

# Notices

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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

## DEPARTMENT OF AGRICULTURE

### Animal and Plant Health Inspection Service

[Docket No. 01-025-1]

#### Monsanto Co.; Availability of Petition and Environmental Assessment for Determination of Nonregulated Status for Cotton Genetically Engineered for Insect Resistance

**AGENCY:** Animal and Plant Health Inspection Service, USDA.

**ACTION:** Notice.

**SUMMARY:** We are advising the public that the Animal and Plant Health Inspection Service has received a petition from Monsanto Company seeking a determination of nonregulated status for cotton designated as Event 15985, which has been genetically engineered for insect resistance. The petition has been submitted in accordance with our regulations concerning the introduction of certain genetically engineered organisms and products. In accordance with those regulations, we are soliciting public comments on whether this cotton event presents a plant pest risk. We are also making available for public comment an environmental assessment for the proposed determination of nonregulated status.

**DATES:** We will consider all comments we receive that are postmarked, delivered, or e-mailed by May 17, 2002.

**ADDRESSES:** You may submit comments by postal mail/commercial delivery or by e-mail. If you use postal mail/commercial delivery, please send four copies of your comments (an original and three copies) to Docket No. 01-025-1, Regulatory Analysis and Development, PPD, APHIS, Suite 3C71, 4700 River Road Unit 118, Riverdale, MD 20737-1238. Please state that your comments refer to Docket No. 01-025-1. If you use e-mail, address your

comment to

[REDACTED] Your comment must be contained in the body of your message; do not send attached files. Please include your name and address in your message and "Docket No. 01-025-1" on the subject line.

You may read the petition, the environmental assessment, and any comments we receive on this notice of availability in our reading room. The reading room is located in room 1141, USDA South Building, 14th Street and Independence Avenue, SW, Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure that someone is available to help you, please call [REDACTED] before coming.

APHIS documents published in the *Federal Register*, and related information, including the names of organizations and individuals who have commented on APHIS dockets, are available on the Internet at <http://www.aphis.gov/ppd/rad/webrepor.html>.

**FOR FURTHER INFORMATION CONTACT:** Dr.

[REDACTED] and [REDACTED]  
[REDACTED] To obtain a copy of the petition or the environmental assessment, contact Ms. [REDACTED]; e-mail: [REDACTED]

**SUPPLEMENTARY INFORMATION:** The regulations in 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are Plant Pests," regulate, among other things, the introduction (importation, interstate movement, or release into the environment) of organisms and products altered or produced through genetic engineering that are plant pests or that there is reason to believe are plant pests. Such genetically engineered organisms and products are considered "regulated articles."

The regulations in § 340.6(a) provide that any person may submit a petition to the Animal and Plant Health Inspection Service (APHIS) seeking a determination that an article should not be regulated under 7 CFR part 340. Paragraphs (b) and (c) of § 340.6 describe the form that a petition for a determination of nonregulated status

must take and the information that must be included in the petition.

On December 7, 2000, APHIS received a petition (APHIS Petition No. 00-342-01p) from Monsanto Company (Monsanto) of St. Louis, MO, requesting a determination of nonregulated status under 7 CFR part 340 for cotton (*Gossypium hirsutum* L.) designated as Bollgard II Cotton Event 15985 (event 15985), which has been genetically engineered for resistance to certain lepidopteran insect pests. The Monsanto petition states that the subject cotton event should not be regulated by APHIS because it does not present a plant pest risk.

As described in the petition, cotton event 15985 has been genetically engineered to express a Cry2Ab insecticidal protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (Btk). The petitioner states that the Cry2Ab protein is effective in providing protection from the feeding of lepidopteran insect pests such as tobacco budworm, pink bollworm, and cotton bollworm. The subject cotton event also expresses the  $\beta$ -D-glucuronidase (GUS) protein used as a selectable marker. Expression of the added genes is controlled in part by gene sequences from the plant pathogens cauliflower mosaic virus and *Agrobacterium tumefaciens*. Particle acceleration technology was used to transfer the added genes into the recipient Delta and Pine Land Company variety 50B (DP50B). Cotton cultivar DP50B expresses a Btk Cry1Ac insecticidal protein and a NPTII selectable marker protein, and was developed from cotton event 531, which was deregulated by APHIS in 1995 (APHIS No. 94-308-01p).

Cotton event 15985 has been considered a regulated article under the regulations in 7 CFR part 340 because it contains gene sequences from plant pathogens. This cotton event has been field tested since 1998 in the United States under APHIS notifications. In the process of reviewing the notifications for field trials of the subject cotton, APHIS determined that the vectors and other elements were disarmed and that the trials, which were conducted under conditions of reproductive and physical containment or isolation, would not present a risk of plant pest introduction or dissemination.

In section 403 of the Plant Protection Act (7 U.S.C. 7701-7772), plant pest is defined as any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: A protozoan, a nonhuman animal, a parasitic plant, a bacterium, a fungus, a virus or viroid, an infectious agent or other pathogen, or any article similar to or allied with any of the foregoing. APHIS views this definition very broadly. The definition covers direct or indirect injury, disease, or damage not just to agricultural crops, but also to plants in general, for example, native species, as well as to organisms that may be beneficial to plants, for example, honeybees, rhizobia, etc.

The U.S. Environmental Protection Agency (EPA) is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. In cases in which genetically modified plants allow for a new use of a pesticide or involve a different use pattern for the pesticide, EPA must approve the new or different use. Accordingly, Monsanto has submitted a request to EPA for registration of Cry2Ab as a plant-incorporated protectant.

When the use of the pesticide on the genetically modified plant would result in an increase in the residues in a food or feed crop for which the pesticide is currently registered, or in new residues in a crop for which the pesticide is not currently registered, establishment of a new tolerance or a revision of the existing tolerance would be required. Residue tolerances for pesticides are established by EPA under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), and the Food and Drug Administration (FDA) enforces tolerances set by EPA under the FFDCA. In response to the filing of Monsanto's pesticide petition, EPA has established a regulation for an exemption from the requirement of a tolerance for residues of *Btk* Cry2Ab and the genetic material necessary for its production in or on all raw agricultural commodities.

FDA published a statement of policy on foods derived from new plant varieties in the *Federal Register* on May 29, 1992 (57 FR 22984-23005). The FDA statement of policy includes a discussion of FDA's authority for ensuring food safety under the FFDCA, and provides guidance to industry on the scientific considerations associated with the development of foods derived

from new plant varieties, including those plants developed through the techniques of genetic engineering. The petitioner has begun consultation with FDA on the subject cotton event.

To provide the public with documentation of APHIS' review and analysis of the environmental impacts and plant pest risk associated with a proposed determination of nonregulated status for Monsanto's cotton event 15985, an environmental assessment has been prepared. The EA was prepared in accordance with (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500-1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372).

In accordance with § 340.6(d) of the regulations, we are publishing this notice to inform the public that APHIS will accept written comments regarding the petition for determination of nonregulated status from interested persons for a period of 60 days from the date of this notice.

We are also soliciting written comments from interested persons on the environmental assessment prepared to examine any environmental impacts of the proposed determination for the subject cotton event 15985. The petition and the environmental assessment and any comments received are available for public review, and copies of the petition and the environmental assessment may be ordered (see the **FOR FURTHER INFORMATION CONTACT** section of this notice).

After the comment period closes, APHIS will review the data submitted by the petitioner, all written comments received during the comment period, and any other relevant information. After reviewing and evaluating the comments on the petition and the environmental assessment and other data and information, APHIS will furnish a response to the petitioner, either approving the petition in whole or in part, or denying the petition. APHIS will then publish a notice in the *Federal Register* announcing the regulatory status of Monsanto's insect-resistant cotton event 15985 and the availability of APHIS' written decision.

**Authority:** 7 U.S.C. 166, 1622n, 7756, and 7761-7772; 31 U.S.C. 9701; 7 CFR 2.22, 2.80, and 371.3.

Done in Washington, DC, this 12th day of March 2002.

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 02-6458 Filed 3-15-02; 8:45 am]

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TEL: [REDACTED]  
FAX: [REDACTED]

December 5, 2000

Dr. [REDACTED]  
Assistant Director, Scientific Services  
U.S. Department of Agriculture, APHIS, PPQ  
4700 River Road, Unit 133  
Riverdale, MD 20737-1236

PETITION FOR DETERMINATION OF REGULATORY STATUS

Dear [REDACTED]

Enclosed is a copy of a petition for determination of non-regulated status for Bollgard II Cotton Event 15985 (*Gossypium hirsutum* L.), which has been modified by particle acceleration transformation of Bollgard cotton variety DP50B to insert the insect control gene *cry2Ab* and the scorable marker gene *uidA*. Cotton event 15985 is currently deemed a "regulated article". Based on the data and information contained in the enclosed petition, we believe that there is no longer "reason to believe" that the modified cotton event should be deemed to be a regulated article. The modified cotton event does not present a plant pest risk and is not otherwise deleterious to the environment. Therefore Monsanto requests a determination from APHIS that cotton event 15985 and all progeny derived from crosses of event 15985 with traditional cotton varieties or transgenic cotton varieties that have also received a determination of non-regulated status no longer be considered regulated articles under 7 CFR Part 340.

30-6581 The enclosed petition contains confidential business information. As the Plant Pest Act does not contain any provisions to shield our data from multinational competitors, we are submitting the petition as **Confidential Information of Monsanto Company**. A separate CBI Deleted version of the petition is also enclosed.

Should you have any questions regarding this request, please contact me at [REDACTED] or Dr. [REDACTED]

Sincerely, [REDACTED]

Regulatory Affairs Manager, Cotton

cc: 00-CT-017U  
[REDACTED]

12/7/2000

00-342-01p  
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**Request for Determination of Non-Regulated Status  
for the Regulated Article:**

**Bollgard® II Cotton Event 15985  
(*Gossypium hirsutum* L.)  
Producing the Cry2Ab Insect Control Protein  
derived from  
*Bacillus thuringiensis* subsp. *kurstaki***

Submitted by:

**Monsanto Company  
600 13<sup>th</sup> Street N.W.  
Suite 660  
Washington, D.C. 20005**

**December 5, 2000**

**00-CT-017U**

**CONFIDENTIAL BUSINESS INFORMATION DELETED**



**Request for Determination of Non-Regulated Status for the Regulated  
Article: Bollgard II Cotton Event 15985 (*Gossypium hirsutum* L.)  
Producing the Cry2Ab Insect Control Protein  
Derived From *Bacillus thuringiensis* subsp. *kurstaki***

**Executive Summary**

Bollgard<sup>®1</sup> cotton, developed by the Monsanto Company, has been adopted broadly by growers since its introduction in 1996 and provides effective protection from the feeding of lepidopteran insect pests such as tobacco budworm, pink bollworm, and cotton bollworm. Growers typically apply significantly less insecticide to control these pests, realize higher yields, and achieve greater profitability using these improved Bollgard cotton varieties as compared to conventional products (Fernandez-Cornejo and McBride, 2000).

Monsanto Company has now developed a new genetically modified cotton event, Bollgard II 15985, using particle acceleration plant transformation procedures to insert the *cry2Ab* insect control gene and the *uidA* scorable marker gene into the Bollgard cotton genome. Event 15985 provides equivalent or increased control of the major insect pests of cotton (tobacco budworm, pink bollworm, and cotton bollworm) with additional control of sporadic pests, such as beet and fall armyworm. Combining the Cry2Ab protein with the Cry1Ac protein already in the marketplace, or using the Cry2Ab protein as a stand alone product, will provide an additional tool to delay the development of lepidopteran resistance to the Cry1Ac protein in cotton, as Cry2 is a different Bt protein class than Cry1Ac. Introduction of Bollgard II cotton, in combination with a refuge and the other components of Monsanto's insect resistance management plan, is expected to significantly delay the development of insect resistance to cotton containing the Cry1Ac protein.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for Bollgard II cotton event 15985 based on research and information used to conduct a safety assessment.

Cotton, *Gossypium hirsutum* L., has been extensively characterized and has a long history of safe agricultural production. Seeds are the only survival structures, and cotton is not likely to survive as a weed due to past breeding selection as a result of its domestication. This is supported by the observation that cotton is not found growing in fence rows, ditches, road sides, or unmanaged habitats in the U.S.

A linear fragment of the transformation vector, PV-GHBK11, containing the *cry2Ab* and *uidA* genes with their respective regulatory sequences, was introduced into the cotton genome by a particle acceleration method to produce Bollgard II cotton event 15985. Molecular characterization has been conducted to establish that Bollgard II cotton event 15985 contains one DNA insertion from the linear fragment of PV-GHBK11. The insert

<sup>1</sup> Bollgard<sup>®</sup> is a trademark of the Monsanto Company.  
MONSANTO BGII 15985 USDA 00-CT-017U

contains one copy each of the *cry2Ab* and *uidA* cassettes. The characterization also determined the composition and structure of the insert, as well as the insert stability across multiple generations. The new insertion resulted in the expression of the Cry2Ab and GUS proteins.

The donor organisms, *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) and *Escherichia coli*, are commonly found in the environment. The proteins produced as a result of the insertion are well characterized. The Cry2Ab protein is highly homologous to the Cry2Aa protein produced by *B.t.k.* The Cry2Aa protein has been widely used in sprayable microbial products and has a long history of environmental safety.

Agronomic, disease, and pest susceptibility observations have been recorded for event 15985 for three years in the United States in more than 200 field trials conducted by Monsanto and academic cooperators, in addition to numerous greenhouse and laboratory studies. Event 15985 cotton is agronomically within the normal range of variability observed in conventional cotton varieties for all parameters measured, except for the intended difference in insect efficacy. Neither the inserted genetic material, nor the proteins produced, have resulted in any observed plant pest characteristics during the course of the trials.

The environmental consequences of the introduction of cotton event 15985 have been considered and there is no reason to believe that event 15985 would have a significant adverse impact. The lack of any significant environmental impact of the *B.t.* family of proteins has been demonstrated in microbial products and in plant-incorporated products including Bollgard cotton. In all cases where the effects of the Cry2Ab protein were determined on non-target organisms, the no observed effect concentration (NOEC) greatly exceeded the maximum environmental concentration, indicating minimal risk to non-target organisms.

The environmental consequences of pollen transfer from cotton event 15985 to other cotton is considered to be negligible due to limited movement of cotton pollen, safety of the introduced proteins, and lack of any selective advantage conferred on the recipient cotton plant. Gene transfer is biologically significant only with other cultivated cotton. Interspecific gene transfer is expected to occur at low levels and diminishing to near-zero or zero with increasing distance of separation for *Gossypium hirsutum*. The potential for outcrossing to sexually compatible species is unlikely as there are no significant populations of sexually compatible related species of cotton in the principle regions of cotton production in the U.S. and its territories. The lack of unintended effects on germination and dormancy confirm that event 15985 is typical of cotton and thus unlikely to become a weed. The agronomic consequences of volunteer cotton plants would be minimal as these plants are easily controlled by mechanical means or by one of a number of herbicides currently registered for cotton.

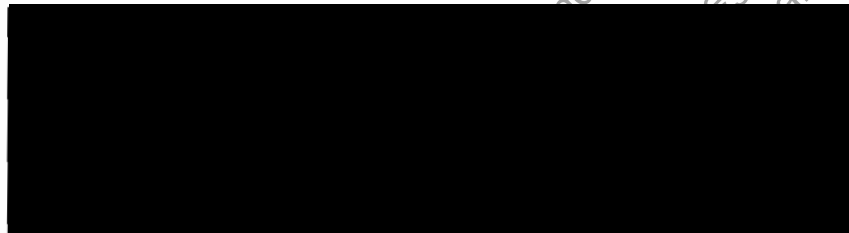
Gianessi and Carpenter (1999) estimated that the planting of Bollgard cotton varieties reduced insecticide applications by two million pounds in 1998 alone, compared to the last year prior to the introduction of Bollgard cotton. Enhanced control of cotton bollworm and armyworm conferred by event 15985 is predicted to further reduce the number of pounds of insecticide used on cotton in the United States, as well as to provide an additional insect resistance management tool to growers.

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Federal Plant Pest Act (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act (7 U.S.C. 151-167) to prevent the introduction and dissemination of plant pests into the U.S. or the interstate movement thereof. The regulations provide that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Data and information provided in this request demonstrate that Bollgard II cotton event 15985 does not represent a unique plant pest risk. Therefore, Monsanto requests a determination of non-regulated status from APHIS that the cotton event 15985, any progenies derived from crosses between this line and other cotton varieties, and any progeny derived from crosses of this line with transgenic cotton varieties that have also received a determination of non-regulated status, no longer be considered regulated articles under regulations in 7 CFR part 340.

### Certification

The undersigned certifies that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner which are unfavorable to the petition.



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### Contributors:



# Request for Nonregulated Status of Bollgard II Cotton Event 15985

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[CBI Deleted] Section 1. Amended Report for Molecular Characterization of Cotton Event 15985. (49 pages)

[CBI Deleted] Section 2. Molecular Analysis of the Stability of Cotton Event 15985. (30 pages)

[CBI Deleted] Section 3. Field Report: Production of Tissue Samples from Insect Protected Cotton Events Grown in 1998 U.S. Field Trials. (41 pages)

- [CBI Deleted] Section 4. Amended Report for MSL-16081: Protein Levels in Insect Protected Cotton Samples Produced in the 1998 U.S. Field Trials. (53 pages)
- [CBI Deleted] Section 5. Assessment of the Equivalence of Proteins Expressed in Cotton Events 15813 and 15985. (78 pages)
- [CBI Deleted] Section 6. Characterization of Insect Protection Protein 2 (IPP2) Produced by Fermentation. (33 pages)
- [CBI Deleted] Section 7. Acute Oral Toxicity Study of Insect Protection Protein 2 (IPP2) in Mice. (209 pages)
- [CBI Deleted] Section 8. Assessment of the *in vitro* Digestibility of Insect Protection Protein 2 (IPP2) Utilizing Mammalian Digestive Fate Models. (34 pages)
- [CBI Deleted] Section 9. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilizing an Allergen Database. (36 pages)
- [CBI Deleted] Section 10. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilizing Toxin and Public Domain Genetic Databases. (231 pages)
- [CBI Deleted] Section 11. Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Honey Bee Larvae. (29 pages)
- [CBI Deleted] Section 12. Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Adult Honey Bees (*Apis mellifera* L.). (35 pages)
- [CBI Deleted] Section 13. Insect Protection Protein 2: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*). (33 pages)
- [CBI Deleted] Section 14. Insect Protection Protein 2: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*). (33 pages)
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- [CBI Deleted] Section 16. Insect Protection Protein 2: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate. (38 pages)

- [CBI Deleted] Section 17. Assessment of Chronic Toxicity of Cotton Tissue Containing Insect Protection Protein 2 to Collembola (*Folsomia candida*). (49 pages)
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- [CBI Deleted] Section 19. Evaluation of Cottonseed Meal Derived From Insect Protected Cotton Lines 15813 and 15985 as a Feed Ingredient for Catfish. (22 pages)
- [CBI Deleted] Section 20. Germination and Dormancy Evaluation of Insect-Protected Cotton Event 15985 for Ecological Assessment of Plant Weediness. (29 pages)

## Abbreviations Used in this Request for a Determination of Non-Regulated Status for Bollgard II Cotton Event 15985

AA	Amino acids
APHIS	Animal and Plant Health Inspection Service
<i>B.t.</i> or <i>B.t.k.</i>	<i>Bacillus thuringiensis</i> organism
Bt	Protein derived from <i>Bacillus thuringiensis</i>
CaMV	Cauliflower mosaic virus
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CFU	Colony-forming units
CPFA	Cyclopropanoid fatty acids; antinutrients in cotton
<i>cry1Ac</i>	Gene in Bollgard Cotton encoding the Cry1Ac insecticidal protein
Cry1Ac	Insecticidal protein produced in Bollgard cotton
Cry2Aa	Insecticidal protein produced by <i>Bacillus thuringiensis</i>
<i>cry2Ab</i>	Gene in Bollgard II Cotton encoding the Cry2Ab insecticidal protein
Cry2Ab, Cry2Ab2, IPP2	Insecticidal protein produced in Bollgard II cotton Event 15985
CTP	Chloroplast transit peptide
CVM	Center for Veterinary Medicine
DW	Dry weight
DP50B	Delta and PineLand Company cotton variety of Bollgard® cotton
<i>E. coli</i>	<i>Escherichia coli</i>
EG7699	Strain of <i>B.t.</i> altered to produce the Cry2Ab protein
ELISA	Enzyme-linked immunosorbent assay
EMBL	European Molecular Biology Laboratory
EPA	United States Environmental Protection Agency
FDA	United States Food and Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
FR	United States Federal Register
fw	Fresh weight
GUS	$\beta$ -glucuronidase protein
HPLC	High Performance Liquid Chromatography
IgEs	Immunoglobulin subclass Epsilon (E)
<i>KpnI</i>	Restriction endonuclease that cuts DNA at specific locations
LOD	Limit of detection
kDa	Kilodaltons
NCPA	National Cottonseed Products Association
NOEL	No Observed Effect Level
NOS 3'	Nopaline synthase 3' polyadenylation sequence
<i>np1H</i> or <i>kan</i>	Gene encoding for the enzyme neomycin phosphotransferase type II
NPTII or Kan	Neomycin phosphotransferase II protein
OECD	Organization for Economic and Co-operation and Development
PCR	Polymerase chain reaction
P-e35S or e-35S	Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region
PIR	Protein Information Resource Database
PV-GHBK11	Plasmid vector
PV-GHBK11L	Linear fragment of the plasmid vector used in transformation of Bollgard II cotton
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid

SOP  
*uidA*  
USDA  
WHO

Standard Operating Procedure  
Gene encoding the GUS protein  
United States Department of Agriculture  
World Health Organization

Standard abbreviations, *e.g.*, units of measure, will be used according to the format described in 'Instructions to Authors' in the Journal of Biological Chemistry.

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## **I. Rationale for the Development of Bollgard II Cotton Event 15985**

Cotton is the leading plant fiber crop produced in the world and the most important in the United States. Cotton production in the United States is located primarily in the tier of fifteen southern states stretching from North Carolina to California, with approximately 14 million acres grown annually. Lepidopteran insects are the main insect pest problem on these acres, including tobacco budworm, pink bollworm and cotton bollworm. During the growing season other insect pests, such as cotton bollweevil, lygus bugs, fleahoppers, spider mites, thrips, armyworms, and aphids, are also present. These insect pests infest the majority of the planted cotton acres and millions of dollars are spent annually for chemical control.

Monsanto Company developed Bollgard cotton, commonly known as "Bt cotton," as a novel approach to controlling insect pest injury in production agriculture (Jenkins *et al.*, 1993; Benedict, 1996; Perlak *et al.*, 1990). The goal was to provide cotton farmers with more environmentally friendly and efficacious insect control at a reduced cost ( [REDACTED] and Altman, 2000). The activity of the protein expressed by the *B.t.* gene present in the cotton genome serves to augment and often replace conventional synthetic insecticide sprays traditionally used to control these major caterpillar pests, providing growers with a highly effective, economically beneficial and environmentally sustainable method of managing insect pests (Adkisson *et al.*, 1999). It also makes it possible for growers to control insect pests currently resistant to certain insecticides and may allow areas that have abandoned cotton production due to economically devastating insect infestations to re-establish their cotton industry (Benedict, 1996).

Bollgard cotton also has value beyond a replacement for insecticide applications for specific pests (Wier *et al.*, 1998). The other direct benefits of Bollgard cotton, supported by data in the current literature, are improved control of target and non-target pests, improved yield, reduced production costs, improved profitability, reduced farming risk, improved opportunity to grow cotton, and improved economic outlook for the cotton industry. There also are a number of proposed indirect benefits associated with the reduction in insecticide use, which includes improved beneficial insect and wildlife populations, reduced runoff of insecticides, reduced air pollution, and reduction of chemical handling for farm workers.

In addition to the continuation or enhancement of the benefits observed from Bollgard cotton, use of the Cry2Ab protein in cotton is expected to provide an additional tool to delay the development of lepidopteran resistance in cotton. This new cotton product, in combination with a refuge and the other components of Monsanto's insect resistance management plan, provides a tool that is expected to significantly delay the development of insect resistance to Bollgard cotton.

Before commercializing Bollgard II cotton event 15985, Monsanto has taken the following actions in the United States:

1. Substances that are pesticides as defined under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. section 136(u)) are subject to EPA's regulatory authority. A request for registration of Cry2Ab as a plant-incorporated protectant was submitted to EPA in April, 2000. Pursuant to section 408(d) of the Federal Food Drug and Cosmetic Act (FFDCA), requests for exemptions from the requirement of tolerances for Cry2Ab and GUS protein were submitted to EPA in the fall of 1999.
2. Bollgard II cotton event 15985 is within the scope of the FDA policy statement concerning regulation of products derived from new plant varieties, including genetically engineered varieties, published in the Federal Register on May 29, 1992. Monsanto provided this summary of the food and feed safety and nutritional assessment of Bollgard II cotton event 15985 to the Agency on June 30, 2000.
3. Under regulations administered by the Animal and Plant Health Inspection Service (APHIS) of USDA (7 CFR 340), Bollgard II cotton event 15985 is currently considered a "regulated article." Monsanto is now requesting a determination of nonregulated status for this cotton event and all progenies derived from crosses between this line and other cotton lines.

## **II. The Cotton Family**

All aspects of the biology, genetics and agronomy of the cotton crop relevant to this petition were previously submitted to the agency by Monsanto as part of the Bollgard and Roundup Ready cotton petitions (94-308-01p and 95-045-01p, respectively).

### **A. Cotton As a Crop**

Cotton production in the United States is located primarily in the tier of 15 southern states stretching from North Carolina to California. It is grown primarily for the value of the fiber, with cottonseed being a by-product. Cotton production in the United States was 13.9 million acres planted in 1999 (USDA, 2000). The primary producing states are: Alabama, Arkansas, Arizona, California, Georgia, Florida, Louisiana, Mississippi, Missouri, New Mexico, North Carolina, South Carolina, Oklahoma, Tennessee and Texas. Of these states, the largest producers are Texas, Mississippi, California, Arkansas, and Louisiana.

Two species of cotton are grown commercially in the United States: *Gossypium barbadense*, commonly called Pima or Egyptian cotton, and *Gossypium hirsutum*, commonly called upland cotton. *G. hirsutum* is noted for its general adaptability and high productivity and is the predominant species in the United States and the world (Lee, 1984). Upland fiber is used for cordage and other non-woven products, as well as for textiles. In addition, upland cotton linters, which are the short fibers removed from seeds prior to crushing, are a major source of industrial cellulose. *G. barbadense* is noted for



the length and quality of its fiber, and its production in the United States is primarily restricted to Arizona, New Mexico and West Texas (Niles and Feaster, 1984). Pima fiber, because of its high quality, is used primarily for sewing threads and luxury fabrics.

Cottonseed oil is a premium quality oil that is used for a variety of food uses, including frying oil, salad and cooking oil, mayonnaise, salad dressing, shortening, margarine, and packing oil. Cottonseed meal and hulls from the seed are not currently used for human consumption in the United States, but are principally sold as feed for livestock. The short fibers on the cottonseed, or linters, consist primarily of cellulose. After extensive processing at alkaline pH and high temperatures, the linters can be used as a high fiber dietary product.

## **B. Taxonomy of Cotton**

Cotton is of the genus *Gossypium* of the tribe Gossypieae of the family Malvaceae of the order Malvales (Fryxell, 1979; Munro, 1987). The genus *Gossypium* is comprised of 39 very diverse species which occur in widely separated parts of the world. Worldwide, four species of cotton are of agronomic importance: the two diploid Old World (or Asiatic) species, *G. arboreum* and *G. herbaceum*; and the two allotetraploid New World species, *G. barbadense* and *G. hirsutum*.

There are four species of cotton in the United States. Two of them, *Gossypium hirsutum* (upland cotton), and *Gossypium barbadense* (sea island cotton, pulpulu haole), are used commercially and escaped plants can be found growing in the wild climates where they can survive in the winter, i.e., southern Florida. In addition, only two native species of *Gossypium* occur in the United States: *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seeman (Brown and Ware, 1958; Fryxell, 1979; Munro, 1987). The former has been described by Kearney and Peebles (1952).

*Gossypium thurberi* Todaro (*Thurberia thespesiodes* Gray) is found in the mountainous regions of southern Arizona. It is found in the following counties: Graham, Gila, Pinal, Maricopa, Cochise, Santa Cruz and Pima. It has also been found in the Bradshaw Mountains (Yavapai County). It is generally found at elevations of 2,500 to 5,000 feet and is isolated from areas of cotton production. Any gene exchange between this species and *G. hirsutum*, if it did occur, would result in triploid ( $3x=39$ ), sterile plants because *G. hirsutum* is an allotetraploid ( $4x=52$ ) and *G. thurberi* is a diploid ( $2x=26$ ). Such sterile hybrids have been produced under controlled laboratory conditions, but they cannot persist in the wild; in addition, fertile allohexaploids ( $6x=78$ ) have not been reported in the wild (██████ 1992).

*G. tomentosum* is a tetraploid and is found on Hawaii (Degener, 1946). The local range is on the larger islands as well as on Nihau and Kahoolawe. It grows on arid, rocky or clay plains not far from the sea. Thus, on the larger islands, it is found chiefly on the dry, leeward side. On Oahu it is common near Koko Crater, and grows scattered between Honolulu and Markus Valley. On Molokai it is extremely common on the southwestern

end; elsewhere it is rare except near Kamalo. Specimens growing near Kaunakakai differ from the typical. On Maui the species may be found from the sea in one of the valleys south of Wailuku.

Hence, only two wild species of cotton are known to inhabit the United States, the *G. thurberi* Todaro as previously listed and the *G. tomentosum* which is endemic to Hawaii. Only the *G. tomentosum* is considered to be capable of crossing with the domesticated *G. hirsutum* and *G. barbedense* and produce fertile offspring; however, neither *G. barbedense* or *G. hirsutum* are grown commercially in Hawaii.

### C. Genetics of Cotton

Based on cytological evidence, seven genomic types, A through G inclusive, many with subtypes, have been identified for the genus *Gossypium* (Endrizzi *et al.*, 1984). Diploid species, AA, BB, *etc.* ( $2n=2x=26$ ), are distributed among tropical and subtropical regions worldwide. As noted above, two of the diploid species, *G. herbaceum* and *G. arboreum*, are of regional agronomic importance.

Worldwide, there are six allotetraploid species ( $2n=4x=52$ ). All of these are of the genomic group AD and euploids are frequently represented as AADD. The allotetraploid species appear to represent the fusion of the A genomic group from the old world with the D genomic group from the new world. Both *G. barbadense* and *G. hirsutum* are of the AD genomic group, as well as *G. tomentosum* (Hawaii).

### D. Pollination of Cotton

Although natural crossing can occur, cotton is normally considered to be a self-pollinating crop (Niles and Feaster, 1984). The pollen is heavy and sticky and transfer by wind is unlikely; however, there are no morphological barriers to cross-pollination based on flower structure. Pollen is transferred instead by insects, in particular by various wild bees, bumble bees (*Bombus* sp.), and honeybees (*Apis mellifera*).

The range over which natural crossing occurs is limited. McGregor (1976) traced movement of pollen by means of fluorescent particles and found that, even among flowers located only 150 to 200 feet from a cotton field that was surrounded by a large number of bee colonies to ensure ample opportunity for transfer of pollen, fluorescent particles were detected on only 1.6% of the flowers. For the sake of comparison, the isolation distances for foundation seed are 1320 feet and for certified cotton seed and registered seed are 660 feet (7CFR§201).

Based on information previously submitted by Monsanto, the agency has stated in the environmental assessment documents for Bollgard and Roundup Ready<sup>®2</sup> cotton that the "potential for gene introgression from genetically engineered cotton lines into wild or cultivated sexually compatible plants is very low" (USDA, 1995a). Importantly, the consequences of such gene flow would be minimal.

<sup>2</sup> Roundup Ready<sup>®</sup> is a trademark of the Monsanto Company.

#### E. Weediness of Cotton

*G. hirsutum* is ineffective as a weed. The USDA has previously determined that "cotton is not considered to be a serious, principal or common weed pest in the U.S." (USDA, 1995b). It appears to be somewhat opportunistic towards disturbed land and appears not to be especially effective in invading established ecosystems. In the continental United States, wild populations of *G. hirsutum* exist only in the southern tip of Florida, due at least in part to the fact that cotton cannot over-winter in those areas where freezing conditions occur.

#### F. Potential Routes of Introgression in Cotton

Three potential routes of gene escape in cotton are considered: (1) by vegetative material; (2) by seed; and (3) by pollen. Cotton does not commonly propagate from vegetative material, and, even if it did, it would be unlikely to survive the freezing winters which occur throughout most of the cotton-growing regions of the United States. It should also be noted that cotton bolls, due to their size and general properties, are unlikely to be dispersed by any of the common mechanisms of seed dispersal such as wind, birds or terrestrial animals.

Escape of genes by pollen is possible only if the pollen finds a *Gossypium* species of the correct chromosomal type. In the case of pollen from *G. hirsutum*, the recipient must be an allotetraploid of AADD genome. *G. thurberi*, the native cotton indigenous to Arizona and nearby Mexico, is not a suitable recipient since it is a diploid of DD genotype.

In the United States there are, in fact, only three *Gossypium* species which can serve as recipients for *G. hirsutum*. These are *G. hirsutum* itself, *G. barbadense*, and *G. tomentosum*, which grows only in Hawaii. *G. barbadense* has not been found growing wild in the United States and, thus, only cultivated plants would be available to be pollinated by *G. hirsutum*. Seed which is intended for planting usually comes from plants which have been segregated from other cotton plants to prevent outcrossing. Thus, if there were such an outcross, it would almost certainly involve plants whose seed was intended for processing rather than planting, since seed production fields are isolated from commercial cotton fields, and any such escape of genes into *G. barbadense* would be very short-lived and of no significance. This would also be true if the genes escaped from *G. hirsutum* into another strain of cultivated *G. hirsutum*. As noted above, *G. hirsutum* grows wild in southern Florida and, while it is possible that genes could escape to a wild *G. hirsutum*, it is unlikely since there is no commercial cotton production within several hundred miles of this area. Escape of genes to *G. tomentosum* in Hawaii is possible; however, this is also not likely to occur since there is no commercial cotton production on these islands.

The low outcrossing potential of cotton is further supported by the Environmental Assessment and Finding of No Significant Impact performed by the United States Department of Agriculture's Animal and Plant Health Inspection Service for Bollgard

Cotton Lines 531, 757 and 1076, which were genetically modified to produce the Cry1Ac protein of *Bacillus thuringiensis* subsp. *kurstaki* (USDA, 1995a).

#### **G. Characteristics of the Parent Cultivar**

The cotton cultivar used as the parental variety for transformation was Delta and Pine Land Company variety 50B (DP50B), derived from Bollgard cotton event 531. This cotton event was commercialized in the United States in 1996 and expresses the Cry1Ac insecticidal protein and the NPTII selectable marker protein. Cotton varieties derived from event 531 were grown on approximately 36% of the cotton acres in the United States in 2000. DP50B is an early maturing variety with smooth leaves that is well adapted to U.S. cotton growing conditions. The DP50B cotton variety was grown on approximately 45,000 acres in the U.S. in 1999, primarily in Louisiana and Texas (National Cotton Council, 2000).

