAS include PBW resistance modeling (Cry1Ac focus) in its product durability analysis.

3. *Consistency in field expression*. It is recommended that DAS statistically analyze its field expression data (Phillips et al. 2002; MRID# 458084-08) to determine whether field expression for Cry1F, Cry1Ac, and Cry1F/Cry1Ac are consistently expressed at high doses throughout the growing season in all plant parts. This will allow the Agency to determine whether there is likely to be any significant drop-off in expression in fruit structures (especially) that may lead to sub-lethal exposure and hence greater selection intensity.

4. *Resistance monitoring.* It is recommended that DAS provide EPA the baseline susceptibility data for the Cry1F and Cry1Ac for the 2002 and 2003 growing season, establish diagnostic/discriminating concentrations for tests for TBW, CBW, and PBW to Cry1F and Cry1Ac, and provide a detailed resistance monitoring plan for both the Cry1Ac and Cry1F ICPs. It is also recommended that the basic resistance monitoring program requirements mandated for Bollgard and Bollgard II, be mandated for WideStrike.with the proviso that they should be specific for the Cry1Ac and Cry1F ICPs (see EPA 2001; EPA 2003). Additionally, it is recommended that DAS coordinate its monitoring efforts for WideStrike with the current resistance monitoring programs for other *Bt* ICPs. The lead for PBW monitoring efforts is Dr. Tim Dennehy, University of Arizona and the lead for the TBW and CBW monitoring efforts is Dr. Carlos Blanco, USDA/ARS, Stoneville, MS. Coordination is essential to a large scale resistance monitoring program, one that potentially covers 5+ million acres of *Bt* cotton.

5. *Remedial Action Plans.* DAS should prepare specific remedial action plans for WideStrike to address TBW, CBW, and PBW resistance if it is suspected or actually does occur as was mandated for Bollgard and Bollgard II cotton with the proviso that they should be specific for the Cry1Ac and Cry1F ICPs (see EPA 2001; EPA 2003). While the general elements of the remedial action plans for suspected and confirmed resistance are noted by DAS, these plans need more detail.

6. *Education and Compliance.* It is recommended that DAS be required to adopt the same education and compliance requirements that are currently required of Monsanto for Bollgard and Bollgard II with the stipulation that an "ABSTC-type" arrangement be made to meet these requirements across all *Bt* cotton products.

7. *Annual Reporting Requirements.* It is recommended that annual reports for research (items in #1), resistance monitoring, grower education, compliance assurance, and sales (for each state and counties within each state) be required.

BACKGROUND:

WideStrike cotton expresses the *Bacillus thuringiensis* Cry1F insecticidal protein (ICP) pyramided with the already registered Cry1Ac ICP (Cry1Ac is the ICP found in Bollgard®, EPA Reg. No. 524-478 and is one of two ICPs (the other one is Cry2Ab) in Bollgard II®, EPA Registration No. 524-522). WideStrike is intended to protect cotton from feeding by tobacco budworm (*Heliothis virescens*, TBW), pink bollworm (*Pectinophora gossypiella*, PBW), cotton bollworm (*Helicoverpa zea*, CBW), cabbage looper (*Trichoplusia ni*, CL), soybean looper (*Pseudoplusia includens*, SL), beet armyworm (*Spodoptera exigua*, BAW), fall armyworm (*Spodoptera frugiperda*, FAW) and southern armyworm (*Spodoptera eridania*, SAW). Based on cotton insect loss data from 1991-2000, the three primary pests, TBW, CBW, and PBW, account for more than 77% of the yield lost and 84% of the insecticide use due to lepidopteran infestation in cotton.

Dow AgroSciences (DAS) transformed Acala cotton line GC510 with plasmids pAGM281 and pMYC3006. Cotton event 281-24-236 (Cry1F) resulted in the insertion from pAGM281 of one intact copy of *cry1F* and one intact copy of *pat* (plant selectable marker gene, phosphinothricin acetyltransferase). Cotton event 3006-210-23 (Cry1Ac) resulted in the insertion from pMYC3006 of one intact copy of *cry1Ac* and one intact copy of *pat*. These two Acala cotton lines, Event 281-24-236 (Cry1F) and Event 3006-210-23 (Cry1Ac) were separately backcrossed three times with cotton line PSC355 followed by one generation of self-pollination to yield the BC3F1generation. The two BC3F1 events were then intercrossed and self-pollinated to the F3 generation, forming cottonseed designated 281-24-236/3006-210-23, which contains the genes for expression of Cry1F, Cry1Ac, and PAT proteins designated as WideStrike (MXB-13).

REVIEW OF DOW'S PRODUCT DURABILITY PLAN

Dow has provided information regarding the scientific basis for the product durability plan (insect resistance management strategy) and the practical implementation of the durability plan. These will both be reviewed.

I. Scientific Basis for the Product Durability Plan

A. Target Pests and Perceived Risk of Resistance to *Bt* cotton

Dow Review. WideStrike cotton (MXB-13) is intended to protect cotton from feeding by three key lepidopteran pests of cotton in their respective geographies: tobacco budworm (*Heliothis virescens*, TBW, Lepidoptera: Noctuidae)), pink bollworm (*Pectinophora gossypiella*, PBW, Lepidoptera: Gelechiidae), and cotton bollworm (*Helicoverpa zea*, CBW, Lepidoptera: Noctuidae). In addition, several other lepidopteran pests are controlled by this product: cabbage looper (*Trichoplusia ni*, CL), soybean looper (*Pseudoplusia includens*, SL), beet armyworm (*Spodoptera exigua*, BAW), fall armyworm (*Spodoptera frugiperda*, FAW) and southern armyworm (*Spodoptera eridania*, SAW) (all Lepidoptera: Noctuidae).

The perceived risks and consequences of adaptation to *Bt* cotton vary by pest **(Table 1)**. TBW, PBW and CBW are regarded as those at highest risk of adapting to transgenic cotton and of greatest consequences as they are the key lepidopteran pests in their respective geographies (Gould and Tabashnik, 1998). TBW and PBW pest populations are thought to be centered on cotton in the US cotton belt while CBW populations exist on corn and cotton where Bt-expressing varieties of both crops are widely deployed. Risks and consequences of adaptation by secondary lepidopteran pests are thought to be smaller due to their broad crop range and wild host ranges **(Table 1.)**. Several of these secondary pests can only overwinter in the extreme south of the US and therefore selection in most of the Cotton Belt is not relevant to inter-seasonal resistance evolution, e.g., FAW. In addition, their populations are sporadic and patchy and do not require seasonal intervention. The secondary pests are also expected to result in much less economic damage and have less environmental impact than TBW, CBW, or PBW and thus, WideStrike cotton would not be impaired if there was adaption to the insecticidal control proteins (ICPs). Population-wide selection pressure for adaptation is

expected to be low for these pests and product durability measures taken to slow adaptation to TBW, CBW and PBW would be expected to be adequate to maintain susceptibility in secondary pests. Hence, the product durability plan focuses on TBW, CBW, and PBW.

EPA Review: DAS has provided an adequate discussion of the target pests of WideStrike cotton and the perceived risks of adaption to *Bt* cotton, such as WideStrike cotton. EPA agrees that the product durability plan should focus on the three primary, most economically and environmentally damaging lepidopteran pests: TBW, CBW, and PBW. Any plan that focuses on TBW, CBW, and PBW should be adequate, to maintain susceptibility in secondary pests, such as FAW, BAW, SAW, SL, and CL.

Table 1 displays the factors driving resistance risk and consequence for target pests of MXB-13 (WideStrike cotton). Resistance risk is considered to be moderate for TBW, CBW, PBW, and BAW and low for FAW, SAW, CL, and SL (=SBL in the table). The resistance consequence is high for TBW, CBW, and PBW; moderate for BAW; and low for FAW, SAW, CL, and SL. A number of factors drive the risk of resistance: host range, geographic range, overwinter range, pest states in US cotton, and dose (whether high or not). These factors will be taken into consideration separately in this review.

Efficacy data was provided by Dow, Pellow et al. (2002); MRID# 45808407, and reviewed separately by EPA (see Memorandum S. Matten to L. Cole, EPA Review of Field Efficacy Data for the Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton, dated February 5, 2004). The degree of efficacy that WideStrike has for each of the target pests (primary and secondary pests) is briefly summarized below.

The results of 19 evaluations in efficacy trials from 2001 to 2002 indicate that Cry1F/Cry1Ac transgenic cotton line MXB-13 provided effective control against the eight cotton insect pests evaluated: tobacco budworm (TBW), *Heliothis virescens* (F.); cotton bollworm (CBW), *Helicoverpa zea* (Boddie); pink bollworm (PBW), *Pectinophora gossypiella* (Saunders); beet armyworm (BAW), *Spodoptera exigua* (Hubner); southern armyworm (SAW), *Spodoptera eridania* (Stoll); fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith); soybean looper (SBL), *Pseudoplusia includens* (Walker); and cabbage looper (CL), *Trichoplusia ni* (Hubner). That is, in all trials and for all insect pests evaluated, MXB-13 (sprayed and unsprayed) provided as good or better control when compared to the sprayed or unsprayed non-transgenic control line, PSC35 (the recurrent parent for both the Cry1F and Cry1Ac transgenic cotton events).

Results of five trials over a two-year span indicate MXB-13 provides a high level of control of TBW. The level of control is at least equal to, and in many cases far superior to optimum chemical spray programs used during ideal environmental conditions. Results also indicate that MXB-13 surpassed the effectiveness of chemical spray programs under non-ideal environmental conditions such as sustained periods of rain. Efficacy against TBW was demonstrated in both the early fruit development stage and in the late season boll maturation stage.

Also, in five trials spanning two years, MXB-13 was shown to effectively control CBW. A total of 80 individual evaluations was made comparing MXB-13 plots to unsprayed PSC355 plots. In 96% of these comparisons, the MXB-13 line exhibited equal to or less damage than the unsprayed control with 53% of the differences being statistically significant. Likewise, MXB-13 plots had equal or less damage in 58% of the comparisons to the sprayed PSC355 plots with optimum insecticide control. There were no evaluations where MXB-13 had significantly more damage or infestation than the chemically controlled PSC355 plants. No significant differences in yield were found between the unsprayed MXB-13 line and the sprayed PSC355.

MXB-13 was shown to have excellent control of PBW with no measurable boll infestation compared with 23-75% for the non-transgenic control variety. In both field trials and bioassays, MXB-13 was effective at controlling various armyworm species including BAW, SAW, and FAW. In addition, data from field trails indicate that MXB-13 controls two species of loopers: SBL and CL

B. Cry1F and Cry1Ac ICPs Mode of Action

Dow Review. Cry1 insecticidal control proteins (ICPs) have been widely studied and the mode of action well understood. Protoxin is ingested by the susceptible insect, proteolytic enzymes cleave the protoxin to the toxin core, which binds to specific receptor molecules on the surface of midgut epithelial cells. Once bound, the receptor/ICP complex causes pores to form in the midgut cells, leading to cell lysis, cessation of feeding and death. Protein-pest specificity is mediated by ICP-binding midgut receptors. Each Cry1 protein binds to a specific set of receptors that are typically present in only a relatively narrow set of Lepidoptera larvae. There is commonly overlap between the sets of receptors for different Cry1 ICPs, although the overlap is often incomplete.

For *Bt* ICPs, two modes of resistance have been observed: detoxification in the midgut lumen by proteases that cleave the ICP and alteration of receptors that prevents binding (Ferré and Van Rie 2002). The later mechanism is the most common. The receptor-site insensitivity is likely to have less fitness costs and is more likely to be mediated by single gene mutations, and thus, is expected to be the faster mechanism to evolve.

Binding of Cry1Ac and Cry1F has been studied in TBW (Jurat-Fuentes and Adang, 2001) and CBW (Adang et al., 2002; Sheets and Storer, 2001). Their findings demonstrate that both ICPs in WideStrike bind to different receptors, a subset of which are shared **(see Figure 1)**. In TBW, Cry1Ac binds to at least three sets of receptors, while Cry1F binds to at least two, only one of which binds Cry1Ac. In CBW, Cry1Ac and Cry1F each bind to at least four sets of receptors, of which two are shared. For CBW, approximately 60% of Cry1Ac binding is to receptors that also bind Cry1F, and the remaining 40% of Cry1Ac binding is to receptors that do not bind Cry1F. Incomplete shared binding is expected to lead to incomplete cross-resistance when resistance is mediated by receptor changes. Thus, a mutation in a gene that codes for a receptor that bind both ICPs will not prevent all binding of either ICP and thus alone will not allow high survival of the insect bearing even two copies of it, on Cry1F/Cry1Ac stack cotton plants. It is likely for high survival, several genetic mutations will be needed. Indeed, the YHD2 colony that is 230,000-fold resistant to Cry1Ac appears to have mutated

alleles at 3 or 4 loci (Ferré and Van Rie, 2002) and at the same time is only 130-fold resistant to Cry1F (Jurat-Fuentes et al., 2001).

EPA Review. Dow has provided adequate information to describe the potential receptor binding patterns of Cry1Ac and Cry1F in TBW and CBW. Results indicate that both Cry1Ac and Cry1F have both shared and unshared receptors. TBW and CBW binding studies involving Cry1F and Cry1Ac each have unique binding sites and share one binding site. TBW and CBW binding studies involving Cry1F and Cry1Ac (summarized in **Figure 1**) indicate that there are at least two, and probably at least six binding sites for these two proteins. As DAS notes, incomplete shared binding is expected to lead to incomplete cross-resistance when resistance is mediated by receptor changes. A single mutation in a gene that codes for a receptor that can bind both Cry1Ac and Cry1F will not prevent binding of either Cry1F or Cry1Ac, singly, and thus will not allow high survival of the insect bearing two copies of it. Multiple genetic mutations are likely to be needed for high survival of cross-resistant insects.

No PBW receptor-site binding information regarding *Bt* toxins was provided by DAS. However, there is some published literature. Karim et al. (2000) examined the receptor binding properties of Cry1Aa, Cry1Ab, Cry1Ac, and Cry2Aa *Bt* toxins to PBW and CBW midgut epithelial membranes. Both Cry1Ab and Cry1Ac toxins showed saturable, high -affinity binding to PBW and CBW brush border membrane vesicles. Cry2Aa and Cry1Aa toxins bound to BBMVs with low binding affinity, but with high binding site concentration. Saturation binding data correlated with toxicity in PBW. That is, the most potent toxins, Cry1Ac and Cry1Ab, showed high affinity saturable binding. Heterologous competition binding assays to investigate binding site cross reactivity showed that Cry1Aa, Cry1Ab, and Cry1Ac recognize the same or common binding sites on PBW and CBW midgut epithelial membranes which is different from Cry2Aa. Ligand blot data showed that Cry1Ac binds to a major 120-kDa BBMV protein in PBW and several proteins, 120 kDa, 140 kDa, and 155 kDa in CBW. Cry1Ab binds to a major 201 kDa BBMV protein in PBW and a 170 k-Da BBMV protein in CBW.

Results from the DAS cotton-insect-pest susceptibility study examining the relative sensitivities of six cotton-feeding insects to the purified Cry1F (synpro) toxin showed that PBW was essentially insensitive to the toxin (Herman and Young, 1999; MRID# 45542307). This suggests that there are few, if any, binding receptors for Cry1F in the PBW midgut. Cross-resistance through modification of binding site receptors of Cry1Ac and Cry1F would therefore not be realistic.

C. Protein Expression Patterns

Dow Review. Phillips et al. 2002, (MRID# 45808408) presented a comprehensive analysis of ICP expression in WideStrike in all cotton plant tissues over time. Cry1F is expressed at 3.5 - 8.2 ng/mg dry weight in tissues fed on by the lepidopteran pests (leaf, flower, square and boll). Cry1Ac is expressed at 0.65 - 1.82 ng/mg dry weight in the same tissues. Expression levels remain constant season long and are not affected by stacking of the two ICPs. This means that the insect pests feeding on WideStrike cotton would be exposed to both of the insecticidal proteins simultaneously at consistent levels throughout the cotton growing season. In this respect, WideStrike is superior as an IRM stack to

Bollgard II® (a stack of Cry1Ac and Cry2Ab) where Cry1Ac expression has been reported to decline over time (Adamcyzk et al., 2001).

EPA Review. Acceptable field expression data have been provided. EPA reviewed the protein expression data for WideStrike in all cotton plant tissues over time under separate cover (MRID # 45808408, Memorandum S. Matten to L. Cole entitled "EPA Review of Additional Product Characterization and Human Health Data in Support of the Section 3 Application for the Mycogen Brand Cry1F (synpro)/Cry1Ac (synpro) Construct 281/3006 Cotton," dated January 20, 2004 and in the Data Evaluation Record, S. Matten, dated January 18, 2004). A summary of the review is provided below. EPA agrees with DAS that the expression is not affected by the stacking of the two ICPS. The field expression data were not statistically analyzed for consistency in expression of each ICP over time in each tissue. Therefore, it is not possible to conclude that WideStrike provides more consistent expression throughout the growing season than Bollgard cotton (Cry1Ac) or Bollgard II cotton (Cry1Ac + Cry2Ab). DAS should statistically analyze its field expression data (Phillips et al., 2002; MRID# 458084-08) to determine whether field expression for Cry1F, Cry1Ac, and Cry1F/Cry1Ac are consistently expressed at high doses throughout the growing season in all plant parts. This will allow the Agency to determine whether there is likely to be any significant drop-off in expression in fruit structures (especially) that may lead to sub-lethal exposure and hence greater selection intensity. Field efficacy data indicate there is excellent control of PBW throughout the growing season (Pellow et al, 2002; MRID# 45808407).

The soluble, extractable Cry1F, Cry1Ac and PAT proteins were measured using ELISA methods with a limit of quantitation ranging from 0.001-0.4 ng protein/mg sample weight. Fresh sample weight was used for cottonseed, pollen, nectar and processed products; and dry sample weight was used for all other tissues. The Cry1Ac and Cry1F proteins were detected in all matrices except nectar, meal and oil. Mean Cry1Ac expression was approximately three- to twenty-times lower than Cry1F expression in leaves, squares, flowers, whole plant, boll, and seed tissue, depending on the tissue. Pollen was the only tissue in which Cry1Ac expression was higher than Cry1F expression. Expression levels of individual Cry1F and Cry1Ac proteins were similar for the single event and stacked cotton lines. PAT proteins were detected in the cotton samples from the Cry1F event and the Cry1F/Cry1Ac stacked event, but generally not detected in the Cry1Ac event samples. Varying expression of Cry1F and Cry1Ac proteins in different plant parts may cause differential selection pressure for insect resistance.

Highest Cry1Ac mean expression was observed in young leaves and squares, 1.82 ng Cry1Ac/mg tissue and in flowers, 1.83 ng Cry1Ac/mg tissue. Mean Cry1Ac expression was 1.31 ng Cry1Ac/mg tissue in terminal leaves, and 0.55 ng Cry1Ac/mg tissue in seeds. Mean Cry1F expression in root tissue ranged from N.D. to 0.2 ng Cry1Ac/mg tissue. Mean Cry1Ac expression in pollen was 1.45 ng Cry1Ac/ mg pollen.

Highest Cry1F mean expression was observed in young leaves 6.81 ng Cry1F/mg tissue and terminal leaves, 8.19 ng Cry1F/mg tissue. Mean Cry1F expression was 4.88 ng Cry1F/mg tissue in squares, 5.44 ng Cry1F/mg tissue in flowers, 3.52 ng Cry1F/mg tissue in bolls, and 4.13 ng Cry1F/mg tissue in

seeds. Mean Cry1F expression in root tissue was 0.5 to 0.9 ng Cry1F/mg tissue. Mean Cry1F expression in pollen was less than the limit of quantitation, <0.15 ng Cry1F/ mg pollen.

D. Pest Adaptation Factors

Dow Review. Pest adaptation to control technologies is an evolutionary process. There are several operational, biological and genetic factors involved in the process. A plan to manage this process must take into account each of the factors and interactions among them. Simulation models generally show that a small subset of these factors are key drivers that determine the appropriate product durability plan. These key factors as they apply to WideStrike cotton (MXB-13) are discussed below.

Operational Factors

Dose and Functional Dominance

Insect resistance management centers on reducing the mean (population wide) selective differential between insects carrying one copy of an allele for adaptation (resistance) at a given locus (*i.e.* RS heterozygotes) and those carrying no such alleles (SS homozygotes). This can be done by expressing ICPs at a dose that is expected to minimize the survival and fitness of heterozygotes. The level of ICP expression in the plants determines the fitness of SS insects in the *Bt* field, and indicates a ranges for the expected fitness of RS insects in the *Bt* field. Doses that cause high mortality of susceptible insect will also cause high mortality of heterozygous insects unless the R allele is dominant. At doses that cause low to moderate mortality of susceptible insects, heterozygous insects are expected to have a lower mortality, even if the R allele is nearly recessive. Because loss of binding is expected to be the primary mechanism of resistance, it is expected that R-allele conferring adaptation to *Bt* ICPs will be incompletely to completely recessive. If one copy of the gene codes for the normal receptor, then some receptor binding will still occur and some mortality will result. Therefore, a high dose leads to the expectation that adaptation will be functionally recessive, with little difference between SS and RS mortality.

Two USEPA Scientific Advisory Panel (SAPs) in 1998 and 2000 decided that a for lepidopteran-active plant-incorporated protectants (PIPs), a high dose is expected to kill a high proportion of heterozygotes. Therefore, high dose is defined as 25 times the dose required to kill 99% of susceptible insects (SAP 1998, 2000). The SAP recommended five imperfect methods for demonstrating high dose and indicated that at least two of them should be used to demonstrate a high dose. Two such methods were employed by Dow to demonstrate that 99.9% of insects in the field were killed by the dose expressed in MXB-13 and that the dose in MXB-13 was sufficient to cause high mortality of instars that are around 25 times more tolerant of the ICP than are neonates (the later instar serves as a surrogate for the heterozygote). Season long expression of both ICPs in MXB-13 (WideStrike cotton) is advantageous since heterozygote survival will remain low for all generations. Dow's expression data (MRID# 45808408) confirms that both ICPs are expressed consistently at high levels throughout the growing season (see earlier discussion).

High dose data for the key targets pests, TBW, CBW and PBW, are summarized below. Additional pests controlled by this product are BAW, FAW, SAW, CL and SL. Further testing is expected to show efficacy against other minor lepidopteran pests.

TBW

Both Cry1Ac and Cry1F ICPs have exhibited activity against TBW in spectrum studies (Herman and Young, 1999, MRID# 45542307; Herman, 2001, MRID# 45542308). Research by three methods indicates that WideStrike meets the high dose definition for TBW (Blanco et al, 2002; MRID# 45808417). First, lyophilized plant tissue diluted 25-fold in diet bioassays was sufficient to cause 100% mortality of neonate TBW in laboratory studies. Thus, WideStrike appears to be at a dose 25 times that which is sufficient to kill all neonates. The single ICP lines also gave very high mortality and growth inhibition in similar bioassays. Second, mortality of second instars that are 25 times tolerant of the ICP in lab bioassays when placed on excised MXB-13 (WideStrike) leaf tissue was 95% compared with mortality on non-transgenic near isoline leaf tissue. These second instars are surrogates for heterozygous neonates that are 25-fold resistant to ICPs, and empirical data on resistance of Lepidoptera to *Bt* toxins shows that heterozygotes are rarely greater than 25-fold resistant unless the inheritance of resistance is dominant (SAP, 2001). Therefore, WideStrike is expected to cause at least 95% mortality of the most tolerant heterozygotes. Third, after extremely heavy field infestation with laboratory reared TBW at three field locations, no surviving larvae larger than neonate were found after extensive sampling and observations of feeding damage to cotton plants was minimal. Thus, WideStrike appears to be at a dose sufficient to kill in excess of 99.99% of susceptible insects in the field.

EPA Review. Dow AgroSciences submitted a study investigating the high dose of MXB-13 cotton against TBW (Blanco et al. 2002; MRID# 45808417). The results of this study are briefly discussed above. Dow AgroSciences employed two laboratory-based and one field-based method to demonstrate high dose of MXB-13 against TBW. Because MXB-13 expresses two insecticidal proteins, Cry1Ac and Cry1F, and because the expected durability of a stack of two proteins is in part dependent on the dose of the individual proteins, it is important to investigate the dose of each protein.

Method 1. Artificial diet containing 4% concentration lyophilized leaf material expressing Cry1F or Cry1Ac or both had a significant impact on the development of the tobacco budworm (lower weight gain and % mortality) compared with the same concentration of lyophilized leaf material not expressing the proteins (Table 2 in MRID# 45808417). The expression of Cry1Ac alone in *Bt* cotton event 3006-210-23 (MXB-7, Cry1Ac only) and the expression of Cry1Ac and Cry1F combined in MXB-13 is at least 25-fold that required to kill susceptible neonates (a high dose). However, expression of Cry1F alone in *Bt* cotton event 281-24-236 is slightly lower than a high dose, 96.9% in MS and 90.6% in NC, as defined by the 1998 and 2000 SAPs. No statistical analysis was performed to quantitatively compare the data.

Method 2. This study is a laboratory study using freshly harvested young leaves from field-grown plants. Across all tests, mortality of neonates and 2-day old larvae were very similar for all of the transgenic cotton lines, MXB-13 (Cry1Ac/Cry1F), MXB-7 (Cry1Ac alone), MXB-9 (Cry1F alone),

although mortality was not 100% for either neonates or 2-day old larvae. Mortality of the 2-day old larvae was approximately 7-12% lower than neonate mortality at Stoneville, MS. At Fresno, CA, there were some problems with the non-transgenic control cotton, PSC255. Mortality for the 2-day old larvae and neonates was virtually identical in the Wayside, MS trial. Across all three locations, the mortality of 2-day old larvae was greater than 95% relative to mortality of neonates indicating that larvae that are 25-fold tolerant of the toxins are extremely unfit on the *Bt* cotton lines and were much higher than the non-transgenic control cotton, PSC355 (Table 3 in MRID# 45808417). Since the 2-day old larvae is a surrogate for heterozygotes, the data suggest that insects that are heterozygous for resistance alleles to the *Bt* proteins will not exhibit significantly higher survival compared to susceptible insects.

During this bioassay, one would not necessarily expect all the insects to actually be dead by the end of the 5- to 7-day bioassay period based on the relatively slow action of the *Bt* proteins once ingested. Mortality in this assay is assumed when the larvae failed to respond when prodded by a probe, while what is relevant in the field, is the ability to develop to a fertile adult. Weight gain information (Tables B4-B5) in MRID# 45808417 show that the transgenic lines have much lower weight gains than the non-transgenic control line (PSC355). In the field, lack of growth results in death (e.g., failure to reach adulthood). The goal of this study is not to show neonate mortality >99.9% rather the field study, Method 3, is the better way to show this. The goal of this study is to predict survival of heterozygote neonates, and the 2-day old larvae represent heterozygotes. Because this study can't be directly translated to field mortality, as noted above, survival of 2-days olds should be expressed relative to survival of neonates, remembering that the goal of a "high dose" is to assure high likelihood of functional dominance being <0.05 (i.e., 95% mortality of RS in the field). Using the across-study means (Table 3 in MRID# 45808417), it is reasonable to conclude from this study that Cry1Ac (MXB-7) and the stack (MXB-13) are at least a high dose of Cry1Ac and Cry1F combined with Cry1Ac to control TBW (RS relative mortality >95%, functional dominance <<0.05), respectively. MXB-9 does not express a high dose of Cry1F, but is close to a high dose (RS relative mortality is close to 95%, functional dominance is approximately equal to 0.04) to control TBW. There may be less certainty in determining a high dose for TBW using Method 2 than in using Methods 1 and 3 because of the variability associated with using leaf tissue. No statistical analysis was performed to quantitatively compare the data.

Method 3. Across all three collection methods, 3,840 squares and 6,400 bolls were examined and beat cloth samples of 9,900 plants were made from MXB-13 plants infested with a total of 270,341 neonates on MXB-13 plants over a period of 56 days at 3 different locations. No larvae, other than 3 neonates compared to 679 larvae from the same sampling regime in the non-*Bt* control plots were found, a greater than 99.5% difference. The field experiments support that MXB-13 provides a high dose against TBW. No statistical analysis was performed to quantitatively compare the data.

Conclusion. Three methods (two laboratory and one field) outlined by USEPA's Scientific Advisory Panel were used to demonstrate that Dow AgroSciences's transgenic cotton line MXB-13 expresses a high dose of two *Bt* insecticidal proteins, Cry1F and Cry1Ac, to control TBW larvae. This dose is high enough to kill nearly all susceptible TBW, and therefore, is expected to cause low survival of neonates

heterozygous for resistance alleles. Using Methods 1 and 2, MXB-7 expresses a high dose of Cry1Ac for control of tobacco budworm. Using Methods 1 and 2, MXB-9 expresses a not quite high dose of Cry1F for control of TBW. That is, the Cry1Ac component of the stack in MXB-13 is by itself a high dose, while the Cry1F component in MXB-13 is not. Methods 1 and 2 both show that the stack, MXB-13, produces a high dose to control TBW. Although Cry1F expression is not quite a high dose, neonate mortality is quite high, >90% based on results from Method 1 and >83% based on results from Method 2. The field experiments (Method 3) support that MXB-13 expresses a high dose against TBW. No larvae, other than 3 neonates compared to 679 larvae from the same sampling regime in the non- *Bt* control plots were found, a greater than 99.5% difference. There may be less certainty in determining a high dose for TBW using Method 2 than in using Methods 1 and 3 because of the variability associated with using leaf tissue. No statistical analysis was performed to quantitatively compare the data. Based on all of the data, MXB-7 and MXB-13 express a high dose of Cry1Ac and Cry1Ac combined with Cry1F, respectively. It is highly likely that resistance to MXB-13 will be functionally recessive, and thus evolve only very slowly in the presence of a structured refuge.

PBW

Only Cry1Ac has shown good activity against PBW. Cry1F is thought to not contribute significantly to the mortality of PBW in the field. Field efficacy trials showed no larvae developing to third instars from 3,450 boll entry holes in WideStrike (Pellow, 2002; MRID# 45808407). Likewise, data from cotton line MXB-7 (which expresses only Cry1Ac) indicated that a single, third instar was found from 6,800 boll entry holes. Thus, it is expected that the mortality of PBW in the field is 99.99% and therefore the resistance to WideStrike is very likely functionally recessive, with very low survival of insects carrying single copies of alleles for adaptation.

EPA Review. Subsequent to the Pellow 2002 (MRID# 45808407) field efficacy studies for TBW, PBW, CBW, and other lepidopteran pests, Dow AgroSciences submitted a study investigating the high dose of MXB-13 cotton against PBW (Storer and Richardson, 2003; MRID# 46071901). Dow employed one laboratory-based and one field-based method to demonstrate that MXB-13 has a high dose for PBW. Because MXB-13 expresses two insecticidal proteins, Cry1Ac and Cry1F, and because the expected durability of a stack of two proteins is in part dependent on the dose of the individual proteins, it is important to investigate the dose of each protein.

Method 1. Results from Method 1 indicate that the expression of Cry1Ac in MXB013 is at least 25-fold that required to prevent development of susceptible insects. This meets one of the high dose criterion. Cry1F provides essentially no control of PBW (see Method 2).

Method 2. Based on this field study, MXB-13 expresses Cry1Ac at a dose in excess of the $LC_{99.99}$ for PBW. This meets one of the high dose criterion. The Cry1F protein provides virtually no control of PBW, as shown by the high survival on MXB-9.

Note: There are some off-genotypes growing inside the plot areas (contaminants) in some test plots. The identity of each plant providing bolls for all bioassays could be assessed through strip tests or Dow's quantitative ELISAs or PCR determinations. By chance, the MXB-13 plants selected for the

assay at random were always the MXB-13 genotype. As a result, the calculations for %efficacy should not be affected by the identified in-field contamination.

Conclusion. The two methods chosen by Dow AgroSciences demonstrate that the MXB-13 cotton plants expressing Cry1Ac and Cry1F meet the definition of high dose for PBW as described by the SAP panels (SAP 1998, 2000). They kill >99.9% of PBW larvae in the field and they express at a dose 25-fold higher than that needed to kill nearly all the susceptible PBW. Cry1Ac (event 3006-21-23) is responsible for essentially all the mortality because the Cry1F protein provides virtually no control. Therefore, it is highly likely that resistance to MXB-13 in PBW will be functionally recessive, and thus resistance will evolve only very slowly in the presence of a structured refuge. If PBW that are 25X resistant to Cry1F/Cry1Ac (such as a heterozygote) feed on Cry1F/Cry1Ac cotton (MXB-13), they will be unlikely to complete development. Tabashnik et al (2002) reported that the F1 hybrid of a 3000-fold resistant strain of PBW to the Cry1Ac protein in MVPII, initially collected from the field, and a susceptible strain were only 5-fold resistant. These results coupled to the results from the Dow study suggest that if there were resistant insects, that presumably heterozygotes would be killed (functionally recessive) on MXB-13.

CBW

Both Cry1F and Cry1Ac exhibit activity against CBW in spectrum studies (Herman and Young, 1999, MRID# 45542307; Herman, 2001, MRID# 45542308). Prior research on efficacy indicated that WideStrike was unlikely to be at a high dose against CBW. Thus, specific trials were not designed to demonstrate high dose for either ICP alone or in the stack. Efficacy trials in field plots measured by sampling (large larvae) of CBW indicate that MXB-9 (expressing only Cry1F) gave ~ 67% control, MXB-7 (expressing only Cry1Ac) gave ~ 93% and WideStrike (Cry1F/Cry1Ac stack) gave ~ 94% control averaged across both the North Carolina and Mississippi trials (Storer and Blanco, 2002; MRID# 45808418).

Based on these studies, neither protein in WideStrike is classified as high dose for CBW, but a moderate dose (Storer and Blanco, 2002; MRID# 45808418). The combination of these proteins in WideStrike provides very high levels of control (~ 94%). Because high dose of the individual toxins is lacking for this species, it is less likely that the resistance will be functionally recessive. The effect on resistance evolution is less intuitively predictable, but the risk of resistance is likely to be higher than for TBW. Therefore, a highly conservative spatially-explicit, stochastic computer model was created to simulate adaptation by CBW to WideStrike, as well as CBW adaptation to Bollgard (this study). The model used a simplified binding pattern for proteins, by reducing the number of receptors from six to two, with one site binding Cry1Ac alone and the other binding both Cry1Ac and Cry1F. For model simulations, it was assumed that alleles for resistance would be functionally additive rather than recessive. Burd et al. (2001) has shown that such alleles may be additive. The model is highly conservative due to the limited number of binding sites considered and particularly because no Cry1F-only binding site was included. Even so, the model indicates that WideStrike is inherently very durable in two realistic landscapes (one representing the Mississippi Delta, the other representing eastern North Carolina).

EPA Review. Because previous efficacy trials (Pellow 2002; MRID# 45808407) indicated that there was significant survival of cotton bollworm on MXB-13, Dow AgroSciences designed a study to more accurately quantify the dose, but not to investigate high dose. Because MXB-13 expresses two insecticidal proteins, Cry1Ac and Cry1F, and because the expected durability of a stack of two proteins is in part dependent on the dose of the individual proteins, it is important to investigate the dose of each protein.