### **III. Description of the Method of Transformation and the Molecular Biology of the Plant**

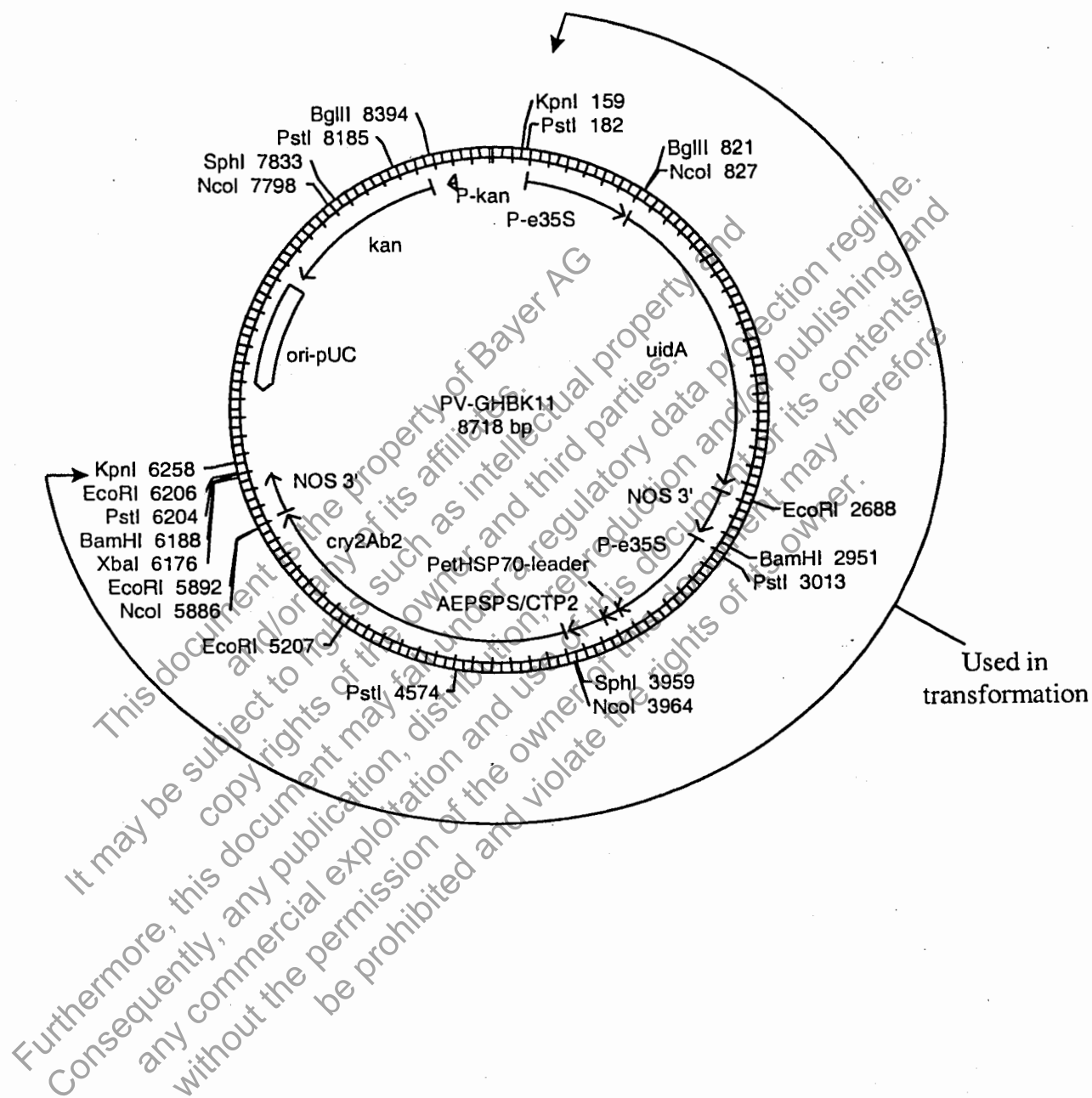
Bollgard II cotton event 15985 was generated using the particle acceleration transformation system. The plasmid vector, PV-GHBK11 (Figure 1), contains two adjacent plant gene expression cassettes: the gene of interest, *cry2Ab*, and the scorable marker gene *uidA*, which encodes for the GUS protein. The vector inserted into the cotton genome was a linearized fragment of the plasmid, designated PV-GHBK11L.

#### **A. The Transformation System**

The plasmid containing the *cry2Ab* and *uidA* gene cassettes, PV-GHBK11, was propagated in *E. coli* and purified from bacterial suspensions using column purification. The gene of interest and the marker gene were purified away from the vector backbone by cutting with a restriction endonuclease *KpnI* (Ausubel *et al.*, 1987) and subsequently separated and purified based on size differences by HPLC. This linear fragment is designated PV-GHBK11L. The purified linear DNA, PV-GHBK11L, was then precipitated onto gold particles using calcium chloride and spermidine, essentially as described by John (1997).

The cotton tissue that was the recipient of the introduced DNA, variety DP50B, is a Delta and Pine Land Company commercial variety containing the Bollgard cotton *cry1Ac* gene. DNA was introduced into the cotton meristems by the particle acceleration method described by John (1997). Germline integration of DNA was detected by histochemical staining for GUS in vascular tissue. Nontransformed tissue was removed over time, thus promoting growth of meristems containing the introduced DNA. The resulting seed from these plants was then screened for the production of the Cry2Ab protein.

**Figure 1. Plasmid Map of PV-GHBK11.**



**Table 1. Summary of DNA Components of the Plasmid PV-GHBK11.**

<b><u>Genetic Element</u></b>	<b><u>Range (bp)</u></b>	<b><u>Function (reference)</u></b>
<i>P-e35S</i>	183-797	The cauliflower mosaic virus (CaMV) promoter (Odell <i>et al.</i> , 1985) with a duplicated enhancer region used to drive expression of the <i>uidA</i> gene.
Intervening Sequence	798-828	Synthetic sequence, polylinker.
<i>uidA</i>	829-2637	The <i>uidA</i> gene from <i>E. coli</i> plasmid pUC19 encoding a $\beta$ -D-glucuronidase (GUS) protein (Gilissen <i>et al.</i> , 1998).
Intervening Sequence	2638-2692	Synthetic sequence, polylinker
NOS 3'	2693-2948	The 3' nontranslated region of the nopaline synthase (NOS) gene from <i>Agrobacterium tumefaciens</i> which terminates transcription and directs polyadenylation (Fraley <i>et al.</i> , 1983).
Intervening Sequence	2949-3013	Synthetic sequence, polylinker.
<i>P-e35S</i>	3014-3627	The cauliflower mosaic virus (CaMV) promoter (Odell <i>et al.</i> , 1985) with the duplicated enhancer region used to drive expression of the <i>cry2Ab</i> gene.
PetHSP70-leader	3628-3727	Heat shock protein 70 gene 5' untranslated leader sequence from petunia.
AEPSPS/CTP2	3729-3959	The N-terminal chloroplast transit peptide from Arabidopsis thaliana EPSPS gene (Van den Broeck, <i>et al.</i> , 1985).
Intervening Sequence	3960-3965	Synthetic linker sequences.
<i>cry2Ab</i>	3966-5873	The synthetic <i>cry2Ab</i> gene based on the sequence from <i>Bacillus thuringiensis</i> (Widner and Whiteley, 1990).
Intervening Sequence	5874-5896	Synthetic linker sequence.
NOS 3'	5897-6152	The 3' nontranslated region of the nopaline synthase (NOS) gene from <i>Agrobacterium tumefaciens</i> which terminates transcription and directs polyadenylation (Fraley <i>et al.</i> , 1983).
Intervening Sequence	6153-6277	Synthetic linker sequence.
Backbone	6278-158	(Vieira and Messing, 1987).
<i>lacZ</i>	6278-6516	A partial <i>lacI</i> coding sequence, the promoter P-lac and a partial coding sequence for $\beta$ -D-galactosidase or <i>lacZ</i> protein.
<i>ori-pUC</i>	6661-7315	A plasmid replication origin which permits propagation of DNA in bacterial hosts such as <i>E. coli</i> .
<i>npII (kan)</i>	7396-8363	The gene for the enzyme neomycin phosphotransferase type II from Tn5, a transposon isolated from <i>Escherichia coli</i> (Beck <i>et al.</i> , 1982). The <i>npII</i> gene also contains a 0.153 kb portion of the 0.378 kb <i>ble</i> gene from Tn5.
<i>P-kan</i>	8452-8501	Promoter for <i>npII</i> gene obtained from Tn5.
Intervening Sequence	159-182	Synthetic linker sequence.

## B. Plant Expression Vector PV-GHBK11

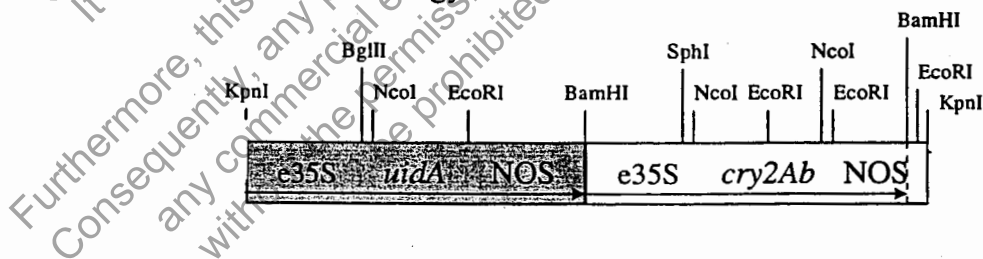
The plasmid vector, PV-GHBK11, is an 8.7 Kb high copy number, pUC-based plasmid. It contains well-characterized DNA elements for selection and replication of the plasmid in bacteria. The host for DNA cloning and vector construction was *E. coli* XL1Blue, a derivative of the common laboratory *E. coli* K-12 strain. The genetic elements in PV-GHBK11 are listed in Table 1; sizes listed here include non-functional DNA needed for the cloning. The ori-pUC is from the plasmid pUC19 (Vieira and Messing, 1987) and it provides the origin for replication and maintenance in *E. coli*.

The chimeric gene cassette that produces the Cry2Ab protein consists of the enhanced 35S promoter (Odell *et al.*, 1985), the fully synthetic *cry2Ab* coding sequence, and the 3' nontranslated region of the nopaline synthase gene from *Agrobacterium tumefaciens* which provides the signal for mRNA polyadenylation. The *cry2Ab* gene cassette was transferred to an intermediate plasmid as a *NotI* fragment. This intermediate plasmid contained the following elements: enhanced 35S promoter, the *E. coli uidA* gene, the 3' nontranslated polyadenylation signal from the nopaline synthase gene of *Agrobacterium tumefaciens* and a multi-cloning site containing a *NotI* site. The plasmid PV-GHBK11 results from the fusion of the *NotI* *cry2Ab*-containing fragment into the *NotI* site of the intermediate plasmid.

The HPLC-isolated linear restriction fragment of the plasmid vector, designated PV-GHBK11L, utilized for transformation of Bollgard II cotton event 15985, contains only the *cry2Ab* and *uidA* plant gene expression cassettes and does not contain the *nptII* selectable marker gene or origin of replication (Figure 2).

### Figure 2. Linear Map of DNA Segment PV-GHBK11L.

The DNA segment, PV-GHBK11L used to generate insect-protected cotton event 15985 by particle acceleration technology.



## IV. Donor Genes and Regulatory Sequences

### A. The *cry2Ab* Gene

Cry2Ab is a protein derived from *Bacillus thuringiensis* and has also been designated Cry2Ab2, CryIIB, CryB2 or CryIIAb (Liang and Dean, 1994; Widner and Whiteley, 1990; Crickmore, *et al.*, 1998) or the Monsanto designation Insect Protection Protein 2 (IPP2). In the current nomenclature scheme for Cry proteins, names are assigned according to amino acid similarity to established holotype proteins as defined by



Crickmore *et al.* (1998). In this nomenclature, Cry proteins with similar amino acid sequences are grouped together. Cry proteins with the same Arabic numeral, *e.g.*, Cry2, share at least a 45% amino acid sequence identity. Those with the Arabic numeral and upper case letter, *e.g.*, Cry2A, share at least a 75% sequence identity. Finally, Cry proteins with the same Arabic numeral, upper case letter and lower case letter, *e.g.*, Cry2Ab, share a greater than 95% sequence identity.

*Bacillus thuringiensis* (B.t.) is a gram-positive bacterium commonly present in soil that has been used commercially in the U.S. since 1958 to produce microbial-derived products with insecticidal activity (EPA, 1988). *Bacillus thuringiensis* subsp. *kurstaki*, present in commercial microbial pest control products such as DIPEL and Crymax, contains both the *cry2Aa* and *cry2Ab* genes. The *cry2Aa* gene is expressed in these commercial products; however, the *cry2Ab* gene is a pseudogene, which even though present is not expressed due to an inefficient *cry2Ab* promoter (Dankocsik *et al.*, 1990). Therefore, the Cry2Ab protein is not naturally produced in soil bacteria or sprayable microbial formulations (Widner and Whiteley, 1990; Crickmore, *et al.*, 1994). Both the *cry2Aa* and *cry2Ab* genes are located on the same 100 MDa plasmid (Donovan, *et al.*, 1988; 1989) and the sequence of the *cry2Ab* gene has been fully characterized (Widner and Whiteley, 1990).

The *cry2Ab* gene that is the subject of this request is a synthetically optimized version of the gene from *Bacillus thuringiensis* subsp. *kurstaki*. Optimization was necessary to provide controlling sequences to allow expression in the cotton plant. The *cry2Ab* gene with the necessary promoter region was cloned into *Bacillus thuringiensis* strain EG7699. The *cry2Ab* gene expression product was then isolated and purified from the modified EG7699 bacterial strain. The Cry2Ab protein product (GenBank Accession No. X55416) is 633 amino acids in length, with an approximate mass of 71 kDa (Widner and Whiteley, 1990; Dankocsik *et al.*, 1990). The deduced amino acid sequence of the Cry2Ab protein introduced into cotton is shown in Figure 3. An additional amino acid (position 2, Figure 3) was introduced to create a restriction enzyme cleavage site for cloning purposes. The Cry2Ab protein that is present as a stable protein product in transgenic cotton plants is predicted to contain an additional three amino acids due to processing of the chloroplast transit peptide (underlined positions 77-79, Figure 3).

The deduced amino acid sequence generated from the coding region of the *cry2Ab* gene in B.t.k. is highly similar to that deduced from the *cry2Aa* gene (Figure 4), sharing 88% amino acid sequence identity (Widner and Whiteley, 1990; Dankocsik *et al.*, 1990) and 97% amino acid similarity (amino acid identities and conservative amino acid substitutions).

**Figure 3. Deduced Cry2Ab Protein Sequence as Produced in Cotton.** The sequence deduced from the DNA used to transform cotton. The chloroplast transit peptide is shown in italics (residues 1-79). The Cry2Ab protein corresponds to residues 80-713. The underlined amino acids (residues 77-79) correspond to the predicted portion of the chloroplast transit peptide remaining after processing. The amino acid at position 81 (D, aspartic acid) corresponds to the residue introduced for cloning purposes.

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1  MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTOQHP RAYPISSSWG
51  LKKSGMTLIG SELRPLKVMS SVSTACMLAM DNSVLNSGRT TICDAYNVAA
101 HDPFSFQHKs LDTVQKEWTE WKKNNHSLYL DPIVGTVASF LLKKVGSLLVG
151 KRILSELRNL IFPSGSTNLM QDILRETEKF LNQRNLNTDTL ARVNAELTGL
201 QANVEEFNRQ VDNFLNPNRN AVPLSITSSV NTMQQLFLNR LPQFQMQGYQ
251 LLLLPLFAQA ANLHLSFIRD VILNADEWGI SAATLRITYRD YLKNYTRDYS
301 NYCINTYQSA FKGLNTRLHD MLEFRTYMFL NVFEYVSIWS LFKYQSLLVs
351 SGANLYASGS GPQQTQSETS QDWPFLYSLF QVNSNYVLNG FSGARLSNTF
401 PNIVGLPGST TTHALLAARV NYSGGISSGD IGASPFNQNF NCSTFLPPLL
451 TPFVRSWLDS GSDREGVATV TNWQTESFET TLGLRSGAFT ARGNSNYFPD
501 YFIRNISGVP LVVRNEDLRR PLHYNEIRNI ASPSGTPGGA RAYMVSVHNR
551 KNNIHAVHEN GSMIHLAPND YTGFTISPIH ATQVNNQTRT FISEKFGNQG
601 DSLRFEQNNT TARYTLRGNG NSYNLYLRVS SIGNSTIRVT INGRVYTATN
651 VNTTTNNDGV NDNGARFSDI NIGNVVASSN SDVPLDINVT LNSGTQFDLM
701 NIMLVPTNIS PLY

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**Figure 4. Deduced Amino Acid Sequences of Cry2Ab and Cry2Aa Proteins as Encoded in *Bacillus thuringiensis* subsp. *kurstaki*.**

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Cry2Ab  MDNSVLNSGRITTCDAYNVAADPFPSFQHKSLDTVQKEWTEWKNNHSLYLDPIVGTVAS
        10      20      30      40      50      60
Cry2Aa  MNVLNSGRITTCDAYNVAADPFPSFEHKSLDTIQKEWMEWKRTDHSLYVAPVVGTVSS
        10      20      30      40      50

        70      80      90      100     110     120
FLLKKVGSVLGKRILSELNRNLIFFSGSTNLMQDILRETEKFLNQRLNTDTLARVNAELTG
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
FLLKKVGSVLGKRILSELWGIIFPSGSTNLMQDILRETEQFLNQRLNTDTLARVNAELIG
60      70      80      90      100     110

        130     140     150     160     170     180
LQANVEEFNRQVDNLFNPNRNVPLSITSSVNTMQQLFLNRLPOFQMGGYQLLLPLFAQ
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
LQANIREFNQVDNLFNPTQNPVPLSITSSVNTMQQLFLNRLPOFQIQGYQLLLPLFAQ
120     130     140     150     160     170

        190     200     210     220     230     240
AANLHLSFIRDVILNADEWGISAATLRTRYRDLKNYTRDYSNYCINTYQSAFKGLNTRLH
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
AANMHLFIRDVILNADEWGISAATLRTRYRDLKNYTRDYSNYCINTYQTAFRGLNTRLH
180     190     200     210     220     230

        250     260     270     280     290     300
DMLEFRTYMFNLVFEYVSIWSLFKYQSLVSSGANLYASGSGPQQTQSFTSQDWPFYLSL
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
DMLEFRTYMFNLVFEYVSIWSLFKYQSLMVSSGANLYASGSGPQQTQSFTAQNWPFYLSL
240     250     260     270     280     290

        310     320     330     340     350     360
FQVNSNYVLNGESGARLSNTFFPNIVGLPGSTTTHALLAARVNYSGGISGSDIGASPFNQ
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
FQVNSNYILSGISGTRLSITFFPNIGGLPGSTTTHSLNSARVNYSGGVSSGLIGATNLNHN
300     310     320     330     340     350

        370     380     390     400     410     420
FNCSTFLPPLLTPFVRSWLDSDREGVATVTNWOTESFETTLGLRSGAFTARGNSNYFP
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
FNCSTVLPPLSTPFVRSWLDSDREGVATSTNWOTESFQTTLSLRCGAFSARGNSNYFP
360     370     380     390     400     410

        430     440     450     460     470     480
DYFIRNISGVPLVVRNEDLRRPLHYNEIRNIASPSGTPGGARAYMVSVHNKNNIHAVHE
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
DYFIRNISGVPLVVRNEDLRRPLHYNQIRNIESPSGTPGGARAYLVSVHNKNNIYAANE
420     430     440     450     460     470

        490     500     510     520     530     540
NGSMIHLAPNDYTGFTISPIHATQVNNQTRTFISEKFGNQGDSLRFEQNNTTARYTLRGN
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
NGTMIHLAPEDYTGFTISPIHATQVNNQTRTFISEKFGNQGDSLRFEQSNNTTARYTLRGN
480     490     500     510     520     530

        550     560     570     580     590     600
GNSYNLYLRVSSIGNSTIRVTINGRVYTATNVNTTTNNDGVNDNGARFSDINIGNVVAS
|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
GNSYNLYLRVSSIGNSTIRVTINGRVYTVSNVNTTTNNDGVNDNGARFSDINIGNIVASD
540     550     560     570     580     590

        610     620     630
NSDVPLDINVTLNSGTQFDLMNIMLVPTNISPLY
|||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
NTNVTLDINVTLNSGTPFDLMNIMFVPTNLPLY
600     610     620     630

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**Legend:** Alignment of the deduced amino acid sequences of Cry2Ab and Cry2Aa proteins. | = identical AA; : = AA conservative substitutions (similarities)

## B. The *uidA* Gene

The development of plant varieties containing useful new traits introduced by plant genetic engineering depends upon an effective means to select for transformed plant cells containing the inserted gene(s) of interest from those plants cells that fail to take up or maintain the added DNA. Therefore, a scorable marker is used to identify the cells to be carried forward through the regeneration process. The  $\beta$ -glucuronidase gene, *uidA*, also known as *gus* or *gusA* gene, is derived from *Escherichia coli* strain K12 (Jefferson, *et al.*, 1986). The sequence has been fully characterized and is available in GenBank (Jefferson, *et al.*, 1986; Schlaman *et al.*, 1994). This gene encodes for the enzyme  $\beta$ -D-glucuronidase (GUS).

$\beta$ -D-glucuronidase is an exohydrolase that catalyzes the hydrolysis of a range of the  $\beta$ -glucuronides into their corresponding acids and aglycones (Oshima *et al.*, 1987), including the artificial substrate p-nitrophenyl- $\beta$ -D-glucuronide. Hydrolysis of this chromogenic compound releases a blue dye that functions as a visible scorable marker in plant transformation processes (Jefferson *et al.*, 1987). The biochemistry and catalytic activity of this protein have been thoroughly studied (Wang and Touster, 1972). The deduced amino acid sequence of the GUS protein as expressed in cotton event 15985 is presented in Figure 5.

The GUS protein was originally isolated from *E. coli* (Stahl and Fishman, 1974). *E. coli* is ubiquitous in the digestive systems of vertebrates, including humans (Jefferson *et al.*, 1986), where primary glucuronidation activity occurs in the liver. Endogenous GUS activity is also observed in other tissues, such as kidney, spleen, breast milk, adrenal glands and the alimentary tract (Gilissen *et al.*, 1998). Glucuronide conjugation increases the water solubility and excreatability of foreign substances from the body (Dutton, 1980). GUS activity is also observed in a large number of other bacteria, including other anaerobic digestive tract bacteria such as *Clostridium* and *Bacteroides* (Hawkesworth *et al.*, 1971), as well as many bacteria (Levy and Marsh, 1959; Ritz *et al.*, 1994). GUS is also present in cattle and in a number of invertebrate species, including nematodes, mollusks, snails, and insects (Gilissen *et al.*, 1998).

GUS-like activity has also been detected in over 50 plant species in various tissues, including embryo, fruit, seed coat and endosperm (Hu *et al.*, 1990). These species include a number of human food sources, such as potato, apple, almond, rye, rhubarb, and sugar beet (Schulz and Weissenbock, 1987; Hodal *et al.*, 1992; Wozniak and Owens, 1994).

**Figure 5. Deduced Amino Acid Sequence of Plant-Produced GUS Protein.**

The sequence deduced from the DNA used to transform cotton.

1	MVRPVETPTR	EIKKLDGLWA	FSLDRENCGI	DQRWWESALQ	ESRAIAVPGS
51	FNDQFADADI	RNYAGNVWYQ	REVFIPKGWA	GQRIVLRFDA	VTHYGKVWVN
101	NQEVMEHQGG	YTPFEADVTP	YVIAGKSVRI	TVCVNNELNW	QTIPPGMVIT
151	DENGKKKQSY	FHDFFNYAGI	HRSVMLYTTP	NTWVDDITVV	THVAQDCNHA
201	SVDWQVVANG	DVSVELRDAD	QQVVATGQGT	SGTLQVWNPH	LWQPGEGLY
251	ELCVTAKSQT	ECDIYPLRVG	IRSAVKGEQ	FLINHKPFYF	TGFGRHEDAD
301	LRGKGFDNVL	MVHDHALMDW	IGANSYRTSH	YPYAEEMLDW	ADEHGIVVID
351	ETAAVGFNLS	LGIGFEAGNK	PKELYSEEAV	NGETQQAHLQ	AIKELIARDK
401	NHPSVVMWSI	ANEPDTRPQA	AREYFAPLAE	ATRKLDPTRP	ITCVNVMFCD
451	AHTDTISDLF	DVLCNRYYG	WYVQSGDLET	AEKVLEKELL	AWQEKLHQPI
501	IITEYGVDTL	AGLHSMYTDI	WSEFYQCWL	DMYHRVFDV	SAVVGEQVWN
551	FADFATSQGI	LRVGGNKKGI	FTRDRKPKSA	AFLLQKRWG	MNFGEKPPQG
601	GKQ				

## V. Genetic Analysis and Agronomic Performance

### A. Characterization of the Inserted Genetic Material

The inserted DNA from Bollgard II cotton event 15985 was characterized by traditional molecular techniques. Southern blot analysis was used to determine the insert number (number of integration loci within the cotton genome), the copy number (the number of transgenes at a single locus), the intactness of the *cry2Ab* and *uidA* coding regions, the intactness of the *cry2Ab* and *uidA* cassettes, and to confirm the absence of plasmid backbone sequence derived from plasmid PV-GHBK11. Plasmid PV-GHBK11, the plasmid backbone, the *cry2Ab* and *uidA* coding regions, the enhanced CaMV 35S promoter, and the NOS 3' polyadenylation sequence were all used as probes. Additionally, the 5' and 3' insert-to-plant junctions were verified using the polymerase chain reaction (PCR).

The data show that Bollgard II cotton event 15985 contains one DNA insertion from the linear fragment of PV-GHBK11 (Table 2). The insert contains one copy each of the *cry2Ab* and *uidA* cassettes. The *cry2Ab* coding region and cassette are complete; however, the restriction site following the NOS 3' polyadenylation sequence in the cassette is no longer present. The *uidA* coding region and its NOS 3' polyadenylation sequence are also complete; however, 260 bp of the 5' end of the enhanced CaMV 35S promoter of the *uidA* cassette is not present in the inserted *uidA* gene cassette. The e35S promoter is still functional despite this truncation, as demonstrated by production of the

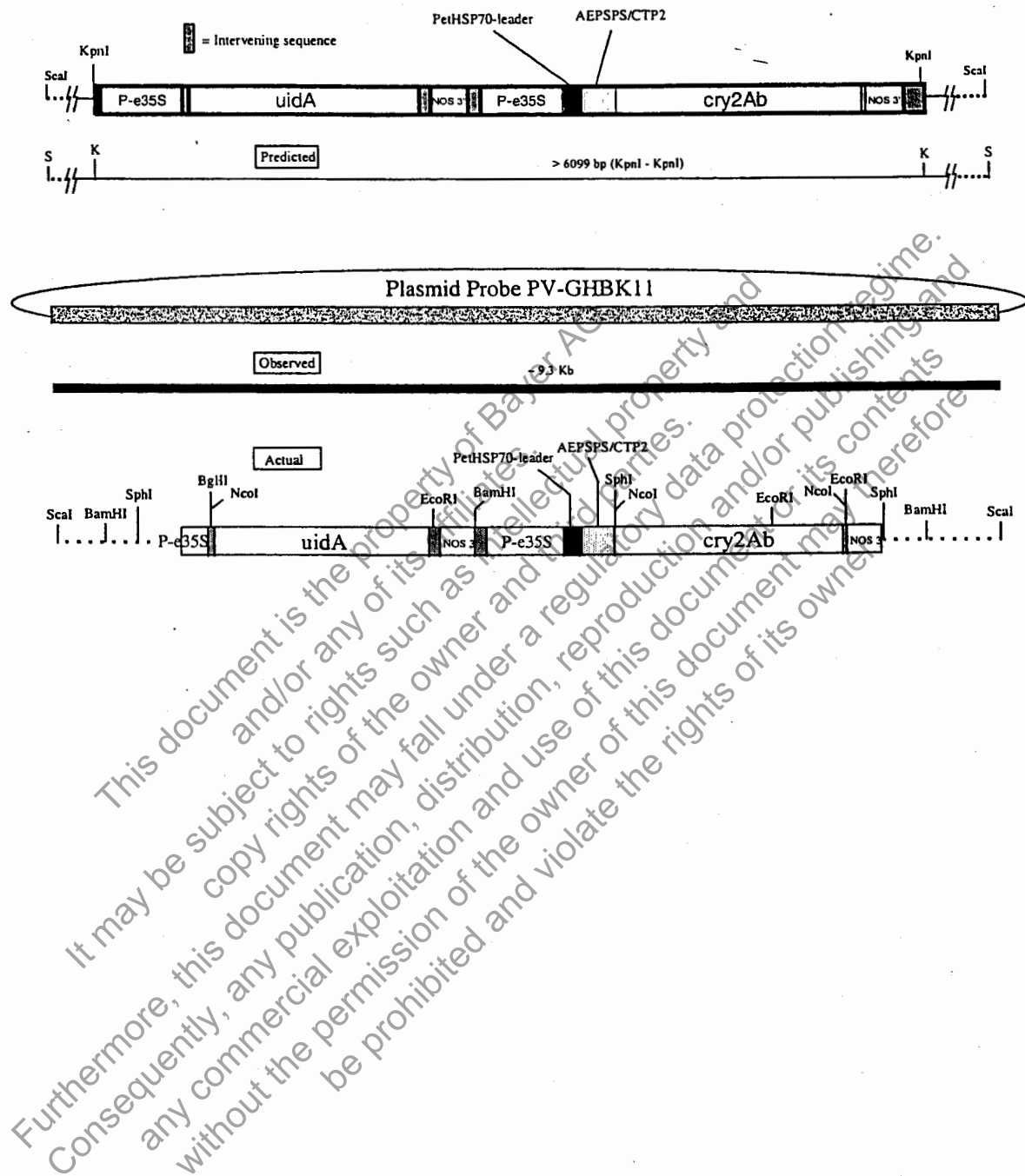
GUS protein. This event does not contain any detectable backbone sequence derived from plasmid PV-GHBK11. It is therefore concluded that full-length Cry2Ab and GUS proteins should be produced in event 15985 as a result of integration of the DNA segment derived from plasmid PV-GHBK11. Production of the full-length Cry2Ab and GUS proteins in cotton event 15985 has been confirmed (Appendix 6, Section 5).

**Table 2. Summary of the Molecular Characterization of Cotton Event 15985.**

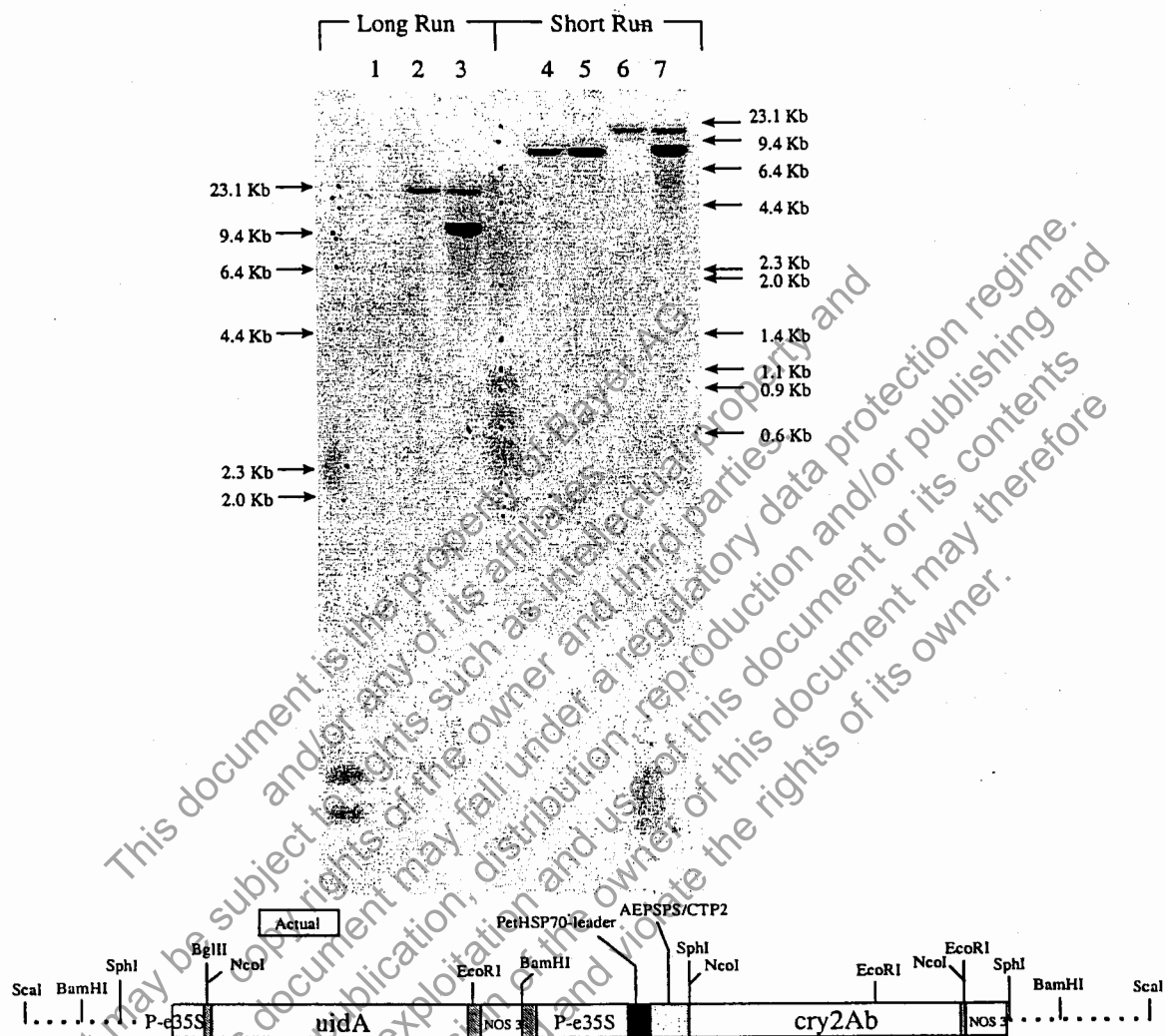
Cotton Event 15985	
# of new insertions	One
# of copies of <i>cry2Ab</i> and <i>uidA</i> cassettes	One of each
Genetic Element	
enhanced CaMV 35S promoter ( <i>uidA</i> )	Intact except for absence of 260 bp from 5' end (~40%)
<i>uidA</i> coding region	Intact
NOS 3' polyadenylation sequence ( <i>uidA</i> )	Intact
enhanced CaMV 35S promoter ( <i>cry2Ab</i> )	Intact
<i>cry2Ab</i> coding region	Intact
NOS 3' polyadenylation sequence ( <i>cry2Ab</i> )	Intact
Backbone DNA	Not detected

### 1. Analysis for Insert Number

For insert number characterization, genomic DNA isolated from the test and control substances and PV-GHBK11 mixed with DP50 DNA samples were digested with the restriction enzyme *ScaI*. *ScaI* does not cleave within the inserted DNA (Figure 1), and should release a genomic segment containing the inserted DNA and adjacent plant DNA. Plasmid PV-GHBK11 DNA (Figure 1) mixed with DP50 DNA was also digested with *XbaI* to linearize the plasmid. The Southern blot (Figure 6) was probed with radiolabeled plasmid PV-GHBK11 (Figure 1). Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced a single band at approximately 8.7 Kb, the size of the entire plasmid. As expected, the probe did not hybridize to the control DP50 DNA. The probe hybridized to *ScaI*-digested DP50B DNA (lanes 2 and 6), producing two bands of approximately 22 Kb and 15 Kb (faint). Since these bands are present in both event 15985 and the DP50B control (and not in DP50), they are considered to be associated with the *cryIAc* event present in DP50B. Event 15985 (lanes 3 and 7) produced one unique hybridizing band not present in either the DP50 or DP50B at ~9.3 Kb. This result suggests that 15985 contains one unique integrated DNA insert.