Results of the two field studies (Storer and Blanco 2002; MRID# 45808418) conducted in NC (naturally-infested) and MS (artificially-infested) indicate that cotton bollworm control (large larvae averaged across both locations) by Cry1Ac (alone, MXB-7, and in the stack, MXB-13) is very high (~ 93% and ~ 94%, respectively), while control by Cry1F (MXB-9) is less effective, only 67% of the control. Based on these studies, MXB-13 expressing Cry1Ac and Cry1F insecticidal proteins does not meet the definition of a high dose as one that is sufficient to kill 99.9% of the insects in the field; however, cotton bollworm control was still high, approximately 88% in the MS study and 96% in the NC study. These mortality levels are higher than those for Bollgard cotton (Lambert et al. 1997).

EPA agrees with Dow AgroSciences's assessment that because a high dose of the individual toxins is lacking for CBW, it is less likely that the resistance will be functionally recessive. The risk of CBW resistance is potentially higher than that of TBW. The DAS spatially-explicit, stochastic CBW resistance model is discussed in more detail later in this review.

ICP Stack

DAS discusses the importance of a stack exhibiting some level of cross-resistance to insect resistance management. Stacks of two proteins that do not exhibit cross-resistance have long been expected to provide very durable host-plant resistance (Gould 1998). The Cry1F/Cry1Ac stack (WideStrike cotton, MXB-13) may exhibit some level of cross-resistance; whereby one resistance allele can provide enhanced fitness on the cotton plants, as indicated by the overlap in binding sites (see above discussion under "Mode of Action"). Based on binding studies, Cry1F and Cry1Ac shared some receptors **(Figure 1)**. However, it is not expected that cross-resistance will be complete. That is, an insect must possess several R-alleles at more than one location (e.g., homozygous at one locus and heterozygous at another and a high dose, or heterozygous at two or more loci and not a high dose of either ICP) to survive on the stack.. It is the functional dominance of any single R-allele that is of importance, and a R-allele for adaptation for one ICP is likely to be functionally recessive in an insect feeding on the stack unless accompanied by R-alleles for adaptation to the other ICP. A mutation in a gene that codes for a receptor that binds both ICPs will not prevent all binding of either ICP; and thus alone will not allow high survival of the insect bearing even two copies of it, on MXB-13 plants.

EPA Review. Cross-resistance occurs when a pest becomes resistant to one *Bt* protein that then allows the pest to resist other, separate *Bt* proteins. Cross-resistance poses a risk to stacked strategies, in which multiple proteins are deployed simultaneously in the same hybrid. To date, the development of cross-resistance has not been shown in insect pests exposed in the field to *Bt* crops producing different *Bt* proteins.

After review of the binding studies, EPA agrees that Cry1F and Cry1Ac share some receptors in TBW and CBW, but cross-resistance is expected to be incomplete **(see Figure 1)**. In TBW, Cry1F and Cry1Ac each have unique receptors, Receptor A and C, respectively, and Cry1Ac can also bind to Receptor A. In CBW, Cry1F binds to at least four receptors and Cry1Ac binds to at least four receptors, two of which are shared by both Cry1F and Cry1Ac. That is, a single mutation in a gene that codes for a receptor that binds both Cry1Ac and Cry1F will not prevent all binding of either one; and thus will not allow high survival of an insect bearing even two copies of it, on MXB-13 plants. The complexity of cross-resistance in MXB-13 is explored in the CBW model described and reviewed later in this document.

Discussions of cross-resistance are complicated due to the fact that the exact nature and genetics of *Bt* resistance are not fully understood. Resistance may vary substantially from pest to pest, adding to the unpredictability of the system. In general, it is possible for resistance to *Bt* proteins to occur through several different mechanisms, some of which may result in cross-resistance to other proteins. As noted earlier, for *Bt* ICPs, two modes of resistance have been seen - detoxification in the midgut lumen by proteases that cleave the ICP and alteration of receptors that prevents binding (Ferré and Van Rie, 2002). Of these, the latter is by far more common. Receptor site insensitivity is likely to have less fitness and is more likely to be mediated by single gene mutations and thus expected to be the faster to evolve. Other mechanisms that may lead to resistance (and ultimately cross-resistance) include protease inhibition, metabolic adaptations, gut recovery, and behavioral adaptations (Heckel 1994, Tabashnik 1994).

The complexity of cross-resistance within a single species or different species is demonstrated by a wealth of experimental evidence. For example, cross-resistance in TBW follows a variable pattern for a closely related group of proteins (Cry1A toxins). An example of a possible shared binding site resulting in cross-resistance was observed with TBW. Gould et al. (1995) selected a TBW strain (YHD2) for a high level of resistance to Cry1Ac (approximately 2000-fold). The YHD2 laboratory-selected strain was found to be cross-resistant to Cry1Aa, Cry1Ab, and Cry1F and showed limited cross-resistance to Cry1B, Cry1C, and Cry2A. Genetic experiments revealed that resistance in the YHD2 strain is partially recessive and is controlled mostly by a single locus or a set of tightly linked loci (Heckel et al. 1997). These results differ from Gould et al. (1992) using a more moderately-resistant laboratory strain of TBW (<50-fold) which showed some broad-spectrum resistance to Cry1Aa, Cry1Ab, Cry1B, Cry1C, and Cry2A. The resistance levels in this TBW strain were low, and subsequent work showed that resistance was inherited as a nearly additive trait (Heckel et al. 1997). Work by Jurat-Fuentes and Adang (2001) indicates that resistance in the YHD2 strain is directed against the homologous domain II loop. Results suggest that it will be difficult to predict what cross-resistance patterns are likely to be in the field because evolutionary responses will depend on the initial frequencies of each resistance allele, the genetic dominance of the alleles, and the mechanism(s) of resistance.

Refugia

DAS discusses the importance of a refuge in insect resistance management. "A refuge is an area of host plants where non-selected individuals can be produced (i.e., there is no selective differential or SS genotypes are favored over RS genotypes) that are available to mate with any RS or RR individuals selected in the *Bt* field." Heterozygous (RS) or homozygous susceptible (SS) individuals (offspring) from matings will be controlled by the *Bt* cotton; thus, the refuge serves to reduce the frequency of R-alleles. This is done in three ways: 1) dilution effect: the refuge serves to provide large numbers of S-alleles; 2) random mating: the refuge serves to limit the production of RR individuals; and 3) high dose: the refuge encourages the production of RS individuals (through mating of rare RR individuals with SS individuals) that will be killed off by the *Bt* cotton. The refuge must provide non-selected insects within the local population at the same time as selected insects are produced on *Bt* cotton, e.g., a patch of non- *Bt* cotton planted close to *Bt* cotton fields and managed the same way. Alternatively, non- *Bt* crop or wild plants that are nearby, attractive, and suitable at the same time as *Bt* cotton may serve as refugia.

EPA Review. The refuge strategies that may be employed by DAS for WideStrike cotton are discussed later in this review.

Adult Effects

DAS notes that adults of the target insects do not feed on plant tissue, except for perhaps some nectar feeding. According to the expression data (Phillips et al., 2002; MRID# 48608408), there are no detectable levels of Cry1F or Cry1Ac proteins in the nectar of MXB-13 (WideStrike cotton). Adult feeding (an additional level of exposure) could in theory intensify selection and decrease the value of the refuge.

EPA Review. EPA agrees with DAS's analysis. Adult effects due to feeding on WideStrike tissue are not expected to have any impact on the selection intensity (i.e., there are no detectable levels of Cry1F or Cry1Ac proteins in the nectar) and thus, will not decrease the value of the refuge.

Technology Adoption Rates and Alternative Controls

DAS's *Bt* cotton (WideStrike) contains both the Cry1F and Cry1Ac proteins. Monsanto's *Bt* cotton, Bollgard and Bollgard II, contains the Cry1Ac protein and the Cry1Ac and Cry2Ab protein, respectively. The proportion of cotton planted to *Bt* cotton varieties that contain Cry1Ac or both Cry1F and Cry1Ac will affect how much of the pest population is exposed to the ICPs, and thus the level of selection pressure for adaptation. MXB-13 (WideStrike cotton) cotton is expected to gain market share over time. While Cry1Ac is common to all three *Bt* cotton products, the presence of a second ICP in the two stacked products, WideStrike (Cry1Ac + Cry1F) and Bollgard II (Cry1Ac + Cry2Ab) will add durability. Insects that may possess alleles allowing enhanced survival on Cry1Ac are likely to still be controlled by the other ICPs.

EPA Review. EPA agrees with the DAS conclusion that the proportion of cotton planted to *Bt* cotton varieties that contain Cry1Ac or both Cry1F and Cry1Ac will affect how much of the pest population is

exposed to the ICPs, and thus the level of selection pressure for adaption. DAS has modeled the impact of MXB-13 market share on resistance evolution. This will be discussed later in this review.

Biological Factors

Along with the various operational factors discussed above, Dow AgroSciences discusses the impact of a number of biological factors that impact the evolution of insect resistance: adult movement, larval movement, alternate hosts, population dynamics, and metapopulation dynamics. These will be briefly discussed below.

Adult Movement

Adult dispersal among patches before mating enables the SS genotypes produced by the refuge to mate with RS or RR individuals that may be produced by the *Bt* cotton. The production of RS individuals rather than RR individuals is important in situations where the *Bt* crop produces a high dose against the pest, thus minimizing the fitness differential between individuals carrying the R alleles and those not carrying such alleles. Post-mating dispersal determines where the eggs are laid. The extent of post-mating dispersal can prevent localized foci of elevated R-allele frequency from forming as the R-alleles are diluted across the landscape by S-alleles. That is, post-mating dispersal will ensure the spread of the R-alleles across the region.

TBW. Adult dispersal is regarded as moderate (Fitt 1989). That is, there is considerable short range movement between fields and within fields, but longer range dispersal is more limited. Dispersal is more extensive in the spring when adults are looking for suitable non-crop hosts before cotton is available (Peck et al., 1999).

PBW. Adult dispersal in PBW is limited in distance, with most insects not dispersing more than ½ mile, though some long-distance dispersal has been observed (Tabashnik et al., 1999).

CBW. CBW dispersal is driven by host plant attractiveness. CBW has many wild and crop hosts, and in all cases, is attracted most to the flowering and fruiting structures. CBW adult populations move considerably in areas and at times when there is diversity in host plant phenology, e.g., corn maturation, cotton flowering. Adult movement is less when crops and crop phenology are more uniform such as an area dominated solely by flowering cotton. CBW also exhibits long range migration (Fitt 1989). It is migration that allows CBW to colonize areas of the Corn Belt where it cannot overwinter. A consequence of migration is that the selection pressure for adaptation in one region may not have much effect on the local population's rate of adaptation, as the population mixes significantly with populations in other regions. That is, the range of host plants available in one geographic area may only represent a subset of all the host plants that the local population utilized.

EPA Review. EPA agrees with the DAS assessment of the adult movement for TBW, PBW, and CBW and the resultant impact on selection pressure.

Carrière et al. (2001a) estimated dispersal distances of PBW by tracking movement of males and females from isolated non-*Bt* cotton refuges (source) in surrounding *Bt* cotton (sink). Because *Bt* cotton acts as a deadly sink, moth moths flying in *Bt* cotton at the end of the growing season (September-November) must originate from refuges. Their results showed that dispersal of females from non-*Bt* cotton to *Bt* cotton was dramatically reduced at only 0.83 km (½ mile) from the border of the refuge. This work confirmed the earlier results regarding adult dispersal found by Tabashnik et al. (1999) discussed above.

Larval Movement

Larval movement can negate the value of the high dose, if it allows partially-adapted heterozygotes to survive better than fully susceptible insects. This situation can arise if the RS insects become established on non-*Bt* plants and then move to *Bt* plants or if the RS insects feed on *Bt* plants and then move to non-*Bt* plants to better survive intoxication than SS insects. Larval movements impacts a moderate or low dose situation much less because there is already a considerable fitness difference between RS and SS larvae.

TBW. Larval movement has been observed in TBW. It is not known whether heterozygous insects would experience better subsequent survival than would homozygous susceptible TBW. In any case, to minimize the amount of movement between *Bt* and non-*Bt* plants (or vice-versa) then mixed plantings of *Bt* and non-*Bt* plants should be avoided.

PBW. The larval stage of PBW is spent entirely within a single boll. Boll to boll or plant to plant movement is minimal. Therefore, mixed plantings of *Bt* and non-*Bt* plants would not affect the effectiveness of the high dose.

CBW. CBW, like TBW, have mobile larvae. However, in the absence of a high dose, the consequence of such larval movement on the population rate of adaption is relatively small, since heterozygote survival is already relatively high compared to SS larvae.

EPA Review. EPA agrees with the DAS assessment of larval movement for TBW, PBW, and CBW and the resultant impact on selection pressure. Because of limited PBW larval movement, EPA has allowed narrow in-field strips, at least one row non-*Bt* cotton, for every six to ten rows of *Bt* cotton in the same field for both Bollgard and Bollgard II.

Alternate Hosts

Utilization by insect populations of hosts other than *Bt* cotton reduces the selection pressure exerted by the host crop. This contribution to the refuge is applicable at all dose levels. Dow AgroSciences used the HOSTS database (a database of the host plants of the world's Lepidoptera at http://www.nhm.ac.uk/entomology/hostplants/) as a means of illustrating the potential hosts of TBW, PBW, and CBW.

TBW. The HOSTS database lists 66 species from 20 families that are hosts of TBW in the Nearctic region. Many of these hosts are common weeds and crops in cotton-growing regions. Early season wild hosts in the Cotton Belt probably serve as the main source of insects that later infest cotton, especially after winter in which adults fly to wild hosts to lay their eggs. In the main cotton production areas, the ability of alternate (non-cotton) hosts to support complete insect development during the summer is unclear.

PBW. The HOSTS database lists 26 species from 5 families that are hosts of PBW in the Nearctic region. Most of these hosts are closely related and are in the same family as cotton, the Malvaceae. Non-cotton hosts are of little importance in determining adaptation rates to *Bt* cotton.

CBW. The HOSTS database lists 108 species from 30 families that are hosts of CBW in the Nearctic region. Like TBW, many of these hosts are common weeds and crops in cotton-growing regions. Because CBW have a tendency for long-distance dispersal then host plants outside the immediate cotton -growing area act as important sources of non-selected populations. These host plants do not represent a structured refuge (by the definition above) because random mating with the local population does not occur at every generation, but rather they represent large areas producing large numbers of non-selected insects that contribute to a reduction in the population-wide selection pressure thus diluting resistance (metapopulation dynamics). Since the insects are capable of large-scale inter-regional dispersal, the population-wide selection pressure is important. Work by Gould et al. (2002) on carbon-isotope ratios in CBW adults collected in the mid-south and southwest US, indicate the more insects emerge from alternate hosts than from cotton for most of the year. As with TBW, within the Cotton Belt, weeds and early-spring crops serve as the main host for CBW in the early spring generations. Also, weeds serve as a primary late season. In very southern latitudes, southern Texas and Mexico, CBW populations can remain active throughout the winter by exploiting wild hosts.

EPA Review. The utilization and effectiveness of alternate hosts has not been sufficient to prove that non-cotton hosts are effective refuges for TBW, PBW, and CBW. For TBW, alternate hosts do exist, but the ability of alternate (non-cotton) hosts to support complete insect development during the summer is unclear. For PBW, alternate hosts are expected to have no bearing on resistance evolution because they are so limited. Alternate hosts are likely to have the biggest impact on CBW resistance management because of the sheer number of possible hosts and the fact that WideStrike does not express a high dose of either Cry1Ac or Cry1F. Therefore, the impact of alternate hosts are of greater importance for non-high dose scenarios, due to possible RS survivors (i.e., the need for susceptible SS immigrants from other sources is greater).

Data indicate that CBW are capable of long-distance dispersal and host plants outside the immediate cotton-growing area may act as important sources of non-selected populations potentially diluting resistance. These hosts may lower the metapopulation-wide selection pressure for adaptation, and contribute non-selected insects to the local populations. Yet, empirical evidence is lacking. Both the 1998 and 2000 FIFRA SAPs Subpanels concluded that there was very little data to support inclusion of alternate hosts as effective refugia (refuge used here in a broad context, supplying SS moths to dilute resistance). The 1998 SAP Subpanel stated that, "until it is shown that non-cotton hosts produce

enough susceptible moths to significantly delay the evolution of resistance in CBW populations exposed to moderate *Bt* doses, non-*Bt* cotton acreage must be considered the primary source of susceptible CBW moths " (SAP 1998) Subsequently, the 2000 SAP Subpanel stated with regard to soybean as a possible refuge that "if there were better empirical data on soybeans, a more realistic model could be developed that accounted for the true year to year variation in the utility of soybean as a refuge" (SAP 2001).

Gould et al. (2002) used stable carbon isotope analysis to assess alternate host use by CBW. They found that non-*Bt* corn in Mexico and the U.S. Corn Belt appears to serve as an important alternate host (non-structured refuge) for CBW. Late-season CBW moths captured in Louisiana and Texas are migrants whose larvae developed on corn in more northern locations. These findings counter the prevailing hypothesis that the majority of late-season moths are produced from larvae feeding on cotton, soybean, and other C_3 plants. The authors conclude that the non-*Bt* corn refuge is probably more critical to CBW resistance management than the relatively small non-*Bt* cotton structure refuge, and this non-*Bt* corn refuge should be maintained.

Work by Gore et al. (2003) examined the temporal and spatial occurrence of CBW on crop hosts including conventional cotton, soybean, grain sorghum, and field corn in the Mississippi Delta. Stable carbon analyses similar to Gould et al. (2002) were performed. Results indicate that field corn and grain sorghum provide a good source of susceptible moths during the early season. Grain sorghum may provide sufficient numbers of susceptible CBW for resistance management during some years. However, soybeans do not appear to produce sufficient numbers of CBW for resistance management. Carbon isotope analysis of moths indicated that a significant percentage of the moth population throughout the season developed on host plants other than cotton. The percentage of moths that developed on C_4 plants (e.g., field corn and grain sorghum) never dropped below 25% and for most of the season was greater than 80%. However, the origin of these moths is not clear and more research is needed to investigate the role of wild hosts and long-range migration on the population dynamics of CBW.

While alternate hosts should be considered when attempting to understand pest adaptation and resistance management, empirical evidence regarding their utilization and effective contribution to the production of SS moths to dilute resistance is not known. DAS makes certain assumptions regarding alternate hosts in its CBW modeling efforts discussed later in this review.

Based on the evidence provided to the Agency, until such time as there is sufficient empirical data that demonstrate that alternate hosts are producing insects in sufficient quantity, temporal synchrony, fitness, and proximity to the resistant insects that would be emerging from *Bt* cotton fields, or that susceptible insect from hosts some distance were lowering selection pressure for adaptation (i.e., immigrating metapopulations), then only non-*Bt* cotton can be used a structure refuge. This uncertainty prompted EPA to require that other *Bt* cotton products (Bollgard and Bollgard II) provide the Agency with additional IRM data to characterize the impact of alternate hosts and supplemental insecticide treatments on refuge effectiveness for CBW, and north-south movement of CBW (EPA 2001, 2003). These same data requirements should also apply to WideStrike cotton. These data would confirm the

DAS CBW modeling predictions and support that external natural refugia in addition to structure refuge reduce the likelihood of CBW adaptation..

Population Dynamics.

Population dynamics in space and time determine the relative sizes of populations infesting *Bt* and non-*Bt* crops, and thus affect the population-level selection pressure for adaptation. Population dynamics is complicated and is affected by weather, density-dependent mortality, density-independent mortality, dispersal, and fecundity. The populations of TBW, CBW, and PBW vary widely from location to location and from season to season, making it difficult to predict actual rates of adaptation. Dow AgroSciences contends that the product durability plan is sufficiently conservative to accommodate this uncertainty.

EPA Review. The importance of population dynamics on insect resistance evolution and resistance management is considered in the DAS product durability plan for WideStrike. The impact of population dynamics on resistance evolution is simulated in the DAS CBW model and and TBW models. The conservative extent of the product durability plan will be discussed later in this review.

Metapopulation Dynamics

Insect metapopulations are more-or-less subdivided into local populations that are linked by dispersal. Host plants across the geographic range of the metapopulation produce insects that through dispersal contribute to local populations. As discussed above for CBW dispersal and utilization of alternate hosts, non-*Bt* hosts outside of the local population do not represent a true refuge, as defined earlier, because random mating with the local population does not occur at every generation, although the dilution effect does apply. These non-*Bt* hosts lower the metapopulation-wide selection pressure for adaptation and contribute on-selected insects to the local population. These hosts are considered when attempting to understand pest adaptation and product durability.

EPA Review. The importance of metapopulation dynamics is considered by DAS in its CBW model. This model will be discussed later in this review.

Genetic Factors

Along with the various operational and biological factors discussed above, Dow AgroSciences discusses the impact of a number of genetic factors that impact the evolution of insect resistance: genetic dominance, initial R frequency, cross-resistance among *Bt* ICPs, and cross-resistance with other control mechanisms. These will be briefly discussed below.

Genetic Dominance

The genetic dominance of an R-allele determines the potential functional dominance. If the R-allele is genetically completely recessive (i.e., dose-response of RS = dose-response of SS), then it will also be

functionally recessive irrespective of the dose of the ICP (i.e., RS survival = SS survival). If an R-allele is completely genetically dominant (i.e., dose-response of RS = dose-response of RR), then it will also be functionally dominant irrespective of the dose of the ICP (RS survival = RR survival). If the RR has low survival on the *Bt* plant, then the selection pressure favoring the R allele will be weak. If the R-allele is nearly completely recessive (i.e., dose-response of RS is close but slightly higher than for SS), then the functional dominance of resistance is likely to be low, even at non-high doses. That is, heterozygote survival will not be much higher than SS survival on the plant. If the R-allele is additive (i.e., dose response of RS is halfway between those for SS and RR), then the functional dominance is highly sensitive to dose if it is not high (i.e., a small change in dose can have a large effect on the difference between RS and SS survival).

For *Bt* ICPs and resistance mediated through a receptor binding change, the expectation is that the R-alleles will be genetically recessive to incompletely recessive, as resistance is mediated through a loss of function. Resistance that is mediated through a gain of function, for example if a digestive enzyme is novel or expressed at much higher levels, then the expectation is that the R-allele will be incompletely dominant. In their review of the binding site modification data related to *Bt* resistance, Ferré and Van Rie (2002) found that resistance was due to a recessive or partially recessive mutation in a major autosomal gene.

EPA Review. EPA agrees with the DAS discussion of genetic dominance of an R-allele and its importance. See modeling discussion below.

Initial R Frequency

A key expectation is that initial Bt-resistance allele frequency will be low in the population. Gould et al. (1997) estimated that the frequency of a Cry1Ac major resistance allele in TBW in NC was in the order of 4.1×10^{-3} (upper bound of the 95% confidence interval) and that resistant larvae did not survive on *Bt* cotton plants. Burd et al. (2001) estimated that resistance was rare. Reports of resistance allele frequency for PBW have been variable, from undetectable to 0.16 (Tabashnik et al. 2000). DAS comments that at a frequency of 0.16 that field damage should have been observed, but this hasn't been the case. A possible explanation is that this R frequency is an artifact of population sampling perhaps collected from close to a *Bt* field before population mixing. However, the assumption that resistance remains rare is still valid.

EPA Review. EPA agrees with the DAS explanation that Bt-resistance allele(s) frequency will likely be low in the population for TBW, CBW, and PBW. As noted above, Gould et al. (1997) estimated that the field frequency of a Cry1Ac major resistance alleles in TBW as 1.5×10^{-3} (4.1×10^{-3} the upper bound of the 95% confidence interval) . Burd et al. (2001) estimated that the frequency of resistance to Cry1Ac was 4.3×10^{-4} indicating that resistance was rare, although inheritance of resistance was incompletely dominant. Reports of resistance allele frequency for PBW have been variable as noted above, from undetectable to 0.16 (Tabashnik et al. 2000). Subsequent work to Tabashnik et al. (2000) explains that the lack of field failure due to PBW resistance in the population is due to fitness costs associated with resistance (reduced overwintering survival and reduced survival on

non- *Bt* cotton plants) and maternal effects (Carrière et al. 2001b and c). Resistance monitoring work in 2001 and 2002 in Arizona has shown that resistant PBW were detected in the field (0.172% survival at the 10 µg/ml), but at much lower frequencies than in 1997 (R-allele frequency = 0.16) and efficacy in the field remained unchanged (Dennehy et al. 2003).

Cross-Resistance Among Bt ICPs

The potential for genes that confer cross-resistance by reduced binding was discussed above (see "ICP Stack" under "Operational Factors"). The Cry1F/Cry1Ac stack may exhibit some level of cross-resistance, whereby one resistance allele can provide enhanced fitness on the cotton plants, as indicated by the overlap in binding sites. However, the cross-resistance is not expected to be complete and thus the likelihood of enhanced survival is expected to be small. Thus, an insect must possess several R-alleles at more than one location (e.g. homozygous at one locus and heterozygous at another and a high dose, or heterozygous at two or more loci and not a high dose of either ICP) to survive on the stack.

EPA Review. See earlier cross-resistance above, "ICP Stack"under "Operational Factors." EPA recognizes the potential for Cry1Ac and Cry1F to confer cross-resistance in TBW and CBW because Cry1F and Cry1Ac share some binding sites. However, the cross-resistance is not expected to be complete because of the number of binding sites involved. PBW is not susceptible to Cry1F and thus cross-resistance to Cry1F is not an issue. The complexity of cross-resistance is discussed in the context of predictive models below.

Cross-Resistance With Other Control Mechanisms

Chemical insecticides have been used to control TBW, CBW, and PBW. These include the following classes: pyrethroids, carbamates, spinosyns, and organophosates, as well as others. Cross-resistance between *Bt* and these other classes has never been documented and is not expected based on the mode-of-action.

EPA Review. EPA agrees with the DAS assessment. Cross-resistance between *Bt* and other chemical insecticide classes is not expected based on differences in mode of action.

E. Resistance Management Models

Computer models can provide an objective synthesis of the complex interaction among the operational, biological, and genetic factors discussed above and provide a scientific basis for understanding the overall impacts of product durability strategies on the rate of pest adaptation. DAS provides an analysis of TBW and CBW resistance management models as they pertain to WideStrike cotton. EPA's review follows the DAS analysis.

TBW

Peck et al. (1999) described a spatially-explicit, stochastic model for TBW adaptation to Cry1Ac-expressing *Bt* cotton in the mid-south and used it to analyze different refuge strategies under a range of assumptions. The model assumed all crop fields are planted to cotton. Peck et al (1999) examined the refuge size and spatial pattern of *Bt* and non-*Bt* plants (such as seed mixes and external refuge) on resistance development, and the effects of varying the spatial pattern each year. They also examined the impact of dispersal, reproductive rates, larval movement, initial R-allele frequency, developmental delays on the model output. Modeling indicated that planting a refuge in the same location each year maintained a source of non-selected insects and was most effective in extending durability. In some model runs, a focus of R-alleles developed which subsequently spread across the region, but these foci did not appear if the refuge was moved from year to year or when the model was adjusted to account for higher spring dispersal. They also found that seed mixes compromised the value of the refuge.

DAS states that the rate of adaptation of TBW to WideStrike will always be slower than that predicted in the Peck et al. model. WideStrike provides a high dose for TBW so the susceptible survival parameter used by Peck (0.01) is higher than is expected for the stacked ICPs in WideStrike. Moreover, DAS has shown that the Cry1Ac expressed in one parent of WideStrike is sufficient for a high dose to TBW, while the Cry1F in the other parent is close to a high dose. Simulations of stacked ICPS (e.g. Roush 1997, Gould 1988) show that adding an additional ICP to the plant always delays the development of resistance to each ICP individually. Peck et al. used an initial R-allele frequency of 0.03 in their runs, a value that is much higher than the frequency of Cry1Ac R-alleles in TBW populations across the Cotton Belt (see earlier discussion, "Initial R Frequency"). Lowering the R-allele frequency (as expected) would slow the population adaptation rate predicted by Peck et al.'s model.

Cry1Ac is expressed in Bollgard (Cry1Ac only), Bollgard II (Cry1Ac + Cry2Ab) and WideStrike (Cry1Ac + Cry1F) cotton lines. Therefore (for WideStrike cotton), the selection pressure for adaptation to Cry1Ac will be more intense than the pressure for adaptation to Cry1F. It is possible for individuals carrying Cry1Ac R-alleles could develop on Bollgard cotton and move to WideStrike cotton where they will be challenged only by Cry1F. There are two possible consequences. The first consequence is that the Cry1F will reduce the survival differential between Cry1Ac-adapted insects and non-adapted insects, thus extending the durability of Bollgard cotton. A second consequence is that the pressure for adaptation to Cry1F will be higher in the component of the population that is adapted to Cry1Ac. Therefore, the presence of Bollgard cotton may be hazardous to the durability of the stack (WideStrike). However, the expectation is that Bollgard II cotton, a stacked product of Cry1Ac and Cry2Ab, will rapidly replace Bollgard cotton. The presence of the second ICP, Cry2Ab, in Bollgard II cotton will slow the adaptation to Cry1Ac. Because the second ICPs in Bollgard II cotton and WideStrike cotton are different, Cry2Ab and Cry1F, respectively, these unique ICPs will lower the chances of cross-resistance. It is expected that neither Bollgard II cotton nor WideStrike cotton will reduce the durability of each other. This concept is explored in the DAS CBW model discussed below.

EPA Review (TBW Model).

EPA reviewed the Peck et al. (1999) stochastic, spatially-explicit, simulation model that examined factored that may influence the regional development of TBW resistance to Cry1Ac (see EPA 2001). A brief summary is provided here. Using this model, they found that the spatial scale and the temporal pattern of refuges can have a strong effect on the development of TBW resistance to *Bt* cotton. Specifically, the time to resistance was significantly longer (49 years) in regions where the same fields were used as a refuge from year to year and adult movement among fields is limited. In regions where the refuge fields are changed randomly from year to year, the region develops resistance more quickly (17 years). Peck et al. (1999) concluded that it would only take a minority of growers who do not employ refuges properly to start a regional resistance problem. These authors found that 20% (sprayed) refuges did delay resistance. They noted that a delay in larval development on *Bt* plants can alter the rate of resistance development to increase or decrease the rate of resistance development. They commented that designing controls to limit the overwintering potential of the last generation may be effective in slowing resistance. Exploring the interaction among parameters is very difficult with this complex model, but this type of model is useful to examine a number of challenges to managing resistance in *Bt* cotton (e.g., how the refuge is managed year to year) and the scale (regional level) of management of resistance. Neither the spatial scale nor temporal pattern of placement of refuges has been investigated in the field.

EPA agrees with DAS's analysis that the rate of adaptation of TBW to WideStrike will always be slower than that predicted in the Peck et al. model. WideStrike (MXB-13) expresses a high dose of Cry1Ac/Cry1F against TBW. Cry1F is expressed at nearly a high dose in MXB-9 and Cry1Ac is expressed at a high dose in MXB-7 (Blanco et al. 2002; MRID#45808417). Thus the survival parameter in the model, 0.01, should be lowered, by at least 10-fold. The resistance allele frequency used in the Peck et al. model was 0.03, a value that is much higher than is estimated. Population adaptation will thus be slower than predicted by the Peck et al. model. Finally, WideStrike expresses two ICPs, rather than a single ICP, as modeled by Peck et al. Modeling predicts that the durability of a two-gene stack will always be greater than a single-gene ICP (Roush 1998, Caprio 1998, Zhao et al. 2003). Zhao et al. (2003) demonstrated that *Bt* broccoli plants expressing two *Bt* toxins will delay diamondback moth (*Plutella xylostella*) resistance more when compared to single toxins used sequentially or in a mosaic.

CBW

EPA has used many CBW models to understand adaptation to *Bt* cotton expressing Cry1Ac in different environments (EPA 2001). These models have indicated that the risks for CBW adaptation are somewhat higher than for TBW under cotton-only and cotton plus corn scenarios.
Dow AgroSciences adapted the Storer et al. (2003) model to account for alternative hosts in different regions and the ICP stack (WideStrike cotton) with incomplete cross-resistance.

Model Input

The DAS CBW model is spatially-explicit and stochastic and was adapted from the CBW model originally described in Storer et al. (2003). The DAS CBW model was extended to include two

additional transgenic cotton ICPs (for a total of three: Cry1Ac, Cry1F, Cry2Ab) and three protein receptors. The model simulates an agroecosystem consisting of the CBW crop hosts soybean, maize, and cotton. The model simulates 15 years of deployment of *Bt* maize and *Bt* cotton. The insects primarily utilize maize for the first two generation and cotton for the second two generations each year. Weed hosts are also utilized in the first and last generations, soybean in the second and third. For these model runs, two agroecosystems are simulated. The first agroecosystem approximates the crop mix in North Carolina **(Figure 2A)**: 50% soybean, 25% maize, and 25% cotton. The soybean and cotton acres are randomly mixed. The second agroecosystem represents the crop mix in the Mississippi Delta **(Figure 2B)**: 62% soybean, 8% maize, and 30% cotton. In the Delta, the soybean and cotton acres are not randomly mixed. Because of the way the region's edges are modeled, the insects at the edge are "bounced back" into the region. This corresponds to a large area dominated by cotton surrounded by soybean acreage (see black box in Figure 2B). The maize is scattered randomly throughout the region. In both agroecosystems, crops are assigned to fields randomly each season. Annual crop phenology, from pre-flowering, through flowering and maturity to harvest follows the statewide averages for crop progress for North Carolina and Mississippi, respectively (USDA-NASS website).

In the North Carolina system, 50% of the maize is planted to hybrids expressing Cry1Ab which for these purposes is assumed to share binding (complete cross-resistance) with Cry1Ac, while the Delta system, none of the maize expresses Cry1Ab. Cotton fields can be sprayed if populations reach threshold (150,000 eggs per ha or 16000 larvae per ha on non-Bt; 1,500,000 eggs per ha on *Bt* cotton). Similarly, soybean can be sprayed if the population reaches 46,000 larvae per ha threshold during flowering or early pod set.

For the Mississippi Delta agroecosystem, early season immigration occurs before the local population emerges from diapause based on published research (e.g., Fitt 1989). This means that the local rate of adaption depends in part on the resistance frequency of the immigrating population, which in turn depends on the selection history of the source population. Immigrating moths are likely coming from southern Texas, Mexico, and the Caribbean; areas in which selection pressure is low under current deployment levels of *Bt* corn and cotton.

ICPs, ICP Binding and Insect Fitness.

The *Bt* ICPs in the model are Cry1F, Cry1Ac, and Cry2Ab. What is understood of the binding of Cry1F and Cry1Ac in TBW and CBW is shown in **Figure 1.** It is assumed that the binding sites of Cry2Ab proteins are not shared with those for Cry1 proteins. **Figure 3** shows the binding map for the purposes of the model and how it is a simplification of what is known for CBW. The simplification is highly conservative, as it only requires changes at three receptors for an insect to be resistant to all three ICPs; whereas from the binding map in **Figure 1B**, changes in upward of seven receptors may be needed for complete cross-resistance.