**Figure 6. Southern Blot Analysis of Event 15985: Insert Number Analysis.**

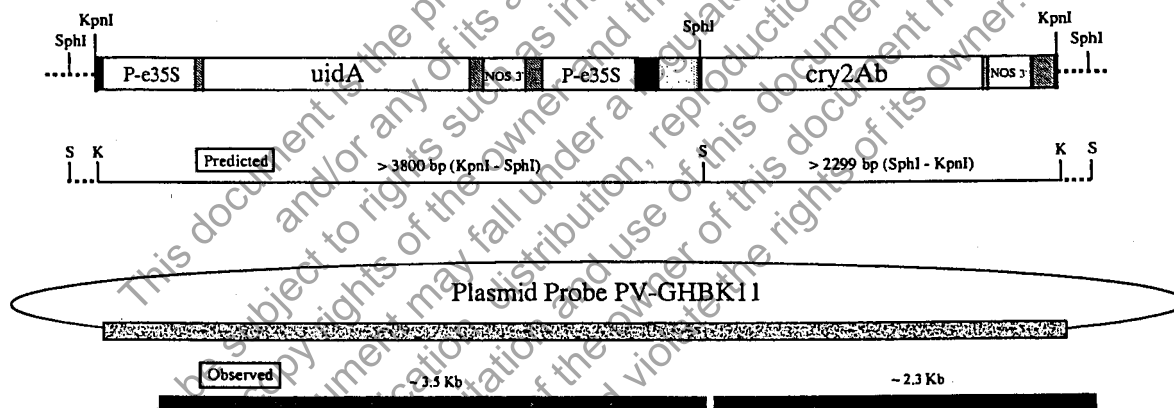
Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *ScaI*. The DP50 short run samples were also digested with *XbaI*. The blot was probed with  $^{32}$ P-labeled PV-GHBK11. Lane designations are as follows:

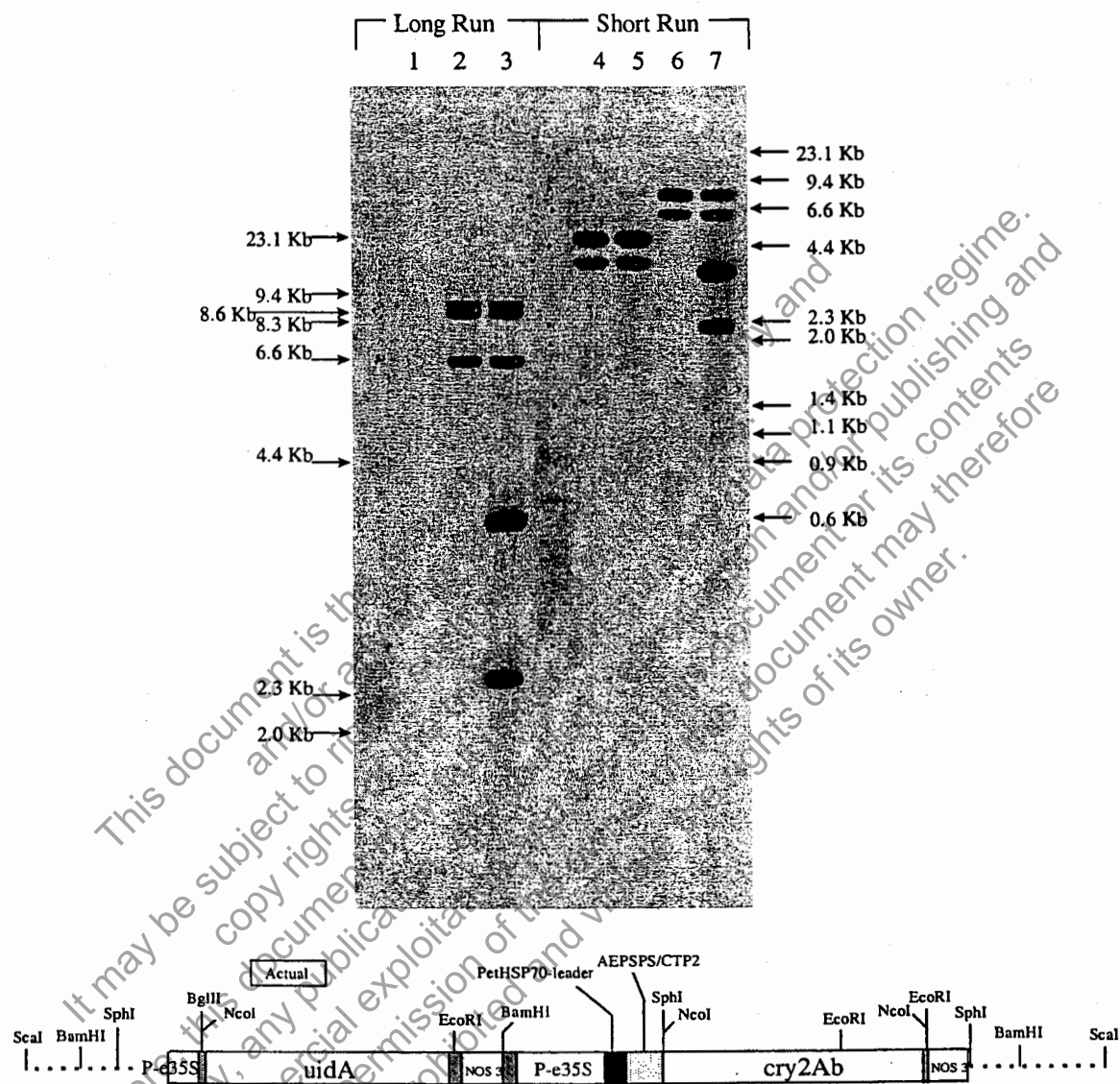
- Lane 1: DP50 (Long Run)
- Lane 2: DP50B (Long Run)
- Lane 3: 15985 (Long Run)
- Lane 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)
- Lane 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)
- Lane 6: DP50B (Short Run)
- Lane 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

## 2. Analysis for Copy Number

For copy number determination, genomic DNA isolated from the test and control substances and PV-GHBK11 mixed with DP50 DNA samples were digested with *Sph*I, which cuts only once in the transforming linear DNA segment (Figure 1). The Southern blot was probed with radiolabeled plasmid PV-GHBK11 (blot shown in Figure 7). As expected, the probe did not hybridize to the non-transgenic control, DP50 (lane 1). Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced bands at approximately 3.9, 4.8 and 8.7 Kb (faint). The faint ~8.7 Kb band is presumably due to undigested plasmid DNA. DP50B (lanes 2 and 6) produced three hybridizing bands at approximately 6.4, 8.3, and 8.6 Kb. Since these bands are present in both event 15985 and the DP50B control they are considered to be associated with the *cry*1Ac event. Two unique bands were apparent in event 15985 (lanes 3 and 7) at approximately 2.3 Kb and 3.5 Kb. Because the enzyme *Sph*I cuts only once within the transformation cassette, this result suggests that 15985 contains one copy of integrated DNA which produces these two unique hybridizing bands.





**Figure 7. Southern Blot Analysis of Event 15985: Copy Number Analysis**

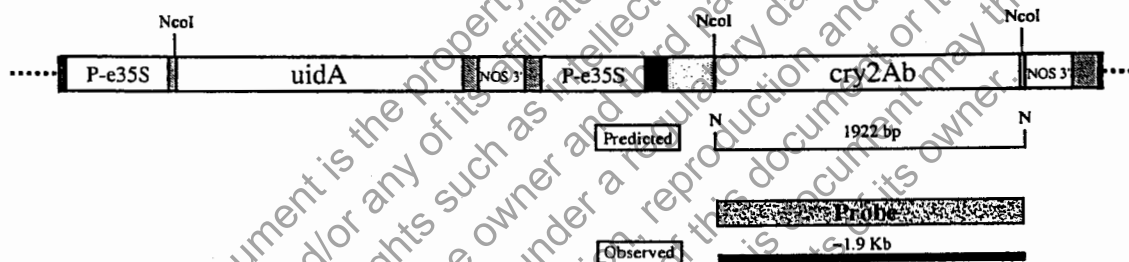
Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Sph*I. The blot was probed with <sup>32</sup>P-labeled PV-GHBK11. Lane designations are as follows:

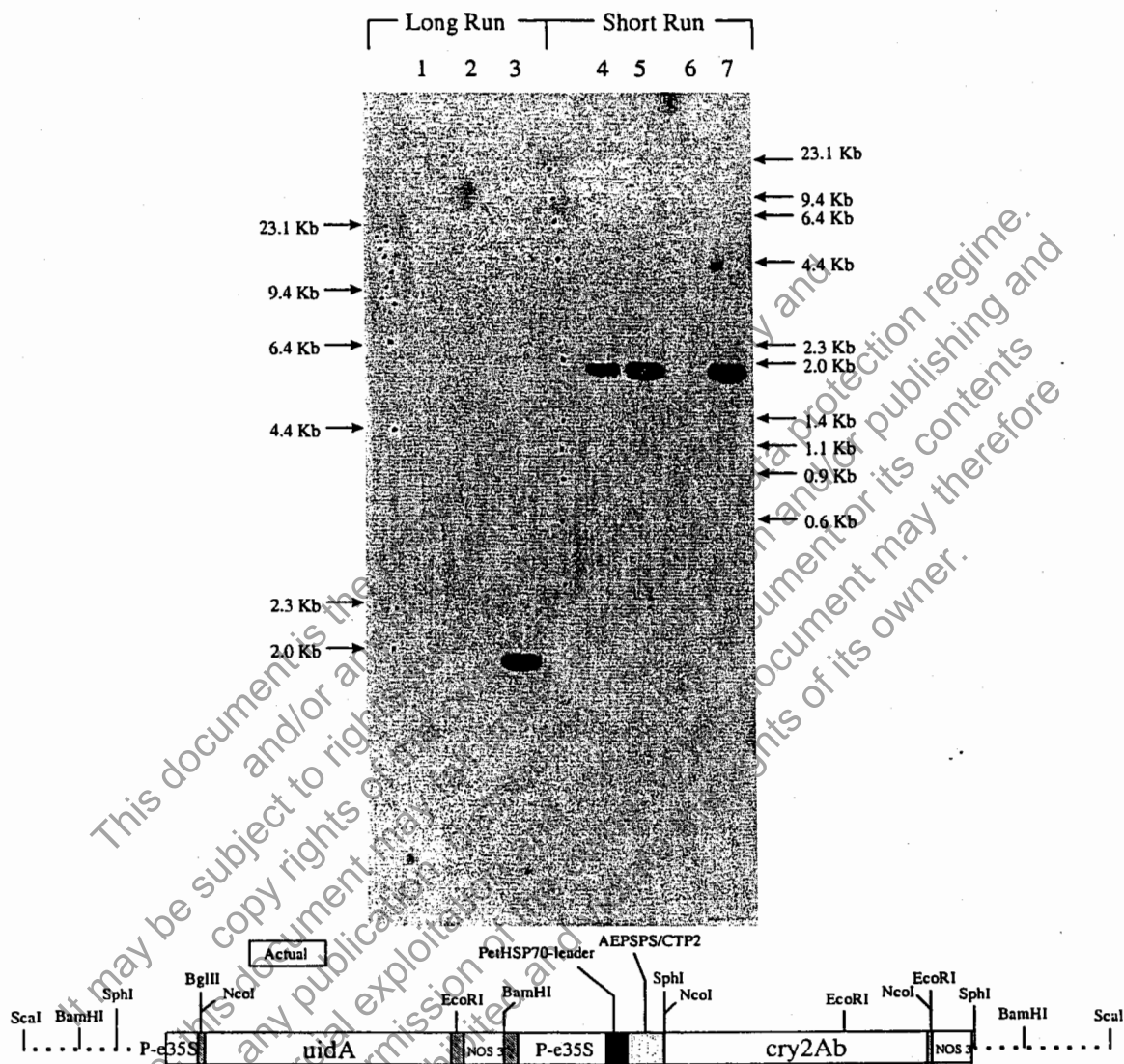
- Lane 1: DP50 (Long Run)  
 2: DP50B (Long Run)  
 3: 15985 (Long Run)  
 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)  
 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)  
 6: DP50B (Short Run)  
 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

### 3. Analysis for *cry2Ab* Coding Region Intactness

Genomic DNA isolated from the test and control substances, and the plasmid PV-GHBK11 mixed with DP50 DNA was digested with *Nco*I to release the *cry2Ab* coding region and assess its intactness. The blot was probed with the full-length *cry2Ab* coding region (see Figure 8). As expected, the DP50 non-transgenic control (lane 1) and the DP50B control (lanes 2 and 6) showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected ~1.9 Kb band which corresponds to the entire *cry2Ab* coding region. A single hybridizing ~1.9 Kb band was also produced in event 15985 (lanes 3 and 7) corresponding to an intact *cry2Ab* coding region. This result supports that event 15985 contains the intact *cry2Ab* region, with no additional detectable bands.





**Figure 8. Southern Blot Analysis of Event 15985: *cry2Ab* Coding Region Intactness**

Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Nco*I. The blot was probed with <sup>32</sup>P-labeled *cry2Ab* coding region. Lane designations are as follows:

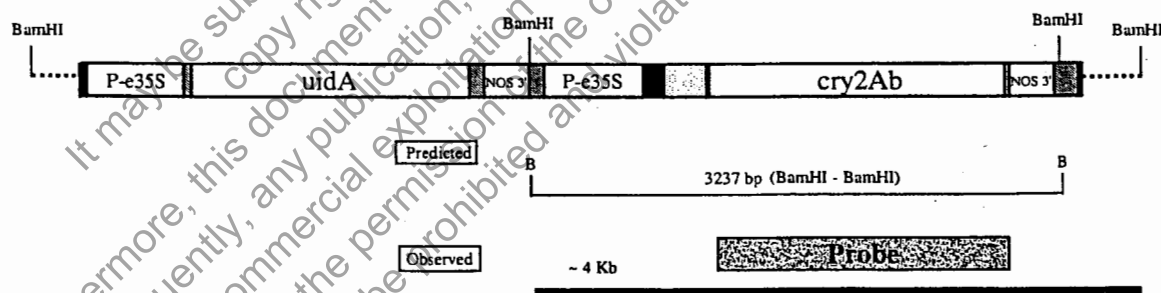
- Lane 1: DP50 (Long Run)  
 2: DP50B (Long Run)  
 3: 15985 (Long Run)  
 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)  
 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)  
 6: DP50B (Short Run)  
 7: 15985 (Short Run)

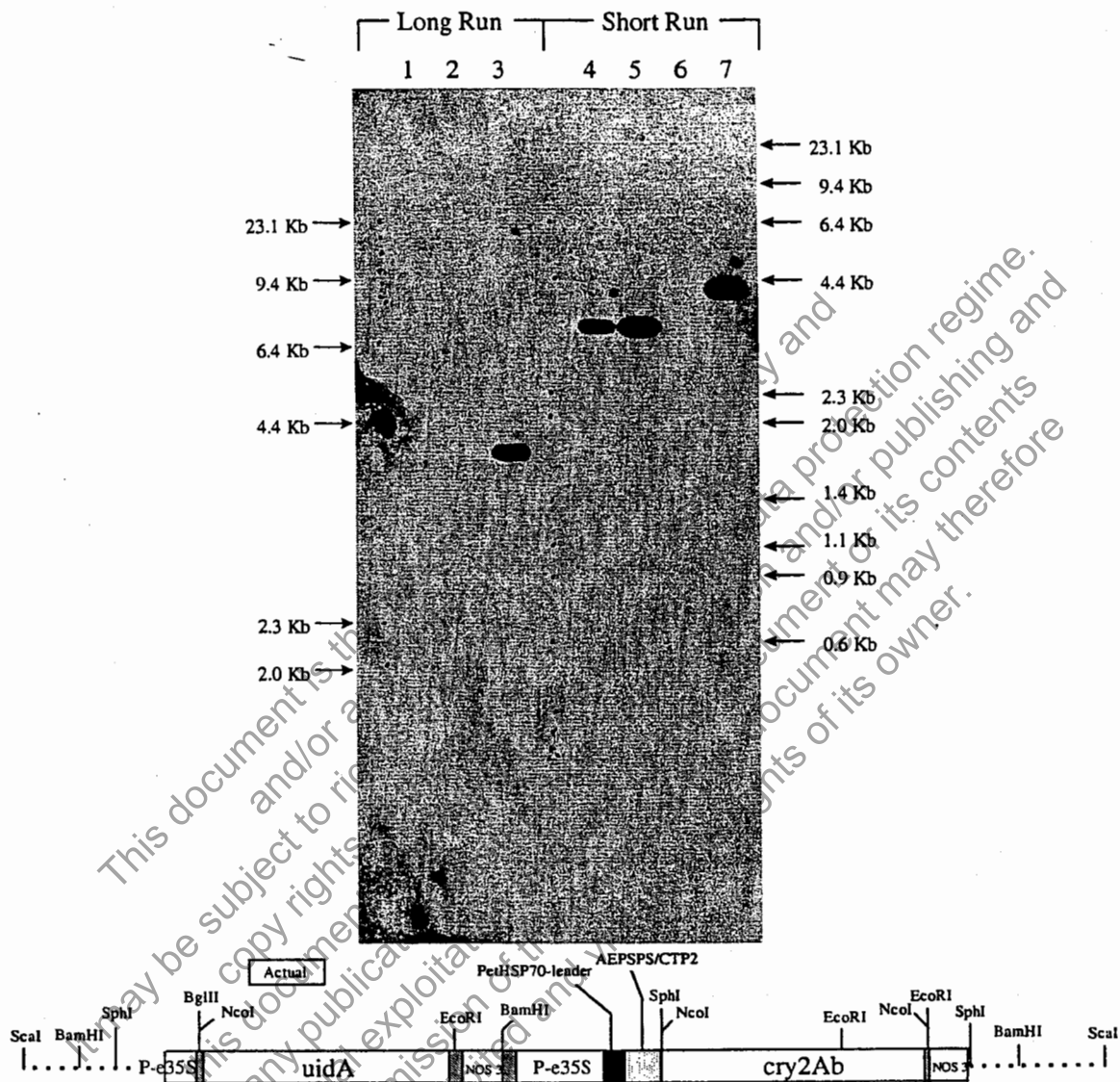
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

#### 4. Analysis for *cry2Ab* Expression Cassette Intactness

The intactness of the *cry2Ab* expression cassette (containing the enhanced CaMV 35S promoter, *cry2Ab* coding region and NOS 3' polyadenylation sequence) was assessed by digesting test and control substances and plasmid PV-GHBK11 mixed with DP50 DNA with the restriction enzyme *Bam*HI which cleaves at the 5' and 3' ends of the *cry2Ab* cassette (Figure 1). The blot was sequentially probed with each radiolabeled element of the cassette.

***Cry2Ab* probe results.** The blot was probed with the full length *cry2Ab* coding region (see Figure 9). As expected, DP50 (lane 1) and DP50B (lanes 2 and 6) showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected ~3.2 Kb band which corresponds to the entire *cry2Ab* cassette. Event 15985 (lanes 3 and 7) produced a band at approximately 4.0 Kb. This result indicates that the 3' end of the transformation cassette lost the *Bam*HI restriction site during the transformation process. The sequence of the 3' insert-to-plant junction at the 3' end of the insert, was previously determined by genome walking and verified by PCR analysis (see Figure 14). Sixty-six base pairs of the 3' end of the transformation cassette including the *Bam*HI restriction site, were shown to have been deleted. More importantly, the deleted nucleotides do not include any of the NOS 3' polyadenylation sequence associated with the *cry2Ab* cassette, but only linker DNA. These results support the conclusion that the *cry2Ab* cassette is intact. No partial *cry2Ab* cassettes were detected.





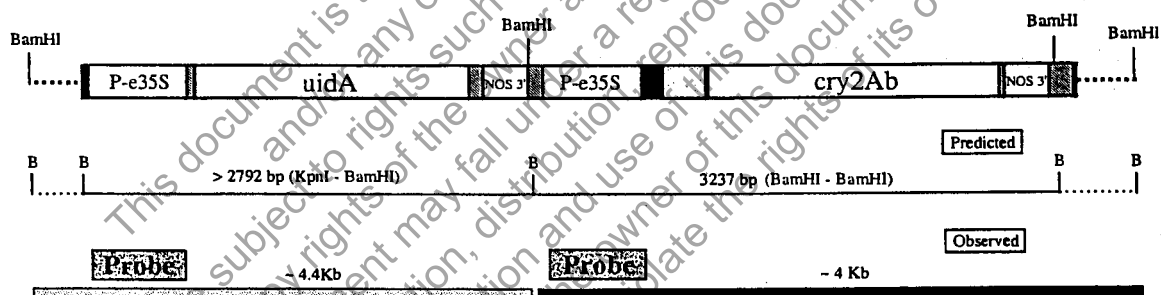
**Figure 9. Southern Blot Analysis of Event 15985: *cry2Ab* Cassette Intactness - *cry2Ab* Probe**

Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Bam*HI. The blot was probed with <sup>32</sup>P-labeled *cry2Ab* coding region. Lane designations are as follows:

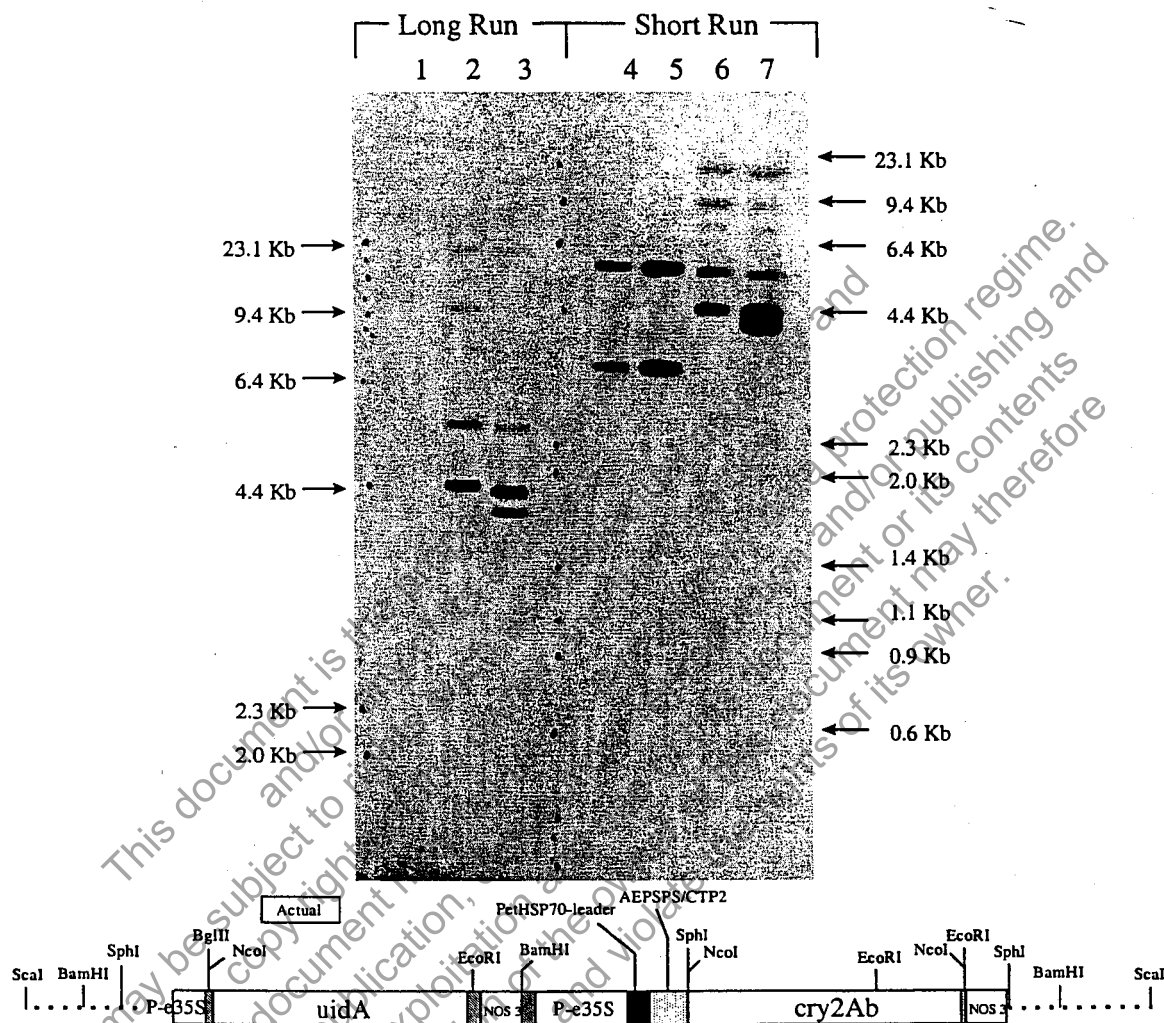
- Lane 1: DP50 (Long Run)  
 Lane 2: DP50B (Long Run)  
 Lane 3: 15985 (Long Run)  
 Lane 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)  
 Lane 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)  
 Lane 6: DP50B (Short Run)  
 Lane 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

**Enhanced CaMV 35S Promoter Probe Results.** The blot was stripped and re-probed with the full length enhanced CaMV 35S promoter (see Figure 10). As expected the probe did not hybridize to the DP50 DNA (lane 1). Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected bands at 5.5 and 3.2 Kb with no additional bands. DP50B (lanes 2 and 6) produced five bands at approximately 4.4, 5.3, 7.5, 9.4, and 22 Kb. Since these bands are present in both event 15985 and the DP50B control they are considered background bands associated with the *cry1Ac* event. Event 15985 (lanes 3 and 7) produced one unique band at approximately 4.0 Kb which is not present in either the DP50 or the DP50B lanes. This corresponds to the segment predicted for the *cry2Ab* cassette and is consistent with the result obtained from the *cry2Ab* coding region probe. A second band in the 15985 lanes resulting from hybridization to the enhanced CaMV 35S promoter associated with the *uidA* cassette is predicted but not apparent in the test lanes. The results of the NOS 3' polyadenylation sequence probe previously discussed, support the conclusion that the enhanced CaMV 35S promoter sequence associated with the *uidA* cassette is present, but that the ~4.4 Kb band co-migrates with a ~4.4 Kb background band on the blot and is therefore not apparent. No extraneous bands were detected.







**Figure 10. Southern Blot Analysis of Event 15985: *cry2Ab* Cassette Intactness - Enhanced CaMV 35S Promoter Probe**

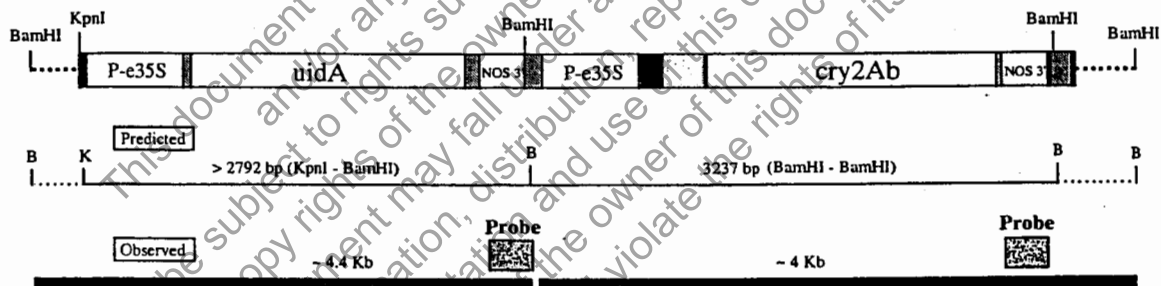
Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Bam*HI. The blot was probed with <sup>32</sup>P-labeled enhanced CaMV 35S promoter probe. Lane designations are as follows:

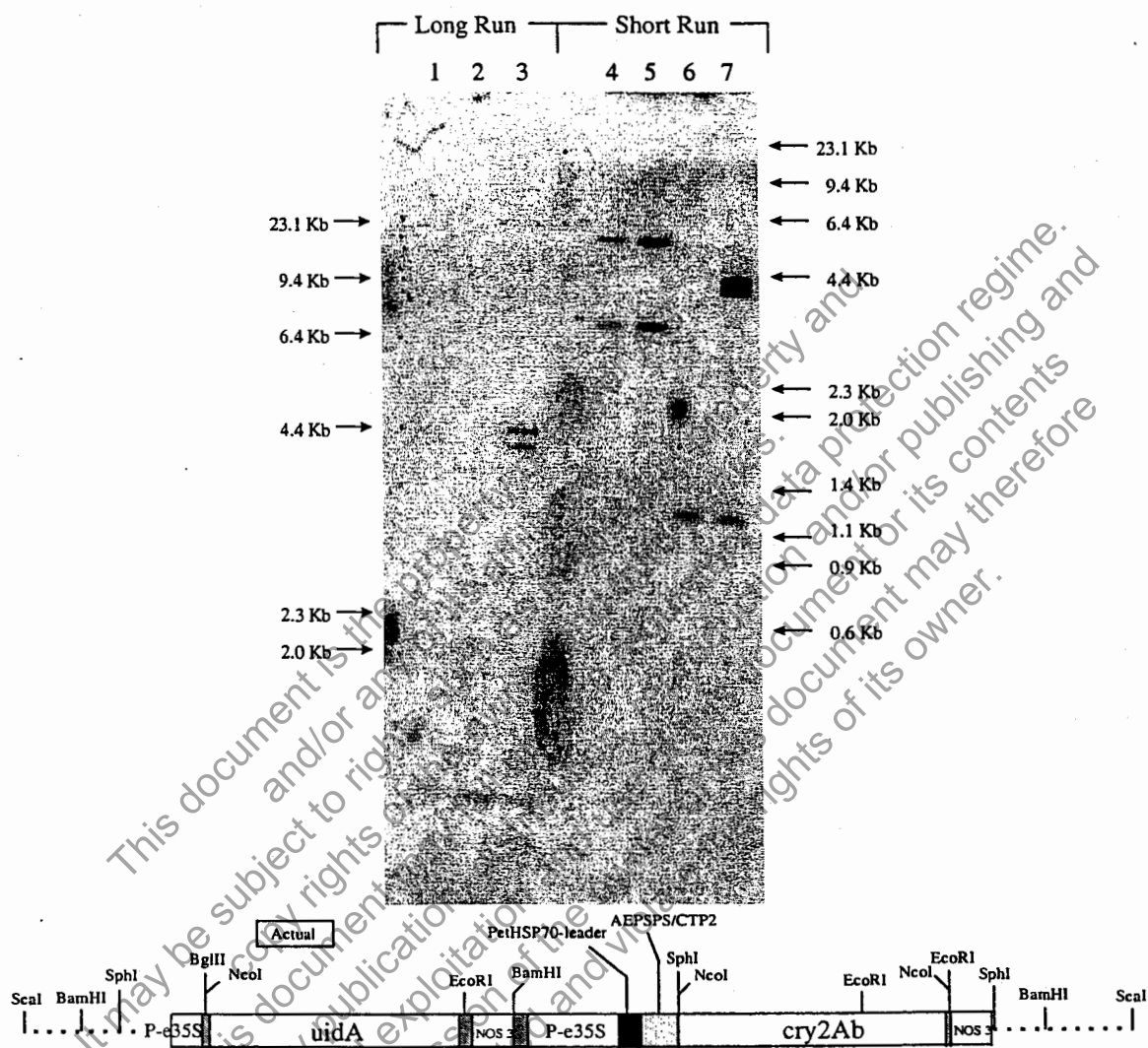
- Lane 1: DP50 (Long Run)
- Lane 2: DP50B (Long Run)
- Lane 3: 15985 (Long Run)
- Lane 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)
- Lane 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)
- Lane 6: DP50B (Short Run)
- Lane 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

**NOS 3' Polyadenylation Sequence Probe Results.** The blot was stripped and re-probed with the full length NOS 3' polyadenylation sequence (see Figure 11). As expected, the probe did not hybridize to the DP50 DNA (lane 1). Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected bands at 5.5 and 3.2 Kb with no additional bands detectable. DP50B (lanes 2 and 6) produced one band at approximately 1.2 Kb. This band is present in event 15985 and DP50B and is considered to be background associated with the *cry1Ac* event. Event 15985 (lanes 3 and 7) produced two additional bands at approximately 4.0 and 4.4 Kb. The ~4.0 Kb band corresponds to the segment predicted for the *cry2Ab* cassette (from the *cry2Ab* probe results). The ~4.4 Kb band (not observed on the CaMV 35S blot) corresponds to the segment predicted for the *uidA* cassette.

Combined with the previous data (CaMV 35S probe), these results support the conclusion that the *cry2Ab* cassette is intact and that there is a deletion of the *Bam*HI site at the 3' end of the transformation cassette. This deletion does not include any of the NOS 3' polyadenylation sequence at the 3' end of the *cry2Ab* cassette. No bands indicative of partial *cry2Ab* cassettes were detected.





**Figure 11. Southern Blot Analysis of Event 15985: *cry2Ab* Cassette Intactness - NOS Probe**

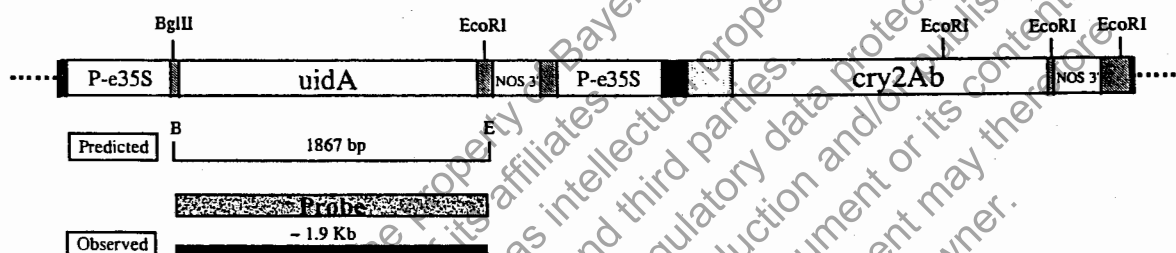
Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Bam*HI. The blot was probed with  $^{32}$ P-labeled NOS 3' polyadenylation sequence. Lane designations are as follows:

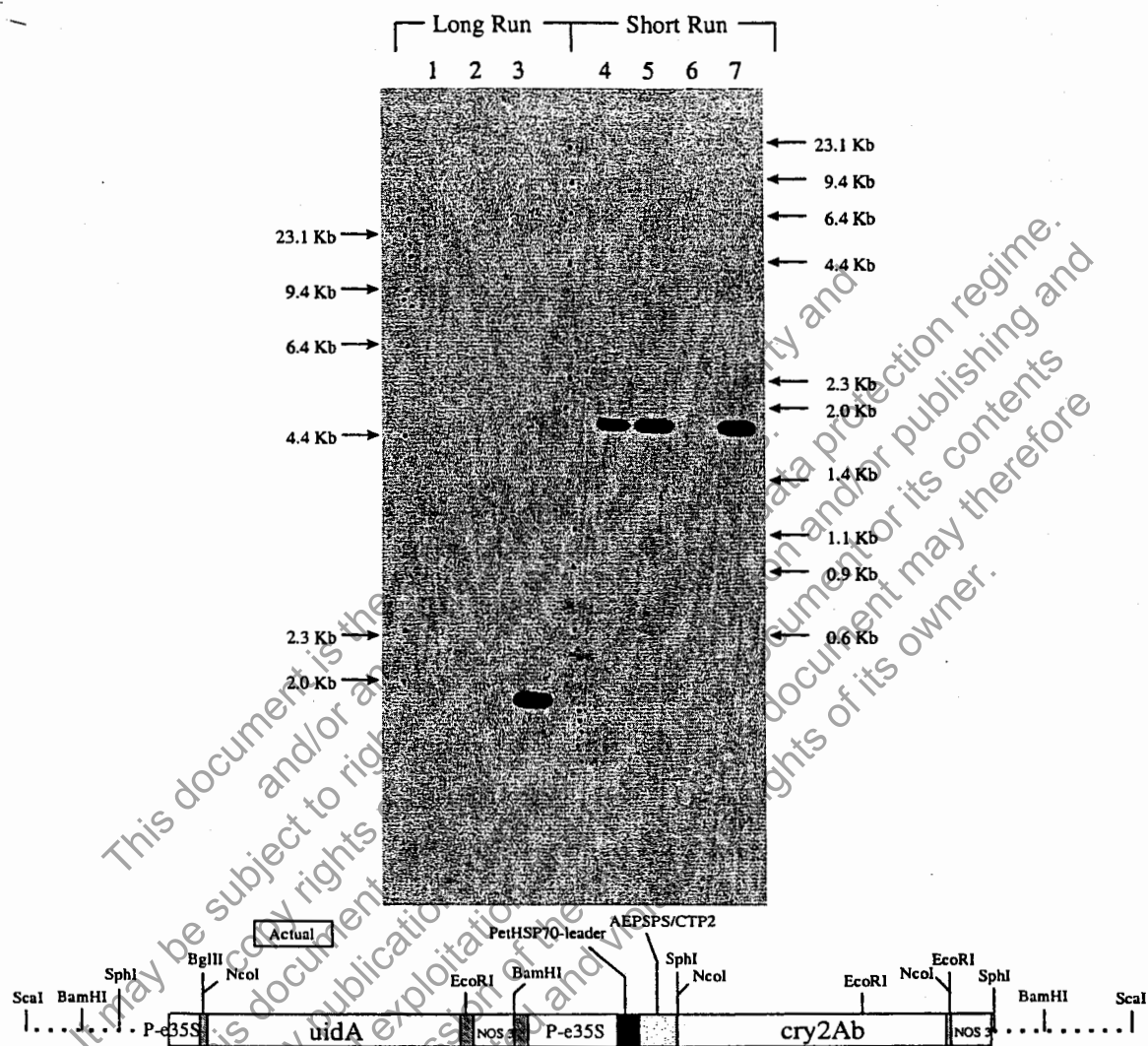
- Lane 1: DP50 (Long Run)
- Lane 2: DP50B (Long Run)
- Lane 3: 15985 (Long Run)
- Lane 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)
- Lane 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)
- Lane 6: DP50B (Short Run)
- Lane 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

## 5. Analysis for *uidA* Coding Region Intactness

Genomic DNA isolated from test and control substances and plasmid PV-GHBK11 mixed with DP50 DNA was digested with *EcoRI* and *BglII* to release the entire *uidA* coding region. The blot shown in Figure 12 was probed with the radiolabeled full-length *uidA* coding region. DP50 (lane 1) and DP50B controls (lanes 2 and 6) showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected ~1.9 Kb band which corresponds to the entire *uidA* coding region. Event 15985 DNA (lanes 3 and 7) also produced a single ~1.9 Kb band which corresponds to the expected size of an intact *uidA* coding region. This result supports that event 15985 contains an intact *uidA* coding region, with no additional bands detected.





**Figure 12. Southern Blot Analysis of Event 15985: uidA Coding Region Intactness**

Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Bgl*III and *Eco*RI. The blot was probed with <sup>32</sup>P-labeled *uidA* coding region. Lane designations are as follows:

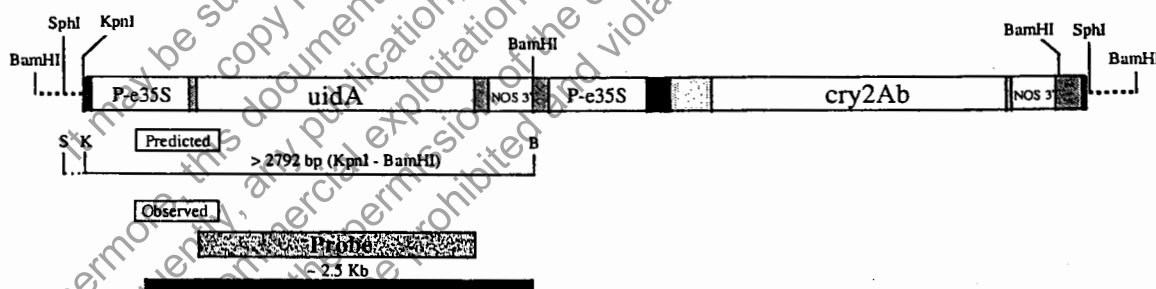
- Lane 1: DP50 (Long Run)  
 2: DP50B (Long Run)  
 3: 15985 (Long Run)  
 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)  
 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)  
 6: DP50B (Short Run)  
 7: 15985 (Short Run)

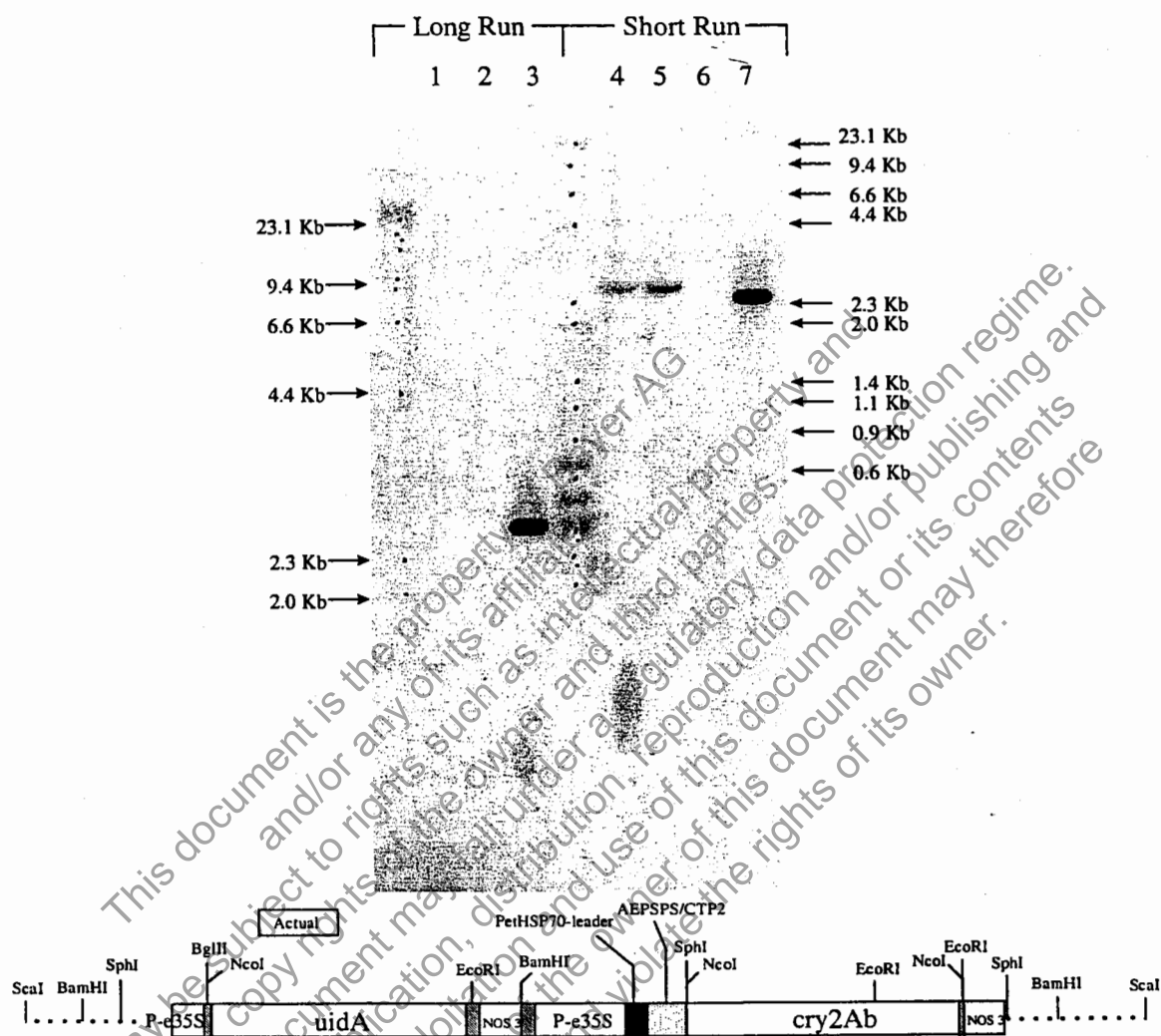
→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

## 6. Analysis For *uidA* Expression Cassette Intactness

Genomic DNA from the test and control substances was digested with *Bam*HI and *Sph*I to release the entire *uidA* cassette (containing the *uidA* coding region, enhanced CaMV 35S promoter and the NOS 3' polyadenylation sequence). Plasmid PV-GHBK11 was digested with *Pst*I and added into the DP50 short run samples (except for the NOS 3' polyadenylation sequence probe blot in which the plasmid PV-GHBK11 was digested with *Bam*HI and *Sph*I). This was done to show the size of an intact full-length *uidA* cassette.

***uidA* Coding Region Probe Results.** The blot was probed with the full length *uidA* coding region (see Figure 13). As expected, the DP50 non-transgenic (lane 1) and DP50B control (lanes 2 and 6) showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected 2.8 Kb band which corresponds to the entire *uidA* cassette. Event 15985 (lanes 3 and 7) produced a ~2.5 Kb hybridizing band. This result indicates that a portion of the *uidA* cassette is not present. The insert-to-plant junction at the 5' and of the insert, previously determined by genome walking, was verified by PCR analysis (see Figure 17). It had been demonstrated previously that 284 bp of the 5' portion of the transformation cassette were deleted. Taken together, these results establish that the *uidA* cassette is missing approximately 260 bp of the 5' promoter sequence and 24 bp of polylinker DNA derived from the multiple cloning site of the plasmid. Odell *et al.* (1985) showed that such a deletion should not affect accurate transcription initiation. No additional bands were detected with the *uidA* coding region probe.





**Figure 13. Southern Blot Analysis of Event 15985: *uidA* Cassette Intactness - *uidA***

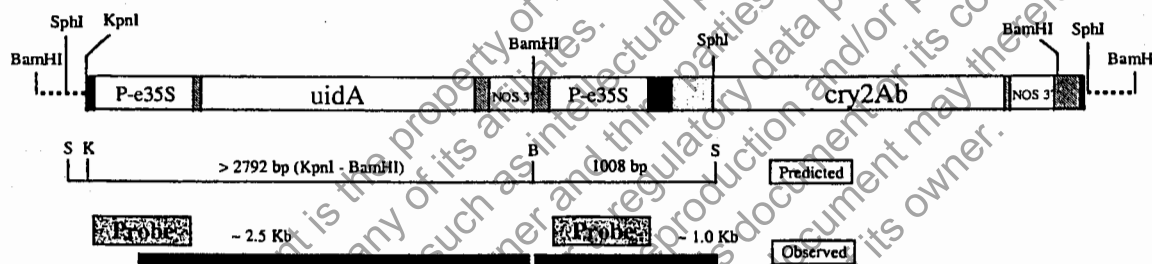
**Probe**

Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Bam*HI and *Sph*I. Plasmid DNA was digested with *Pst*I and spiked into the DP50 genomic samples prior to precipitation. The blot was probed with <sup>32</sup>P-labeled *uidA* coding region. Lane designations are as follows:

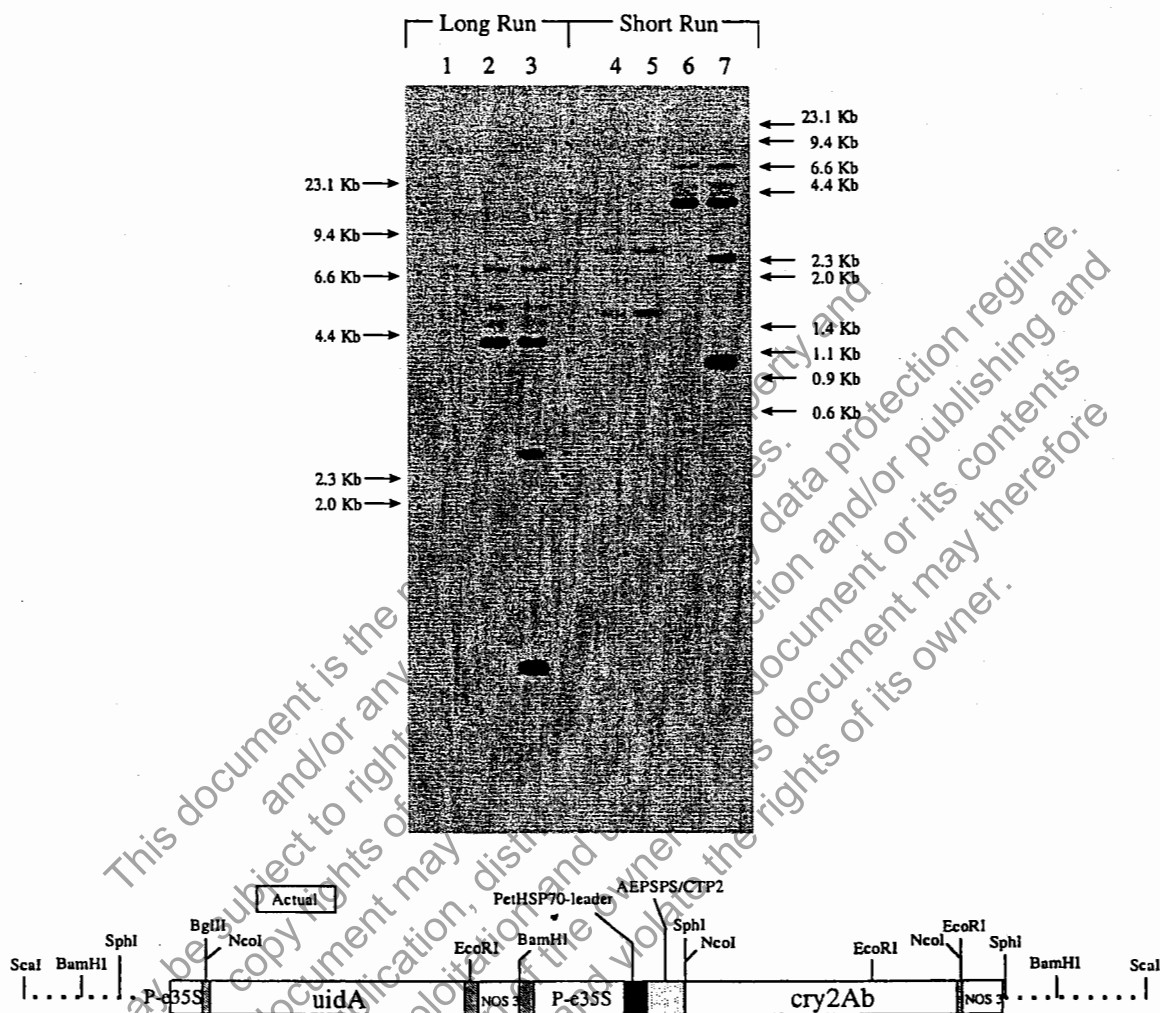
- Lane 1: DP50 (Long Run)  
 2: DP50B (Long Run)  
 3: 15985 (Long Run)  
 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)  
 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)  
 6: DP50B (Short Run)  
 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

**Enhanced CaMV 35S Promoter Probe Results.** The blot was stripped and re-probed with the full length enhanced CaMV 35S promoter (see Figure 14). DP50 (lane 1) did not hybridize to the probe. *Pst*I-digested plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected size bands at 1.5 and 2.8 Kb with no additional bands detected. DP50B (lanes 2 and 6) produced five bands at approximately 4.3, 4.6, 5.0, 6.6, and 8.5 Kb. Since these bands are present in both event 15985 and the DP50B control, they are considered background bands associated with the *cryIAC* event. Two unique bands were apparent in event 15985 (lanes 3 and 7) at approximately 2.5 and 1.0 Kb not present in DP50 or DP50B. The ~2.5 Kb band corresponds to the segment predicted for the *uidA* cassette. The ~1.0 Kb band results from the enhanced CaMV 35S promoter associated with the *cry2Ab* cassette. No extraneous bands were detected with the CaMV 35S probe.







**Figure 14. Southern Blot Analysis of Event 15985: *uidA* Cassette Intactness - Enhanced CaMV 35S Promoter Probe**

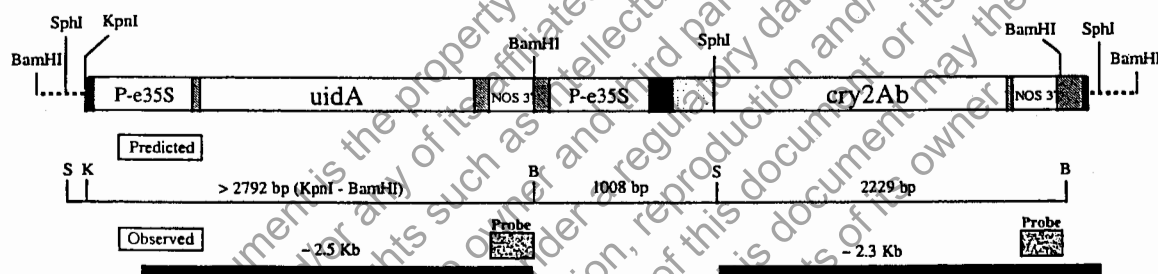
Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *Bam*HI and *Sph*I. Plasmid DNA was digested with *Pst*I and spiked into the genomic samples prior to precipitation. The blot was probed with <sup>32</sup>P-labeled enhanced CaMV 35S promoter probe. Lane designations are as follows:

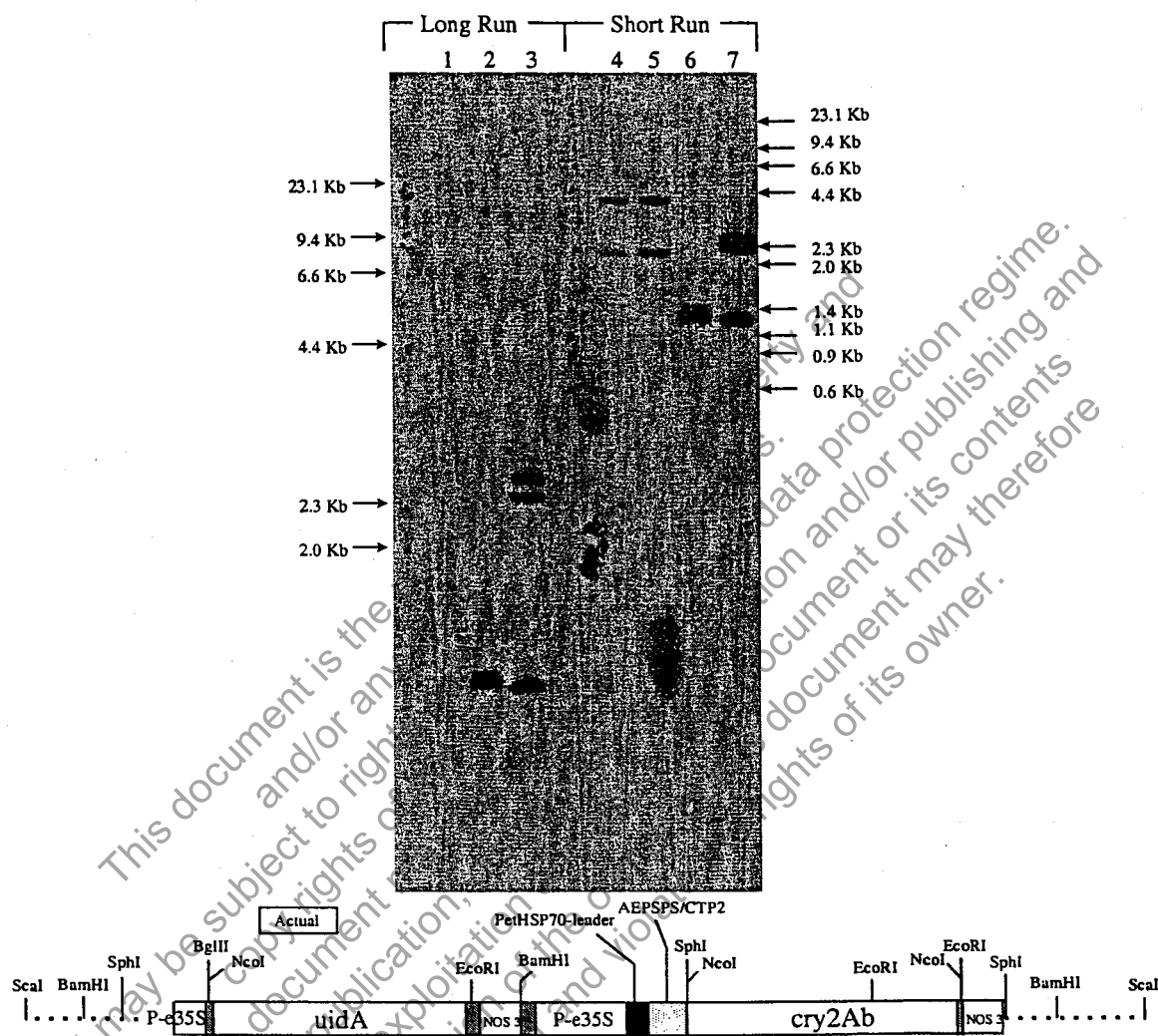
- Lane 1: DP50 (Long Run)
- Lane 2: DP50B (Long Run)
- Lane 3: 15985 (Long Run)
- Lane 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)
- Lane 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)
- Lane 6: DP50B (Short Run)
- Lane 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

**NOS 3' Polyadenylation Sequence Probe Results.** The blot was probed with the full length NOS 3' polyadenylation sequence (see Figure 15). DP50 (lane 1) did not hybridize to the probe. PstI-digested plasmid PV-GHBK11 mixed with DP50 DNA (lanes 4 and 5) produced the expected size bands at 3.8 and 2.2 Kb with no additional bands detected. DP50B (lanes 2 and 6) produced one band at approximately 1.2 Kb. This band is present in event 15985 and DP50B and is considered background associated with the *cryIAc* event. Event 15985 (lanes 3 and 7) produced two unique bands hybridizing with the probe not present in the controls at approximately 2.5 and 2.3 Kb. The ~2.5 Kb band corresponds to the predicted segment associated with the *uidA* cassette. The ~2.3 Kb band corresponds to the predicted segment associated with the *cry2Ab* cassette.

These results taken with the previous data support that the *uidA* cassette is missing a portion of the 5' end of the enhanced CaMV 35S promoter but is otherwise intact.





**Figure 15. Southern Blot Analysis of Event 15985: *uidA* Cassette Intactness - NOS Probe**

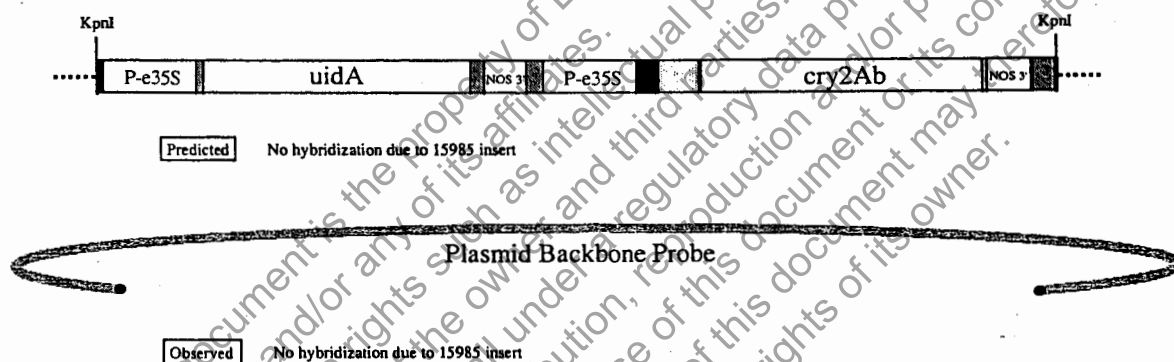
Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue (15985 and DP50B samples) and seed (DP50 sample) were digested with *Bam*HI and *Sph*I. The blot was probed with <sup>32</sup>P-labeled NOS 3' polyadenylation sequence. Lane designations are as follows:

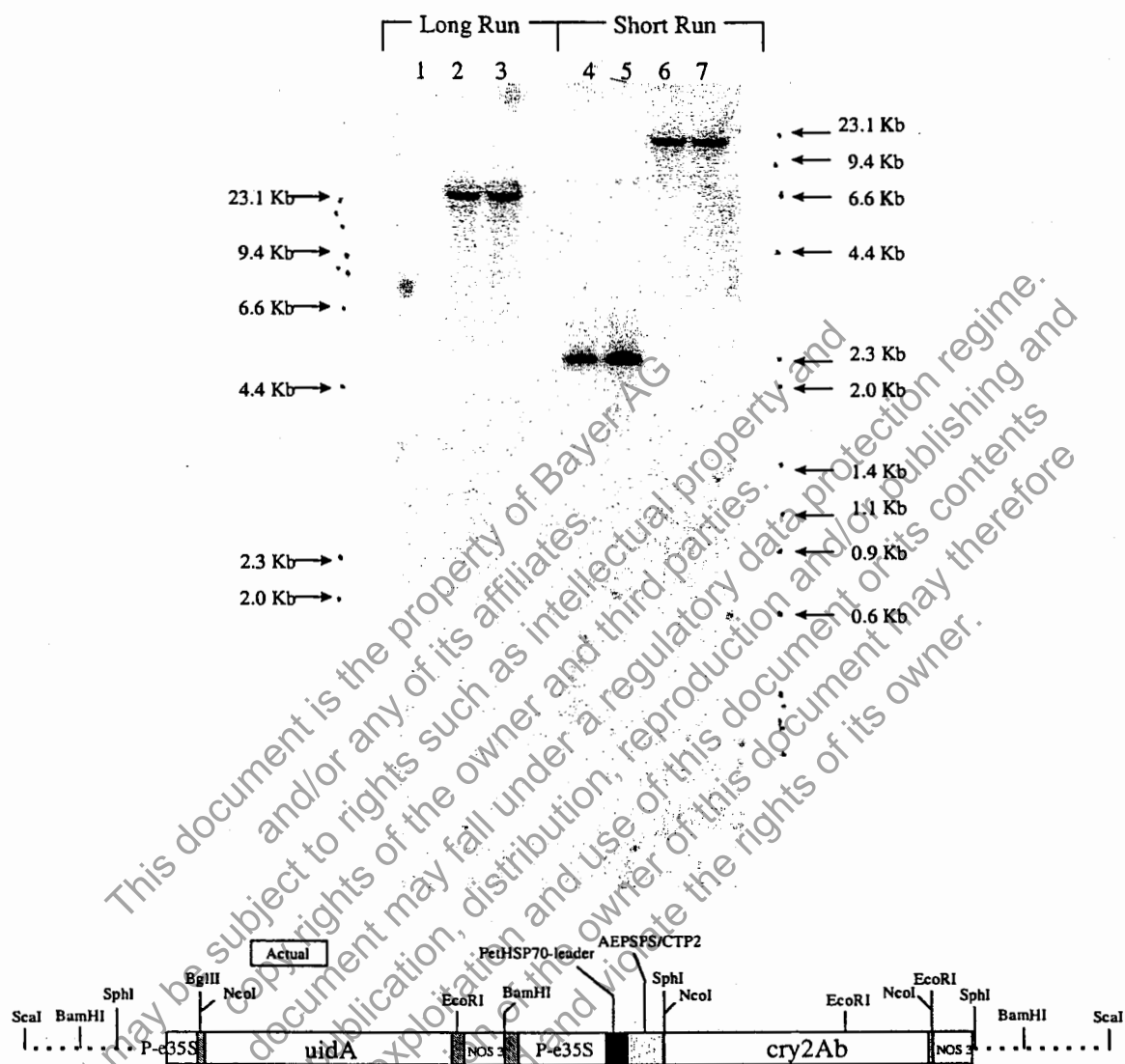
- Lane 1: DP50 (Long Run)  
 2: DP50B (Long Run)  
 3: 15985 (Long Run)  
 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)  
 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)  
 6: DP50B (Short Run)  
 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers .

## 7. Analysis for Presence of Plasmid Backbone

Genomic DNA isolated from test and control substances and plasmid PV-GHBK11 mixed with DP50 DNA was digested with *KpnI*. The blot presented in Figure 16 was probed with the entire backbone sequence (see Figure 1). DP50 (lane 1) showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 (lanes 4 and 5) produced one band at the expected size of 2.6 Kb representing the entire backbone. DP50B (lanes 2 and 6) produced a single band at approximately 22 Kb. This band is present in both event 15985 and DP50B and is considered background associated with the *cryI*Ac event. Event 15985 (lanes 3 and 7) contained the ~22 Kb background band with no additional hybridization. This result supports the conclusion that event 15985 does not contain detectable plasmid backbone sequence resulting from the *cry2Ab* transformation.





**Figure 16. Southern Blot Analysis of Event 15985: Analysis for Backbone Sequences**

Ten micrograms of DP50, DP50B and 15985 genomic DNA isolated from leaf tissue were digested with *KpnI*. The blot was probed with  $^{32}$ P-labeled full length backbone sequence. Lane designations are as follows:

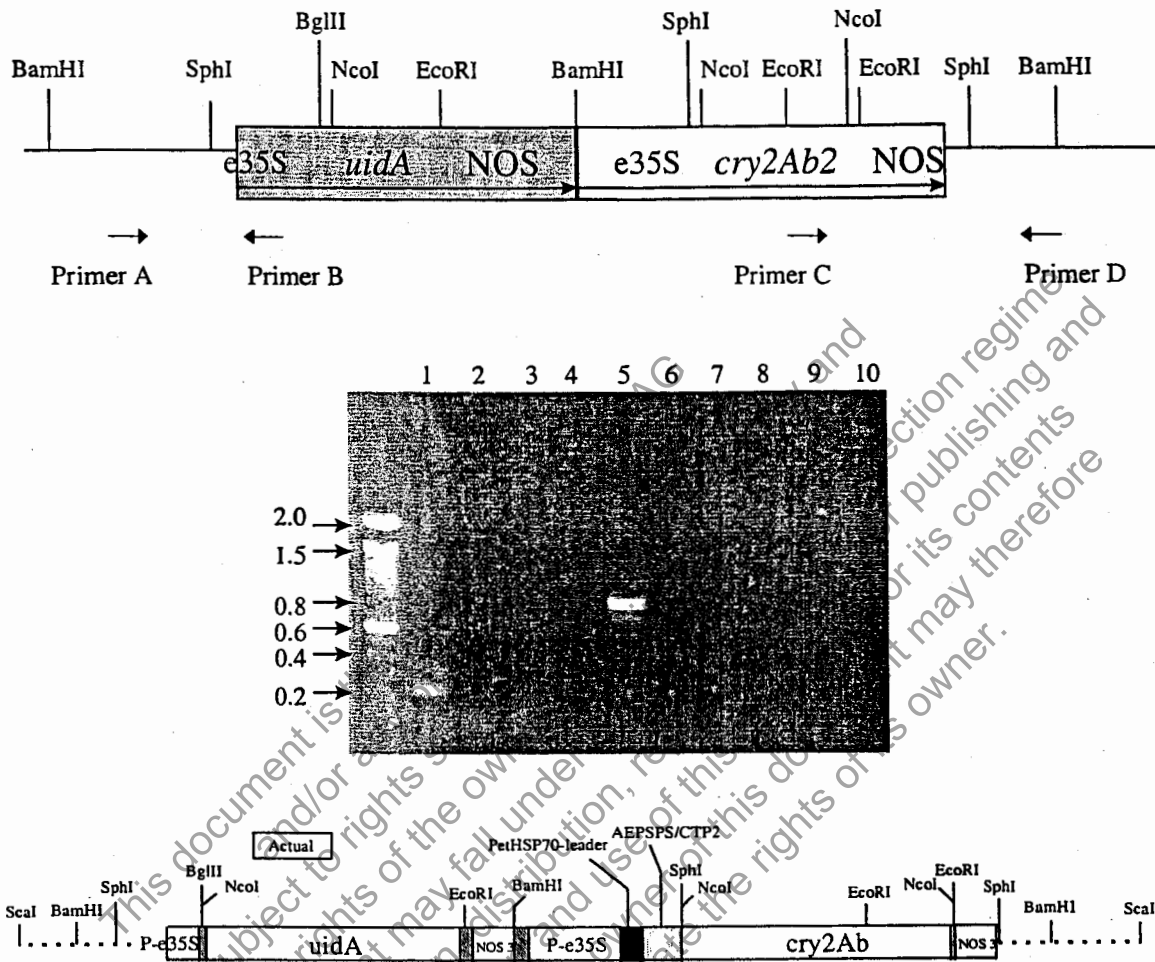
- Lane 1: DP50 (Long Run)
- Lane 2: DP50B (Long Run)
- Lane 3: 15985 (Long Run)
- Lane 4: DP50 spiked with 5.15 pg of PV-GHBK11 (Short Run)
- Lane 5: DP50 spiked with 10.3 pg of PV-GHBK11 (Short Run)
- Lane 6: DP50B (Short Run)
- Lane 7: 15985 (Short Run)

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

#### **8. Analysis of Plant DNA Sequence Flanking the Insert**

PCR was performed on genomic DNA to confirm the insert-to-plant junction sequences at the 5' and 3' ends of the Bollgard II cotton event 15985 insert (see Figure 17). As expected, the non-transgenic samples did not yield a PCR product when either the 5' or 3' primer set was used (lanes 3 and 7). The DP50B sample (*cry1Ac* control event) did not yield products with either primer pair (lanes 4 and 8), as expected. An alternate *cry2Ab*-containing cotton event, 15813, which is not the subject of this petition, also did not yield products when either primer set was used (lanes 2 and 6). Event 15985 yielded the expected size products of 230 bp at the 5' end using primers A and B (lane 1) and 869 bp for the 3' end using primers C and D (lane 5). This PCR analysis confirmed the 5' and 3' border sequences of event 15985.

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**Figure 17. PCR Confirmation of the 5' and 3' Border Sequences of the 15985 Insert**

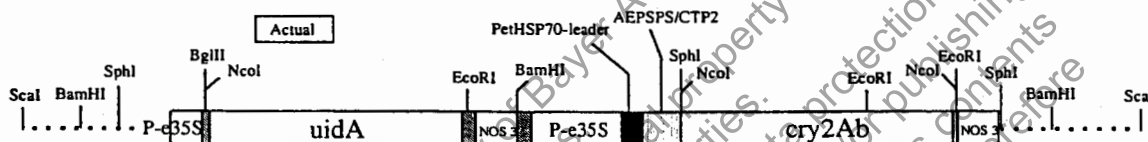
PCR was performed using primers specific to the 5' and 3' border sequences for 15985 on genomic DNA isolated from leaf tissue from DP50 (non-transgenic control), DP50B (Cry1Ac control), an alternate *cry2Ab*-containing transgenic event, 15813 which is not the subject of this petition, and cotton event 15985. DNAs were amplified with primers A and B from the 5' end of cotton event 15985 and primers C and D from the 3' end of 15985 (see below). Lane designations are as follows:

- Lane 1: 10 µl of 5' 15985 reaction product
- 2: 10 µl of 5' alternate Cry2Ab reaction product
- 3: 10 µl of 5' DP50 (non-transgenic) negative control reaction product
- 4: 10 µl of 5' DP50B (Cry1Ac) negative control reaction product
- 5: 10 µl of 3' 15985 reaction product
- 6: 10 µl of 3' alternate Cry2Ab reaction product
- 7: 10 µl of 3' DP50 (non-transgenic) negative control reaction product
- 8: 10 µl of 3' DP50B (Cry1Ac) negative control reaction product
- 9: 10 µl of 5' no template negative control reaction product
- 10: 10 µl of 3' no template negative control reaction product

→ Symbol denotes size of DNA, in kilobase pairs, obtained with MW markers.

## 9. Conclusions of Molecular Characterization of Bollgard II Cotton Event 15985

The insect-protected cotton event 15985 was produced by particle acceleration technology using a *KpnI* DNA segment of plasmid PV-GHBK11 containing the *cry2Ab* and *uidA* expression cassettes. The 15985 event contains one new DNA insert. This insert is located on a 9.3 Kb *ScaI* segment. This insert contains one complete copy of the *cry2Ab* cassette linked to one copy of the *uidA* cassette, which is missing approximately 260 bp at the 5' end of the enhanced CaMV 35S promoter. PCR was used to verify the 5' and 3' junction sequences of the insert with the plant genome. Event 15985 does not contain any detectable plasmid backbone sequence resulting from the *cry2Ab* transformation. A restriction map of the insert is shown below.



### B. Mendelian Inheritance and Insert Stability

To determine the stability of Bollgard II cotton event 15985 across generations, a series of progeny tests were conducted based on a qualitative Cry2Ab enzyme-linked immunosorbent assay (ELISA) of four generations as shown in Figure 18. The results are reported in Table 3. Statistical significance for the segregation data was determined using Chi square analysis.

All generations segregated as expected for a single insertion site. The R1 progeny of Bollgard II cotton event 15985 yielded the expected segregation ratio of 3:1 with respect to the detection of Cry2Ab protein. Progenies of event 15985 backcrossed to commercial cotton cultivars yielded the expected segregation ratio of approximately 1:1 with respect to the Cry2Ab protein. The Chi square analysis of the segregation results showed that the segregation pattern was consistent with a single active site of insertion into the genomic cotton DNA and segregates according to Mendelian genetics. These data confirm that the DNA insert in Bollgard II cotton event 15985 contains a DNA insert of a single locus that segregates according to Mendelian genetics and therefore remains stably integrated in the plant genome over selfed generations and over successive backcross generations.



Figure 18. Progeny Map of Cotton Event 15985 Generations Used for Testing.

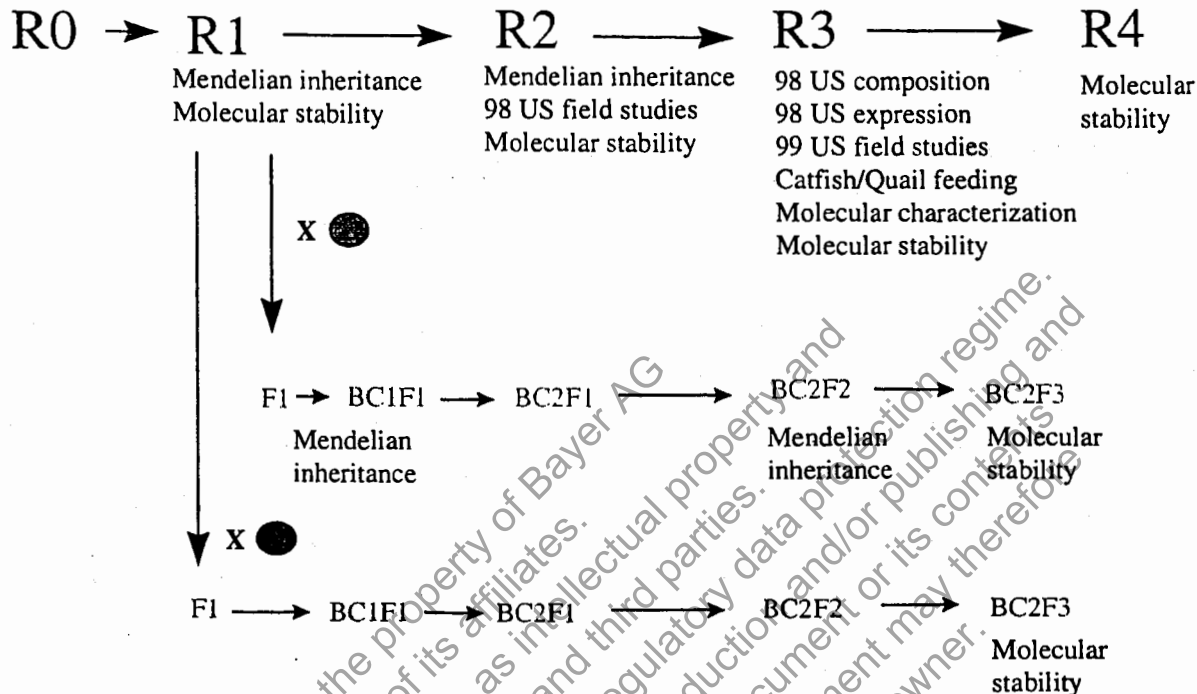


Table 3. Segregation Data and Analysis of Progeny of Bollgard II Cotton Event 15985.

Generation <sup>2</sup>	Expected		Observed <sup>1</sup>		ChiSq
	Positive	Negative	Positive	Negative	
R1 (3:1)	202.5	67.5	210	60	1.11 <sup>ns</sup>
R2 (3:1)	45	15	43	17	0.356 <sup>ns</sup>
BC1F1 (1:1)	199	199	213	185	1.970 <sup>ns</sup>
BC2F2 (3:1)	568	189	549	208	2.477 <sup>ns</sup>

1: Data expressed as number of positive and negative plants based on Cry2Ab qualitative ELISA.

2: R1 seed was from the initial R0 transformant in a DP50B background.

R2 seed was pooled from heterozygous R1 plants in a DP50B background.

BC1F1 and BC2F2 plants were pooled from five different elite cultivar backgrounds.

ns: Not significant at p=0.05 (chi square = 3.84, 1 df).