The amount of cotton that is planted to four different *Bt* cotton types is varied in the model. The four types are: a) varieties expressing Cry1Ac alone (e.g., Bollgard); b) varieties expressing Cry1Ac plus Cry2Ab with no cross-resistance (e.g., Bollgard II); c) varieties expressing Cry1F plus Cry1Ac

(MXB13); and d) non-*Bt* varieties. Type a) (Cry1Ac alone) is assumed to kill 80% of the susceptible CBW based on published field data (e.g., Lambert et al. 1997). Type b) is assumed to kill 96% of the susceptible CBW, whereby the second ICP (Cry2) kills 80% of the survivors of Cry1Ac. Mortality of type c), MXB-13, depends on the mortality inflicted by each ICP alone and on the degree of shared binding. For the purposes of the model, the Cry1F line (MXB-9) is assumed to inflict 67% mortality, the Cry1Ac line (MXB-7) is assumed to inflict 99% mortality, and the stack is assumed to inflict 97% mortality (Storer and Blanco 2002, MRID# 45808418). The mortality of the Cry1F/Cry1Ac stack (MXB-13) is determined by the degree of shared binding. Results from Sheets and Storer (2001) indicate that around 60% of Cry1Ac binding is to molecules that also bind Cry1F (receptor A in **Figure 3**), while the remaining 40% binds to receptors that do not bind Cry1F (receptor B in **Figure 3**). If there was no shared binding, MXB-13 would kill 99% of the susceptible; if there was complete overlap (complete cross-resistance), the combination would kill 97.1% of the susceptibles. Simulations were also run using field data from Mississippi (Storer and Blanco 2002; MRID#45808418) as inputs, but model was not sensitive to input values for mortality.

Understanding the mortality of insects carrying one or more R-alleles is important to understanding the durability of the product. Mortality depends on the functional dominance of resistance on each *Bt* cotton type, and the value of *x* in **Figure 3.** Sheets and Storer (2001) indicated that around 60% of Cry1Ac binding is to molecules that also bind Cry1F (receptor A in **Figure 3**), while the remaining 40% binds to receptors that do not bind Cry1F (receptor B in **Figure 3**).

The R-alleles are assumed to be functionally additive (i.e., functional dominance = 0.5) on *Bt* cotton due to the lack of a high dose against CBW. There are two loci at which R-alleles can lead to adaptation to MXB-13; one for receptor A and one for receptor B. A mutation at the locus for receptor B will not affect Cry1F and Cry2Ab binding, but will affect Cry1Ac binding. A mutation at the locus for receptor A will not affect Cry2Ab binding, but will affect Cry1Ac and Cry1F binding. A mutation at the locus for receptor C will not affect Cry1Ac and Cry1F binding, but will affect Cry2 binding. For complete resistance to Cry1F, only the locus for receptor A must be homozygous for R-alleles. For complete resistance to Cry1Ac, both loci - receptors A and B, must be homozygous for R-alleles. On Cry1F cotton, the survival only depends on the genotype for receptor A. On Cry2Ab cotton, the survival depends only on the genotype for receptor C. On Cry1Ac cotton, survival depends on the genotype for receptors A and B. For Bollgard II cotton, the fitness of each genotype is the product of its fitness on Bollgard and on Cry2Ab (no shared binding). On MXB-13 cotton expressing both Cry1F and Cry1Ac, the fitness is calculated as the product of the survival of binding at each receptor since some of the Cry1Ac activity overlaps with the Cry1F activity. **Appendix A** gives tables for fitness of all 27 insect genotype on each cotton type at three different levels of shared binding.

It is assumed in this study that all specific binding of Cry1Ac is functional. That is, all binding events are followed by incorporation of the ICP into the gut membrane which results in a functional pore leading to cell lysis. Non-functional binding of the ICP to a receptor with formation of a functional pore is also possible, but this would make the analysis considerably more complicated. Adaptation to the ICPs is assumed to be caused by mutations to the midgut receptors that were identified in the ligand-binding study (i.e., Sheets and Storer 2001) and that each receptor requires a different mutation. Therefore, to

be completed adapted to both Cry1Ac and Cry1F, an insect would have to be homozygous to two receptor mutations. Furthermore, insect heterozygous for an adaptation allele will have a fitness exactly half-way between that of homozygous susceptible insects and that of homozygous resistant insects (i.e., dominance of the adaptation trait is additive).

The DAS model represents a worst case scenario because it assumes that there are only three protein binding receptors, fewer than observed in binding studies. In the absence of field resistance to either Cry1Ac or Cry1F, it is impossible to predict with any accuracy how insects carrying one or more R-alleles will survive on MXB-13.

Initial Gene Frequency. As discussed above, it is expected that initial frequency of the R-alleles will be rare. In the model, the initial (unmutated) R-allele frequency for each receptor was assumed to be 0.001. Assuming Hardy-Weinberg equilibrium before selection (no mutation, no fitness costs), 1 in 1,000,000 individuals will be homozygous for the mutated form of one of the receptors. Similarly, 4 in 1,000,000 ($2*0.001^2*(1-0.001^2)$) will be heterozygous for the mutated form of two receptors.

Model Output

Model output is expressed as population fitness on *Bt* cotton after 15 years of deployment. As with other models, this should not be regarded as predictive as there are many uncertain process (e.g., weather) that are not included in the model. The model output is used for comparative purposed to examine the effects of certain parameters and scenarios on pest adaptation and thus, to examine the effectiveness of different product durability programs. Although resistance to Cry2Ab is included in the model runs, rates of adaptation to Cry2Ab-expressing cotton are not reported.

Model Runs and Results

Level of Shared Binding

Shared binding sites by Cry1F and Cry1Ac leads to the expectation of some level of cross resistance, i.e., individuals carrying R-alleles at the locus for the shared binding site will show enhanced survival against both Cry1Ac and Cry1F. However, in the absence of resistance in CBW, it is unclear how much each binding site contributes to mortality. A sensitivity analysis was conducted.

Figure 4 shows the effect of the level of shared binding on the durability of the product, as measured by the change in mean population fitness on MXB13 (WideStrike) after 15 years of deployment alongside Bollgard. These runs were conducted in the North Carolina scenario, with 40% of cotton planted to WideStrike, 40% to Bollgard and 20% as refuge non- *Bt* cotton. As anticipated, when there is completely shared binding ($x = 1$) of Cry1F and Cry1Ac, adaptation to WideStrike occurs most quickly since only one locus needs to be resistant. At completely independent binding ($x = 0$), adaptation occurs significantly more slowly since two R-alleles at two loci are required. In this situation, resistance to Cry1Ac is selected for on Bollgard cotton, WideStrike cotton and on *Bt* corn, whereas resistance to Cry1F is selected on only WideStrike. Since the vast majority of insects that are

heterozygous for resistance to Cry1F are still fully susceptible to Cry1Ac (while Cry1Ac resistance is rare) there is little survival of these insects and the Cry1F R-allele does not increase frequency.

At intermediate levels of shared binding, selection at both loci occurs on all *Bt* cotton and *Bt* corn. Selection pressure exerted by Cry1Ac is greater than that exerted by Cry1F because Cry1Ac is present in all *Bt* cotton and the presence of *Bt* corn (both Cry1Ab and Cry1F are expressed in different hybrids). Resistance to Cry1Ac requires more than one receptor change so it evolves more slowly than when x was set to 1 or 0. At these intermediate levels, adaptation to both Cry1Ac and Cry1F occurs most slowly. As noted earlier, binding data indicate that these intermediate levels are appropriate for this pair of molecules. For the remaining runs of the model, the default value for x is 0.6.

Additional model simulations were run using the dose data from Mississippi (Storer and Blanco, 2002; MRID# 45808418) as input parameters for mortality on WideStrike ICPs. In this field study, mortality of the Cry1F-expressing parent line (MXB-9) was around 70%, while mortality on the Cry1Ac-expressing parent line was about 88%, and the mortality on the stack was around 92%. Results of these runs indicated a similar change in population fitness over 15 years to that for the default mortality parameters (i.e., MXB-9 67% mortality, MXB-7 99% mortality, and the stack 97% mortality. These results indicate that the model is not very sensitive to the actual dose of the two ICPs, given that they are not high dose against CBW.

In all ligand-blot binding studies of Cry1 proteins, each protein has been shown to bind to more than one receptor in the target insects. By stacking two ICPs (e.g., Cry1F + Cry1Ac), the range of receptors involved in resistance is expanded, and the selection pressure for resistance at one receptor is reduced. The binding map used in the model is an oversimplification that assumes that there are no additional receptors involved in toxicity of either protein.

Market Share Modeling

It is expected that WideStrike will share the *Bt* cotton market with Bollgard, Bollgard II or both. As discussed above, the complex of ICPs involved in these products reduces the selection pressure for resistance to any one, especially given the complexity of binding receptors. Modeling **(Figure 5)** showed that market share of WideStrike versus Bollgard had little effect on the rate at which CBW may adapt in either agroecosystem - the WideStrike stack of the two ICPs with incomplete cross-resistance ensures that resistance alleles only increase in frequency slowly.

The impact of WideStrike market share on fitness of Bollgard after 15 years was very small. In the North Carolina agroecosystem, the 15-year population fitness increases somewhat with market share of MXB-13. Conversely, in the Mississippi Delta agroecosystem, the 15-year population fitness decreases with market share of WideStrike. This is due to a decrease in the population surviving each year and a resulting increase in the influence of the immigrant population (which is unselected in the model runs).

Modeling of market share of WideStrike in competition with Bollgard II resulted in slower adaptation than market share with Bollgard as insects are faced with three different *Bt* ICPs. This indicates that as Bollgard II replaces Bollgard, the rate of adaptation to WideStrike will be slowed (that is, lower fitness on WideStrike after 15 years in the presence of Bollgard II than after 15 years in the presence of Bollgard). Correspondingly, a slower rate of adaptation to Bollgard II is anticipated to occur in the presence of WideStrike than with Bollgard as the sole PIP within the cotton market.

Refuge Size and Treatment

Simulations were conducted to determine the effect of refuge size and spray treatment on durability of WideStrike. For these runs, it was assumed that *Bt* cotton was 50% WideStrke, 25% Bollgard and 25% Bollgard II. Modeling **(Figure 6)** runs indicate that the effect of refuge size, whether sprayed or unsprayed, was very small in North Carolina and indiscernible in the Mississippi Delta. Again, this was due to the combination of ICPs in *Bt* cotton, and the number of binding sites involved, coupled with migratory behavior of the pest and the large amount of crop acreage planted to non-cotton host plants. In the Delta, the structured refuge only supplies a small proportion of the non-selected CBW because immigration of non-selected population is high and reduces the local rate of adaptation.

Figure 7 show that R-alleles for resistance at receptor A (Cry1Ac and Cry1F shared receptor) are concentrated in the cotton region of the Delta agroecosystem. However, constant dispersal of adults across the region prevents the R-allele frequency from increasing greatly in the 15 year time period.

Sensitivity Analysis

A sensitivity analysis was conducted to investigate the effect of many of the parameters of this model (results not shown). The effects of the following parameters were found to be most important: the amount of insect product from soybean fields, soybean flowering dates, immigration of non-selected populations, initial R-allele frequency, and fitness costs of R-alleles. The parameters with moderate effects were: functional dominance of R-alleles on each crop, dispersal probability, and larval development duration.

In the Delta agroecosystem, additional sensitivity analyses were conducted. These analyses showed that the R-allele frequency of the immigrating population can overwhelm local selection and act as a driver for adaptation (**Figure 8**) since local adaptation occurs very slowly. At the default setting where immigrant population is at the pre-selection R-allele frequency, the size of the immigrant population, even zero, did not affect local adaptation. This means that local resistance evolution in the Delta agroecosystem is very slow due to the binding patterns and alternate hosts. Other parameters with significant effects in the Delta were: the proportion of soybean in the region, survival of the final, fall population on cotton, and the spray threshold for cotton.

CBW Modeling Conclusions

Modeling indicates that the durability of WideStrike in terms of efficacy after 15 years of deployment, is greater than that for Bollgard. Indeed, the model shows that durability of WideStrike will be very long and much more similar to the durability of Bollgard II, which also expresses two ICPs and attacks multiple binding sites. It further indicates that refuge size is not very important since WideStrike is a stack of two ICPs with limited cross-resistance and is inherently durable when coupled with the natural refuge from alternative hosts. Thus, a 20% sprayable refuge is likely to be more than adequate for prolonging durability against CBW, and will be most important in areas where there is little immigration or where the productivity of CBW from alternative crop hosts is limited. The 5% unsprayed option alone is not as effective as the 20% sprayed under the assumptions and parameter settings used here. However, given that the model is highly conservative, and that there is little change in population fitness after 15 years, this option also should be highly durable.

In regions where immigrating populations contribute significantly to local populations, such as the Mississippi Delta, the local selection pressure may be less important than the metapopulation-wide selection pressure, which given the large host range and large geographic range of the pest, will almost always be lower than is modeled here. Thus, adaptation to WideStrike in these areas is likely to be slower than in similar regions (such as North Carolina) that do not have a significant influx of moths each spring.

The DAS CBW modeling effort has provided insights into how insects may adapt to non-high dose ICPs in different combinations of stacks and mosaics. Being highly conservative in its assumptions about binding and in parameter values as well as *Bt* crop development levels, while being reflective of realistic agricultural ecosystems, the model indicates that we can have high confidence that there will not be a significant change in efficacy of WideStrike in a 15-year time horizon. By extension, for TBW, which exhibits similar patterns in binding studies **(Figure 1)**, against which WideStrike is a high dose and against which the Cry1Ac component alone is a high dose, durability will be even greater than is predicted here for CBW. Additional model testing and development will be conducted as more becomes known about the various crops in the model, the biology of the insect in the complex cropping system, and the manner in which the various *Bt* cotton (and *Bt* corn) crops are used.

EPA Review (CBW Model)

EPA has used several CBW models to understand adaptation to *Bt* cotton expressing Cry1Ac in different environments (EPA 2001; Matten and Reynolds, 2003). Each of these models indicates that the risks for CBW adaptation are somewhat higher than for TBW under cotton-only and cotton plus corn scenarios.

One modeling effort in particular, Storer et al. (2003), was the basis for the DAS CBW model detailed in this submission (MRID# 45808415). The Storer et al. model was adapted from Peck et al. (1999). The Storer et al. (2003) spatial, stochastic computer model was developed to simulate the evolution of resistance in *H. zea* (CEW/CBW) to *Bt* cotton in an agroecosystem that includes both *Bt* corn and *Bt* cotton, such as eastern North Carolina. Using this model, the authors found that selection for resistance is more intense in *Bt* cotton fields than in *Bt* corn fields. For example, the R-allele

frequency if 75% of cotton is *Bt* and 25% of corn is *Bt* increased more rapidly than if 25% of cotton is *Bt* and 75% of corn is *Bt*. Storer et al. concluded that the greater importance of *Bt* cotton with regard to resistance development was due to spraying of non-*Bt* cotton fields when they reached economic threshold levels which reduced the effective refuge size. The spatial distribution of transgenic and non-transgenic plantings can affect both the region-wide evolution of resistance and, especially when the on-farm refuge size is small, the resistance levels in sub-populations. They concluded that farm-level refuge requirements are important even for a highly mobile pest such as *H. zea*. Once established, *H. zea* resistance could spread to farms in regions that do not use *Bt*.

The DAS CBW model was extended from the Storer et al. (2003) model to include two additional transgenic cotton ICPs (for a total of three: Cry1Ac, Cry1F, Cry2Ab) and three protein receptors. The model simulates two agroecosystems, North Carolina and the Mississippi Delta, consisting of the CBW crop hosts soybean, maize, and cotton in varying amounts. The model simulates 15 years of deployment of *Bt* maize and *Bt* cotton. Assumptions are detailed earlier in the review. Sensitivity analyses indicated the following input parameter were most critical: amount of insect product from soybean fields, soybean flowering dates, immigration of non-selected populations, initial R-allele frequency, and fitness costs of R-alleles. The DAS CBW model represents a worst case scenario because it assumes that there are only three protein binding receptors, fewer than observed in binding studies. In the absence of field resistance to either Cry1Ac or Cry1F, it is impossible to predict with any accuracy how insects carrying one or more R-alleles will survive on WideStrike. Based on the conservative nature of the assumptions in the DAS CBW model, these efforts show that we can have high confidence that there will not be a significant change in population fitness of CBW on WideStrike in a 15-year time horizon even without a high dose and incomplete cross-resistance (20 to 60% maximum shared binding) **(Figure 4)**. Resistance evolves more slowly under conditions of incomplete cross-resistance than when there is no cross-resistance ($x = 0$) or when there is complete cross-resistance ($x = 1$). Binding data indicate that thse intermediate levels are appropriate for Cry1F and Cry1Ac. In North Carolina agroecosystem **(Figure 5)**, the 15-year population fitness increased somewhat with market share of MXB-13. While in the Delta agroecosystem **(Figure 5)**, the 15-year population fitness decreases with market share of MXB-13 due to the influence of the immigrant population (**Figure 8**). Increasing WideStrike market share resulted in slower adaptation with Bollgard II than Bollgard because insects are faced with a multitude of *Bt* ICPs. Refuge size **(Figure 6)** had no significant impact on CBW population fitness on MXB-13 after 15 years in either the North Carolina agroecosystem or the Delta agroecosystem.

Previous modeling efforts by Roush (1998), Caprio (1998), and Zhao et al. (2003), have predicted that the durability of a two-gene stack will always be greater than a single-gene ICP. In addition, a bioeconomic model by Livingston et al. (2002) predicts that the addition of a second protein to an existing single protein variety decreases the risk of resistance to the initial protein, while increasing the risk of resistance to the new protein. DAS's CBW modeling efforts confirm the same conclusions as derived from the previous modeling efforts. DAS's efforts indicate that WideStrike (a pyramid for TBW and CBW) will have predicted advantages over a single protein product even whether there is some cross-resistance and when there is somewhat less than a high dose for either protein.