Genetic stability of cotton event 15985 was confirmed by southern blot analysis of the inserted DNA across multiple plant breeding generations as well. Genomic DNA samples from the R1, R2, R3, R4 generations and two different second-generation lines of backcrossing (BC2F3) were digested, blotted, and probed with the entire *cry2Ab* coding region to assess the stability of the inserted DNA over time and breeding generations. The restriction enzyme *SphI* was selected because it generates a unique Southern blot banding pattern fingerprint for event 15985 when probed with the *cry2Ab* coding region. The results are presented in Appendix 6, Section 2. The non-transgenic

control DNA and the parental control DNA produced no hybridization to *cry2Ab*, as expected. The data show no differences in *Cry2Ab* hybridization banding pattern among DNA extracted from any of the five plant breeding generations. This demonstrates that the DNA insert is stable in the plant genome across five plant breeding generations.

### C. Expression of the Inserted Genes

Levels of the *Cry2Ab* and GUS proteins were estimated in samples collected from eight regulatory field trial locations in 1998, which were representative of the major U.S. cotton production regions and a variety of environmental conditions. Locations in Texas and Arizona represented 'plains' type cotton culture and locations in Mississippi, South Carolina, Louisiana and Alabama were chosen for typical southern and southeastern cotton environmental conditions. Bollgard II cotton event 15985 and control lines were successfully grown and harvested under conditions typical for each region.

The trials were planted in a single block with two 15-foot row plots at Winnsboro, LA; Florence, SC; and Corpus Christi, TX; and in four replicate blocks of the same sized plots at Leland, MS; Loxley, AL; Bossier City, LA; and Maricopa, AZ. At the Starkville, MS location, the test and control events were planted in a single block in plots consisting of one 30-foot row. Sampling of the various plant tissues is described as follows:

**Young Leaf:** At each site, the first newly-expanded leaves of approximately 25 cm<sup>2</sup> size from six plants per plot were collected from each plot at 28 days after planting. Subsamples were ground on dry ice prior to analysis.

**Cottonseed:** Bulk seed cotton (2 kg) was collected from each location. The cottonseed was ginned and the cottonseed acid delinted at Monsanto research facilities in St. Louis prior to analysis. Subsamples were ground on dry ice prior to analysis.

**Overseason Leaf:** Young terminal, fully-expanded leaves were collected from six plants per plot approximately every four weeks only at the Loxley, AL and Leland, MS sites. In addition to the young leaf samples at 28 days, samples were also taken at 55, 85 and 108 days after planting. Subsamples were ground on dry ice prior to analysis.

**Whole Plant:** Four whole plants, including the leaves, roots, stem but not bolls, were collected from the test and control events at the Loxley, AL and Leland, MS sites just prior to application of the defoliant. Whole plants were cut into pieces of 2-3 inches. Subsamples were ground on dry ice prior to analysis.

**Pollen:** Samples of pollen were collected only at the Loxley, AL and Leland, MS sites. Pollen was collected from approximately 80 plants into a labeled graduated tube and pooled across replicates at each site to obtain sufficient material for analysis.

Samples collected from event 15985 and the parental control line, DP50B, were received in good condition and stored under conditions to preserve the integrity of the sample. Samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) to

estimate the protein levels present. A description of the methods employed and the descriptive features of the ELISAs developed to measure the Cry2Ab and GUS protein levels in the various tissues are summarized in Appendix 5, Section 4, along with information relating to the assay validations. Levels of the Cry2Ab and GUS proteins were measured in the newly expanded leaf tissue and cottonseed. Additionally, Cry2Ab protein levels were measured in leaves collected throughout the growing season, whole plants, and pollen due to the bioactivity of this protein.

Results of the analyses show the levels of Cry2Ab and GUS protein expressed by Bollgard cotton event 15985 comprise an extremely small percentage of the total fresh weight of leaf and seed tissue from each of the field sites (Tables 4-10). No Cry2Ab or GUS proteins were detected in any of the control tissues.

### **1. Cry2Ab Protein Production**

Levels of the Cry2Ab protein were measured in newly-expanded leaf tissue, leaves collected throughout the growing season, whole plants, pollen, and cottonseed using validated ELISA. Cry2Ab protein in cotton event 15985 was detected at low levels in various plant tissues at a number of times throughout the growing season (Tables 4-8). The levels of Cry2Ab protein in young leaves was consistent across all plots and field locations, with a range from 10.1 to 33.3  $\mu\text{g/g}$  fwt, and a mean across all locations of  $23.8 \pm 6.3$   $\mu\text{g/g}$  fwt (Table 4). The mean levels and ranges of Cry2Ab protein in leaf tissue for each location are summarized in Table 5. The mean level of Cry2Ab protein production in leaf samples peaked at 55 days after planting and subsequently declined over the growing season to a mean of 16.7  $\mu\text{g/g}$  fwt at 108 days after planting (Table 6). No Cry2Ab protein was detected in the control line DP50B or the nontransgenic control DP50 at any location (LOQ = 2.5  $\mu\text{g/g}$  fwt).

Levels of Cry2Ab protein in cottonseed tissue were also consistent across all locations, ranging from 31.8 to 50.7  $\mu\text{g/g}$  fresh weight, with a mean of  $43.2 \pm 5.7$   $\mu\text{g/g}$  (Table 4). No Cry2Ab protein was detected in the control line DP50B or the nontransgenic control DP50. The mean levels and ranges of Cry2Ab protein in cottonseed for the two locations where samples were taken are summarized in Table 7.

In whole plant tissues, the mean levels of Cry2Ab protein were  $8.80 \pm 1.20$   $\mu\text{g/g}$  fwt, with range across locations of 7.28 - 10.46  $\mu\text{g/g}$  (Table 4). No Cry2Ab protein was detected in the control line DP50B or the nontransgenic control DP50. The mean levels and ranges of Cry2Ab protein in whole plant tissue for each location are summarized in Table 8.

In pollen, the Cry2Ab protein was not detected above the limit of detection for the assay (0.25  $\mu\text{g/g}$ ) at either location in either the test or control samples.

**Table 4. Levels of Cry2Ab and GUS Protein in Leaf and Seed Samples Collected in the 1998 Field Season.**

Mean Protein Levels ( $\mu\text{g} / \text{g fwt}$ )<sup>1</sup>  $\pm$  Standard Deviation<sup>2</sup>  
(Range)<sup>3</sup>

	Leaf	Seed	Whole Plant	Pollen
<b>Cry2Ab<sup>4</sup></b>				
15985	23.8 $\pm$ 6.3 (10.1-33.3)	43.2 $\pm$ 5.7 (31.8-50.7)	8.80 $\pm$ 1.20 (7.3-10.5)	<0.25
DP50B	<2.65	<2.31	<1.24	<0.25
DP50	<2.65	<2.31	<1.24	<0.25
<b>GUS<sup>5</sup></b>				
15985	106 $\pm$ 32 (51.7-176)	58.8 $\pm$ 13.0 (37.2-82.3)	NA	NA
DP50B	<0.91	<4.42	NA	NA
DP50	<0.91	<4.42	NA	NA

NA = Not Analyzed

- 1: Protein levels are reported as microgram of protein per gram fresh weight of tissue and have been corrected for overall assay bias.
- 2: The mean and standard deviation were calculated from the analyses of plant samples, one from each of eight field sites except for tissues collected from single site.
- 3: Minimum and maximum values from the analyses of samples across sites.
- 4: The Limit of Detection for the Cry2Ab assay is 2.65  $\mu\text{g}/\text{g}$  in leaf tissue and 2.31  $\mu\text{g}/\text{g}$  in seed tissue. The Limit of Quantification for the Cry2Ab assay is 1.24  $\mu\text{g}/\text{g}$  in whole plant tissue and 0.25  $\mu\text{g}/\text{g}$  in pollen tissue.
- 5: The Limit of Detection for the GUS assay is 0.91  $\mu\text{g}/\text{g}$  in leaf tissue and 4.42  $\mu\text{g}/\text{g}$  in seed tissue.

**Table 5. Levels of Cry2Ab Protein in Leaf Samples from Cotton Event 15985 at Each Location in the 1998 Field Season.**

Site	Mean Cry2Ab ( $\mu\text{g/g}$ fwt)	% CV	Range ( $\mu\text{g/g}$ fwt)	Standard Deviation
Winnsboro, LA <sup>1</sup>	20.2	N.A.	N.A.	N.A.
Florence, SC <sup>1</sup>	14.0	N.A.	N.A.	N.A.
Corpus Christi, TX <sup>1</sup>	33.3	N.A.	N.A.	N.A.
Leland, MS <sup>2</sup>	15.9	19.7	12.4 - 20.0	3.1
Loxley, AL <sup>3</sup>	21.0	23.4	15.5 - 24.9	4.9
Bossier City, LA <sup>2</sup>	14.8	14.2	12.2 - 16.7	2.1
Maricopa, AZ <sup>2</sup>	10.7	5.7	10.1 - 11.3	0.6
Starkville, MS <sup>1</sup>	27.3	N.A.	N.A.	N.A.

1: Percent CV, range or standard deviation are not reported since there was only one plot.

2: The %CV, range and standard deviation for this site are from four replicate plots.

3: The %CV, range and standard deviation for this site are from three replicate plots.

**Table 6. Levels of Cry2Ab Protein in Leaf Samples from Cotton Event 15985 Collected Over the 1998 Field Season.**

**Mean Cry2Ab Protein Levels ( $\mu\text{g/g}$  fwt)<sup>1</sup>  $\pm$  Std Dev.<sup>2</sup>  
(Range)<sup>3</sup>**

	28 DAP	55 DAP	85 DAP	108 DPA
15985	21.0 $\pm$ 4.9 (15.5-24.9)	40.1 $\pm$ 6.5 (34.6-49.4)	19.7 $\pm$ 2.7 (15.9-21.8)	16.7 $\pm$ 0.6 (15.8-17.3)
DP50B	<2.65	<2.65	<2.65	<2.65
DP50	<2.65	<2.65	<2.65	<2.65

1: Protein levels are reported as microgram of protein per gram fresh weight of tissue and corrected for overall assay bias. The value was estimated from the analyses of four samples from the Loxley, AL site. The Limit of Detection for the Cry2Ab assay is 2.65  $\mu\text{g/g}$  in leaf tissue.

2: The mean and standard deviation were calculated from the analyses of plant samples, one from each of eight field sites, except for tissues collected from single site.

3: Minimum and maximum values from the analyses of samples across all eight sites.

**Table 7. Levels of Cry2Ab Protein in Seed Samples from Cotton Event 15985 at Each Location in the 1998 Field Season.**

Site	Mean Cry2Ab ( $\mu\text{g/g}$ fwt)	% CV	Range ( $\mu\text{g/g}$ fwt)	Standard Deviation
Winnsboro, LA <sup>1</sup>	46.7	N.A.	N.A.	N.A.
Florence, SC <sup>1</sup>	34.3	N.A.	N.A.	N.A.
Corpus Christi, TX <sup>1</sup>	48.9	N.A.	N.A.	N.A.
Leland, MS <sup>2</sup>	41.6	8.7	37.3 - 46.2	3.6
Loxley, AL <sup>2</sup>	42.6	20.0	31.8 - 50.5	8.5
Bossier City, LA <sup>2</sup>	42.3	11.2	36.7 - 47.9	4.7
Maricopa, AZ <sup>2</sup>	47.4	9.7	40.7 - 50.7	4.6
Starkville, MS <sup>1</sup>	39.3	N.A.	N.A.	N.A.

1: Percent CV, range or standard deviation are not reported since there was only one plot.

2: The %CV, range and standard deviation for this site are from four replicate plots.

**Table 8. Levels of Cry2Ab Protein in Whole Plant Samples from Cotton Event 15985 at Each Location Sampled in the 1998 Field Season.**

Site	Cotton Event or Line	Mean Cry2Ab ( $\mu\text{g/g}$ fwt)	% CV	Range ( $\mu\text{g/g}$ fwt)	Standard Deviation
Leland, MS <sup>1</sup>	15985	8.89	14.8	7.27 - 10.5	1.31
	DP50B	< 1.24	N.A. <sup>2</sup>	< 1.24	N.A. <sup>2</sup>
	DP50	< 1.24	N.A. <sup>2</sup>	< 1.24	N.A. <sup>2</sup>
Loxley, AL <sup>1</sup>	15985	8.72	14.5	7.31 - 9.87	1.27
	DP50B	< 1.24	N.A. <sup>2</sup>	< 1.24	N.A. <sup>2</sup>
	DP50	< 1.24	N.A. <sup>2</sup>	< 1.24	N.A. <sup>2</sup>

1: The %CV, range and standard deviation for this site are from four replicate plots.

2: Percent CV or standard deviation is not reported since levels were below the limit of detection.

## 2. GUS Protein Production

Levels of the GUS protein were measured in newly expanded leaf and cottonseed using validated ELISA. GUS protein in cotton event 15985 was detected at low levels in these plant tissues (Table 4). The levels of GUS protein production in young leaves ranged from 51.7 to 176  $\mu\text{g/g}$  fwt, with a mean across all locations of  $106 \pm 32$   $\mu\text{g/g}$  (Table 4). The mean levels and ranges of GUS protein in leaf tissue for each location are summarized in Table 9. No GUS protein was detected in the control line DP50B or the nontransgenic control DP50 at any location.

Levels of GUS protein in cottonseed tissue ranged from 37.2 to 82.3  $\mu\text{g/g}$  fresh weight, with a mean of  $58.8 \pm 13.0$   $\mu\text{g/g}$  (Table 4). The mean levels and ranges of GUS protein in leaf tissue for each location are summarized in Table 10. No GUS protein was detected in the control line DP50B or the nontransgenic control DP50.

**Table 9. Levels of GUS Protein in Leaf Samples from Cotton Event 15985 at Each Location in the 1998 Field Season**

Site	Mean GUS ( $\mu\text{g/g}$ fwt)	% CV	Range ( $\mu\text{g/g}$ fwt)	Standard Deviation
Winnsboro, LA <sup>1</sup>	92.1	N.A.	N.A.	N.A.
Florence, SC <sup>1</sup>	101	N.A.	N.A.	N.A.
Corpus Christi, TX <sup>1</sup>	176	N.A.	N.A.	N.A.
Leland, MS <sup>2</sup>	119	12.3	101 - 135	15
Loxley, AL <sup>3</sup>	61.4	13.8	51.7 - 67.1	8.5
Bossier City, LA <sup>3</sup>	100	19.2	79.5 - 126	19
Maricopa, AZ <sup>2</sup>	103	10.5	92.0 - 116	11
Starkville, MS <sup>1</sup>	168	N.A.	N.A.	N.A.

1: Percent CV, range or standard deviation are not reported since there was only one plot from this site.

2: The %CV, range and standard deviation for this site are from four replicate plots.

3: The %CV, range and standard deviation for this site are from three replicate plots.

**Table 10. Levels of GUS Protein in Seed Samples from Cotton Event 15985 at Each Location in the 1998 Field Season.**

Site	Mean GUS ( $\mu\text{g/g}$ fwt)	% CV	Range ( $\mu\text{g/g}$ fwt)	Standard Deviation
Winnsboro, LA <sup>1</sup>	50.6	N.A.	N.A.	N.A.
Florence, SC <sup>1</sup>	46.5	N.A.	N.A.	N.A.
Corpus Christi, TX <sup>1</sup>	71.3	N.A.	N.A.	N.A.
Leland, MS <sup>2</sup>	64.6	13.8	58.0 - 77.7	8.9
Loxley, AL <sup>2</sup>	51.8	19.8	37.2 - 60.6	10.3
Bossier City, LA <sup>2</sup>	54.5	23.7	44.2 - 73.4	12.9
Maricopa, AZ <sup>2</sup>	71.0	16.2	59.2 - 82.3	11.5
Starkville, MS <sup>1</sup>	39.6	N.A.	N.A.	N.A.

- 1: Percent CV, range or standard deviation are not reported since there was only one plot from this site.  
2: The %CV, range and standard deviation for this site are from four replicate plots.  
3: The %CV, range and standard deviation for this site are from three replicate plots

In summary, the levels of the Cry2Ab and GUS proteins expressed in tissues from Bollgard II cotton event 15985 are low. The control samples from all tissues were below the limit of detection for both Cry2Ab and GUS proteins, as expected due to the absence of the genetic insert.

#### **D. Disease, Pest and Agronomic Characteristics**

Bollgard II cotton event 15985, transformed with the linear fragment of plasmid vector PV-GHBK11, has been tested in over 250 field trials in the United States, Puerto Rico, Argentina, South Africa, Costa Rica and Australia since 1998. Table 11 lists the approved U.S. field release notifications for cotton event 15985. Field trials in the United States were completed at eight locations in 1998 (number of locations limited by seed availability) and 90 locations in 1999 to assess the agronomic performance and insect efficacy of cotton event 15985. The 1998 field trials were conducted under USDA notifications and the 1999 and 2000 field trials were conducted under both USDA notifications and EPA Experimental Use Permit 524-EUP-89.

Trials were completed in every state where cotton is a major crop. Quantitative agronomics assessments were conducted at eight locations in 1998 as described earlier (Section V.C.). Both qualitative and quantitative assessments of agronomic performance



were made through cooperation with academics, crop consultants and in state variety trials.

In 1999, most field locations involved randomized complete block arrangements of four rows from 30-60 feet in length. Both qualitative and quantitative assessments of agronomic performance were made through cooperation with academics, crop consultants and in state variety trials. Detailed monitoring for growth and development characteristics and disease incidence of this new cotton event versus control cotton plants was performed at least monthly during the growing season and in some cases more frequently. Monitoring was done on a weekly basis from the onset of lepidopteran larvae infestations. Damage ratings were based upon inspection of ten random plants per center row (20 plants per plot) from each test plot at identified periods of infestation in the non-transgenic check plots (DP50). Evaluations included egg and larvae counts, as well as terminal, square and boll damage. Plots were also harvested for seedcotton yield either by hand-picking a minimum of 15 feet of row from the two center rows of each plot or by machine harvesting. The observations were obtained from a wide variety of individuals familiar with cotton agronomics, including cotton breeders, agronomists, academics, crop consultants, state variety trials officials, private growers, entomologists, field cooperators, and Monsanto field researchers. The quantitative and qualitative observations collected in these trials were typical of those taken routinely to detect the presence and magnitude of a disease or insect infestation and to assess varietal performance in cotton. The USDA field reports for the trials conducted in 1998 and 1999 have been submitted to the Agency; field reports for the 2000 trials will be submitted following analysis of the data.

**Table 11. Notifications for Field Testing of Bollgard II Cotton Event 15985.**

USDA #	Sites Approved	Counties	Report Status
98-084-22n	6	AL: Baldwin AR: Crittenden LA: Franklin MS: Oktibbeha, Washington SC: Florence	Complete
98-084-23n	1	TX: Nueces	Complete
98-085-19n	1	AZ: Pinal	Complete
99-057-05n	4	AZ: Graham, Pima, Pinal, Yuma	Complete
99-061-11n	1	TX: San Patricio	Complete
99-061-12n	6	TX: Fort Bend, Hidalgo, Nueces, San Patricio, Willacy	Complete
99-061-13n	1	CA: Fresno	Complete
99-061-14n	2	TX: Austin, Fort Bend	Complete
99-061-15n	2	TX: Ellis	Complete
99-071-15n	1	AZ: Pinal	Complete
99-095-19n	3	AZ: Pinal MS: Bolivar	Complete
99-102-18n	28	LA: Bossier, Franklin, Morehouse, Rapides, Tensas MS: Bolivar, Coahoma, Grenada, Holmes, Leflore, Oktibbeha, Rankin, Senatobia, Sharkey, Washington OK: Jackson VA: City of Suffolk	Complete
99-102-19n	1	LA: Bossier	Complete

USDA # (cont'd)	Sites Approved	Counties	Report Status
99-102-20n	5	AL: Baldwin TX: San Patricio AZ: Pinal MS: Washington SC: Florence	Complete
99-102-21n	25	NC: Bertie, Edgecombe, Onslow, Perquimans, Sampson, Washington SC: Barnwell, Darlington, Florence, Hampton, Lee, Marlboro TN: Fayette, Hardeman, Madison, Shelby TX: Hale, Lubbock, Pecos, Tom Green	Complete
99-102-22n	14	AL: Autauga, Baldwin, Lee, Limestone, Macon AR: Desha, Jackson, Jefferson AZ: Pinal CA: Fresno	Complete
99-102-23n	16	FL: Jackson, Santa Rosa GA: Burke, Decatur, Macon, Mitchell, Pulaski, Seminole, Sumter, Terrell, Tift MO: Pemiscot MS: Washington	Complete
99-110-19n	10	AR: Mississippi, Monroe, Poinsett GA: Burke NC: Edgecombe, Johnston, Martin, Washington TN: Gibson, Madison	Complete
99-110-22n	1	MS: Washington	Complete
99-110-24n	1	MS: Washington	Complete
99-110-23n	1	AL: Baldwin	Complete
99-252-07n	1	PR: Yauco	Complete
00-040-02n	33	TX: Bexar, Brazos, Burleson, Dallas, Dawson, Deaf Smith, Ellis, Fort Bend, Gaines, Glasscock, Hale, Haskell, Hidalgo, Hill, Jackson, McLennan, Nueces, Pecos, Refugio, Robertson, San Patricio, Tom Green, Uvalde, Wharton, Willacy, Williamson	In Progress
00-041-05n	14	AZ: Graham, Pinal, Yuma	In Progress
00-046-06n	13	AL: Auburn, Autauga, Baldwin, Henry, Lawrence, Lee, Limestone, Macon	In Progress
00-046-07n	10	AR: Crittenden, Desha, Drew, Jefferson, Lincoln, Lonoke, Marion, Mississippi, Poinsett	In Progress
00-046-08n	3	CA: Fresno, Imperial, Kern	In Progress
00-047-01n	19	GA: Burke, Colquitt, Decatur, Macon, Mitchell, Pulaski, Seminole, Sumpter, Terrell, Tift	In Progress
00-047-02n	12	LA: Bossier, Catahoula, Concordia, East Carroll, Franklin, Morehouse, Point Coupee, Rapides, Tensas	In Progress
00-055-04n	1	FL: Jackson	In Progress
00-059-04n	2	OK: Jackson	In Progress
00-060-02n	9	NC: Edgecombe, Hoke, Johnston, Martin, Washington, Wilson	In Progress
00-062-02n	25	MS: Bolivar, Coahoma, Grenada, Hinds, Leflore, Noxubbe, Oktibbeha, Rankin, Sharkey, Tallahatchie, Tate, Washington	In Progress
00-063-14n	23	SC: Aiken, Bamberg, Barnwell, Calhoun, Darlington, Florence, Hampton, Lee, Lexington, Marlboro, Orangeburg, Saluda	In Progress

USDA # (cont'd)	Sites Approved	Counties	Report Status
00-063-15n	5	AR: Jackson MO: Pemiscot TN: Madison VA: City of Suffolk	In Progress
00-063-17n	7	TN: Dyer, Fayette, Hardeman, Lauderdale, Obion	In Progress
00-146-05n	2	PR: Guerrero, Montanyas	Not Planted
00-146-06n	2	PR: Guerrero, Montanyas	Not Planted or Destroyed

**Diseases:** Disease symptoms were generally scouted once per month during the growing season at each location. Plots were visually inspected for the appearance of possible disease symptoms such as damping off, boll rot, spotted leaves, leaf necrosis, stunted or distorted plants or wilting. These symptoms are indicative of, but not limited to *Rhizoctonia solani*, *Fusarium spp.*, *Xanthomonas campestris*, *Thielaviopsis basicola*, *Phomopsis malvacearum*, and *Pythium spp.* Observations for diseases were made over 490 times throughout the two field seasons, with approximately 13% of the field locations documenting symptoms of disease. Symptoms indicative of *Rhizoctonia solani* were the most commonly observed. No differences between cotton event 15985 and the DP50B control were observed in the incidence or severity of disease symptoms. Details of the observations are located in the field reports in Appendix 5 and a summary is given below in Table 12.

**Table 12. Evaluation of Disease and Insect Susceptibility of Cotton Event 15985.**

USDA #	State	County	# Obs Made for Disease	Disease Differences Noted	# Obs Made for Insects	Insect Differences Noted
98-084-22N	AL	BALDWIN	4	no	4	no
98-084-22N	LA	FRANKLIN	1	no	1	no
98-084-22N	MS	OKIBBEHA	3	no	3	no
98-084-22N	MS	WASHINGTON	5	no	5	no
98-084-22N	SC	FLORENCE	4	no	4	no
98-084-23N	TX	NUECES	4	no	4	no
98-085-19N	AZ	PINAL	4	no	4	no
99-057-05N	AZ	GRAHAM	5	no	5	no
99-057-05N	AZ	PIMA	8	no	8	no
99-057-05N	AZ	PINAL	5	no	5	yes <sup>1</sup>
99-057-05N	AZ	YUMA	8	no	8	no
99-061-11N	TX	SAN PATRICIO	5	no	5	yes <sup>1</sup>
99-061-12N	TX	HIDALGO	3	no	3	yes <sup>1</sup>
99-061-12N	TX	WILLACY	4	no	4	no
99-061-13N	CA	FRESNO	5	no	5	yes <sup>1</sup>
99-061-14N	TX	AUSTIN	4	no	4	yes <sup>1</sup>

USDA # (cont'd)	State	County	# Obs Made for Disease	Disease Differences Noted	# Obs Made for Insects	Insect Differences Noted
99-061-14N	TX	FORT BEND	4	yes <sup>2</sup>	4	yes <sup>1</sup>
99-061-15N	TX	ELLIS	4	no	4	no
99-061-15N	TX	ELLIS	4	no	4	no
99-071-15N	AZ	PINAL	5	no	5	no
99-095-19N	AZ	PINAL	4	no	4	yes <sup>3</sup>
99-095-19N	AZ	PIMA	6	no	6	no
99-095-19N	MS	BOLIVAR	4	no	4	yes <sup>1</sup>
99-102-18N	LA	BOSSIER	2	no	9	yes <sup>1</sup>
99-102-18N	LA	BOSSIER	4	no	4	no
99-102-18N	LA	FRANKLIN	4	no	4	yes <sup>1</sup>
99-102-18N	LA	FRANKLIN	6	no	6	no
99-102-18N	LA	MOREHOUSE	5	no	5	no
99-102-18N	LA	RAPIDES	4	no	4	no
99-102-18N	LA	TENSAS	6	no	6	yes <sup>1</sup>
99-102-18N	MS	BOLIVAR	5	no	5	no
99-102-18N	MS	COAHOMA	6	no	6	no
99-102-18N	MS	GRENADA	4	no	4	no
99-102-18N	MS	HOLMES	4	no	4	yes <sup>1</sup>
99-102-18N	MS	LEFLORE	4	no	4	yes <sup>1</sup>
99-102-18N	MS	OKTIBBEHA	4	no	4	yes <sup>1</sup>
99-102-18N	MS	OKTIBBEHA	4	no	4	no
99-102-18N	MS	RANKIN	5	no	5	yes <sup>1</sup>
99-102-18N	MS	TATE	3	no	4	no
99-102-18N	MS	SHARKEY	6	no	6	no
99-102-18N	MS	WASHINGTON	6	no	6	no
99-102-18N	MS	BOLIVAR	5	no	5	no
99-102-18N	MS	WASHINGTON	4	no	4	no
99-102-18N	OK	JACKSON	5	no	5	no
99-102-18N	OK	JACKSON	4	no	4	no
99-102-18N	VA	CITY OF SUFFOLK	6	no	6	yes <sup>1</sup>
99-102-18N	VA	CITY OF SUFFOLK	6	no	6	yes <sup>1</sup>
99-102-19N	LA	BOSSIER	4	no	4	no
99-102-20N	SC	FLORENCE	5	no	5	yes <sup>1</sup>
99-102-20N	TX	SAN PATRICIO	7	no	7	yes <sup>1</sup>
99-102-20N	AL	BALDWIN	5	no	5	yes <sup>1</sup>
99-102-20N	AZ	PINAL	5	no	5	no

USDA # (cont'd)	State	County	# Obs Made for Disease	Disease Differences Noted	# Obs Made for Insects	Insect Differences Noted
99-102-20N	AZ	PINAL	5	no	5	no
99-102-20N	MS	WASHINGTON	6	no	6	yes <sup>1</sup>
99-102-20N	MS	WASHINGTON	5	no	5	yes <sup>1</sup>
99-102-21N	NC	PERQUIMANS	6	no	6	yes <sup>1</sup>
99-102-21N	SC	BARNWELL	5	no	5	yes <sup>1</sup>
99-102-21N	SC	BARNWELL	3	no	5	yes <sup>1</sup>
99-102-21N	SC	DARLINGTON	5	no	5	no
99-102-21N	SC	FLORENCE	4	no	4	no
99-102-21N	SC	HAMPTON	5	no	5	no
99-102-21N	SC	LEE	4	no	4	no
99-102-21N	SC	MARLBORO	5	no	5	no
99-102-21N	TN	HARDEMAN	1	no	6	yes <sup>1</sup>
99-102-21N	TN	MADISON	1	no	5	no
99-102-21N	TN	SHELBY	6	no	6	no
99-102-21N	TX	HALE	5	no	5	no
99-102-21N	TX	LUBBOCK	5	no	5	no
99-102-21N	TX	TOM GREEN	6	no	6	no
99-102-22N	AL	AUTAUGA	5	no	5	yes <sup>1</sup>
99-102-22N	AL	AUTAUGA	6	no	0	not applicable
99-102-22N	AL	BALDWIN	5	no	5	yes <sup>1</sup>
99-102-22N	AL	LEE	4	no	4	no
99-102-22N	AL	LIMESTONE	5	no	5	yes <sup>1</sup>
99-102-22N	AL	LIMESTONE	1	no	7	no
99-102-22N	AL	MACON	4	no	4	no
99-102-22N	AR	DESHA	17	no	17	yes <sup>1</sup>
99-102-22N	AR	JACKSON	4	no	4	no
99-102-22N	AR	JEFFERSON	3	no	3	yes <sup>1</sup>
99-102-23N	FL	JACKSON	4	no	4	no
99-102-23N	FL	SANTA ROSA	5	no	5	no
99-102-23N	GA	BURKE	2	no	7	yes <sup>1</sup>
99-102-23N	GA	DECATUR	4	no	4	yes <sup>1</sup>
99-102-23N	GA	DECATUR	5	no	5	yes <sup>1</sup>
99-102-23N	GA	MITCHELL	5	no	5	no
99-102-23N	GA	DODGE	4	no	4	no
99-102-23N	GA	PULASKI	4	no	4	no
99-102-23N	GA	SEMINOLE	5	no	5	yes <sup>1</sup>
99-102-23N	GA	SUMTER	10	no	10	no
99-102-23N	GA	TERRELL	6	no	6	yes <sup>1</sup>

USDA # (cont'd)	State	County	# Obs Made for Disease	Disease Differences Noted	# Obs Made for Insects	Insect Differences Noted
99-102-23N	GA	TIFT	4	no	4	no
99-102-23N	GA	TIFT	3	no	3	yes <sup>1</sup>
99-102-23N	GA	TIFT	4	no	4	no
99-102-23N	MO	PEMISCOT	5	no	5	no
99-110-19N	AR	POINSETT	5	no	5	no
99-110-19N	AR	MISSISSIPPI	1	no	19	no
99-110-19N	NC	EDGECOMBE	5	no	5	yes <sup>1</sup>
99-110-19N	AR	MONROE	5	no	5	no
99-110-19N	GA	BURKE	5	no	5	no
99-110-19N	NC	WASHINGTON	5	no	5	yes <sup>1</sup>
99-110-19N	NC	MARTIN	5	no	5	yes <sup>1</sup>
99-110-24N	MS	WASHINGTON	4	no	4	yes <sup>1</sup>
99-110-24N	MS	WASHINGTON	4	no	4	yes <sup>1</sup>
99-110-24N	MS	WASHINGTON	4	no	4	yes <sup>1</sup>
99-110-23N	AL	BALDWIN	4	no	4	yes <sup>1</sup>
99-252-07N	PR	YUACO	3	no	3	no

- 1 Efficacy on target insect, soybean looper, and/or armyworms greater in 15985 than in non-transgenic control DP50.
- 2 One 15985 cotton plant exhibited symptoms of copper top disease
- 3 Fleahoppers, lygus and whitefly more prevalent on 15985

**Insects:** Insects were observed throughout the 1998 and 1999 trials. Insect monitoring was extensive in these trials. Monitoring was done on a weekly basis from the onset of lepidopteran larvae infestations. The primary insect pests monitored were *Heliothis virescens*, *Helicoverpa zea*, *Pectinophora gossypiella*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Spodoptera ornithogolli*, *Psuedoplusia includens*, *Trichoplusia ni*, *Lygus lineolaris*, *Anthonomus grandis* and *Aphis spp.*

Qualitative observations for insects were made over 530 times throughout the two field seasons, with approximately 41% of the field locations documenting target insect differences, as expected. Other insect observations accounted for 24% of the field locations and showed no differences in thrips, aphids, stinkbugs, plant bugs, boll weevil and red spider mites, with thrips being the most commonly observed. No substantial differences in non-target infestation or severity were noted between the event 15985 and control plants at any of the sites. Details of the observations are located in the field reports in Appendix 5 and a summary is given below in Table 12.

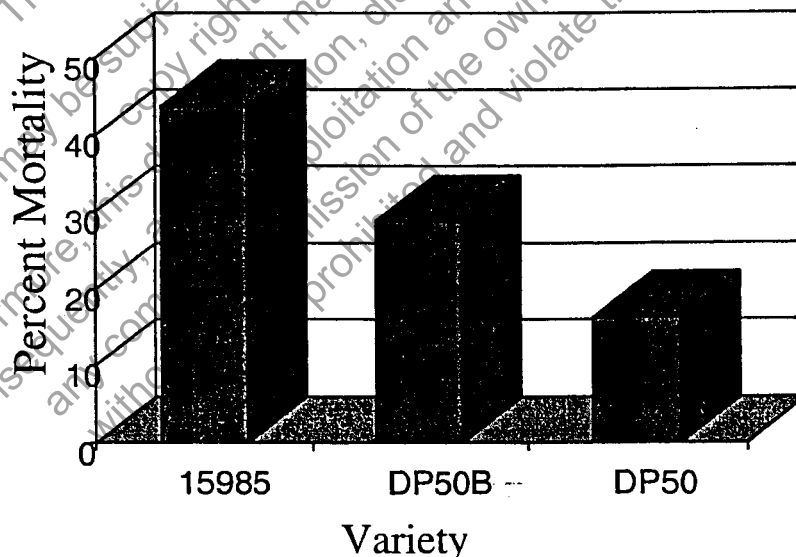
Damage rating data was generated from a combination of both natural and artificial insect infestations. Damage ratings were based upon inspection of ten random plants per center

row (20 plants per plot) from each test plot at identified periods of infestation in the non-transgenic check plots (DP50). Data collected to determine damage ratings included some or all of the following:

- eggs and/or egg masses
- number of beet armyworm (*Spodoptera exigua*) "hits"
- live larvae identified by species and the location found on the plant
- damaged terminals and the suspected species causing the damage
- estimated % defoliation and the suspected species causing the damage
- damaged squares and the suspected species causing the damage
- damaged white blooms and the suspected species causing the damage
- damaged bolls and the suspected species causing the damage

Results of research in 1998 and 1999 clearly show that event 15985 has improved efficacy relative to Bollgard cotton on the target insects: cotton bollworm, tobacco budworm and pink bollworm. Representative data generated by academic cooperators is shown in Figures 19-22 and published results are provided in Appendices 3 and 4. Laboratory results from [REDACTED] at Louisiana State University showed that leaf tissue from event 15985 had improved efficacy on cotton bollworm (Figure 19). Since Bollgard cotton leaf tissue provides control of bollworm under field conditions, these data demonstrate that event 15985 will continue to provide excellent control.

**Figure 19. Percent Mortality of Cotton Bollworm 72 Hours After Infestation on Field Generated Leaf Tissue**

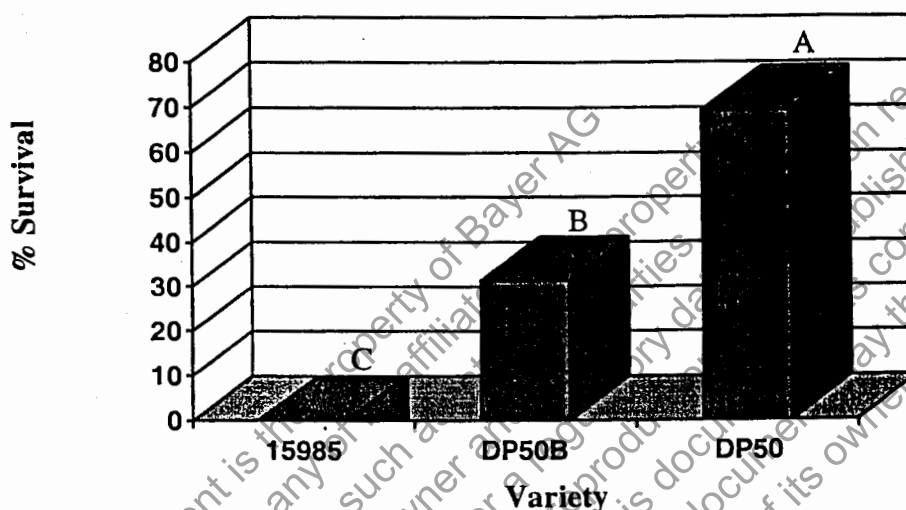


[REDACTED] Louisiana State University

Tobacco budworm is another important target pest for Bollgard cotton. Since Bollgard provides essentially complete control of this species under field conditions it is not possible to improve field efficacy. However, relative efficacy was evaluated in a 1998

laboratory study by Dr. Jenkins at the USDA lab at Mississippi State University. These results show that square tissue from cotton event 15985 provides greater efficacy against budworm than Bollgard cotton tissue (Figure 20).

**Figure 20. Percent Tobacco Budworm Survival After Six Days of Feeding on Cotton Square Tissue Samples.** Data with the same letter are not statistically different.



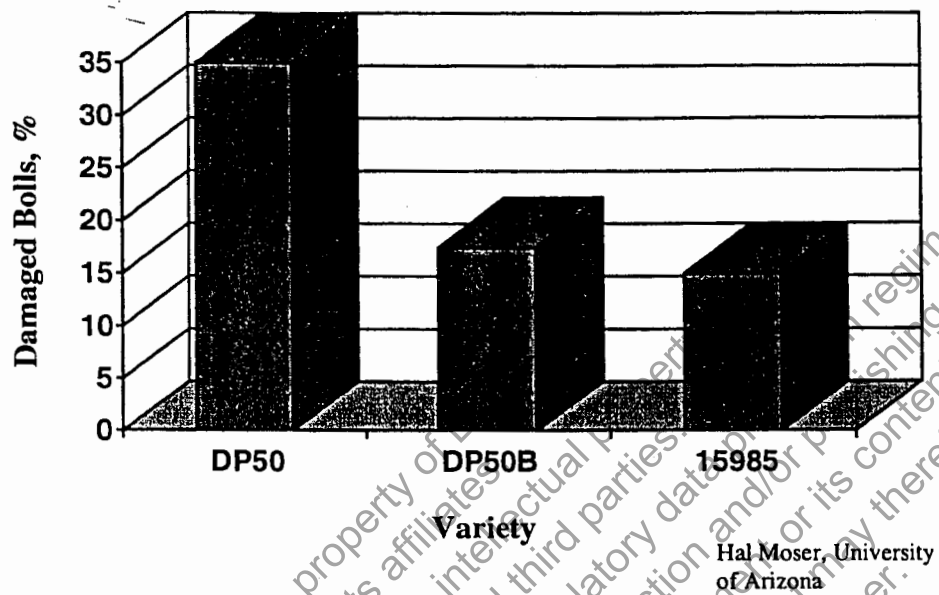
Johnie Jenkins, USDA, Mississippi State

Pink bollworm is an important economic pest in Arizona. Field trials conducted by Dr. Moser at the University of Arizona in 1999 demonstrated that Bollgard and cotton event 15985 each provide excellent protection from boll damage (Figure 21). Exit holes left by pink bollworms and percent of bolls infested with pink bollworms are reduced in event 15985 cotton, whereas insecticide sprays were only marginally effective against this insect pest on the control cotton.

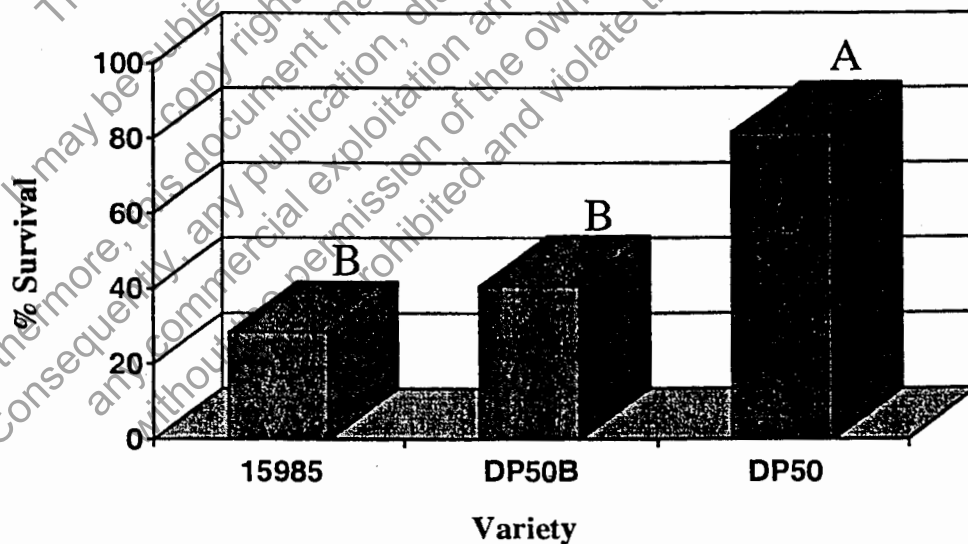
These results are representative of the data establishing the efficacy of event 15985 on the key cotton insect pests and show equivalent or improved control relative to Bollgard cotton. In addition, Bollgard II cotton event 15985 showed an increased spectrum of activity relative to the Bollgard control cotton plants by providing control of beet and fall armyworm and soybean looper, as shown by representative data provided in Figures 9 and 10. Bollgard cotton has marginal activity against these sporadic species and improved control would add value to cotton growers. Fall armyworm efficacy on leaf tissue was evaluated in a laboratory study in Louisiana in 1999. Fall armyworm survival on cotton event 15985 leaf tissue was not significantly lower than for Bollgard cotton, however the larval weights of the survivors were significantly reduced (Figure 22). Even though there was some survivorship on event 15985, the worms were not able to grow and thrive. This indicates that cotton event 15985 has greater efficacy on fall armyworm than Bollgard cotton.



**Figure 21. Percentage of Pink Bollworm Damaged Bolls in Arizona Field Trial in 1999.**



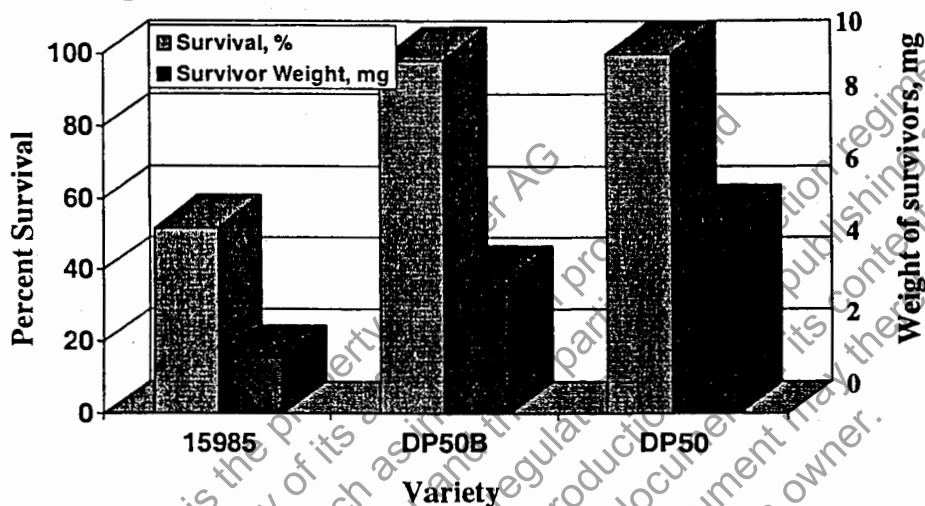
**Figure 22. Survival and Weight of Fall Armyworms Fed For Seven Days on Bollgard II Cotton Event 15985 and Control Leaf Tissue.**  
Data with the same letter are not statistically different.



Johnie Jenkins, USDA, Mississippi State

Soybean looper efficacy on leaf tissue was evaluated in a laboratory study conducted by [REDACTED] at Louisiana State University. Survival and body weights were both lower for cotton event 15985, as compared to DP50B Bollgard cotton and DP50 conventional cotton (Figure 23).

**Figure 23. Survival and Weight of Soybean Looper Fed For Seven Days on Bollgard II Cotton Event 15985 and Control Leaf Tissue.**



[REDACTED] Louisiana State University

**Agronomics:** Weather conditions were typical of those found across cotton growing regions in 1998 and 1999, with the exception of hurricane conditions in Alabama in 1998 producing significant rainfall and wind. Agronomic criteria were measured at multiple locations each year across all fifteen major cotton growing states to assure equivalence to the parental cultivar (Figures 24-25). Three types of agronomic equivalence criteria were measured, which are typical of measurements taken in traditional cotton breeding: yield, morphology and maturity, and fiber quality. Yield, and morphology and maturity determinations are typically obtained using any of a number of different observations, whereas fiber quality is typically conducted by high-volume instrument (HVI) classing alone, including measurements of fiber length, strength and micronaire.

Detailed analyses were conducted in 1998 at eight locations in six states in the regulatory trials described in Section V.C. Additionally, more than 85 agronomic and efficacy trials were conducted in 1998 and 1999 combined, throughout the 15 major cotton growing states. These trials were primarily qualitative in nature, however some aspects of the trials included quantitative data collection, such as yield. Representative quantitative data collected by academic cooperators is presented below from these trials. In addition, some of the research has been published by Mahaffey, *et al.* (2000) and a copy of the paper is attached in Appendix 1. Among all parameters measured, there were no differences observed in cotton event 15985 that were unusual for the DP50 cotton variety. These data support the conclusion that cotton event 15985 is typical of traditional cotton in terms of growth and agronomic performance.

## Yield

Yield components were measured at multiple locations to assess equivalence to parental cultivars. No statistical differences between cotton event 15985 and the DP50B control were found in lint percent (weight of lint as a percentage of lint plus seed), seed index (weight in grams of 100 seed), or boll size (average weight of bolls harvested from a given area in the plant), as shown in Figures 24 and 25. Data in Figure 24 are from 41 trials. Data in Figure 25 were collected from seven locations plant-mapped for maturity and boll set. Seedcotton samples were collected at 10 locations to determine the percent lint, seed index and boll size.

Even though there were no significant differences from the Bollgard control in fruit retention, there is a trend toward increased retention for both Bt cotton events relative to conventional DP50 cotton. This result is expected since there is greater protection from insect damage in insect-protected cotton varieties. The greater boll set for plants containing a Bt protein is likely to be a result of the full-time, in-plant protection from damage by lepidopteran insects. Conventional plants often suffer a low level of sustained damage from insects that is below the threshold for treatment with insecticide or can also sustain damage between insecticide sprays, each of which can result in reduced boll retention. This trend is reflected in the yield for both years (Figures 24 and 25), where there is a trend toward higher yields for cotton event 15985. A separate study conducted by Allen, *et al.* (2000) found no statistically significant differences in fiber yield between cotton event 15985 and the controls.

**Figure 24. Lint Yield in Pounds per Acre Averaged Across Locations in the 1998 and 1999 Field Trials.**

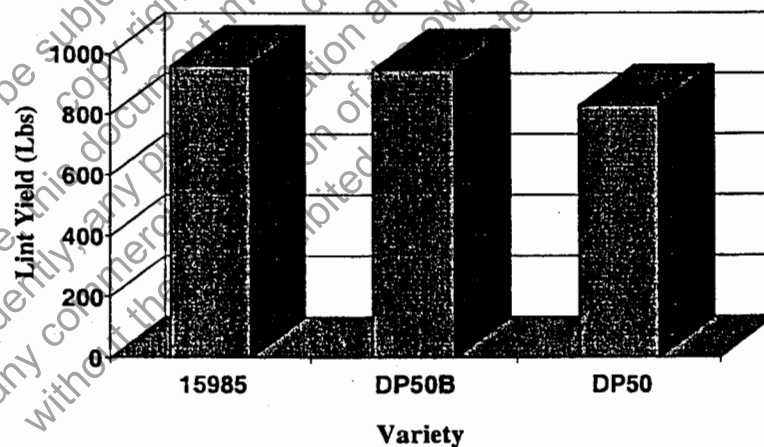
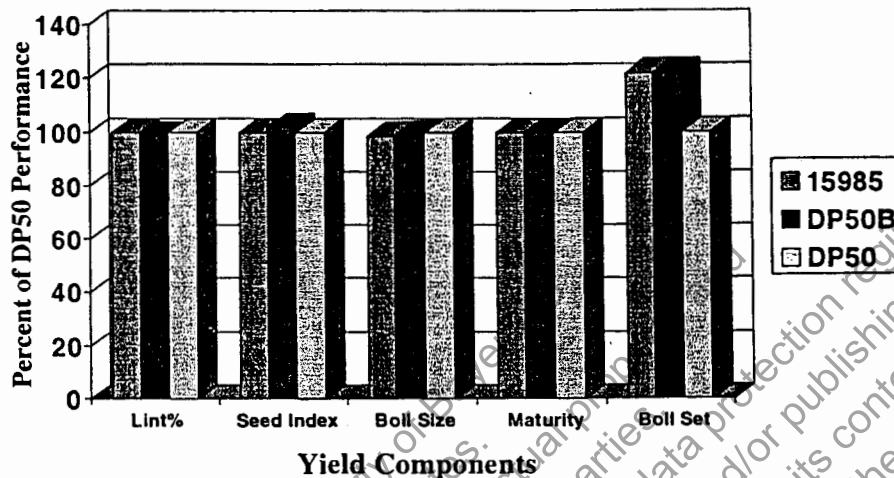


Figure 25. Yield Characteristics as a Percentage of DP50 Performance in the 1998 and 1999 Field Trials.



#### ***Morphology and Maturity***

Several criteria were measured to determine morphology and maturity, including: general plant appearance, days to emergence, seedling vigor, plant stand counts, height-to-node ratio, days to first white flower, days to first cracked boll, days to 50% open bolls, fruit retention (the percentage of first position fruit retained in the 95% zone), plant mapping, and days to harvest. Qualitatively among all 1998 and 1999 field trials, no significant differences were noted in appearance between event 15985 and DP50 control plants that were outside of the norm for the variability of the DP50 variety.

Crop development, growth, and vigor were not significantly different between cotton event 15985 and the DP50B control plants at any of the locations tested, as observed by height:node ratio measurements, flowering dates, and boll counts. Detailed observations made at eight locations in 1998 in the regulatory trials determined that flowering began at all locations between July 20 and August 2, 1998 (Table 13) and the mean number of days to peak bloom was 15-16 for all lines (Table 14). Mean height:node ratio at cut-out ranged from 1.70 to 1.77 across all eight sites (Table 14). First cracked boll counts across all sites appeared between August 31 and September 21, 1998 (Table 13), with the mean total number of bolls per plot ranging from 284 to 431 across all eight sites (Table 14). The number of cracked bolls for the nontransgenic control was lower than the transgenic lines due to insect damage, which led to boll reduction and yield loss. Due to the effects of hurricane "George" at the Alabama site, boll counts and yield were reduced across all cotton events. Therefore, the cracked boll counts from the AL site were not used to generate the mean cracked boll counts. In addition, plant mapping was performed in September 1998 at the AZ field site and recorded first fruiting branch position, number of missing fruit positions, length of top five nodes, and nodes above white flower. No differences were observed between event 15985 and DP50B control cotton plants.

**Table 13. Summary of Mean Emergence, Flowering, and Harvest Dates for Bollgard II Cotton Event 15985 at Eight Locations in the United States in 1998.**

Site Code	Event or Line #	Percent Seedlings Emerged (7 days)	Percent Seedlings Emerged (14 days)	First White Flower Observed	Date of First Cracked Boll Counts	Harvest Date
Arizona 1	15985	71	82	7/21/98	9/8/98	10/16/98
	DP50B	80	85	7/21/98	9/8/98	10/16/98
	DP50	70	73	7/21/98	9/8/98	10/16/98
Arizona 2	15985	51	72	7/27/98	9/8/98	11/18/98
	DP50B	70	76	7/23/98	9/8/98	11/18/98
	DP50	49	62	7/23/98	9/8/98	11/18/98
Louisiana 1	15985	52	68	8/2/98	9/21/98	10/27/98
	DP50B	48	56	7/31/98	9/21/98	10/27/98
	DP50	48	67	7/31/98	9/21/98	10/27/98
Louisiana 2	15985	73	82	7/20/98	8/31/98	10/14/98
	DP50B	68	71	7/20/98	8/31/98	10/14/98
	DP50	54	56	7/20/98	8/31/98	10/14/98
Mississippi 1	15985	70	75	7/30/98	9/4/98	10/19/98
	DP50B	75	76	7/30/98	9/4/98	10/19/98
	DP50	78	62	7/30/98	9/4/98	10/19/98
Mississippi 2	15985	63	78	7/20/98	9/8/98	10/5/98
	DP50B	76	73	7/20/98	9/8/98	10/5/98
	DP50	74	69	7/20/98	9/8/98	10/5/98
South Carolina	15985	88	94	7/23/98	9/9/98	10/27/98
	DP50B	77	87	7/23/98	9/9/98	10/27/98
	DP50	60	78	7/23/98	9/9/98	10/27/98
Texas <sup>1</sup>	15985	73	93	7/23/98	9/2/98	9/28/98 +
	DP50B	83	83	7/20/98	9/2/98	9/28/98 +
	DP50	64	69	7/23/98	9/2/98	10/9/98

1: Bolls were harvested on two dates at the TX site due to excessive moisture which would have increased boll rot.

**Table 14. Summary of Mean Height:Node Ratio, Number of Days to Peak Bloom, and Total Cracked Boll Counts for Bollgard II Cotton Event 15985 at Eight Locations in the United States in 1998.**

Event or Line #	Height:Node ratio	Mean number of days to peak bloom	Mean total number of cracked bolls /plot
15985	1.70	15.29	407
DP50B	1.77	15.03	431
DP50	1.72	15.77	284

Germination studies of event 15985 were performed on seed from two locations in 1998 by Delta and PineLand Seed Company (Table 15). Seed germination ranged from 72-77% for event 15985, which was consistent with each of the control seed germination

rates (80-83% DP50B; 82-89% DP50) and was within the normal expected range for cottonseed.

Germination and dormancy characteristics of cotton event 15985 seed were also evaluated relative to the parent Bollgard variety, the non-transgenic DP50 and ten reference varieties (Appendix 6, Section 20). The study was conducted by BioDiagnostics, Inc. using standards established by the Association of Official Seed Analysts using eight temperature regimes. Test and control seed samples were obtained from three geographically diverse sites in 1999 field trials: Portland, TX; Florence, SC; and Bossier City, LA. Reference seed varieties were obtained from commercial seed stocks and included Phytogen 952, STN474, SG125, SG821, DP5305, DP5690, FiberMax 989, PM1560, DP5409 and DP5415. The number of germinated and degenerated seeds were counted periodically throughout the 12-day study period. Seeds remaining on the final day were tested for viability using a tetrazolium test and characterized as hard or firm-swollen seed. The results of the study indicate that there were no differences for dormant seed between the test event 15985 and the control DP50B (Table 16). Five differences were identified for the other three parameters tested: percentage of germinated seed (pgerm), percent viable firm-swollen seed (pfms) and the percent degenerated seed (pdegen). These differences revealed no observable trends and were within the range of values determined for the reference cottonseed.

**Table 15. Germination and Seedling Vigor Tests on Seed Harvested from Two Locations in 1998.**

Event or Line #	% Germination Day 4	% Germination Day 9	% Cool Germination at 18°C Day 7
15985	76	77	72
DP50B	83	83	80
DP50	88	89	82

#### **E. Compositional Analyses of Bollgard II Cotton Event 15985**

In addition to agronomic performance, analyses of 44 separate components of cottonseed were evaluated by Covance Laboratories, Inc. (Madison, WI) to assess nutritional parameters relevant to public health and to sample for unintended effects on plant metabolism due to the insertion event or expression of the new genes. This data was submitted to the Food and Drug Administration's premarket notification procedure for engineered foods and feeds. This data was presented in a summary to FDA and Monsanto is currently in consultation with the Food and Drug Administration following their policy, "Foods Derived from New Plant Varieties," on the food and feed safety of Bollgard II cotton event 15985.

As described in Section V.C., field trials were conducted at eight U.S. locations within six states in 1998 (Texas, Arizona, Mississippi, South Carolina, Louisiana and Alabama) as described previously. Compositional analyses of seed samples collected in 1998 U.S.

**Table 16. Germination and Dormancy Results for Cotton Event 15985 on Seed Harvested from Three Locations in 1999.**

Temp.	Variety <sup>1</sup>	Mean pvhs (Dormant) <sup>2</sup> (%)	Mean pgerm <sup>2</sup> (%)	Mean pfms <sup>2</sup> (%)	Mean pdegen <sup>2</sup> (%)
5° C	15985	1.2	0.0	95.1	4.1
5° C	DP50B	0.0	0.0	95.2	5.4
5° C	Ref. Range	(0-41)	(0-1)	(53-99)	(1-20)
10° C	15985	0.0	1.2	73.9*	26.4*
10° C	DP50B	0.0	1.3	78.5	21.7
10° C	Ref. Range	(0-28)	(0-3)	(38-91)	(9-62)
20° C	15985	0.0	95.4	0.0	5.4*
20° C	DP50B	0.0	97.4	0.0	3.1
20° C	Ref. Range	(0-6)	(74-100)	(0-13)	(0-26)
30° C	15985	0.0	93.9*	0.0	6.6*
30° C	DP50B	0.0	98.6	0.0	2.2
30° C	Ref. Range	(0-0)	(83-100)	(0-0)	(0-17)
40° C	15985	0.0	85.9	0.0	14.9
40° C	DP50B	0.0	89.3	0.0	11.1
40° C	Ref. Range	(0-0)	(70-96)	(0-0)	(4-30)
5/20° C	15985	0.0	NC	NC	NC
5/20° C	DP50B	0.1	NC	NC	NC
5/20° C	Ref. Range	(0-29)	NC	NC	NC
10/20° C	15985	0.0	NC	1.9	7.5
10/20° C	DP50B	0.0	NC	1.2	5.8
10/20° C	Ref. Range	(0-18)	NC	(0-79)	(1-31)
20/30° C	15985	0.0	NC	0.0	5.1
20/30° C	DP50B	0.0	NC	0.0	3.7
20/30° C	Ref. Range	(0-2)	NC	(0-1)	(0-17)

\* Indicates level of significant difference from DP50B at  $P \leq 0.05$ .

NC = no comparison of combined means possible due to significant variety by site interaction at  $P \leq 0.05$ .

<sup>1</sup> There were 12 observations for both event 15985 and DP50B, in addition to 44 observations for reference varieties in each temperature regime.

<sup>2</sup> pvhs = percent viable hard seed, pgerm = percent germinated seed, pfms = percent viable firm-swollen seed, pdegen = percent degenerated seed.

trials were conducted to measure proximates (protein, fat, ash, carbohydrate, moisture, fiber, calories), amino acids, fatty acids, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), gossypol, cyclopropanoid fatty acids and aflatoxin content of seed. Seed collected from Bollgard II cotton event 15985, the parental line DP50B, the non-transgenic control line DP50 and ten commercially available cotton varieties were analyzed. A summary of the data is provided in Appendix 2.



Statistical evaluation of the composition data included 44 comparisons from the eight U.S. trials demonstrating the equivalence of cottonseed composition in event 15985 and the parental variety DP50B (Appendix 2). There were only six instances where the mean values for event 15985 was statistically different from the Bollgard (DP50B) parental line (Table 17). These few differences were all in levels of fatty acid components and were within the 95% confidence interval, as well as within the range of analyses for commercial reference cotton varieties tested. Furthermore, the statistically different means were not observed at all locations, demonstrating the impact of environmental conditions on variability. Therefore, these differences are not considered biologically relevant and it is concluded that event 15985 is not materially different from other commercially available cotton varieties.

#### F. Toxicants

Cottonseed samples were collected from all eight regulatory trial locations in the 1998 U.S. trials and seed analyses were conducted by Covance Laboratories, Inc. Three different toxicant classes naturally occurring in conventional cotton were assessed for Bollgard II cotton event 15985 relative to control and commercial cotton varieties: gossypol and cyclopropenoid fatty acids produced in cottonseed, and aflatoxins produced by infectious agents.

Gossypol is classified as a terpenoid aldehyde, and is one of a family of terpenoid compounds produced by genera in the plant tribe *Gossypiae* (Fryxell, 1979). Gossypol is produced in lysigenous glands of the seed, leaf, stem and root of the cotton plant, and provides natural insect protection to the plant. Total gossypol levels were measured in cottonseed from all test and control lines collected across all eight field test locations. There were no statistically significant differences in the gossypol levels obtained for cotton event 15985 compared to the Bollgard DP50B control and the mean value was within the nontransgenic and commercial reference ranges (Appendix 2).

The cyclopropenoid fatty acids (sterculic, dihydrosterculic and malvalic acids) are unique fatty acids common in cotton and are considered to be undesirable, anti-nutritional compounds. Statistically significant differences were observed for the mean values of malvalic, dihydrosterculic and sterculic acids between cotton event 15985 and control DP50B (Table 17). All mean differences for event 15985 were within the 95% confidence interval for each true mean difference and mean values were within the nontransgenic and commercial reference ranges (Table 17), as well as literature ranges (Berberich *et al.*, 1996). Additionally, none of the four replicated field locations showed statistically significant differences between 15985 and the control when the data is compared on a site-by-site basis. Therefore the differences were not considered biologically meaningful.

Aflatoxins are a group of mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* that may contaminate food and feed products (Jorgensen and Price, 1981). Cottonseed is one of the commodities most commonly contaminated by aflatoxins (Bagley, 1979). The levels of four primary aflatoxins (B1, B2, G1, G2) were undetected



in the cottonseed for cotton event 15985, control DP50B and the DP50 nontransgenic control and all commercial cotton reference lines at a LOD of 0.1 mg/g fwt.

**Table 17. Summary of Statistically Significant Differences in Composition for Bollgard II Event 15985 Cottonseed Samples from the 1998 U.S. Field Trials.**

Significant Parameter	15985 Mean	DP50B (Control) Mean	Mean Difference	Number of Sites with Significant Differences <sup>1</sup>	Commercial Range <sup>2</sup>	p Value	95% Confidence Interval
myristic acid	1.26	0.92	0.33	2	0.64-2.40	0.004	0.11 - 0.56
stearic acid	2.63	2.38	0.25	3	2.06-3.11	<0.001	0.18 - 0.32
linoleic acid	52.52	53.1	-0.58	1	46-57.10	0.038	(-1.13) - (-0.035)
malvalic acid	0.45	0.39	0.058	0	0.17-0.61	0.024	0.0084 - 0.11
dihydro-sterculic	0.18	0.15	0.03	3	0.11 - 0.22	<0.001	0.021 - 0.051
sterculic acid	0.30	0.25	0.054	0	0.13-0.66	0.034	0.0041 - 0.10

1: Data is from four replicated sites out of the total eight regulatory field locations in 1998.

2: Range includes data from 10 commercial varieties of cotton as listed in Appendix 2.

Therefore, the toxicant levels in Bollgard II event 15985 cottonseed are within the range of levels found in the control and commercial cottonseed tested. This data further establishes that event 15985 is not materially different from other commercially available cotton varieties.

## **VI. Environmental Consequences of Introduction**

### **A. Cry2Ab Protein**

*Bacillus thuringiensis* are crystalliferous, spore-forming gram-positive bacterium that have been used commercially for nearly 40 years to control insects. They are found naturally in soil worldwide at significant levels. The Cry2Ab protein has a high degree of sequence similarity (97%) to the Cry2Aa protein produced in commercial *B.t.k.* products. The proteins produced in these products have an established history of environmental safety, as documented in the EPA 1998 Registration Eligibility Decision Document (EPA, 1998). To confirm the environmental safety of Cry2Ab protein in Bollgard II

cotton event 15985, thirteen studies were conducted on bird, fish, and beneficial terrestrial invertebrate species. These data were submitted to the Environmental Protection Agency in April 2000 to support a request for registration of the Cry2Ab protein as produced in cotton and are provided in Appendix 6 of this petition.

Non-target organisms were exposed to leaf or seed tissue from event 15985 cotton plants or to Cry2Ab protein incorporated into the diet for five days to eight weeks, depending on the study. The doses were set to exceed the predicted environmental exposure. The results discussed below, together with the history of safe use of *B.t.* proteins in general, demonstrate that Cry2Ab proteins in event 15985 cotton pose no foreseeable risks to non-target organisms. No adverse effects were observed at concentrations significantly greater than the predicted environmental concentrations (Table 18). In all cases, the no observed effect concentration (NOEC) greatly exceeded the maximum environmental concentration indicating minimal risk.

Bobwhite quail and channel catfish fed cotton event 15985 cottonseed at 10% and 20% of their diets, respectively, exhibited no mortality and no adverse effects on survival, growth or behavior. The quail study was conducted by Wildlife International Laboratories and the catfish study was conducted at the Thad Cochran National Warmwater Aquaculture Center at Mississippi State University. These data indicate that birds exposed to Cry2Ab protein from consumption of cottonseed or insects or fish fed cottonseed meal as part of their diet will not be adversely affected.

Studies were also conducted to determine whether non-target species of insects and other terrestrial invertebrates are susceptible to Cry2Ab protein. Cry2Ab protein was evaluated in the earthworm, as well as five species of beneficial terrestrial invertebrates representing classes of insects that could be exposed to Cry2Ab protein from event 15985 cotton: adult and larval honey bees (*Apis mellifera*), collembola (*Folsomia candida*), green lacewing (*Chrysoperla carnea*), ladybird beetle (*Hippodamia convergens*) parasitic wasp (*Nasonia vitripennis*), and earthworm (*Eisenia fetida*). These studies were conducted at either Wildlife International Laboratories, California Agricultural Research Inc. or Springborn Laboratories Inc. Results of the studies indicate that Cry2Ab poses minimal risk to these beneficial non-target organisms. No adverse effects were observed at the maximum predicted environmental concentration to which the organisms would be exposed. In most of the studies, the No Observed Effect Concentration (NOEC) exceeded the maximum predicted environmental concentration by 10- to over 100- fold, demonstrating a wide margin of safety for these organisms (Table 18). Field observations of non-target populations conducted during the numerous field trials of Bollgard II cotton event 15985 and documented in the USDA final reports also support this conclusion. ...

**Table 18. Summary of Cry2Ab Protein Studies on Non-Target Organisms**

<u>Test Organism</u>	<u>Results</u>	<u>Test Substance</u>	<u>Conclusions</u> <sup>1</sup>
Bobwhite Quail	No mortality or toxic effects in birds consuming Cry2Ab cottonseed at 10% of diet	Event 15985 cottonseed	Event 15985 cottonseed poses minimal risk
Channel Catfish	No effects on growth or survival in fish consuming Event 15985 cottonseed at 20% of diet	Event 15985 cottonseed	Event 15985 cottonseed can be used in catfish diet at up to 20%, the highest level tested, with no adverse effects
Adult Honey Bee	NOEC = 68 µg Cry2Ab/ml diet	Cry2Ab protein	NOEC > 56X predicted maximum Cry2Ab concentration in cotton
Larval Honey Bee	NOEC = 170 µg Cry2Ab/ml, single dose	Cry2Ab protein	NOEC > 139X predicted maximum Cry2Ab concentration in cotton
Ladybird Beetle	NOEC = 4500 µg Cry2Ab/ml diet	Cry2Ab protein	NOEC > 88X predicted maximum Cry2Ab concentration in cotton leaf tissue
Collembola	NOEC = 69.5 µg Cry2Ab/g diet	Event 15985 leaf tissue	NOEC > 17X maximum predicted environmental exposure to Cry2Ab protein from cotton in soil
Green Lacewing Larvae	NOEC = 1100 µg Cry2Ab/g diet	Cry2Ab protein	NOEC > 22X maximum predicted environmental exposure to Cry2Ab protein from cotton leaf tissue
Parasitic Hymenoptera (Wasp)	NOEC = 4500 µg Cry2Ab/ml diet	Cry2Ab protein	NOEC > 3700X maximum environmental concentration predicted in cotton pollen
Earthworm	NOEC = 330 mg Cry2Ab/kg dry soil	Cry2Ab protein	NOEC ≥ 83X maximum estimated environmental exposure from cotton in soil

<sup>1</sup> Calculations were based upon the highest expression value determined from overseason cotton leaf tissue, pollen or soil, as appropriate to the test animal exposure.

Considered in total, data provided in this submission and discussed above establish the safety of the Cry2Ab protein and *Bt* crops in general for beneficial and other non-target insects commonly found in cotton fields. The absence of toxic effects in the non-target organism studies even at Cry2Ab levels considerably above the maximum predicted environmental exposure demonstrate that Cry2Ab will not have adverse impacts on these and related non-target organisms, including endangered and threatened species.

The potential of Cry1 and Cry2 proteins to effect non-target lepidopterans is well known, including the larvae of butterflies like the monarch, as well as endangered and threatened Lepidoptera. Cry-expressing plant products pose no risk to endangered or threatened Lepidoptera, and negligible risk toward other non-target Lepidoptera, because such species will not be exposed to significant amounts of the proteins. None of these lepidopterans deliberately feed on cotton plants, or tissues from such plants.

Consequently, the only possible route of exposure to Cry2Ab for these species is through cotton pollen drifting onto their host plant and being inadvertently consumed by the larvae. This requires that a species be sensitive to the Cry2Ab protein, be in the larval stages during the short 7-10 day period of pollen shed, and that the larval host plant be close enough to cotton fields for pollen to be deposited on that plant. Since cotton is not considered to be a wind-pollinated crop, deposition of pollen on host plants is unlikely. In addition, data provided in this submission demonstrate that the levels of Cry2Ab protein in cotton pollen are very low and only substantial pollen deposition could cause any adverse effects to even an extremely sensitive species.

## **B. GUS Protein**

The GUS protein has no insecticidal effect and there is no evidence of this protein producing environmental harm.

## **C. Current Agronomic Practices and the Impact of Bollgard II Cotton Event 15985 on Pest Management**

### **1. Potential Impact of Bollgard II Cotton Event on Agronomic Practices**

A significant and anticipated effect of Bollgard cotton on agronomic practices has been the reduction in use of conventional synthetic insecticide sprays and associated total pounds of insecticide active ingredient for control of lepidopteran species. In a poll conducted among U.S. growers in 1997, 79 percent of respondents considered potential savings in insecticide applications an important factor in their decision to grow Bollgard cotton (ReJesus *et al.*, 1997). In this poll, the growers' main reason for adopting the technology was the potential savings in insecticide sprays.

Numerous studies, conducted across the United States and in Australia, China, Mexico, and Spain with Bollgard cotton, have demonstrated an overall reduction in insecticide sprays for lepidopteran pests has occurred as a result of the introduction of Bollgard cotton (Benedict and Altman, 2000; [REDACTED] 1999; Mullins and Mills, 1999; Novillo *et al.*, 1998; Obando-Rodriguez *et al.*, 1999; Xia *et al.*, 1999; Wier *et al.*, 1998; Bachelier *et al.*, 1997; Bryant *et al.*, 1997; ReJesus *et al.*, 1997; Roof and DuRant, 1997; Stark 1997; Mitchner, 1996; Davis *et al.*, 1995). The number of spray reductions ranges from 1.0 to 7.7 sprays. Of the research reviewed, an average reduction of 3.6 sprays per acre is achieved when a grower uses Bt varieties versus non-Bt varieties. Table 19 provides information on the reduction in the number of insecticide sprays by geographic region.

Reducing the number of sprays of insecticides translates into a total reduction of pounds of active ingredient used to control insects in cotton and related costs to the grower. Using a

more conservative average reduction of 2.2 insecticide applications per acre, Benedict and Altman (2000) demonstrated that insecticide concentrates were reduced by 28 fluid ounces per acre due to the use of Bollgard cotton. When extrapolated out to the estimated 2.4 million acres of Bollgard cotton varieties planted in the United States in 1998, cotton growers reduced insecticide concentrate use by over 600,000 gallons, which translates into a reduction of over two million pounds of insecticide active ingredient across all U.S. acres of Bollgard cotton (Benedict and Altman, 2000).

**Table 19. Reduction in Insecticide Applications on Bollgard Cotton Varieties Relative to Conventional Cotton.**

Location	Reduction in number of sprays per year	Source
Australia	7.7	[REDACTED] 1999
Spain	5.6	[REDACTED] <i>et al.</i> , 1998
Mississippi	5.5	Davis <i>et al.</i> , 1995
Arkansas	5.0	Bryant <i>et al.</i> , 1997
Spain	4.4	Novillo <i>et al.</i> , 1999
South Carolina	4.0	ReJesus <i>et al.</i> , 1997
South Carolina	3.6	Roof and DuRant 1997
Arkansas	3.0	Bryant <i>et al.</i> , 1997
South Carolina	2.9	Roof and DuRant 1997
Georgia	2.5	Stark 1997
North Carolina	2.5	Bacheler <i>et al.</i> , 1997
Southern and Southeastern United States	2.4	Mullins and Mills 1999
MidSouth and Southeast	2.2	Benedict and Altman 2000
Georgia	2.0	Carlson <i>et al.</i> , 1998
Mexico	1.0	Obando-Rodriguez <i>et al.</i> , 1999
Average across studies	3.6	

Gianessi and Carpenter (1999) showed a two million pound reduction in insecticide usage by comparing the pounds of insecticide active ingredient used before and after the introduction of Bollgard cotton, which is supported by similar findings in a study conducted by the Economic Research Service/USDA (Fernandez-Cornejo and McBride, 1999). Gianessi and Carpenter (1999) looked at 12 insecticides and their usage rates in Arkansas, Arizona, Louisiana, Mississippi, and Texas. Of the 12 insecticides, nine showed a decrease in use and three showed a slight increase.