EPA Review of PBW Modeling. DAS does not include any discussion of PBW resistance models. PBW models for purposes of examining resistance evolution under a variety of mitigating strategies do not currently exist. Carrière et al. (2003) used multiple regression and two different population dynamics models (one deterministic, the other stochastic, similar to Peck et al. 1999, described earlier) to show that high use of *Bt* cotton (threshold = 0.65 or 65% *Bt* cotton) led to regional PBW population declines in Arizona. This is important work because, as the authors note, insecticide sprays have not caused long-term suppression of PBW in Arizona. The authors conclude that long-term regional suppression of PBW may further reduce insecticide use and enhance implementation of the EPA-mandated refuge requirements for *Bt* cotton. It is recommended that DAS include pink bollworm PBW resistance modeling in its product durability analysis to determine the relative expected efficacy of its proposed IRM strategy for PBW.

II. Practical Implementation of the Durability Plan

A. IRM Tools.

The above analysis indicates that the IRM tools available for managing product durability (insect resistance management) vary by pest. Key points are summarized based on the analysis above.

TBW and CBW binding studies involving Cry1F and Cry1Ac (summarized in **Figure 1**) indicate that there are at least two, and probably at least six binding sites for these two proteins. For TBW, WideStrike is at a high dose against TBW, the Cry1Ac-expressing parent line is also at a high dose, and the Cry1F-expressing parent line is highly efficacious. The Peck et al. (1999) model showed that a high dose of a single ICP with a 20% refuge is a durable plan for TBW. The addition of a second ICP (Cry1F + Cry1Ac stacked in MXB-13) makes the 20% refuge even more durable and reduces the refuge size needed for the same level of protection across a 15-year time horizon. For CBW, neither Cry1F nor Cry1Ac is expressed at a high dose in WideStrike; although Cry1Ac mortality is much higher in WideStrike than for Bollgard cotton and Cry1F efficacy is moderate. This pest has numerous alternate hosts and is highly migratory. This reduces the role of local selection pressure at the local population level and increases the role of metapopulation-wide selection pressure. The planting of non- *Bt* cotton refugia in or close to all *Bt* cotton fields contributes to lowering the metapopulation selection pressure.

For PBW, MXB-13 (WideStrike) is a high dose for Cry1Ac. A small structured refuge in combination with the high dose, planted as close as practicable to the *Bt* cotton, would increase the WideStrike durability.

EPA Review.

EPA agrees with the DAS analysis.

Cry1F has no apparent control of PBW based on field efficacy data (Pellow 2002; MRID# 45808407) and high dose data (Storer and Richardson 2003; MRID# 46071901) and thus,

WideStrike effectively expresses a single ICP (Cry1Ac) to control PBW. A structured refuge planted in very close proximity to WideStrike cotton will manage PBW resistance. As discussed above, the complex of ICPs involved in WideStrike, Bollgard, and Bollgard II reduces the selection pressure for TBW or CBW resistance to any one ICP, especially given the complexity of binding receptors. However, with regard to PBW, there are only two effective ICPs, Cry1Ac and Cry2Ab. WideStrike will put more selection pressure on Cry1Ac, the ICP that is common to all three commercial *Bt* cotton products. Only Cry1Ac in WideStrike is effective against PBW, Cry1F is not. It is recommended that DAS include pink bollworm (*Pectinophora gossypiella*, PBW) resistance modeling in its product durability analysis to determine the relative expected efficacy of its proposed IRM strategy for PBW.

For TBW, the addition of a second ICP, makes the 20% refuge even more durable than for a single ICP expressed at a high dose and reduces the refuge size (as compared to a single, high dose ICP) needed for the same level of protection as predicted by Peck et al. (1999) across the same time horizon. Further refinement of the Peck et al. (1999) is recommended.

For CBW, neither Cry1F nor Cry1Ac is expressed at a high dose in WideStrike; although Cry1Ac mortality is much higher in WideStrike (96% in NC; Storer and Blanco 2002; MRID# 45808418) than for Bollgard cotton (68% in NC; Lambert et al. 1997) and Cry1F efficacy is moderate (approximately 70%, Storer and Blanco 2002; MRID# 45808418). Modeling runs indicate that WideStrike durability over a 15-year time horizon will be higher than for Bollgard and similar to the durability of Bollgard II, a product which also expresses two ICPs and attacks multiple binding sites. Population fitness is lowest with intermediate levels of shared binding of Cry1F and Cry1Ac (**Figure 4**). Some level of shared binding is expected for Cry1F and Cry1Ac based on binding studies (**Figure 1**). Sheets and Storer (2001) indicate that 60% of Cry1Ac binds to the Cry1F receptor in CBW. Even in the simplification of binding receptors, an insect would have to be homozygous for two receptor mutations (**A and B in Figure 3)** to be completely adapted to both Cry1F and Cry1Ac. Given that the model assumes fewer binding sites than were observed in binding studies, complete adaptation in the field would require at least six or more receptor mutations. Modeling runs also indicate that refuge size is not very important to management of CBW resistance since WideStrike is a stack of two ICPs with limited cross-resistance and is inherently durable when coupled with the natural refugia from alternative hosts. More empirical data need to collected to validate the effectiveness of nature refugia from alternate hosts. Thus, a 20% sprayable refuge is likely to be more than adequate for prolonging durability against CBW, and will be most important in areas where there is little immigration (as in North Carolina) or where the productivity of CBW from alternative crop hosts is limited (as in the Mississippi Delta). The 5% unsprayed option alone is not as effective as the 20% sprayed under the assumptions and parameter settings used in the model. However, given that the model is highly conservative, and that there is little change in population fitness after 15 years, this option should also be considered durable.

B. IRM Plan for WideStrike Cotton

Based on all of the data discussed above, Dow AgroSciences believes that for CBW and especiallyTBW, the IRM plan in place for single-ICP *Bt* cotton should provide protection of the durability of WideStrike that exceeds that afforded to the single-ICP *Bt* cotton (Bollgard) for which it

was designed. The presence of multiple ICPs (Cry1Ac, Cry2Ab, Cry1F) afforded by the commercial availability Bollgard, Bollgard II, and WideStrike means the selection pressure against each is eased. For PBW, the IRM plan in place should provide at least equal durability protection. For the sake of clarity of the IRM plan to growers, consultants, extension entomologist, seed dealers and others, Dow AgroSciences proposes the same refuge requirements as are currently in place for Bollgard cotton even though it believes this plan is very conservative for WideStrike cotton. The refuge requirements for Bollgard cotton are detailed in EPA (2001) and are briefly described below.

1. 5% external unsprayed refuge option. Five percent of the cotton fields must be planted to non- *Bt* cotton and not be treated with any lepidopteran-control technology. The refuge must be at least 150 ft. wide (preferably 300 ft.) and within ½ mile (preferably adjacent or within 1/4 mile or closer) of the *Bt* cotton.

2. 20% external sprayable refuge option. Twenty percent of the cotton fields must be planted to non-*Bt* cotton and may be treated with lepidopteran-active insecticides (or other control technology) except for microbial *Bt* formulations. The refuge must be within 1 mile (preferably within ½ mile or closer) of the *Bt* cotton fields.

3. 5% embedded refuge option (for TBW and CBW). Five percent of a cotton field (or fields) must be planted with non- *Bt* cotton as a block within a single field, at least 150 ft. wide (preferably 300 ft. wide) or single field blocks within a one mile squared field unit. The refuge may be treated with lepidopteran-active insecticides (or other control technology) only if the entire field or field unit is treated at the same time.

4. Embedded (in-field strip) refuge option for PBW. One single row of a non- *Bt* cotton variety must be planted for every 6 to 10 rows of *Bt* cotton. This can be treated with lepidopteran-active insecticides (or other control technology) only if the entire field is treated at the same time.

5. Community refuge option. Farmers can combine neighboring fields within a one-mile squared field unit that act as a 20% sprayable refuge or the 5% unsprayed refuge. Participants in the community refuge option must have a community refuge coordinator and appropriate documentation is required.

EPA Review.

EPA agrees with the DAS analysis and recommendation that the same refuge options currently mandated by EPA for Bollgard and Bollgard II cotton should be appropriate for insect resistance management to WideStrike and will afford clarity and consistency of the IRM to growers, consultants, extension entomologists, seed dealers, and others that need to understand and implement it. EPA also agrees with DAS's analysis that the durability of WideStrike should be equal to or greater than that afforded to Bollgard cotton, a single ICP *Bt* cotton for which the refuge options were originally designed.

The DAS CBW modeling efforts show that we can have high confidence that there will not be a significant change in population fitness of CBW on WideStrike in a 15-year time horizon even without a

high dose and incomplete cross-resistance (20 to 60% maximum shared binding) **(Figure 4)**. Market share analysis of WideStrike versus Bollgard or Bollgard II had little effect on the rate at which CBW may adapt in either the North Carolina or Mississippi Delta agroecosystem. **(Figure 5)**. Refuge size, whether sprayed or unsprayed, **(Figure 6)** had no significant impact on CBW population fitness on WideStrike (MXB-13) after 15 years. In the Delta the immigrating non-selected population further reduces the local rate of adaptation **(Figure 8)**. The local structured refuge only supplies a small proporation of the non-selected insects in the Delta. For TBW, which exhibits similar patterns in binding studies **(Figure 1)**, against which WideStrike is a high dose and against which the Cry1Ac component alone is a high dose, durability will be even greater than is predicted for CBW and that which was predicted using the TBW model by Peck et al. (1999). For PBW, WideStrike expresses a high dose of Cry1Ac, just as does Bollgard (Cry1Ac) cotton. Current refuge options mandated for management of PBW resistance to Bollgard cotton should be appropriate for WideStrike.

Although WideStrike selects for R-alleles at the genes encoding receptors for Cry1Ac, this is balanced by the presence of Cry1F reducing survival of Cry1Ac-resistant insects. The precise population biology in any given area in any given year greatly influences the balance of these competing forces. The same affect applies equally to TBW and CBW. Because the model does not include any Cry1F-only receptors (which are known to exist), it underestimates the mortality of Cry1Ac-resistant individuals on WideStrike and therefore underestimates the magnitude of the Cry1F effect delaying resistance to Cry1Ac. Just as predicted evolution of resistance to Cry1Ac is greatly delayed when the number of Cry1Ac binding sites is increased from one to two, so the evolution of resistance to Cry1F is predicted to be similarly delayed when additional Cry1F receptors are included in the model. Under typical cotton production practices, it is expected that the Cry1F in WideStrike will be durable and will reduce the rate at which Cry1Ac-resistance evolves in TBW and CBW. WideStrike will thereby protect the durability of other Cry1Ac-expressing *Bt* cotton (both Bollgard and Bollgard II).

It is also important to note that recent labeling schemes encouraged by EPA and the chemical insecticide industry encourage growers to use multiple modes of action in controlling insects in order to reduce the likelihood of insects evolving resistance to any one control agent. Following this principle, use of WideStrike in an agroecosystem where other control measures are also used reduces the selection pressure for resistance to each measure. Likewise, the use of new, insecticides such as spinosad against Lepidoptera in cotton further enhances the durability of *Bt* cotton especially WideStrike. WideStrike gives higher levels of control of bollworm than are reported for Bollgard cotton and thus fewer chemical insecticide treatments will be needed which in turn reduces the selection for resistance to chemicals.

See additional comments above, "IRM Plan for WideStrike Cotton."

C. Grower Implementation (Education and Compliance)

DAS notes that ensuring growers plant and manage refuges in the required manner is an important element of their product durability plan, especially for managing adaptation to TBW and PBW because of their comparatively limited host range, limited adult dispersal, and the high dose. Achieving 100%

grower compliance is not a necessary goal based on the conservatism built into the plan, but achieving high levels of grower compliance is important. DAS will implement a multi-pronged effort to educate growers and measure the level of refuge implementation.

Education

DAS will build up the familiarity that cotton growers already have with IRM for *Bt* cotton. By unifying the requirements with those already in place for Bollgard and Bollgard II, DAS can build upon the messages growers have already received. The DAS education program will encompass the extensive efforts to be undertaken by DAS individually, as well as coordinated efforts among the other *Bt* cotton registrants and other stakeholders, such as the National Cotton Council and cooperative extension services. It includes the following general aspects:

- Training sales representatives on IRM principles and requirements;
- References to IRM in seed catalogues, seed bag tags, and promotional materials;
- Articles on IRM published in seed company magazines and web sites;
- Distribution of news release to, and the placement of educational materials in, farm media, informing growers of IRM requirements.
- Emphasis on IRM guidelines in grower guides supplied to growers who purchase *Bt* cotton seed.

Compliance

DAS states that it is necessary to take steps to ensure that individual cotton farmers who purchase WideStrike cotton seed are aware of their IRM obligations and are implementing them correctly. To this end, DAS will implement a compliance assurance program similar to that being established for *Bt* corn by the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) and the EPA. This program will include the following elements:

- Grower agreements to be signed by all growers who purchase MXB-13 seed;
- A system to ensure grower agreements are on file for all purchasers of MXB-13 seed;
- A system whereby growers will annually affirm their IRM obligations;
- An anonymous grower survey to measure the level of IRM adherence;
- Grower visits to assist with, and assess adherence to, IRM requirements;
- Education and warnings to bring non-compliant growers to compliance; and
- Denial of MXB-13 *Bt* cotton seed to growers who repeatedly and willfully ignore their IRM obligations.

EPA Review.

Education and compliance with IRM requirements are critical elements for successful resistance management. Significant non-compliance with IRM among growers may increase the risk of resistance

for *Bt* cotton. However, it is not known what level of grower non-compliance will compromise the risk protection of current refuge requirements. While DAS may not believe 100% compliance with IRM requirements to be necessary because of the conservative nature of the IRM product durability (IRM) plan, EPA believes that while 100% compliance may not be obtainable, it is the right goal. *Bt* cotton grower education has been reviewed in EPA's White Paper (EPA 1998) and was emphasized at the EPA/USDA Workshop on *Bt* cotton IRM held in August 1999 (EPA/USDA 1999). The 2000 SAP Subpanel stressed the importance of grower education and its impact on grower compliance (SAP 2001). Because of the recommendations made by the SAP and many stakeholders, EPA subsequently required specific grower education and compliance programs as terms and conditions of the Bollgard and Bollgard II registrations (see EPA 2001, 2003).

DAS briefly summarizes their education and compliance programs for WideStrike IRM and their importance to achieving IRM. Both education and compliance are central to the success of any IRM program. They indicate they will build upon existing IRM education and compliance programs for *Bt* cotton that convey to growers and other stakeholders the importance of complying with the IRM program for WideStrike. DAS states it will implement a compliance assurance program similar to that being established for *Bt* corn by the ABSTC and the EPA. DAS has correctly noted the appropriate elements for the current compliance assurance program requirements for other *Bt* corn and *Bt* cotton products. It has also identified critical information sources that are key to successful grower education. However, the most appropriate compliance assurance program model for WideStrike is that which is currently required for Bollgard and Bollgard II, but incorporates the logistical and legal issues in which multiple registrants are presumably cooperating to meet high compliance goals. Therefore, it is recommended that DAS be required to adopt the same education and compliance requirements that are currently required of Monsanto for Bollgard and Bollgard II with the stipulation that an "ABSTC-type" arrangement be made to meet these requirements across all *Bt* cotton products.

D. Baselines, Resistance Monitoring and Mitigation (Remedial Action)

DAS proposes two key aspects to its monitoring program. First, population collections will be made from across the Cotton Belt, concentrating on areas of highest use of WideStrike. Second, a system will be set up for growers, consultants or others to report cases of unexpected damage that may be caused by resistant insects. While the first program will be targeted at the target pests of greatest concern (TBW, CBW and PBW), the second program will address all target pests.

In the first program, 15 to 20 populations of TBW and CBW will be collected from across their range in the Cotton Belt. These will be bioassayed against Cry1F, Cry1Ac and a mixture of the two ICPs. Four to six populations of PBW will be collected from across its range (Arizona, New Mexico and California) and bioassayed against Cry1Ac. Two years of baseline data will be available prior to commercialization, from populations collected in 2002 and 2003. From the baseline data, we will attempt to establish a discriminating dose for identifying putative partially-resistant insects. Should a discriminating dose be established, subsequent monitoring will rely primarily on this. Any individuals or populations showing statistically significant survival or growth in the bioassays will be investigated further to confirm resistance.

In the case of changes in pest susceptibility detected by this program before field failure, the resistance will be characterized in order to develop a scientifically based program to manage the resistance. The management options for such a situation may involve changes to refuge area, changes to refuge or *Bt* crop management, increased monitoring or other measures. The precise program will depend on the characteristics and frequency of the resistance detected.

In the second program, growers, crop consultants, extension groups and company representatives will be educated as to what to expect in terms of insect survival and damage on WideStrike, based on efficacy data and experience with DAS and Phytogen. These groups will also be educated on scouting WideStrike cotton for damage. DAS will establish a system for these groups to report incidents of unexpected damage. All reports will investigated to determine if WideStrike is expressing the ICPs at the normal levels. If these elements suggest that a resistant population may be responsible, collections of the insects from the damaged and surrounding fields will be made to allow bioassay of the population in the laboratory. Also, growers will be required to terminate plant growth, shred the stalks within 1 month and plow the fields in order to eliminate any resistant insects which remain in the field.

If laboratory bioassays show that the insects have an enhanced survival in dose-response tests, and are able to survive and develop on *Bt* cotton tissue in the laboratory, the resistance will be deemed confirmed. In the case of confirmed resistance, sales to the affected area of WideStrike cotton will be stopped, and other registrants of *Bt* cotton that share one or other ICP will be informed. A remedial action plan will be devised working with other stakeholders, including farmers, extension groups, the EPA, and the USDA.

EPA Review.

Resistance monitoring. The need for proactive resistance detection and monitoring is critical to the survival of *Bt* PIP technologies. Early detection of significant changes in resistance allele frequency (that will lead to field resistance) is necessary. This will allow IRM plans to be potentially altered prior to field failure.