Bollgard cotton not only reduces the number of insecticide sprays necessary, it also impacts total production costs associated with insect control. The technology makes it possible for a

cotton grower to lower his investment in supplies, equipment, and labor (Benedict and Altman, 2000; ReJesus *et al.*, 1997; Benedict, 1996; Benedict *et al.*, 1996). For every spray that is eliminated, a grower reduces the number of spray trips and the related fuel, machinery, and labor costs. This further translates into potentially lower annual loan requirements to support the farm and less interest to pay to the bank each year (2000). Thirty-nine percent of U.S. Bollgard cotton users surveyed perceived a cost advantage related to labor and equipment (Marketing Horizons, 1999).

The locations where growers plant cotton also factor in the use of Bt cotton. Acres that have been difficult to farm with conventional varieties and spray regimes, as well as acres that are adjacent to environmentally sensitive areas, urban/suburban areas, or rural neighbors, are now more manageable. According to ReJesus *et al.* (1997), choosing "where to plant" Bt cotton is a major decision for farmers, such that 50 percent of the respondents to their survey indicated that the characteristics of their fields determined the varieties planted. Distance to the fields, type of soil, and whether the land was irrigated were important factors. The distance factor was significant because the farther away a field is from machinery storage and work areas, the greater savings in the labor and machinery costs associated with fewer insecticide applications. Growers interviewed stated they used Bollgard cotton in fields that were logistically difficult to spray either because of configuration of the field or distance required to move equipment. Bollgard cotton allows the growers to plant in irrigated lands, which get muddy and limit the ability to apply insecticides when conditions are wet and insect activity is high. Soil type was a consideration because it is difficult to spray during muddy, wet conditions, thus fewer insecticide applications are appealing on those soils. In environmentally sensitive areas, Bollgard cotton is particularly attractive where control of tobacco budworms, bollworms, and pink bollworms is needed but conventional insecticide sprays are avoided or restricted (Benedict, 1996). These areas include fields along waterways or near lakes where there is a reduced or eliminated use of synthetic insecticides and in restricted areas around homes and businesses where foliar insecticides cannot be applied.

All of these effects on agronomic practices resulting from the use of insect-protected cotton are expected to continue or become enhanced with the use of Bollgard II cotton event 15985.

## **2. Impact of Insect-Protected Cotton on Pest Management**

Bollgard cotton has provided effective control of the three major caterpillar pests in cotton (Jenkins *et al.*, 1993). U.S. growers surveyed in 1999 perceived that they had "much better/somewhat better" control of tobacco budworms (77 percent), bollworms (66 percent), and pink bollworms (57 percent) (Marketing Horizons, 1999) when comparing Bollgard to conventional cotton pest control systems. In Texas, Moore *et al.* (1997) estimated that Bollgard cotton varieties provided 95 percent control over tobacco budworm, 90 percent control over bollworm (pre-bloom), and 99 percent control over pink bollworm.

For many growers, insecticide application decisions are based on the level of infestation of certain pests. At certain low levels of infestation, it is not economically feasible to spray insecticides even though yield-reducing insect activity is occurring. With Bollgard, plant protection is available throughout the growing season and is provided irrespective of that

threshold level of infestation. Therefore, yield that a grower would normally give up to low-level infestations is maintained by Bollgard, resulting in an overall improvement of yield for the grower (Benedict, 1996; Benedict *et al.*, 1989). In general, economic infestations of target insect pests are slower to develop or do not develop at all in Bollgard cotton compared to cotton varieties without built-in lepidopteran insect resistance (Adkisson *et al.*, 1999).

Bollgard cotton varieties have been shown to decrease overall insecticide applications for lepidopteran pests (see previous section). However, when supplemental sprays are applied for insect control, their efficacy is higher on Bollgard varieties than conventional cotton varieties. Mann and Mullins (1999) showed a 54 percent higher insecticide efficacy related to bollworm feeding on Bollgard versus non-Bollgard cotton. In field tests in 1997 and 1998, Mann and Mullins demonstrated that insecticides like Karate<sup>®</sup>, Pirate<sup>®</sup>, and Tracer<sup>®</sup><sup>3</sup> exhibited enhanced efficacy, thus improving the overall control of pests in Bollgard cotton.

Given the improved target insect control of cotton event 15985 relative to the Bollgard parent variety DP50B (Section V.D.), it is anticipated that these trends in improved pest management will continue, especially in areas of heavy cotton bollworm infestations or in instances of sporadic armyworm damage where Bollgard II performance relative to Bollgard is most significant.

#### **D. Development of Pest and Resistance Management Strategies for Bollgard II Cotton Event 15985**

Monsanto is committed to appropriate stewardship of all our products, including Bt insect-protected crops. In 1995, Monsanto voluntarily submitted an insect resistance management plan (IRM) to EPA as part of our stewardship program for Bollgard cotton. This plan was developed in consultation with cotton entomologists across the cotton-producing states and was based on scientific data, as well as the growers ability to implement the plan logistically and economically. EPA endorsed the plan as a means to delay resistance.

Monsanto believes strongly that the scientific and grower-related parameters on which the original IRM plan was developed are still valid today. In 2000, Bollgard was planted on approximately 36% of the total U.S. cotton acres. Bollgard has performed as expected with no incidence of resistance observed in the field. Growers place a high value on Bollgard, particularly in those areas active in boll weevil eradication, areas of high lepidopteran infestations, and areas of pyrethroid-resistant cotton bollworms and/or tobacco budworms.

Because of concerns over the potential for the development of insect resistance to Bollgard cotton, it has been a major focus of Monsanto to add a second gene for lepidopteran insect control to the cotton marketplace. Laboratory bioassay studies with the Cry2Ab protein produced from the genetic material in *Bacillus thuringiensis*, when used alone or combined with Cry1Ac, showed potential for improved control of target insects, improved spectrum of

<sup>3</sup> Karate 1E, Pirate 3F, and Tracer 4F are registered trademarks of Zeneca Ag Company, American Cyanimid Company, and Dow AgroSciences Company, respectively.



activity, and would likely provide a good insect resistance management tool. The increased control of lepidopteran insect pests of cotton provided by Bollgard II cotton event 15985 (Section V.D.) has demonstrated that Bollgard II cotton will provide an additional tool to delay the development of lepidopteran resistance to Bt proteins in cotton.

#### **E. Cross Pollination of Cultivated and Native Species of Cotton**

Outcrossing to wild relatives is also not expected to result in effects on threatened or endangered animal or plant species either through direct toxic effects or competition. Outcrossing will not occur because cultivated cotton varieties do not exist in the wild in the United States, nor are there wild relatives that can readily interbreed with cotton in the areas of the United States where these crops are grown. Based on these observations, USDA deregulation and commercialization of cotton expressing the Cry2Ab protein will pose minimal risk to the environment with no predicted effects on threatened or endangered species. A detailed discussion of the potential for gene escape via pollen transfer is addressed in Part II of this Petition for Determination of Non-Regulated Status.

#### **F. Potential for Bollgard II Cotton Event 15985 to Become a Weed**

*G. hirsutum* is ineffective as a weed and does not appear to be especially effective in invading established ecosystems. In the continental United States, wild populations of *G. hirsutum* exist in the southern tip of Florida, due at least in part to the fact that cotton cannot over-winter in those areas where freezing conditions occur. There is little probability that the Bollgard II cotton event 15985 or any *Gossypium* species crossing with it could become a weed.

Cotton is not considered to have weedy characteristics as an annual plant grown in the United States. It does not possess any of the attributes commonly associated with weeds, such as seed dormancy, long soil persistence, germination under diverse environmental conditions, rapid vegetative growth, a short life cycle, high seed output, high seed dispersal, or long distance dispersal of seeds. These characteristics of weeds are controlled by multiple not single genes.

The only difference one would expect between the modified and non-modified cultivated cotton would be that the modified cotton would be better able to withstand damage from foliar-eating insects. A general consensus of the traits common to many weeds was developed by Baker (1974). Not all weeds have all of the characteristics, but in general they include:

1. germination requirement fulfilled in many environments
2. discontinuous germination and great longevity of seed
3. rapid growth through vegetative phase to flowering
4. continuous seed production for as long as growing conditions permit
5. self-compatibility but not completely autogamous and apomictic
6. when cross-pollinated, unspecialized visitors or wind pollinated
7. high seed output in favorable environments and some seed production in a wide range of environments
8. adaptation for short- and long-distance dispersal
9. if perennial like cotton, vegetative production or regeneration from fragments and brittleness, so not easily removed from the ground



10. ability to compete interspecifically by special means, such as rosette formation and presence of allelochemicals.

Cotton does not possess the characteristics of plants that are notably successful weeds, as listed above. The seed is not dormant and is not able to persist in the soil for long periods of time. In fact, only in the southernmost parts of the U.S. cotton growing regions can the seed successfully over-winter and germinate the next spring. As discussed in Section II, cotton has no weedy relatives in the continental United States to which it can cross, and therefore it is not expected to cross with other species. Monitoring of plots during and after harvest for the past two years of Bollgard II cotton event 15985 field trials has not revealed any differences in survivability and competitiveness relative to other varieties of cotton. Expression of the gene products (Cry2Ab and GUS proteins) in event 15985 cotton plant has not changed any of the above listed attributes, as described in Section V.D. Therefore, Bollgard II cotton event 15985 is not expected to have any different weedy characteristics than other cotton grown in the United States.

#### **VII. Statement of Grounds Unfavorable**

The results of all field studies and laboratory tests establish that there are no unfavorable grounds associated with Bollgard II cotton event 15985 developed using the linear fragment of plasmid vector PV-GHBK11.

#### **VIII. Conclusions**

Cotton (*Gossypium hirsutum* L.) has been extensively characterized, and has a long history of safe agricultural production. It is technically an exotic species, introduced to the U.S. as a source of fiber. The species has been highly domesticated through traditional breeding over the last 60 years. To date, there is no evidence of cotton acting as a plant pest in managed and non-managed ecosystems. Seeds are the only known survival structures, and cotton is not capable of surviving as a weed due to past selection as a result of its domestication. Cotton is not found growing in fence rows, ditches, road sides, or unmanaged habitats in the U.S.

The transformation vector, PV-GHBK11, containing the *cry2Ab* and *uidA* genes with their respective regulatory sequences, was introduced into the Bollgard cotton genome by a particle acceleration method to produce Bollgard II cotton event 15985. Molecular characterization has been conducted to establish that Bollgard II cotton event 15985 contains one DNA insertion from PV-GHBK11. The insert contains one copy each of the *cry2Ab* and *uidA* cassettes. The characterization also determined the composition and structure of the insert, as well as the insert stability across multiple generations. The insertion resulted in the expression of only the Cry2Ab and GUS proteins.

The donor organisms, *Bacillus thuringiensis* subsp. *kurstaki* and *Escherichia coli*, are commonly found in the environment and are not known to be harmful. The proteins produced as a result of the insertion are well characterized. The Cry2Ab protein is highly homologous to the Cry2Aa protein produced by *B.t.k.* The Cry2Aa protein has been widely used in sprayable microbial *B.t.* products and has a long history of environmental safety.

Agronomic, disease, and pest susceptibility observations have been recorded for event 15985 for three years in the United States in more than 200 field trials conducted by Monsanto and academic cooperators, in addition to numerous greenhouse and laboratory studies. Event 15985 cotton is within the normal range of variability observed in conventional cotton varieties for all agronomic and physiological parameters measured, except for the intended difference in insect efficacy. Neither the inserted genetic material, nor the proteins produced, have resulted in observed plant pest characteristics during the course of the trials.

The environmental consequences of the introduction of cotton event 15985 have been considered and there is no indication that event 15985 would pose a significant risk. On the contrary, there is evidence to support the expectation that Bollgard II cotton event 15985 will reduce environmental impact by reduction of the dependence on synthetic insecticides and reduction in the risk of the development of resistance in lepidoptera to Bt. The lack of any significant environmental impact of the *B.t.* family of proteins has been demonstrated in both microbial products and in plant-incorporated products including YieldGard<sup>5</sup> corn and Bollgard cotton. In all cases where the effects of the Cry2Ab protein were determined on non-target organisms, the no observed effect concentration (NOEC) greatly exceeded the maximum environmental concentration, indicating minimal risk to non-target organisms.

The environmental consequences of pollen transfer from cotton event 15985 to other cotton is considered to be negligible due to limited movement of cotton pollen, safety of the introduced proteins, and lack of any selective advantage conferred on the recipient cotton plant. Gene transfer is only expected to occur with other cultivated cotton and then only at low levels biologically normal for *Gossypium hirsutum*. The potential for outcrossing to sexually compatible species is unlikely as there are no significant populations of sexually compatible related species of cotton in the U.S. and its territories where cotton is grown. The lack of unintended effects on germination and dormancy, the predominant factors limiting the weediness of cotton in the U.S., confirm that event 15985 is unlikely to become a weed. The agronomic consequences of volunteer cotton plants would be minimal as these plants are easily controlled by mechanical means, or by one of a number of herbicides currently registered for cotton.

Data and information in this request demonstrate that Bollgard II cotton event 15985 does not represent a unique plant pest risk. Cotton event 15985 has been shown through extensive field testing to be equivalent to the agronomic performance of traditional cotton varieties which are well established as having no plant pest risk. Therefore, Monsanto requests a determination of non-regulated status from APHIS that the cotton event 15985, any progenies derived from crosses between this line and other cotton varieties, and any progeny derived from crosses of this line with transgenic cotton varieties that have also received a determination of non-regulated status, no longer be considered regulated articles under regulations in 7 CFR part 340.

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<sup>5</sup> YieldGard is a registered trademark of the Monsanto Company.

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**Appendix 1. "The Agronomic Performance of One Bollgard II Donor Variety"**  
**by Mahaffey *et al.*, 2000.**

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## THE AGRONOMIC PERFORMANCE OF ONE BOLLGARD II™ DONOR VARIETY

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### Abstract

An experimental cotton variety, DPX 985 BGII, was planted in a series of 11 trials across the cotton belt. Trials were monitored via plant mapping at intervals through the season, machine picked yields, and fiber quality analysis. The responses of DPX 985 BGII were well within the acceptable range in all measured categories, indicating that this line could be successfully used in varietal development programs for the future.

### Introduction

Beginning with the 1996 cropping season, transgenic cotton plants containing the Bollgard™ gene by Monsanto have been utilized by cotton growers across the U.S. cotton belt. These plants express a gene coding for the Cry IA(c) protein from the soil-borne bacterium *Bacillus thuringiensis* (Berliner) subsp. *Kurstaki*. This protein exhibits insecticidal properties against certain lepidopterous species including *Helicoverpa zea* (cotton bollworm) and *Heliothis virescens* (tobacco budworm) (Beegle and Yamamoto 1992). The adoption of this new class of transgenic pest control tool has enabled many growers to virtually eliminate input costs associated with control of insecticide resistant pests such as *H. virescens* and to reduce costs associated with *H. zea* control (Mullins and Mills 1999).

One of the major concerns surrounding the utilization Bollgard containing cotton varieties in cotton cropping systems concerns the management of resistance to the Cry toxins. In fact, approval of the transgenic varieties by certain governmental agencies hinged on the development of a resistance management plan for these toxins. Plans were developed by Monsanto in conjunction with USDA and University scientists. These multi-faceted plans include restrictions on the ratio of Bollgard/non-Bollgard cotton which may be planted by a given grower, stipulations on management of the crops throughout the season, and monitoring of plan compliance (USDA 1999). The primary objective of this plan is to generate moths which are susceptible to the Cry toxin and allow those moths to mate with any resistant individuals which may emerge from the Bollgard fields.

One resistance management tactic which has been discussed by many authors is the utilization of multiple toxins with dissimilar modes and/or sites of action (i.e. insertion of multiple of traits in the same plant or "stacking") (USDA 1999). This use of two dissimilar toxins should greatly decrease the likelihood of resistance manifestation.

In trials planted during 1999, D&PL endeavored to evaluate the agronomic performance of two cotton lines containing both the Bollgard™ and the Bollgard II™ genes which could be used as donor parents in breeding programs to develop "stacked" cotton varieties.

### Materials and Methods

The Bollgard™ II gene was inserted into the commercially available cotton variety DP 50B via particle gun bombardment ("gene gun" insertion). Plants were regenerated from these transformations. All transformation and regeneration work was done by Monsanto. These plants were evaluated for gene purity and moved in self pollination and seed increase programs.

Upon availability of sufficient amounts of seed, trials were undertaken to compare the agronomic acceptability of DPX 985 BGII to currently available BG and conventional varieties. The trials described in this paper were performed for that purpose.

Trials were set up as randomized complete blocks with four replications at 11 locations across the cotton belt. Plot size ranged from small, research plot size (four rows by 30 feet long) to larger length-of-the-field sized (4 rows X 600 feet long) plots. All agronomic practices were performed as typical for the area in which the plot was planted. Non-lepidopteran insects were controlled in the plots. No treatments were made for lepidopteran pests.

Plant mapping was initiated on 5 plants from each plot at intervals throughout the season. The primary purpose of the plant mapping was to monitor varieties for aberrant growth characteristics and to measure varietal response to the 1999 testing environments.

Final data collection included machine picking, ginning in a commercial-style gin, and HVI testing of fiber samples.

### Results

Plant growth monitoring results are presented in Table 1 with appropriate statistics (means, probability levels, and LSD's). Significant differences existed among varieties in all growth parameters measured. However, none of the tested varieties deviated outside the normal range which could be expected among commercially available cotton varieties.

Fiber quality results are presented in Table 2 with appropriate statistics (means, probability levels, and LSD's). Significant differences existed among varieties when comparing micronaire, strength and length. Probability levels for the variety by location interaction also indicate that location had a significant effect on fiber properties during 1999 and that not all varieties responded in a similar manner to a given environment.

The last line of Table 2 is labeled "Contrast DPX 985 BGII vs. DP 50 XX". This data is the result of the orthogonal contrast of mean fiber properties from DPX 985 BGII to the same properties of the varieties DP 50, DP 50 B, and DP 50 B/RR when grouped collectively, for a given characteristic. Note that some significant differences were indicated by this test among the groups. DPX 985 BGII had significantly higher micronaire and longer fiber than the collective DP 50 XX group. No significant difference was indicated when contrasting the fiber strength of the two groups. However, none of the variety means are outside of the normal range of fiber quality found among cotton varieties.

Table 3 contains the percent lint (turnout) and lint yield data with appropriate statistics (means, probability levels, and LSD's) from the 1999 trials. Note that significant differences were indicated among varieties when comparing both turnouts and lint yields. Also, the location by variety interaction indicates that not all of the tested varieties responded to the 1999 testing environments in a similar manner.

The last line of Table 3 is labeled "Contrast DPX 985 BGII vs. DP 50 XX". This data is the result of the orthogonal contrast of mean turnouts and lint yields from DPX 985 BGII to the same properties of the varieties DP 50, DP 50 B, and DP 50 B/RR when grouped collectively, for a given characteristic. No significant differences were seen in lint turnouts among the groups. The contrast for lint yields indicates that the DPX 985 BGII variety yielded significantly more lint than the other "DP 50-type" varieties which were included in the trials.

### Summary

Throughout the course of the 1999 trials no grossly unacceptable characteristics in plant growth, yield, or fiber properties were observed in any of the tested varieties. Although a range of response was measured in all of the measured parameters, none of the tested varieties fell outside of the acceptable range for commercial cotton varieties. Also, location by variety interactions were significant across almost every measured parameter. This indicates that the environment which a variety is planted into has a significant effect on the performance of that variety.

DPX 985 BGII performed very well throughout this series of tests. In all of the measured characteristics this variety responded much the same as other non-BGII varieties. However, some significant differences were observed when comparing the DPX 985 BGII to the other "DP50-type" varieties. Even though these varieties are very similar in their lineage, they were not identical in their response to the 1999 testing environments. This indicates that even though new transgenic, or for that matter, non-transgenic varieties, are derived from well known parents, adequate testing must be performed to accurately quantify their characteristics prior to commercial introduction.

The fact that DPX 985 BGII performed in a similar manner to other commercially available varieties in this series of tests indicates that it may successfully be used in varietal development programs for the future.

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Table 1. Plant growth monitoring results from 10 trials conducted by Delta and Pine Land Company for evaluation of BG II lines during 1999. All data are taken from end of the season plant maps.

Variety	Height <sup>1</sup>	#Nodes <sup>2</sup>	#FB <sup>3</sup>	#N95 <sup>4</sup>	FR 95% <sup>5</sup>
DPX 985 BGII	32.7	17.5	12.2	15.1	56.1
DP 450 B/RR	35.0	17.5	12.2	15.5	54.3
DP 50	33.8	17.6	12.2	15.7	47.2
DP 50 B	33.5	17.2	12.1	14.9	56.5
NuCOTN 33B	35.1	18.4	12.8	15.9	48.5
Variety p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD p<0.05	0.8	0.3	0.3	0.3	2.2

<sup>1</sup> Total plant height

<sup>2</sup> Total number of nodes

<sup>3</sup> Total number of fruiting branches

<sup>4</sup> Total number of nodes accounting for 95% of the harvestable yield

<sup>5</sup> Percent first and second position fruit retention in the fruiting zone containing 95% of the harvestable yield

Table 2. Fiber property results from 9 trials conducted by Delta and Pine Land Company for evaluation of BG II lines during 1999.<sup>1</sup>

Variety	Micronaire	Strength	Length
DPX 985 BGII	4.51	29.15	1.16
DP 409 B/RR	4.16	28.81	1.14
DP 428 B	4.49	28.47	1.15
DP 450 B/RR	4.41	28.34	1.14
DP 451 B/RR	4.50	28.55	1.15
DP 50	4.27	28.99	1.14
DP 50B	4.25	29.28	1.17
NuCOTN 33B	4.39	30.11	1.14
PM 1218 BG/RR	4.55	28.14	1.10
PM 1560 BG/RR	4.05	29.83	1.15
SG 501 B/RR	4.76	30.73	1.13
Variety - p value	<0.0001	<0.0001	<0.0001
LSD (p<0.05)	0.09	0.33	0.007
V X L - p value	<0.0001	0.0025	0.0001
Contrast DPX 985 BGII vs. DP 50 XX <sup>2</sup>	0.0001	0.1348	0.0180

<sup>1</sup> All fiber properties were derived via standard HVI testing.

<sup>2</sup> This statistic is an orthogonal contrast of the DPX 985 BGII mean value for a given parameter versus the varieties DP 50, DP 50 B, and DP 50 B/RR (collectively grouped as "DP 50 XX") for the same parameter. The value is the probability that the varieties are not different.

Table 3. Lint turnout and yield results from 9 trials conducted by Delta and Pine Land Company for evaluation of BG II lines during 1999.

Variety	% Turn Out <sup>1</sup>	Lint Yield
DPX 985 BGII	30.1	819
DP 409 B/RR	32.9	838
DP 428 B	31.2	818
DP 450 B/RR	30.0	793
DP 451 B/RR	30.7	824
DP 50	30.1	689
DP 50B	29.7	777
NuCOTN 33B	32.4	777
PM 1218 BG/RR	34.9	825
PM 1560 BG/RR	33.4	743
SG 501 B/RR	33.3	824
Variety - p value	<0.0001	<0.0001
LSD (p<0.05)	0.34	32
V X L - p value	<0.0001	<0.0001
Contrast DPX 985 BGII vs. DP 50 XX <sup>2</sup>	0.5781	0.0012

<sup>1</sup> Turnouts determined through ginning of plot samples.

<sup>2</sup> This statistic is an orthogonal contrast of the DPX 985 BGII mean value for a given parameter versus the varieties DP 50, DP 50 B, and DP 50 B/RR (collectively grouped as "DP 50 XX") for the same parameter. The value is the probability that the varieties are not different.

**Appendix 2. Summary of Compositional Analysis Results of  
Cotton Event 15985 Seed from the 1998 Season.**

Component	Event 15985 (test)	DP50B (parental control)	DP50 (non-transgenic control)	Commercial reference range <sup>1</sup>
<b>Protein, %</b>	26.13 (21.45-28.82)	26.06 (21.93-28.15)	25.96 (21.76-27.79)	21.76-28.15
<b>Fat, %</b>	20.52 (17.54-27.42)	20.37 (16.04-23.48)	19.74 (15.44-23.64)	15.44-23.83
<b>Ash, %</b>	4.36 (3.93-4.81)	4.38 (4.06-4.67)	4.34 (3.76-4.85)	3.76-4.85
<b>Fiber, crude %</b>	16.83 (14.93-17.95)	17.17 (15.42-19.69)	17.19 (15.38-19.31)	15.38-20.89
<b>Carbohydrate, %</b>	49.09 (42.97-52.69)	49.23 (46.85-51.93)	49.94 (45.64-52.44)	45.64-53.62
<b>Calories/100g DW</b>	485.33 (468.50-520.01)	484.45 (463.09-498.71)	481.57 (457.77-499.84)	457.77-500.49
<b>Moisture, %</b>	5.99 (4.34-7.59)	6.05 (4.22-7.28)	6.03 (3.97-7.26)	3.97-8.47

<b>aspartic acid</b> (% total AA)	10.02 (9.74-10.49)	9.98 (9.76-10.39)	9.95 (9.78-10.45)	9.75-10.45
<b>threonine</b> (% total AA)	3.56 (3.37-3.77)	3.56 (3.40-3.90)	3.55 (3.38-3.73)	3.38-3.90
<b>serine</b> (% total AA)	4.77 (4.23-5.04)	4.77 (4.21-5.20)	4.78 (4.16-5.08)	4.16-5.20
<b>glutamic acid</b> (% total AA)	20.82 (20.09-21.27)	20.95 (20.09-21.68)	20.93 (20.24-21.25)	20.09-21.68
<b>proline</b> (% total AA)	4.17 (4.03-4.46)	4.14 (4.00-4.50)	4.12 (3.93-4.38)	3.93-4.50
<b>glycine</b> (% total AA)	4.61 (4.51-4.72)	4.62 (4.51-4.88)	4.60 (4.54-4.68)	4.50-4.88
<b>alanine</b> (% total AA)	4.32 (4.20-4.48)	4.31 (4.18-4.60)	4.27 (4.15-4.41)	4.15-4.60
<b>cystine</b> (% total AA)	1.79 (1.68-2.03)	1.85 (1.46-2.12)	1.87 (1.67-1.99)	1.46-2.12
<b>valine</b> (% total AA)	4.97 (4.77-5.34)	4.94 (4.72-5.34)	4.89 (4.72-5.22)	4.72-5.34
<b>methionine</b> (% total AA)	1.71 (1.55-1.97)	1.75 (1.46-2.03)	1.75 (1.49-1.98)	1.46-2.03
<b>isoleucine</b> (% total AA)	3.58 (3.47-3.79)	3.56 (3.45-3.78)	3.53 (3.38-3.71)	3.38-3.78
<b>leucine</b> (% total AA)	6.58 (6.45-6.86)	6.56 (6.44-6.94)	6.52 (6.43-6.65)	6.38-6.94
<b>tyrosine</b> (% total AA)	2.85 (2.73-2.91)	2.85 (2.66-3.05)	2.83 (2.72-2.96)	2.66-3.05

<b>Component (continued)</b>	<b>Event 15985 (test)</b>	<b>DP50B (parental control)</b>	<b>DP50 (non-transgenic control)</b>	<b>Commercial Reference range</b>
<b>phenylalanine</b> (% total AA)	5.68 (5.54-5.79)	5.70 (5.58-5.84)	5.66 (5.51-5.75)	5.51-5.84
<b>lysine</b> (% total AA)	5.10 (4.81-5.46)	5.08 (4.84-5.50)	5.11 (4.90-5.55)	4.83-5.55
<b>histidine</b> (% total AA)	3.07 (3.00-3.13)	3.09 (3.01-3.23)	3.09 (3.06-3.12)	3.01-3.23
<b>arginine</b> (% total AA)	11.37 (10.69-11.95)	11.24 (6.88-11.96)	11.49 (10.98-11.80)	6.88-12.17
<b>tryptophan</b> (% total AA)	1.02 (0.95-1.23)	1.03 (0.93-1.20)	1.03 (0.94-1.22)	0.93-1.26
<b>myristic (14:0)</b> (% total FA)	1.26 (0.88-2.94)	0.92 (0.74-1.91)	1.02 (0.77-2.15)	0.64-2.40
<b>palmitic (16:0)</b> (% total FA)	25.80 (24.50-27.90)	25.92 (24.90-27.60)	25.81 (24.30-28.10)	23.40-28.10
<b>palmitoleic (16:1)</b> (% total FA)	0.56 (0.33-0.65)	0.58 (0.43-0.68)	0.63 (0.43-0.98)	0.43-0.98
<b>stearic (18:0)</b> (% total FA)	2.63 (2.41-3.10)	2.38 (2.24-2.60)	2.30 (2.06-2.71)	2.06-3.11
<b>oleic (18:1)</b> (% total FA)	15.58 (13.60-18.10)	15.59 (13.30-18.10)	15.40 (12.90-17.40)	12.90-20.10
<b>linoleic (18:2)</b> (% total FA)	52.52 (47.70-55.50)	53.10 (49.00-55.80)	53.31 (49.50-57.10)	46.00-57.10
<b>linolenic and gamma linoleic (18:3) (% total FA)</b>	0.13 (0.050-0.29)	0.14 (0.05-0.55)	0.11 (0.05-0.31)	0.05-0.55
<b>arachidic (20:0)</b> (% total FA)	0.30 (0.25-0.43)	0.29 (0.25-0.36)	0.27 (0.24-0.34)	0.24-0.36
<b>lignoceric (24:0)</b> (% total FA)	0.14 (0.05-0.26)	0.12 (0.05-0.26)	0.14 (0.05-0.29)	0.05-0.29
<b>Total gossypol</b> (% DW)	1.00 (0.79-1.29)	0.97 (0.78-1.27)	0.96 (0.72-1.23)	0.71-1.24
<b>CPFA malvalic (C-17)</b> (% total fatty acids)	0.45 (0.26-0.71)	0.39 (0.22-0.51)	0.39 (0.17-0.61)	0.17-0.61
<b>CPFA sterculic (C-18)</b> (% total fatty acids)	0.30 (0.21-0.58)	0.25 (0.16-0.44)	0.24 (0.13-0.43)	0.13-0.66
<b>CPFA (C-19) dihydrosterculic</b> (% total fatty acids)	0.18 (0.12-0.22)	0.15 (0.11-0.17)	0.16 (0.12-0.19)	0.11-0.22
<b>Aflatoxin B1</b> (ppb)	<1.00	<1.00	<1.00	<1.00
<b>Aflatoxin B2</b> (ppb)	<1.00	<1.00	<1.00	<1.00



Component (continued)	Event 15985 (test)	DP50B (parental control)	DP50 (non-transgenic control)	Commercial Reference Range
<b>Aflatoxin G1</b> (ppb)	<1.00	<1.00	<1.00	<1.00
<b>Aflatoxin G2</b> (ppb)	<1.00	<1.00	<1.00	<1.00
<b>calcium</b> (% DW)	0.15 (0.13-0.19)	0.15 (0.13-0.20)	0.15 (0.12-0.20)	0.12-0.33
<b>copper</b> (mg/kg DW)	7.18 (4.27-10.12)	7.24 (4.39-9.51)	7.48 (4.39-10.35)	4.39-10.35
<b>iron</b> (mg/kg DW)	50.83 (43.92-57.56)	51.13 (41.84-60.76)	54.13 (42.57-72.15)	41.84-72.15
<b>magnesium</b> (% DW)	0.41 (0.37-0.47)	0.41 (0.37-0.49)	0.41 (0.37-0.47)	0.37-0.49
<b>manganese</b> (mg/kg DW)	14.11 (11.96-16.53)	14.10 (11.17-16.81)	14.11 (12.16-16.39)	11.17-18.31
<b>phosphorus</b> (% DW)	0.70 (0.58-0.83)	0.71 (0.61-0.88)	0.73 (0.63-0.86)	0.61-0.88
<b>potassium</b> (% DW)	1.16 (1.07-1.24)	1.15 (1.09-1.22)	1.15 (1.08-1.23)	1.08-1.25
<b>sodium</b> (% DW)	0.14 (0.067-0.21)	0.15 (0.039-0.30)	0.14 (0.04-0.25)	0.0054-0.30
<b>zinc</b> (mg/kg DW)	40.30 (27.70-52.50)	41.06 (27.39-51.20)	40.97 (31.66-48.62)	27.39-51.20

Underlined values are statistically significant relative to the DP50B control ( $p \leq 0.05$ ).

Values represent samples taken from eight U.S. regulatory field sites in 1998.

- 1: Range includes data from ten commercially available transgenic and nontransgenic cotton varieties (DP50, DP51, DP20, DP5409, DP50B, DP5415RR, DP436RR, SG125BR, PM1220BR and DP458BR).

**Appendix 3. "Bollgard II Efficacy: Quantification of Total Lepidopteran Activity  
in a 2-Gene Product"  
by Greenplate, *et al.*, 2000.**

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# BOLLGARD II EFFICACY: QUANTIFICATION OF TOTAL LEPIDOPTERAN ACTIVITY IN A 2-GENE PRODUCT

Monsanto, Agricultural Sector  
St. Louis, MO

## Abstract

A 4 field site study was performed in which Bollgard II (containing 2 lepidopteran active Bt proteins: CryIAc and CryX) and DP50B (original Bollgard® containing only CryIAc) cotton tissue samples were collected throughout the growing season and evaluated for total lepidopteran bioactivity using a sensitive *Heliothis virescens* quantitative bioassay which utilized purified CryIAc as the quantitative standard. In addition, protein-specific ELISA assays were performed upon the same tissue samples to determine relative levels of both insect control proteins. Total lepidopteran bioactivity, expressed in CryIAc equivalents, was greatly increased in Bollgard II tissue samples. Overall means were four times as great for Bollgard II as for DP50B (the "parent" Bollgard® variety); overall means were 3 times as great for terminal foliage and 6 times as great for square tissue. This relative increase in lepidopteran activity was observed at every sampling time (from 4-leaf stage to 6 weeks after first bloom) and at every field site. ELISA evaluations showed that the presence of the second protein (CryX) had no deleterious effect on the levels of the first Bollgard® protein (CryIAc) as measured in DP50B. Also, relative levels of the two Bt proteins remained relatively constant over time and across field sites. A main-effect ANOVA determined that, in addition to the Bollgard II-Bollgard® difference, field site, sampling time, and plant tissue type were all significant sources of variability among levels of lepidopteran bioactivity; although the tissue type variability was due solely to differences between terminals and squares within DP50B; when evaluated alone, there was no statistical difference in the lepidopteran activity between Bollgard II squares and terminals. These data strongly suggest that the greatest single effect of the addition of the CryX protein to Bollgard® to produce Bollgard II is likely to be greatly increased lepidopteran activity, especially in reproductive tissues.

## Introduction

In the development of the second generation of Bollgard® products, a second insect control gene encoding another Bt protein, qualitatively different from CryIAc (called CryX by Monsanto), was used to transform tissue from the current

Bollgard® variety DP50B (Delta & Pineland). Cloned plants regenerated from the transformed tissue expressed both the CryIAc protein and the CryX protein. These genes also segregated independently. The proposed name for the 2-gene product is Bollgard II; it has not yet received EPA registration. In this study, quantitative evaluations were made to determine levels of lepidopteran activity in specific Bollgard II tissues over time and in comparison with its Bollgard® "parent" variety (DP50B).

## Materials and Methods

Cotton tissue samples from 4 field sites were collected and shipped to Monsanto laboratories where they were processed and evaluated in a sensitive quantitative bioassay which utilized purified CryIAc as a standard and took advantage of the extreme sensitivity of *Heliothis virescens* to the CryIAc protein (Greenplate, 1999). Tissue sample effects on *H. virescens* larval development were compared with effects of known concentrations of CryIAc; lepidopteran activity levels in cotton tissues were thereby estimated and expressed as "CryIAc equivalents". Within each site, several plants within 4 replicate plots were sampled at 2 week intervals beginning at 4-leaf stage and ending at 6 weeks after first bloom. The specific tissues sampled were main terminal foliage, and pre-candle squares (1" position square 2-3 nodes below main terminal); terminal tissue was sampled from 4-leaf stage to 6 weeks after first bloom; square tissue was sampled from 2 weeks pre-bloom to 3 weeks after first bloom. The JMP® (version 3.1) statistical software (SAS Institute, Cary NC) was used to perform the statistical evaluations on lepidopteran bioactivity values. A main effect ANOVA was used to test the influence of field site, sampling time, tissue type, and replicate plot (within site) on variability among mean CryIAc levels. Subsequent mean comparisons were made using Tukey-Kramer HSD (Kramer, 1956). The cotton tissue samples were further evaluated in CryIAc- and CryX-specific ELISA tests (Sims *et al* 1996) to determine relative levels of the two proteins over time and across field sites.