DAS has described the basic elements of its proposed resistance monitoring program. The proposed program has a route for reporting and investing suspected cases of resistance and one for confirmed resistance. DAS proposed to collect 15 to 20 populations each of TBW and CBW and perform laboratory bioassays to determine whether there are any changes to the susceptibility of these insects to either Cry1F and/or Cry1Ac. Sampling will be focused in areas of highest adoption. The current resistance monitoring programs for Bollgard and Bollgard II mandate that at least 20 populations each of TBW and CBW be collected and analyzed. Similarly, 4 to 6 populations of PBW will be collected across Arizona, New Mexico, and California and examined for changes in insect susceptibility. EPA agrees with DAS that the resistance monitoring program should be focused in areas of highest adoption in which selection pressure is expected to be highest. Baseline susceptibility data for WideStrike collected during the 2002 and 2003 are still being analyzed for TBW, CBW, and PBW. Based on the baseline data, a discriminating dose for Cry1F and Cry1Ac will be established.

The currently required resistance detection method for *Bt* resistance is the discriminating dose/diagnostic dose bioassay system that would distinguish between resistant and susceptible phenotypes. However, such tests have been criticized as being too insensitive to be able to provide early detection before resistance develops or can spread very far, especially if the alleles for resistance are rare in the insect population. Discriminating dose bioassays are most useful when resistance is common (homozygous recessive alleles, i.e., field failure levels) or conferred by a dominant allele when the resistance allele frequency is greater than 0.01 (Andow and Alstad, 1998; Andow et al., 1998). It is currently considered as one of the central components of any monitoring plan, but other monitoring methods, such as the F_2 screen and DNA markers, may have value in conjunction with the discriminating concentration assay. Diagnostic concentration assays are already in use for the Cry1Ac toxin (Bollgard) for testing for resistance development in TBW, CBW, and PBW.

It is recommended that DAS provide EPA the baseline susceptibility data for the Cry1F and Cry1Ac for the 2002 and 2003 growing season, establish diagnostic/discriminating concentrations for tests for resistance to Cry1F and Cry1Ac, and provide a detailed resistance monitoring plan for both the Cry1Ac and Cry1F ICPs. It is also recommended that the basic resistance monitoring program requirements mandated for Bollgard and Bollgard II, be mandated for WideStrike.with the proviso that they should be specific for the Cry1Ac and Cry1F ICPs (see EPA 2001 and 2003). Additionally, it is recommended that DAS coordinate its monitoring efforts for WideStrike with the current resistance monitoring programs for other *Bt* ICPs. The lead for PBW monitoring efforts is Dr. Tim Dennehy, University of Arizona and the lead for the TBW and CBW monitoring efforts is Dr. Carlos Blanco, USDA/ARS, Stoneville, MS. Coordination is essential to a large scale resistance monitoring program, one that potentially covers 5+ million acres of *Bt* cotton.

Remedial action plans. EPA required remedial action plans be developed by Monsanto for Bollgard and Bollgard II cotton in the unfortunate situation that resistance is suspected or actually does develop (EPA 2001, 2003). These plans define not only suspected and confirmed resistance, but also the key steps and actions needed if and when resistance develops. The Arizona *Bt* cotton Working Group has produced "A Remedial Action Plan for PBW Resistance to *Bt* cotton in Arizona" (EPA 2001, Appendix 1). An interim remedial action plan is currently required and is being revised to address TBW and CBW resistance to *Bt* cotton, key economic pests of cotton in the mid-South and Southeastern US (see EPA 2001, Appendix 2). A revised remedial action plan for TBW and CBW resistance management to *Bt* cotton was submitted to the Agency and reviewed.

DAS should prepare specific remedial action plans for WideStrike to address TBW, CBW, and PBW resistance if it is suspected or actually does occur as was mandated for Bollgard and Bollgard II cotton with the proviso that they should be specific for the Cry1Ac and Cry1F ICPs (see EPA 2001 and 2003). While the general elements of the remedial action plans for suspected and confirmed resistance are noted by DAS, these plans need more detail.

Generally, if resistance is confirmed, the farmers involved will treat their *Bt* crop with alternative pest control measures. This might be a chemical pesticide known to be highly effective against the insect or it might mean measures such as crop destruction. In addition, the sales and distribution of the *Bt* crop

would be suspended in that area and the surrounding area until it can be determined that insects in that area have regained their susceptibility to the *Bt* ICP. Other registrants with the same (or similar) *Bt* ICPs would be notified. There would also need to be increased monitoring to define the remedial action area(s). Other remedial action strategies include increasing refuge size, changing dispersal properties, use of sterile insects, or use of other modes of actions. Geospatial surveys would help define the scale of remedial action and where to intensify monitoring. Because no *Bt* field resistance has yet been found, all of these tactics are untested. The greatest concern with remedial action plans is that they will not work either to eradicate resistance or mitigate it. This concern was noted by the 2000 SAP Subpanel (SAP 2001).

E. On-going Research

DAS states that the WideStrike product durability is conservative and is designed to accommodate uncertainties in target insect biology and the characteristics of *Bt* resistance. DAS indicates that researchers and EPA have determined that the current IRM plan for *Bt* cotton expressing one protein; whereas, WideStrike expresses two proteins. Additional research will be conducted that will improve the understanding of several elements that may affect resistance development.

North-south Migration of CBW. DAS indicates that there is ongoing research examining the impact of north-south migration of CBW from the Corn Belt to the Cotton Belt that will be submitted to the EPA by DAS and other *Bt* corn registrants via ABSTC when it is complete. Preliminary results indicate that the CBW reverse migration will in nearly all realistic circumstances reduce the selection pressure for adaptation by bollworm to *Bt* cotton, as it brings insects that have been in agroecosystems with less *Bt* ICP used in host crops.

Development of Bt-resistant Colonies. Two independent academic groups have been attempting to develop colonies of CBW that are resistant to Cry1F proteins and there are several colonies of TBW that have been similarly developed. These colonies will be used to better understand the cross-resistance patterns among Cry1Ac, Cry1Ab, and Cry1F, and thereby improve estimates of the fitness parameters of the different genotypes on different crop types. This information can be inputted to the DAS CBW model to help understand more clearly the impact on potential adaptation to WideStrike.

EPA Review.

EPA recommends that DAS provide the Agency with relevant IRM research applicable to WideStrike IRM such as that described above: north-south migration of CBW and its impact on both *Bt* corn and *Bt* cotton resistance management and development of Bt-resistant colonies to better understand cross-resistance patterns. ABSTC has submitted their final report to the Agency regarding north-south migration of CBW and it is under review. Other IRM research is desirable to refine TBW and CBW resistance models and to develop PBW resistance models. To support alternate hosts as effective refuges of CBW (TBW, PBW), DAS would need to supply published information or data regarding the timing and production of larvae and adults on each alternate host, mating behavior, origin of moth production (i.e., which alternate hosts) both locally and regionally, proximity of alternate host

production to *Bt* cotton, survival and fecundity of each host, and fitness of adults coming of alternate hosts. Similarly, DAS should provide appropriate data regarding the effectiveness of supplemental insecticide treatment of *Bt* cotton fields to control putative resistant CBW. This research will improve the strength and reliability of an IRM plan to effectively reduce the likelihood that TBW, CBW, or PBW will become resistant to the Cry1Ac and Cry1F ICPs.

Carbon isotope work by Gould et al. (2002) and Gore et al. (2003) indicates that a significant portion of the CBW population in *Bt* cotton areas arose from alternate hosts other than cotton. These findings support the importance of the non-*Bt* corn refuge in the Corn Belt. While alternate hosts should be considered when attempting to understand pest adaptation and resistance management, empirical evidence regarding their utilization and effective contribution to the production of SS moths to dilute resistance is not known. DAS makes certain assumptions regarding alternate hosts in its CBW modeling efforts. However, empirical data are needed to validate these assumptions. Further research is needed on the origin of the moths from different alternate hosts throughout the growing season, mating dynamics, and fitness of the CBW moths emerging from different crops.

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Table 1. Summary of factors driving resistance risk and consequence for target pests of WideStrike cotton. (See MRID# 45808415, p. 48-49)

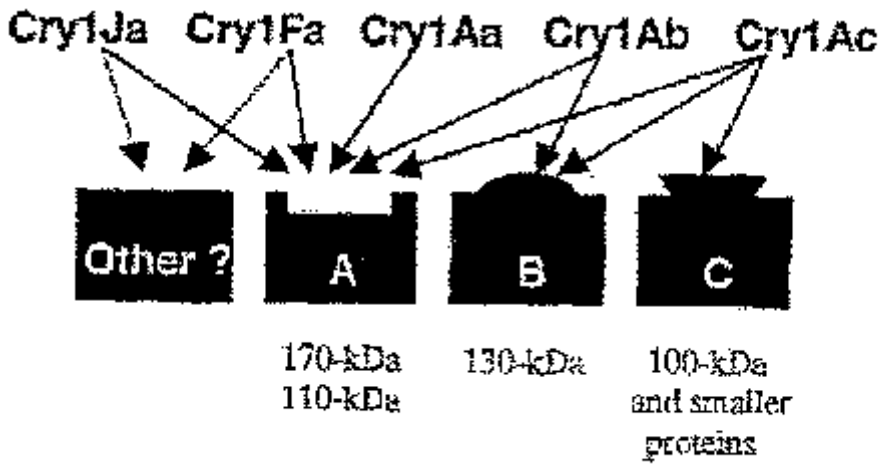
Pest	Host Range[a]	Geographic Range[b]	Over-Winter Range[b]	Importance of Cotton as a Host[c]	Pest Status in Cotton	Effective ICPs in MXB-13	High Dose[d]	Resistance Risk[e]	Resistance Consequence[f]
TBW	66 species 20 families	east, southeast, midsouth, southwest	southeast, midsouth, southwest	high	key pest	Cry1F, Cry1Ac	yes	moderate	high
CBW	108 species 30 families	all US	south	moderate	key pest	Cry1F, Cry1Ac	unproven	moderate	high
PBW	26 species 5 families	southwest	southwest	high	key pest locally	Cry1Ac	yes	moderate	high
BAW	37 species 18 families	south, west	AZ, FL, TX	moderate	irregular infestation, patchy outbreaks	Cry1F, Cry1Ac	no	moderate	moderate
FAW	108 species 31 families	midwest, east, south	south FL, south TX	low	irregular infestation, isolated outbreaks	Cry1F, Cry1Ac	unproven	low	low
SAW	67 species 29 families	south	FL, MX, CA	low	irregular infestation, isolated outbreaks	not known	unproven	low	low
CL	63 species 23 families	all US	south	low	irregular and patchy	Cry1F, Cry1Ac	no	low	low
SBL	39 species 19 families	midwest, east, south	south FL, south TX	low	patchy, late season	Cry1F, Cry1Ac	no	low	low

[a] Host range in North America from HOSTS database
[b] Geographic range within USA

[c] Importance of cotton in the life system of the pest is based on host range, host preferences, number of generations in cotton, etc.
[d] High dose as defined by USEPA, 1998
[e] Resistance risk is based on geographic range, host range, importance of cotton, ICP activity and dose
[f] Resistance consequence is based on pest status in cotton
(Table taken from MRID# 45808415, pg. 48).

Figure 1. Binding map for Cry1 proteins in TBW (top graphic) and CBW (bottom graphic). (Original references Jurat-Fuentes and Adang (2001) and Adang et al. (2002) [See MRID# 45808415, pg. 50).]

Tobacco Budworm



Cotton Bollworm

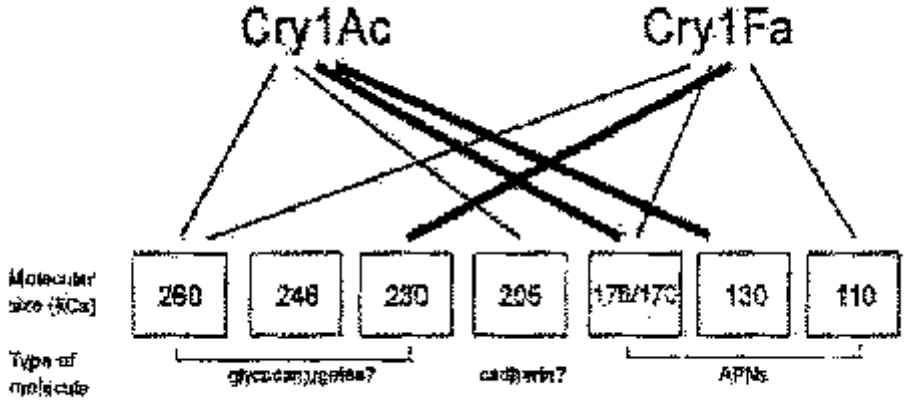


Figure 2. Crop Distribution for Two Agroecoystems.North Carolina (A) and the Mississippi Delta (B) are depicted. In each, the center 10 x 10 fields are actually modeled, while the surrounding area is assumed to be identical for North Carolina and a mirror image for the Delta. (See, MRID# 45808415, 51)

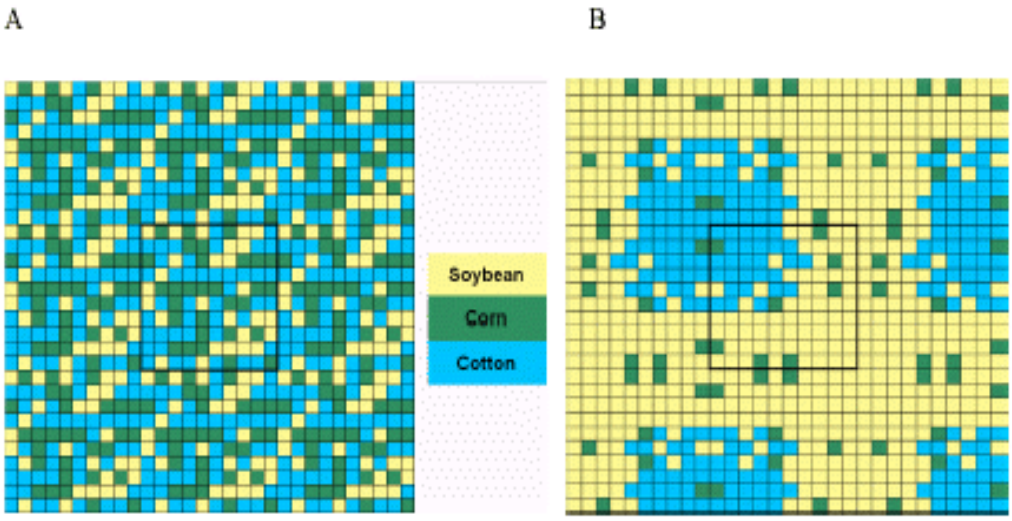


Figure 3. Simplification of Binding Map for Cry1 Proteins Employed in CBW Model. Uppercase-lettered receptors (binding indicated by solid lines) are included in the model, lowercase-lettered receptors (binding indicated by dotted lines) are not. The degree to which each protein binds to each receptor in the model is shown; *x*% represents the proportion of Cry1Ac that binds to receptor A as opposed to receptor B. (see MRID# 45808415, p. 52)

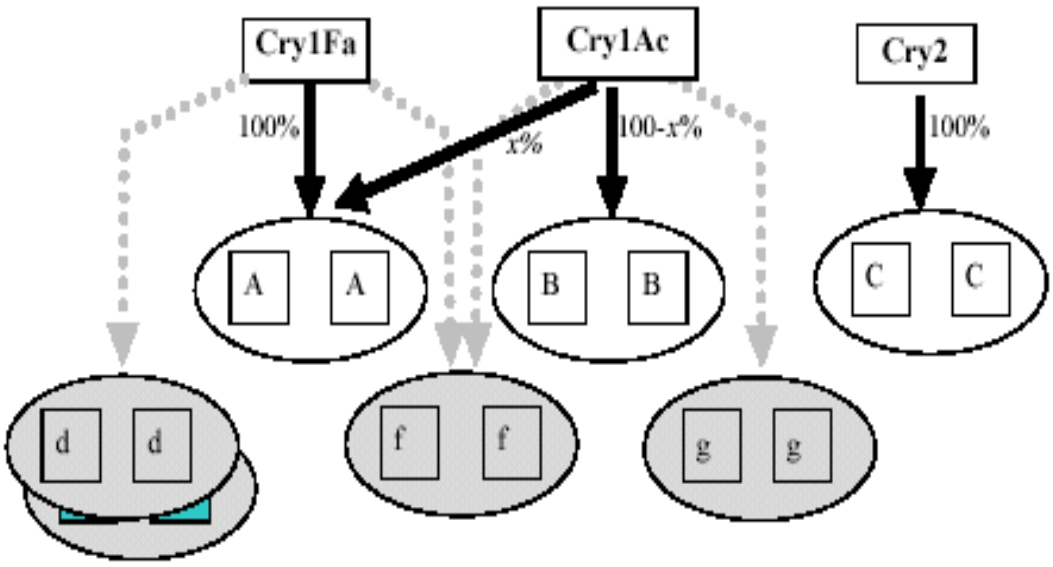


Figure 4. Effect of Different Levels of Shared Binding on the Change in Population Fitness on MXB-13 and Bollgard Cotton in 15 Years.
Runs were made using the North Carolina agroecosystem with 40% of cotton planted to MXB-13, 40% to Bollgard and a 20% no-*Bt* refuge. 50% of *Bt* corn was planted to Yieldgard, and 50% to non-Bt. Each data point is the average (with standard deviation) of 5 runs. (See MRID# 45808415, p. 53)

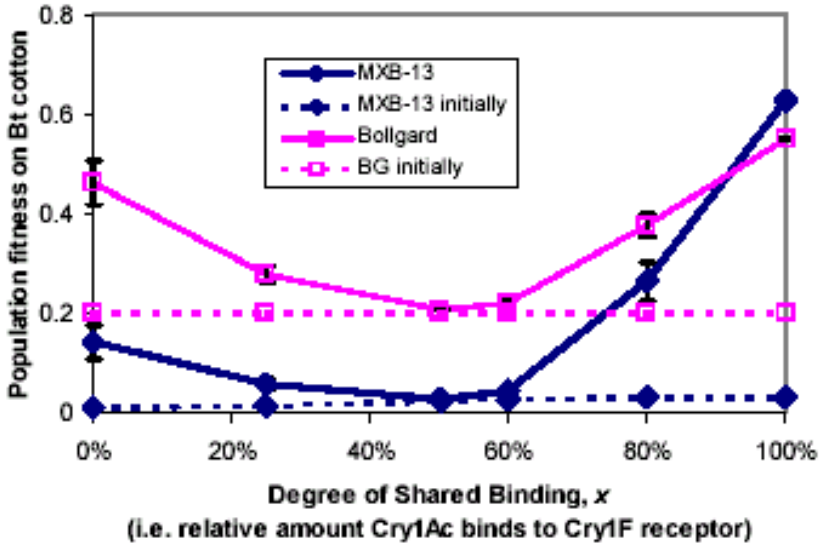


Figure 5. Effect of Market Share of MXB-13 With Either Bollgard or Bollgard II on the Change in Population Fitness in 15 Years.
On the left is for the North Carolina agroecosystem with a 20% non-Bt cotton refuge, on the right is the Delta agroecosystem. Each data point is the average (with standard deviation) of 5 runs. (See MRID# 45808415, p. 54)

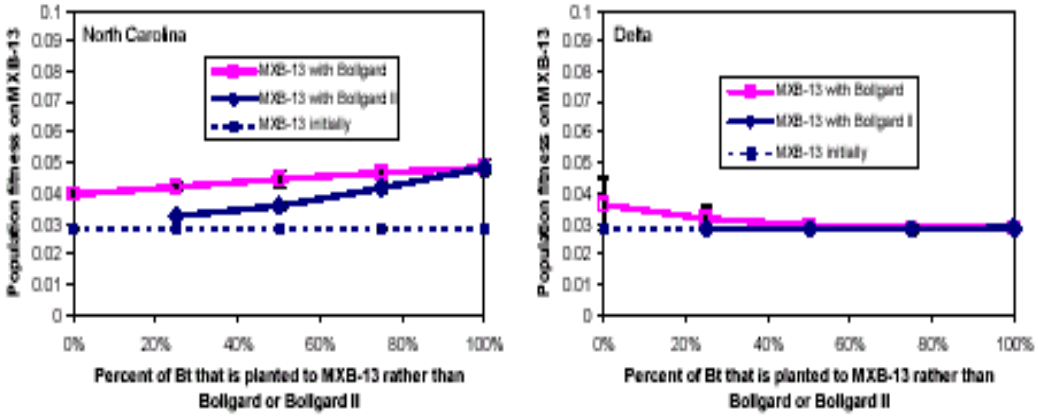


Figure 6. Effect of Refuge Size on the Change in Population Fitness on MXB-13 in 15 Years.

On the left is for the North Carolina agroecosystem and on the right is the Delta agroecosystem, both planted to a combination of Bt cottons (50% MXB-13, 25% Bollgard and 25% Bollgard II). Each data point is the average (with standard deviation) of 5 runs. (See MRID# 45808415, p. 55)

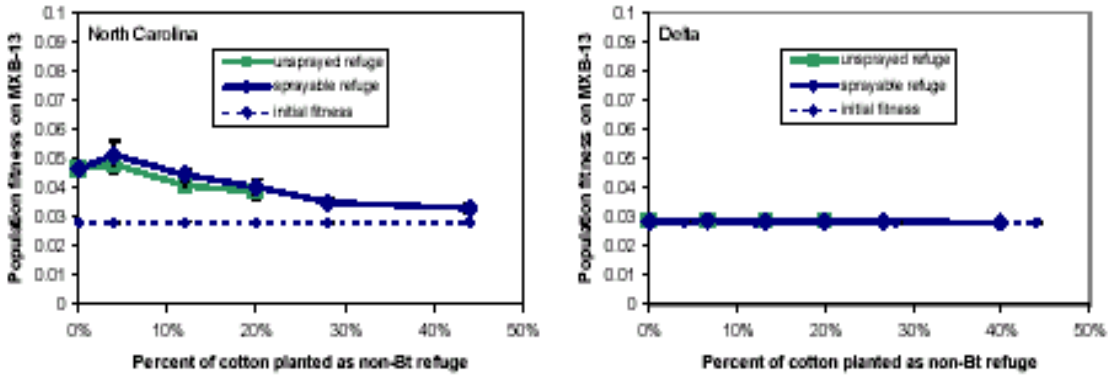


Figure 7. Spatial Distribution across the Field Grid of R-Alleles for Receptors A and B in Mississippi Delta Default Runs.
The refuge size is 20%, 50% of Bt cotton was planted to MXB-13, 25% to Bollgard, and 25% to Bollgard II. Each column represents a field. Cotton is concentrated in the far corner of the region; soybean occupies the rest of the region. (See MRID# 45808415, p. 56)

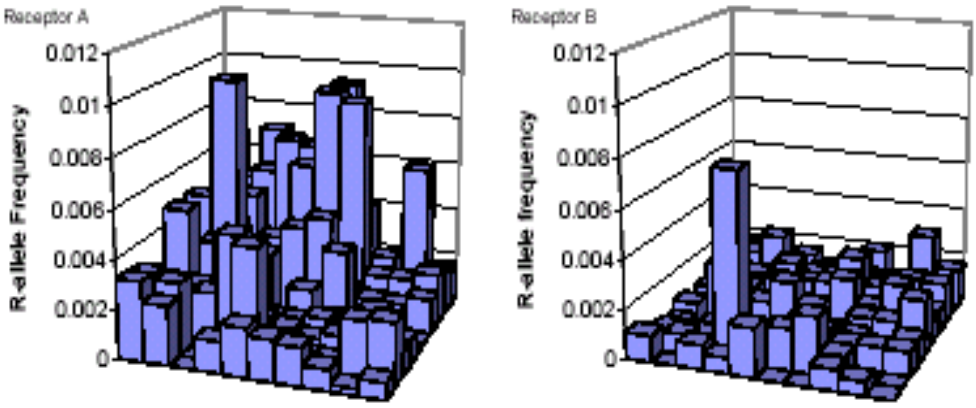
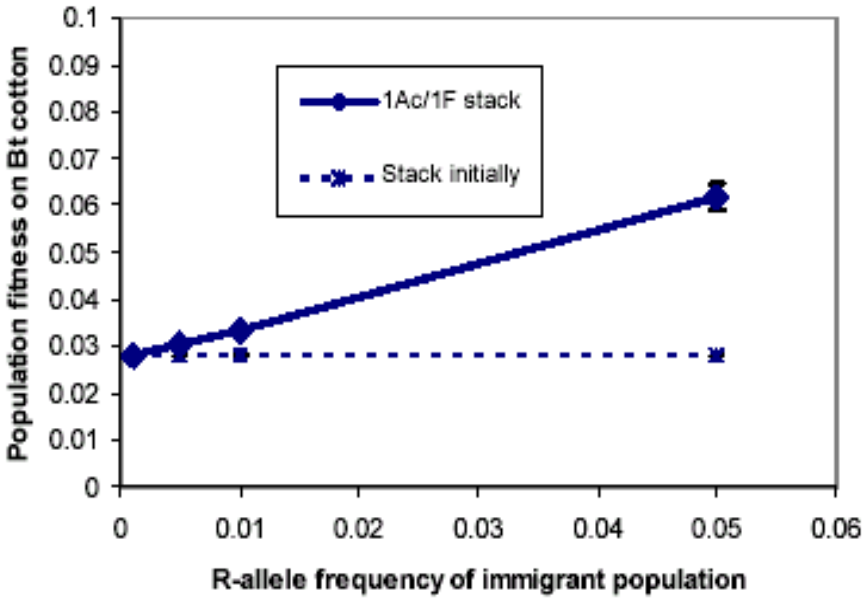


Figure 8. Effect of the R-allele Frequency of the Immigrant Population on the 15-Year Population Fitness of the Local Population in the Mississippi Delta.
Each data point is the average (with standard deviation) of 5 runs. (See MRID# 45808415, p. 57)



Appendix A – Sample fitness tables for CBW model.

SA is the wild-type allele for receptor A, while RA is a mutated allele for receptor A that prevents ICP binding. Similar symbols are used to denote the genotype for receptors B and C. Fitness values indicate the survival probability of each genotype on each Bt cotton type. Fitness of all genotypes on non-Bt cotton is 1.000. Each table gives the fitness values for different levels of Cry1Ac binding to Receptor A rather than receptor B. All Cry1F binds to receptor A; all Cry1Ab binds to receptor C. The functional dominance of resistance at each receptor is 0.5.

Appendix Table 1. Fitness Table for CBW Model. $x = 0.0$; All Cry1Ac Binds to Receptor B; No Cross-Resistance

Insect genotype			Cotton genotype				
Receptor A	Receptor B	Receptor C	Cry1F alone	Cry1Ac alone	MXB-13 stack	Bollgard	Bollgard II
S_AS_A	S_BS_B	S_CS_C	0.330	0.030	0.010	0.200	0.040
R_AS_A	S_BS_B	S_CS_C	0.665	0.030	0.020	0.200	0.040
R_AR_A	S_BS_B	S_CS_C	1.000	0.030	0.030	0.200	0.040
S_AS_A	R_BS_B	S_CS_C	0.330	0.515	0.170	0.600	0.120
R_AS_A	R_BS_B	S_CS_C	0.665	0.515	0.342	0.600	0.120
R_AR_A	R_BS_B	S_CS_C	1.000	0.515	0.515	0.600	0.120
S_AS_A	R_BR_B	S_CS_C	0.330	1.000	0.330	1.000	0.200
R_AS_A	R_BR_B	S_CS_C	0.665	1.000	0.665	1.000	0.200
R_AR_A	R_BR_B	S_CS_C	1.000	1.000	1.000	1.000	0.200
S_AS_A	S_BS_B	R_CS_C	0.330	0.030	0.010	0.200	0.120
R_AS_A	S_BS_B	R_CS_C	0.665	0.030	0.020	0.200	0.120
R_AR_A	S_BS_B	R_CS_C	1.000	0.030	0.030	0.200	0.120
S_AS_A	R_BS_B	R_CS_C	0.330	0.515	0.170	0.600	0.360
R_AS_A	R_BS_B	R_CS_C	0.665	0.515	0.342	0.600	0.360
R_AR_A	R_BS_B	R_CS_C	1.000	0.515	0.515	0.600	0.360
S_AS_A	R_BR_B	R_CS_C	0.330	1.000	0.330	1.000	0.600
R_AS_A	R_BR_B	R_CS_C	0.665	1.000	0.665	1.000	0.600
R_AR_A	R_BR_B	R_CS_C	1.000	1.000	1.000	1.000	0.600
S_AS_A	S_BS_B	R_CR_C	0.330	0.030	0.010	0.200	0.200
R_AS_A	S_BS_B	R_CR_C	0.665	0.030	0.020	0.200	0.200
R_AR_A	S_BS_B	R_CR_C	1.000	0.030	0.030	0.200	0.200
S_AS_A	R_BS_B	R_CR_C	0.330	0.515	0.170	0.600	0.600
R_AS_A	R_BS_B	R_CR_C	0.665	0.515	0.342	0.600	0.600
R_AR_A	R_BS_B	R_CR_C	1.000	0.515	0.515	0.600	0.600
S_AS_A	R_BR_B	R_CR_C	0.330	1.000	0.330	1.000	1.000
R_AS_A	R_BR_B	R_CR_C	0.665	1.000	0.665	1.000	1.000
R_AR_A	R_BR_B	R_CR_C	1.000	1.000	1.000	1.000	1.000

Appendix Table 2. Fitness Table for CBW Model. *x* = 0.6; 50% of Cry1Ac Binds to Receptor A, 40% to Receptor B.

Insect genotype			Cotton genotype				
Receptor A	Receptor B	Receptor C	Cry1F alone	Cry1Ac alone	MXB-13 stack	Bollgard	Bollgard II
S_AS_A	S_BS_B	S_CS_C	0.330	0.030	0.028	0.200	0.040
R_AS_A	S_BS_B	S_CS_C	0.665	0.208	0.182	0.384	0.077
R_AR_A	S_BS_B	S_CS_C	1.000	0.385	0.385	0.568	0.114
S_AS_A	R_BS_B	S_CS_C	0.330	0.054	0.050	0.276	0.055
R_AS_A	R_BS_B	S_CS_C	0.665	0.373	0.327	0.530	0.106
R_AR_A	R_BS_B	S_CS_C	1.000	0.693	0.693	0.784	0.157
S_AS_A	R_BR_B	S_CS_C	0.330	0.078	0.072	0.352	0.070
R_AS_A	R_BR_B	S_CS_C	0.665	0.539	0.472	0.676	0.135
R_AR_A	R_BR_B	S_CS_C	1.000	1.000	1.000	1.000	0.200
S_AS_A	S_BS_B	R_CS_C	0.330	0.030	0.028	0.200	0.120
R_AS_A	S_BS_B	R_CS_C	0.665	0.208	0.182	0.384	0.230
R_AR_A	S_BS_B	R_CS_C	1.000	0.385	0.385	0.568	0.341
S_AS_A	R_BS_B	R_CS_C	0.330	0.054	0.050	0.276	0.166
R_AS_A	R_BS_B	R_CS_C	0.665	0.373	0.327	0.530	0.318
R_AR_A	R_BS_B	R_CS_C	1.000	0.693	0.693	0.784	0.470
S_AS_A	R_BR_B	R_CS_C	0.330	0.078	0.072	0.352	0.211
R_AS_A	R_BR_B	R_CS_C	0.665	0.539	0.472	0.676	0.406
R_AR_A	R_BR_B	R_CS_C	1.000	1.000	1.000	1.000	0.600
S_AS_A	S_BS_B	R_CR_C	0.330	0.030	0.028	0.200	0.200
R_AS_A	S_BS_B	R_CR_C	0.665	0.208	0.182	0.384	0.384
R_AR_A	S_BS_B	R_CR_C	1.000	0.385	0.385	0.568	0.568
S_AS_A	R_BS_B	R_CR_C	0.330	0.054	0.050	0.276	0.276
R_AS_A	R_BS_B	R_CR_C	0.665	0.373	0.327	0.530	0.530
R_AR_A	R_BS_B	R_CR_C	1.000	0.693	0.693	0.784	0.784
S_AS_A	R_BR_B	R_CR_C	0.330	0.078	0.072	0.352	0.352
R_AS_A	R_BR_B	R_CR_C	0.665	0.539	0.472	0.676	0.676
R_AR_A	R_BR_B	R_CR_C	1.000	1.000	1.000	1.000	1.000

Appendix Table 3. Fitness Table for CBW Model. $x = 1.0$; All Cry1Ac Binds to Receptor A; Receptor B is Not Involved

Insect genotype			Cotton genotype				
Receptor A	Receptor B	Receptor C	Cry1F alone	Cry1Ac alone	MXB-13 stack	Bollgard	Bollgard II
S_AS_A	S_BS_B	S_CS_C	0.330	0.030	0.029	0.200	0.032
R_AS_A	S_BS_B	S_CS_C	0.665	0.515	0.456	0.600	0.102
R_AR_A	S_BS_B	S_CS_C	1.000	1.000	1.000	1.000	0.200
S_AS_A	R_BS_B	S_CS_C	0.330	0.030	0.029	0.200	0.032
R_AS_A	R_BS_B	S_CS_C	0.665	0.515	0.456	0.600	0.102
R_AR_A	R_BS_B	S_CS_C	1.000	1.000	1.000	1.000	0.200
S_AS_A	R_BR_B	S_CS_C	0.330	0.030	0.029	0.200	0.032
R_AS_A	R_BR_B	S_CS_C	0.665	0.515	0.456	0.600	0.102
R_AR_A	R_BR_B	S_CS_C	1.000	1.000	1.000	1.000	0.200
S_AS_A	S_BS_B	R_CS_C	0.330	0.030	0.029	0.200	0.097
R_AS_A	S_BS_B	R_CS_C	0.665	0.515	0.456	0.600	0.307
R_AR_A	S_BS_B	R_CS_C	1.000	1.000	1.000	1.000	0.600
S_AS_A	R_BS_B	R_CS_C	0.330	0.030	0.029	0.200	0.097
R_AS_A	R_BS_B	R_CS_C	0.665	0.515	0.456	0.600	0.307
R_AR_A	R_BS_B	R_CS_C	1.000	1.000	1.000	1.000	0.600
S_AS_A	R_BR_B	R_CS_C	0.330	0.030	0.029	0.200	0.097
R_AS_A	R_BR_B	R_CS_C	0.665	0.515	0.456	0.600	0.307
R_AR_A	R_BR_B	R_CS_C	1.000	1.000	1.000	1.000	0.600
S_AS_A	S_BS_B	R_CR_C	0.330	0.030	0.029	0.200	0.162
R_AS_A	S_BS_B	R_CR_C	0.665	0.515	0.456	0.600	0.512
R_AR_A	S_BS_B	R_CR_C	1.000	1.000	1.000	1.000	1.000
S_AS_A	R_BS_B	R_CR_C	0.330	0.030	0.029	0.200	0.162
R_AS_A	R_BS_B	R_CR_C	0.665	0.515	0.456	0.600	0.512
R_AR_A	R_BS_B	R_CR_C	1.000	1.000	1.000	1.000	1.000
S_AS_A	R_BR_B	R_CR_C	0.330	0.030	0.029	0.200	0.162
R_AS_A	R_BR_B	R_CR_C	0.665	0.515	0.456	0.600	0.512
R_AR_A	R_BR_B	R_CR_C	1.000	1.000	1.000	1.000	1.000