## Results

A main-effect ANOVA determined that variety (Bollgard® vs Bollgard II), field site, sampling time, and plant tissue type were all significant sources of variability among levels of lepidopteran bioactivity (Table 1); although the tissue type variability was due solely to differences between terminals and squares within DP50B; when evaluated alone, there was no statistical difference in the lepidopteran activity between Bollgard II squares and terminals (Table 3). There was no significant plot effect. Table 2 shows that overall means of lepidopteran activity were 4 times as great in Bollgard II as in Bollgard® (65 and 17 µg of CryIAc equivalents per g dry wt, respectively). There was no significant difference between levels of lepidopteran activity in terminals and

squares of Bollgard II (67 and 62 µg of CryI Ac equivalents per g dry wgt, respectively), while Bollgard® terminals contained twice as much lepidopteran activity as corresponding squares (22 and 10 µg of CryI Ac equivalents per g dry wgt, respectively) (Table 3). Tables 4 and 5 show that overall levels of lepidopteran bioactivity remained significantly higher in Bollgard II tissues at all sampling times and at all field sites.

ELISA evaluations were used to measure relative levels of individual proteins (Figures 1-3). The addition of the second protein (CryX) to Bollgard® to create Bollgard II appeared to have no deleterious effect on levels of the original Bollgard® protein (CryI Ac) overall (Figure 1), or at various sampling times (Figure 2), or field sites (Figure 3).

### Discussion

In this 4 field-site study, total lepidopteran bioactivity, expressed in CryI Ac equivalents, was greatly increased in Bollgard II tissue samples. Overall means were four times as great for Bollgard II as for DP50B (the "parent" Bollgard® variety); overall means were 3 times as great for terminal foliage and 6 times as great for square tissue (Table 2; Table 3). This relative increase in lepidopteran activity was observed at every sampling time (from 4-leaf stage to 6 weeks after first bloom) and at every field site (Table 4; Table 5). A main-effect ANOVA of total lepidopteran bioactivity determined that, in addition to the Bollgard II-Bollgard® difference, field site, sampling time, and plant tissue type were all significant sources of variability (Table 1), although the tissue type variability was due solely to differences between terminals and squares within DP50B; when evaluated alone, there was no statistical difference in the lepidopteran activity between Bollgard II squares and terminals (Table 3).

Protein-specific ELISA evaluations showed that the presence of the second protein (CryX) had no deleterious effect on the levels of the first Bollgard® protein (CryI Ac) as measured in DP50B (Figure 1). Also, relative levels of the two Bt proteins remained relatively constant over time and across field sites (Figure 2; Figure 3). It may be observed that the ELISA values for CryI Ac in DP50B were somewhat lower than CryI Ac values as estimated in the quantitative bioassay (Table 2; Figure 1). This can be explained to a large degree by the ability of the ELISA to measure only soluble protein. The ability of the ELISA procedure to extract and solubilize CryI Ac is never complete; some remains insoluble and, therefore, undetected (Sachs *et al* 1998). In addition, combined ELISA values for CryX and CryI Ac in Bollgard II are considerably greater than values (estimated in CryI Ac equivalents) for total lepidopteran bioactivity (Table 2; Figure 1); this, although apparently inconsistent, can also be explained. The approximate 10X higher level of CryX over

CryI Ac in Bollgard II (Figure 1) did not result in a 10X (or greater) difference in bioactivity over Bollgard® (Table 2) because the CryX protein is less potent than CryI Ac against *H. virescens* (Monsanto internal communication). Instead, in Bollgard II the original CryI Ac with 10X CryX added combined to result in the reported 3-6X increase in the *H. virescens* bioactivity.

As measured in this study, the greatest single effect of the addition of the CryX protein to Bollgard® to produce Bollgard II was greatly increased lepidopteran activity, especially in reproductive tissues.

### Acknowledgments

The authors wish to thank the following collaborators in this study: Roger Leonard - Northeast Research Sta., Winnsboro LA; Johnie Jenkins - USDA/ARS, Mississippi State MS; Lloyd May, John DuRant - USDA/ARS, Florence SC; John Benedict - Texas A&M Research Ctr., Corpus Christi TX

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Table 1. ANOVA main effects table.

Source	DF	Sum of Squares	F Ratio	Prob>F
Sampling Time	5	23463.43	11.588	<.0001
Line	1	170885.02	421.9796	<.0001
Replicate[Field Site]	12	1563.16	0.3217	0.9851
-Tissue Type	1	8863.61	21.8876	<.0001
Field Site	3	11436.97	9.4141	<.0001

Table 2. Mean lepidopteran activity levels (MLA), expressed as  $\mu$  CryI Ac equivalents/g dry weight, for Bollgard® and Bollgard II. Means with different letters are statistically different at  $P = 0.05$  as measured by Tukey-Kramer HSD.

Variety	MLA	SEM	
Bollgard II	64.94	2.63	a
Bollgard	16.89	1.04	b

Table 3. Mean lepidopteran activity (MLA), expressed as  $\mu$  CryI Ac equivalents/g dry weight, for Bollgard® and Bollgard II terminals and squares. Within columns (tissue type), means with different lower-case letters are statistically different at  $P = 0.05$  as measured by Tukey-Kramer HSD. Within rows (variety), means with different upper-case letters are statistically different at  $P = 0.05$  as measured by Tukey-Kramer HSD.

Variety	Terminal MLA	SEM		Square MLA	SEM	
Bollgard II	66.89	3.91	a A	62.08	3.02	a A
Bollgard	21.55	1.49	b A	10.05	0.7	b B

Table 4. Mean lepidopteran activity (MLA), expressed as  $\mu$  CryI Ac equivalents/g dry weight, for Bollgard® and Bollgard II at various sampling times. For every sampling time, Bollgard II and Bollgard® means are statistically different at  $P = 0.05$  as measured by Tukey-Kramer HSD.

Sampling Time	Bollgard II MLA	SEM	Bollgard MLA	SEM
4 leaf	57.24	9.76	27.59	4.72
Pre-bloom	78.45	5.30	15.14	10.62
1st bloom	65.67	4.71	18.56	2.07
2 Weeks	65.96	5.52	13.88	1.57
3-4 Weeks	66.11	6.61	16.50	3.08
6 Weeks	29.27	4.54	15.34	3.15

Table 5. Mean lepidopteran activity (MLA), expressed as  $\mu$  CryI Ac equivalents/g dry weight, for Bollgard® and Bollgard II at various field sites. For every field site, Bollgard II and Bollgard® means are statistically different at  $P = 0.05$  as measured by Tukey-Kramer HSD.

Field Site	Bollgard II MLA	SEM	Bollgard MLA	SEM
LA	83.34	5.84	17.29	2.07
MS	66.42	3.40	21.87	1.51
SC	35.73	3.67	8.59	0.53
TX	73.35	4.39	19.52	2.68

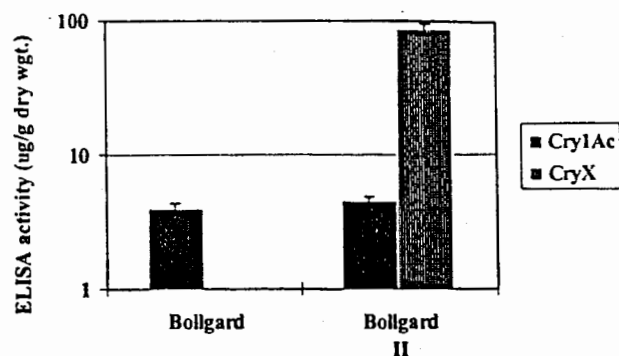


Figure 1. Overall mean concentrations (as measured by ELISA) of CryI Ac in Bollgard and Bollgard II tissues and CryX in Bollgard II tissues.

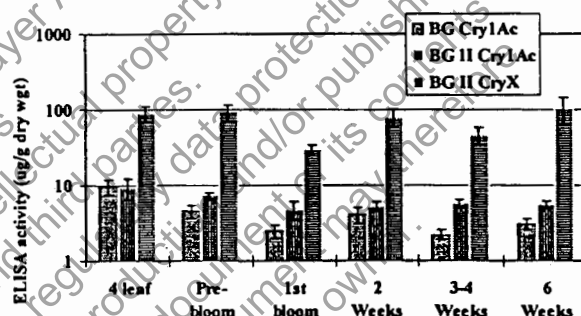


Figure 2. Mean concentrations, from specific sampling times, of CryI Ac in Bollgard and Bollgard II tissues and CryX in Bollgard II tissues. All means represent ELISA-derived values.

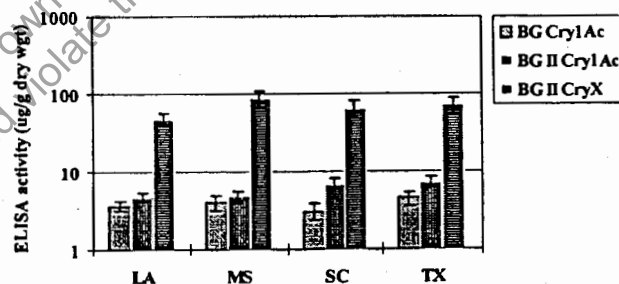


Figure 3. Mean concentrations, from individual field sites, of CryI Ac in Bollgard and Bollgard II tissues and CryX in Bollgard II tissues. All means represent ELISA-derived values.

**Appendix 4. "Effectiveness of Bollgard II Cotton Varieties Against Foliage and  
Fruit Feeding Caterpillars in Arkansas"  
by Allen, *et al.*, 2000.**

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# EFFECTIVENESS OF BOLLGARD II COTTON VARIETIES AGAINST FOLIAGE AND FRUIT FEEDING CATERpillARS IN ARKANSAS

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## Abstract

The Bollgard II technology tested showed excellent promise in protecting cotton from caterpillar pests. More data is needed on all species of caterpillar pests on cotton to confirm these findings.

Additional work on improving the agronomics of varieties with Bollgard II genetics appears to be needed before the varieties are released commercially.

## Introduction

Bollgard cotton varieties became commercially available in 1996. They have provided cotton growers an alternative to foliar insecticides for controlling some of the caterpillar pests of cotton. And, they have removed some of the natural selection for resistance to foliar insecticides. Since their release in 1996, cotton losses from caterpillar pests have not declined in the U.S. or in Arkansas, however (Williams, 1994-9). Nationally, losses to caterpillars 1996-8 were about the same as in the previous three years, 4.5% and 4.4%, respectively. In Arkansas, losses were higher from 1996-8 than from 1993-5, 5.4% and 2.5%, respectively. Certainly, there is room for improvement of the caterpillar management technology.

Bollgard II technology incorporates two *Bacillus thuringiensis* toxins into the cotton plant. It is hoped that the two toxin technology will provide broader spectrum caterpillar control and will slow the development of resistance in caterpillar pests to Bt toxins.

This study was conducted to gain a better understanding of the effectiveness of the Bollgard II technology against caterpillar pests and to investigate the agronomic characteristics and yield potential of these varieties.

## Materials and Methods

This study was conducted on the Southeast Branch Experiment Station at Rohwer, AR. Eight replications of four

treatments were planted in 4 row x 40 foot plots on 5-21-99. Standard production practices were used except that no insecticides for caterpillar control were used. Treatments were the cotton varieties which were planted. The varieties were, 15813 (Bollgard II), 15985 (Bollgard II), DPL 50B and DPL 50.

The plots were sampled weekly from mid-July to mid-August by counting the plant bugs, boll weevils and boll weevil damage, and Heliothine larvae and damage on 25 terminals, 25 squares and 25 small bolls per plot. On 8-5-99, eight beet armyworm egg masses were stapled to lower canopy leaves in each plot. On 8-16-99 whole plots were searched for beet armyworm hits (hatching egg masses) and larvae. Soybean and cabbage looper populations increased in the plots in September. Six foot beet sheet counts were taken in each plot on 9-15-99. An infestation of Heliothine larvae occurred on late season small bolls. Fifty uppermost small bolls were inspected for the presence of worm damage and larvae on 9-24-99. Larvae found were collected and identified under a dissecting microscope.

The data collected was processed using Agriculture Research Manager and Costat Statistical Software. The data were analyzed using Analysis of Variance and LSD ( $P \leq .05$ ).

## Results and Discussion

Bollworm and tobacco budworm populations were low in mid-season this study, therefore no useable bollworm/budworm data were collected during July and August.

Beet armyworm data (after the introduction of egg masses) and late season tobacco budworm data are shown in Table 1. Significantly fewer beet armyworm hits and larvae were seen in the Bollgard II plots as compared with the Bollgard (DPL 50 B) or conventional (DPL 50) plots. No beet armyworm larvae were found in either of the Bollgard II varieties.

The Heliothine larvae collected from bolls in September were 94% *Heliothis virescens*. Significantly fewer tobacco budworm larvae or tobacco budworm damaged bolls were seen in the Bollgard II and Bollgard plots as compared with the conventional cotton. Low level boll damage from tobacco budworm was observed in the DPL 50 B (Bollgard) and 15813 (Bollgard II) plots, while no tobacco budworm damage was seen in the 15985 (Bollgard II) plots.

Looper infestations and damage are shown in Table 2. Significantly fewer cabbage looper larvae were found in the Bollgard II varieties than in the Bollgard or conventional varieties. Very low levels of cabbage loopers were seen in the Bollgard II varieties, however.



Significantly fewer soybean loopers were seen in the Bollgard II cotton than in the Bollgard or conventional cotton. A very low level of soybean looper presence was observed in the 15813 Bollgard II cotton, however.

Looper damage was significantly lower in the Bollgard II cotton than in the Bollgard or conventional cotton. Bollgard cotton had less damage than the conventional cotton, however.

### Conclusions

The Bollgard II varieties tested showed good promise in protecting cotton from caterpillar larvae. The data collected in this study shows that these varieties were protected from beet armyworm, tobacco budworm, soybean looper and cabbage looper. No data was collected on the efficacy of this technology against bollworm. The agronomic characteristics of these varieties are still questionable. In summary, more study is needed on the effectiveness of Bollgard II varieties against caterpillar pests in cotton, and more work needs to be done to get Bollgard II varieties agronomically ready for release to growers.

### Acknowledgments

The authors wish to thank Monsanto for supporting this work by providing the seed, grant support, and beet armyworm egg masses. Also, we wish to thank the staff at the Southeast Branch Experiment Station at Rohwer, AR, for their assistance. Finally, we wish to thank Fran Tomerlin, Amy Gibson, Miranda Greer, Ashley Lucas, Sheila Willis, Cori Treat, Cari Russel and Brad Allen, the student workers who worked on this project.

### Literature Cited

Williams, M.R. 1994-9. Cotton Insect Losses 1994-9. Proceedings Beltwide Cotton Conference.

Table 1. Beet armyworm and late season tobacco budworm larvae and damage on Bollgard II, Bollgard, and conventional cotton varieties<sup>1</sup>. Rohwer, AR. 1999.

	Beet Armyworm		Tobacco Budworm	
	Hits per Plot <sup>1</sup>	Larvae per Plot <sup>1</sup>	Larvae per 100 Small Bolls	% Damaged Bolls
15985	0.0 a	0.0 a	0.0 a	0.0 a
15813	0.0 a	0.0 a	0.0 a	0.5 a
DPL 50 B	5.8 b	6.6 b	0.0 a	0.8 a
DPL 50	6.5 b	8.1 b	2.2 b	10.2 b

<sup>1</sup>Means followed by the same letter are not significantly different ( $P \leq .05$ ).

<sup>2</sup>Plots were 4 rows x 40 feet (160 row feet).

Table 2. Cabbage and soybean looper counts<sup>1</sup> and damage<sup>2</sup> on Bollgard II, Bollgard and conventional varieties<sup>3</sup>. Rohwer, AR. 1999.

	Cabbage Loopers per 6 row ft.	Soybean Loopers per 6 row ft.	Looper Damage Rating <sup>1</sup>
15895	0.1 a	0.0 a	0.0 a
15813	0.4 a	0.1 a	0.0 a
DPL 50 B	27.4 b	40.5 b	2.3 b
DPL 50	23.9 b	47.9 b	3.4 c

<sup>1</sup>6 foot beat sheet sample.

<sup>2</sup>Rating 0-5; 0 = no damage, 5 = severe defoliation.

<sup>3</sup>Means followed by the same letter are not significantly different ( $P \leq .05$ ).

Table 3. Agronomic characteristics and yield of Bollgard II, Bollgard and conventional varieties. Rohwer, AR. 1999.

	Stand Counts Plants/A <sup>1</sup>	Seedling Vigor Rating <sup>2</sup>	Yield Lbs Lint/A
15985	54,736 b	2.3 b	747 ab
15813	67,346 a	1.6 a	668 b
DPL 50 B	35,023 b	2.3 b	847 a
DPL 50	60,735 ab	1.9 ab	785 a

<sup>1</sup>Means followed by the same letter are not significantly different ( $P \leq .05$ ).

<sup>2</sup>Counts made on 3 row feet/plot on 6-3-99.

<sup>3</sup>Rating 1-5; 1 = very good, 5 = poor.



## Appendix 5. Field Test Reports for Cotton Event 15985

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**1998 Cotton Field Trial Report**  
**USDA #98-084-22N/Mons #98-163XR**

**Washington County, MS**

Planting Date: June 3, 1998

Harvest Date: October 19, 1998

Vector Construct: PV-BHBK13 (Line 16120)

PV-GHBK11 (Lines 15813, 15835, 15985, 16019, 16072)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. The trial was monitored on June 17, July 1, July 15, July 30 and August 17, 1998.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility. The trial was monitored on June 17, July 1, July 15, July 30 and August 17, 1998.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 17, July 1, July 15, July 30 and August 17, 1998.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 17, July 1, July 15, July 30 and August 17, 1998.

**Monitoring for Volunteers:** Monitoring for volunteers occurred on November 19 and December 18, 1998, January 15, February 15, March 15, April 15, May 14 and June 7, 1999. No volunteers were observed.

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1998 Cotton Field Trial Report  
USDA #98-084-22N/Mons #98-163XR

Oktibbeha County, MS

Planting Date: June 1, 1998

Harvest Date: October 21, 1998

Vector Construct: PV-BHBK13 (Line 16120)

PV-GHBK11 (Lines 15813, 15835, 15985, 16019, 16072)

Actual Line Numbers Planted: 15813, 15985 (Varieties DP50, DP50B, DP33B)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. The trial was monitored on June 29, July 20, and August 19, 1998.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility. The trial was monitored on June 29, July 20 and August 19, 1998.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 29, July 20 and August 19, 1998.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 29, July 20 and August 19, 1998.

**Monitoring for Volunteers:** The winter weather was the method used to destroy volunteers. In the Spring of 1999, a herbicide was applied to burn down winter vegetation weeds. Tilled field, re-hydrated beds, ran do-all machine and planted to transgenic cotton on May 13 and May 18, 1999. The field was free of any volunteer cotton when planted in 1999.

**1998 Cotton Field Trial Report**  
**USDA #98-084-22N/Mons #98-163XR**

**Franklin County, LA**

Planting Date: June 10, 1998

Harvest Date: October 27, 1998

Vector Constructs: PV-BHBK13 (Line 16120)

PV-GHBK11 (Lines 15813, 15835, 15985, 16019, 16072)

Actual Line Numbers Planted: 15813, 15985 (Varieties DP50, DP50B)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. The trial was monitored on July 12, 1998.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility. The trial was monitored on August 4, 1998.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on August 25, 1998.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on September 15 and October 2, 1998.

**Monitoring for Volunteers:** Monitoring for volunteers occurred on November 20 and December 15, 1998, January 10, February 1, February 26, March 22, April 12, May 6, June 15, and July 3, 1999. No volunteers were observed during the monitoring period.

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**1998 Cotton Field Trial Report**  
**USDA #98-084-22N/Mons #98-163XR**

**Baldwin County, AL**

Planting Date: June 1, 1998 and June 3, 1998  
Harvest Date: October 16, 1998 and October 19, 1998  
Vector Constructs: PV-BHBK13 (Line 16120)  
PV-GHBK11 (Lines 15813, 15835, 15985, 16019, 16072)  
Actual Line Numbers Planted: 15813, 15985 (Varieties DP50, DP50B)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants.

**Monitoring for Volunteers:** The dates for monitoring for volunteers were November 18, and December 16, 1998, January 15, February 19, March 18, April 14, May 12 and June 15, 1999. Observation made on one plant per square feet on November 18, 1998. The field was disked and cultivated on time. After the November 18, 1998 field disk, there were no more volunteers observed.

**Crittenden County, AR**

The Monsanto Farm at this location was closed in early 1998. This field site location was not used.

1998 Cotton Field Trial Report  
USDA #98-084-23N      Monsanto #98-164XR

November 3, 2000

Monsanto Company

Location

[ CBI DELETED ]

County

Nueces

State

Texas

Nueces County, TX

Planting Date: June 2, 1998

Harvest Date: October 9, 1998

Vector Construct: PV-GHBK13 (Line 16120)

PV-GHBK11 (Lines 15813, 15835, 15985, 16019, 16072)

Purpose: Evaluation of Insect Resistant Cotton Lines Containing Genes Expressing the *Bt* Protein.

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. In-season monitoring occurred on July 2, August 3, September 2 and October 5, 1998.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility. In-season monitoring occurred on July 2, August 3, September 2 and October 5, 1998.

**Field Monitoring for Plant Growth Characteristics:** No differences were noted in the general appearance and growth of the transgenic and nontransgenic plants. In-season monitoring occurred on July 2, August 3, September 2 and October 5, 1998.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. In-season monitoring occurred on July 2, August 3, September 2 and October 5, 1998.

**Monitoring for Volunteers:** Monitoring for volunteers was conducted on October 30, 1998, thousands of volunteers were observed and the area was disked and chisel plowed. On November 30, 1998, hundreds of volunteers were observed, 10-20 per row foot. The plot area was disked. On December 30, 1998, no volunteers were observed. A heavy rainfall occurred in October, but no irrigation was needed.

1998 Cotton Field Trial Report  
USDA #98-085-19N      Monsanto #98-166XR

November 3, 2000

Monsanto Company

Location

County

State

CBI DELETED

Pinal

AZ

Pinal County, AZ

Planting Date: June 2, 1998

Harvest Date: November 18, 1998

Vector Construct: PV-GHBK13 (Line 16120)

PV-GHBK11 (Lines 15813, 15835, 15985, 16019, 16072)

Actual Lines Planted: 15813, 15985, DP50B, DP50

Purpose of Field Trial: Evaluation of Insect Protected Cotton Lines

Field Monitoring for Disease Susceptibility: There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines.

Field Monitoring for Insect Susceptibility: No differences were noted between the transgenic lines and their respective parent for insect susceptibility.

Field Monitoring for Plant Growth Characteristics: It appeared that the DP50 plants were deficient (in growth and appearance) when compared to the transgenic lines, although the differences were not statistically significant.

Field Monitoring for Weediness Characteristics: The germination of transgenic plants was no different from non-transgenic plants.

1999 Cotton Field Trial Report  
USDA #99-057-05n Monsanto #99-143XRAB

November 3, 2000

Monsanto Company

Location

CBI DELETED

County

Pima  
Pinal  
Graham  
Yuma

State

AZ  
AZ  
AZ  
AZ

CBI DELETED

Planting Date: April 23, 1999  
Harvest Date: November 16, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 21, June 1, July 1, July 14, August 3, August 24, September 21 and October 28, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 21, June 1, July 1, July 14, August 3, August 24, September 21 and October 28, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 21, June 1, July 1, July 14, August 3, August 24, September 21 and October 28, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 21, June 1, and July 1, 1999.



1999 Cotton Field Trial Report  
USDA #99-057-05n/Mons #99-143XRAB

[ CBI DELETED ]

Planting Date: April 16, 1999  
Harvest Date: October 14, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 7, May 25, June 11, June 29 and July 28, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 7, May 25, June 11, June 29 and July 28, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 7, May 25, June 11, June 29 and July 28, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 7 and May 25, 1999.

**NOTE:** These plots were checked on August 13, August 27, September 6, September 21 and at harvest on October 14, 1999. There were no differences in disease susceptibility, no differences in non-target insect species, or in the general appearance and growth.

[ CBI DELETED ]

Planting Date: April 23, 1999  
Harvest Date: November 10, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 27, July 2, July 28, September 27 and October 10, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 27, July 2, July 28, September 27 and October 10, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 27, July 2, July 28, September 27 and October 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 27 and July 2, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-057-05n/Mons #99-143XRAB**

[ CBI DELETED ]

Planting Date: April 19, 1999  
Harvest Date: October 19, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 5, May 24, June 3, June 29, August 9, August 31, September 10 and September 20, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 5, May 24, June 3, June 29, August 9, August 31, September 10 and September 20, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 5, May 24, June 3, June 29, August 9, August 31, September 10 and September 20, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 5 and May 24, 1999.

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1999 Cotton Field Trial Report  
USDA #99-061-11n Monsanto #99-097XRAB

November 3, 2000

Monsanto Company

Location

County

State

CBI DELETED

San Patricio

TX

San Patricio County, TX

Planting Date: May 1, 1999

Harvest Date: September 13, 1999

Destruction Date: September 25, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. The trial was monitored on May 28, June 11, July 9, August 4 and September 1, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility. The trial was monitored on May 28, June 11, July 9, August 4 and September 1, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 28, June 11, July 9, August 4 and September 1, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 28, June 11, July 9, August 4 and September 1, 1999.

**Plant Stand:** Observation showed there was a difference in plant stand between the transgenic and the non-transgenic plots. Plant stand count for Line 15813 was lower than the control but considered to be within normal variation for cotton plants. There was no difference in stand count between 15985 and the control.

**Disposition of Seed:** The leftover seed was soaked in gasoline and burned on May 15, 1999.

**General Results of Field Trial:** The two stacked lines had fewer insects/damage than DP50B and DP50. There were not many differences between the stacked lines.

1999 Cotton Field Trial Report  
USDA #99-061-12n Monsanto #99-135XRAB

November 3, 2000

Monsanto Company

Location

CBI DELETED

County

San Patricio  
Hidalgo  
Fort Bend  
Hildago  
Willacy  
Nueces

State

Texas  
Texas  
Texas  
Texas  
Texas  
Texas

San Patricio County, TX

This site was not planted.

Hidalgo County, TX

Planting Date: April 16, 1999

Harvest Date: August 20, 1999

Vector Constructs: PV-GHBK11

Lines Planted: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. This trial was monitored on May 10, June 10 and July 10, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility. This trial was monitored on May 10, June 10 and July 10, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were differences in the general appearance and growth of the transgenic and the non-transgenic plants, but the differences were considered to be within the normal variation range for cotton plants. This trial was monitored on May 10, June 10 and July 10, 1999. The growth was taller in the transgenic than DPL-50.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trial was monitored on May 10, June 10 and July 10, 1999.

1999 Cotton Field Trial Report  
USDA #99-061-13n Monsanto #99-150XRAB

[REDACTED]  
November 3, 2000

Monsanto Company

Location

[ CBI DELETED ]

County

Fresno

State

CA

Fresno County, CA

Planting Date: April 26, 1999

Harvest Date: November 5, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 3, June 30, July 14, July 29 and September 10, 1999.

**Field Monitoring for Insect Susceptibility:** Insect monitoring and controlled evaluations were conducted on a regular basis throughout the season. A beet armyworm bioassay was conducted on cotton leaves sampled on June 24 and July 26. Each evaluation showed high mortality to early instar forms introduced on both Bollgard II plant types, while DP50B, DP50 and an adjacent California Acala cotton standard, Maxxa, showed high worm survival on both dates.

On August 10, 1999 field inspection, beet armyworm hits on leaves were counted and found to be greatest on DP50 while Maxxa and DP50B showed lower but still moderate feeding on plants. Very low worm feeding was observed in 15813 and 15985.

Pink bollworm was not shown to be a significant problem at this field site and is not currently a major California cotton pest. Of the nearly 5,000 boll samples, only one was shown to have bollworm feeding. Nearby pink bollworm traps confirmed the near absence of this pest from these trials.

Overall, no consistent differences were observed in cotton insect pests during the season.

1999 Cotton Field Trial Report  
USDA #99-061-14n Monsanto #99-152XRAB

November 3, 2000

Monsanto Company

Location

CBI DELETED

County

Austin  
Fort Bend

State

Texas  
Texas

Austin County, TX

Planting Date: April 21, 1999

Harvest Date: September 10, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** The trial was monitored during the season. There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines.

**Field Monitoring for Insect Susceptibility:** The trial was monitored during the season. Lines 15813 and 15985 exhibited very good loopier control when compared to the DP50B.

**Field Monitoring for Plant Growth Characteristics:** The trial was monitored during the season. Appearance and growth were like the DPL50. However, 15813 matured earlier and 15985 was slower in maturing when compared to DPL50. The maturity difference noted was considered to be within the normal variation range for cotton plants.

**Field Monitoring for Weediness Characteristics:** The trial was monitored during the season. No differences were observed in weediness characteristics of the transgenic plants compared to the non-transgenic plants.

**Plant Stand:** The trial was monitored during the season. The quality of the DPL50 seed provided was of very poor quality.

**Destruct Date and Method:** The material left after harvest was shredded and stalks were pulled.

**Monitoring for Volunteers:** The trial area was monitored on November 9 and December 17, 1999 and on January 12 and February 17, 2000. No volunteers were observed. The harvest as well as the fall and early winter were unseasonably dry. The test site had been shredded and then plowed.

1999 Cotton Field Trial Report  
USDA #99-061-15n Monsanto #99-151XRAB

November 3, 2000

Monsanto Company

Location  
CBI DELETED

County

Ellis  
Ellis

State

Texas  
Texas

CBI DELETED

Planting Date: April 21, 1999  
Harvest Date: August 25, 1999  
Vector Construct: PV-GHBK11  
Lines: 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Plant Growth Characteristics:** During the monitoring observation on April 29, 1999, line number 15985 expressed more vigor in the transgenic line. During the monitoring observations on May 12, May 26 and June 9, 1999, there were no differences in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Plant Stand:** During the monitoring observation on April 29, 1999, the plant stand was 43,500 for the transgenic plants and 44,500 for the non-transgenic plants. Observations on May 12, May 26 and June 9, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On August 30, 1999, the trial area was shredded and plowed.

**Disposition of Seeds:** The seeds were buried 1-1/2 feet in the plot area.

**1999 Cotton Field Trial Report**  
**USDA #99-061-15n/Mons #99-151XRAB**

**General Results of Field Trial:** Mean seasonal fruit injury from bollworm was significantly higher in conventional non-BollGard DP50. No significant fruit injury between BollGard DP50 and BollGard II 15985. The highest yield was observed from BollGard II 15985.

**CBI DELETED**

**Planting Date:** April 19, 1999  
**Harvest Date:** August 27, 1999  
**Vector Construct:** PV-GHBK11  
**Lines:** 15813, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Plant Growth Characteristics:** During the monitoring observation on April 29, 1999, line number 15813 expressed more vigor in the transgenic line. During the monitoring observations on May 12, May 26 and June 9, 1999, there were no differences in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Plant Stand:** During the monitoring observation on May 12, 1999, the plant stand was 45,500 for the transgenic plants and 47,000 for the non-transgenic plants. Observations on April 29, May 26 and June 9, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On September 7, 1999, the trial area was shredded and plowed.

**Disposition of Seeds:** The seeds were buried 1-1/2 feet in the plot area.

**General Results of Field Trial:** Mean seasonal fruit injury from bollworm was significantly higher in conventional non-BollGard DP50. No significant fruit injury between BollGard DP50 and BollGard II 15813. The highest yield was observed from BollGard II 15813.



1999 Cotton Field Trial Report  
USDA #99-071-15n Monsanto #99-223XRAB

[REDACTED]  
November 3, 2000

Monsanto Company

Location

[ CBI DELETED ]

County

Pinal

State

AZ

Pinal County, AZ

Planting Date: April 23, 1999

Harvest Date: October 13, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

1999 Cotton Field Trial Report  
USDA #99-095-19n Monsanto #99-262XR

November 3, 2000

Monsanto Company

Location

CBI DELETED

County

Pinal

Pima

Bolivar

State

Arizona

Arizona

Mississippi

Pinal County, AZ

Planting Date: Mid-June, 1999

Harvest Date: January 5, 2000

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored in June, July, August, and September, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were observed between the transgenic plants and the non-transgenic plants when the trial was monitored in June, July, and August, 1999. No differences between transgenic plants and non-transgenic plants were observed when monitoring occurred in September, 1999. In June, 1999, the transgenic plants had a moderate amount of fleahoppers. During the July, 1999 monitoring, Lygus was observed. During August, 1999, whitefly, moderate to heavy, was observed in the transgenic plants compared to the non-transgenic plants.

**Field Monitoring for Plant Growth Characteristics:** No differences were observed between the transgenic plants and the non-transgenic plants when the trial was monitored in June, July, August, and September, 1999. The cotton was very largated due to weather and insect pressure and the late planting date. There was heavy square shed through August, 1999.

**Field Monitoring for Weediness Characteristics:** No differences were observed in weediness characteristics when the transgenic plants were compared to the non-transgenic plants. The trial was monitored for weediness characteristics during, June, July, August, and September, 1999.

**Plant Stand:** No differences were observed in plant stand between the transgenic plants and the non-transgenic plants. The trial was monitored for plant stand during June, July, August, and September, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-095-19n/Mons #99-262XRAB**

**Destruct Date and Method:** January 15, 2000. Gin trash returned to field and plowed under.

**Disposition of Seeds:** Seed from trial was provided to ☐ ☐ CBI DELETED ☐

**Pima County, AZ**

**Planting Date:** May 9-May 16, 1999

**Harvest Date:** December 3, 1999

**Vector Construct:** PV-GHBK11

**Lines:** 15813, 15985

**Field Monitoring for Disease Susceptibility:** No differences in disease susceptibility between the transgenic plants and non-transgenic plants were observed. This trait was monitored in June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Field Monitoring for Insect Susceptibility:** No differences in insect susceptibility between transgenic plants and non-transgenic plants were observed. Monitoring occurred on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Field Monitoring for Plant Growth Characteristics:** No differences were observed in plant growth characteristics between the transgenic plants and the non-transgenic plants. Monitoring occurred on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Field Monitoring for Weediness Characteristics:** No differences were observed in weediness characteristics when the transgenic plants were compared to the non-transgenic plants. The trial was monitored for weediness characteristics on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Plant Stand:** No differences were observed in plant stand between the transgenic plants and the non-transgenic plants. The trial was monitored for plant stand on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Destruct Method:** By stalk cutter.

**Disposition of Seeds:** Seed from trial was provided to ☐ ☐ CBI DELETED ☐

1999 Cotton Field Trial Report  
USDA #99-102-18n Monsanto #99-249XRAB

November 3, 2000

Monsanto Company

Location

County

State

CBI DELETED

Franklin

Louisiana

CBI DELETED

Franklin

Louisiana

CBI DELETED

Franklin

Louisiana

CBI DELETED

Franklin

Louisiana

CBI DELETED

Bossier

Louisiana

CBI DELETED

Bossier

Louisiana

CBI DELETED

Tensas

Louisiana

CBI DELETED

Rapides

Louisiana

CBI DELETED

Morehouse

Louisiana

CBI DELETED

Bolivar

Mississippi

CBI DELETED

Sharkey

Mississippi

CBI DELETED

Washington

Mississippi

CBI DELETED

Washington

Mississippi

CBI DELETED

Bolivar

Mississippi

CBI DELETED

Leflore

Mississippi

CBI DELETED

Tate

Mississippi

CBI DELETED

Coahoma

Mississippi

CBI DELETED

Grenada

Mississippi

CBI DELETED

Washington

Mississippi

CBI DELETED

Rankin

Mississippi

CBI DELETED

Oktibbeha

Mississippi

CBI DELETED

Holmes

Mississippi

CBI DELETED

Oktibbeha

Mississippi

CBI DELETED

Jackson

Oklahoma

CBI DELETED

Jackson

Oklahoma

CBI DELETED

City of Suffolk

Virginia

CBI DELETED

City of Suffolk

Virginia

CBI DELETED

City of Suffolk

Virginia

1999 Cotton Field Trial Report  
USDA #99-102-19n      Monsanto #99-279XRAB

November 3, 2000

Monsanto Company

Location

CBI DELETED

County

Bossier

State

Louisiana

Bossier County, LA

Planting Date: May 21, 1999

Harvest Date: October 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. In observations on June 4, 1999, noted that less than 3% of the transgenic and the non-transgenic plants damping off occurred field wide, the percentage is small. Observations on July 7 and August 5 noted no differences. In observations on September 10, 1999 there were no differences noted, but a small amount of boll rot, less than 2% appeared fairly uniform in all plots.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. In observations on June 4, 1999, noted that less than 3% of the transgenic and the non-transgenic plants had cutworm/armyworm damage field wide. Observations on July 7, August 5 and September 10, 1999 noted no differences in the plants.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 4, July 7, August 5 and September 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 4, July 7, August 5 and September 10, 1999.

**Plant Stand:** Observations on June 4, 1999 noted that line 15813 7-DAP plants stand low but 14-DAP plant stands similar to 15985 and control. The transgenic and the non-transgenic plots observed on July 7, August 5 and September 10, 1999 noted no differences in plant stand.

**Destruct Date and Method:** The remaining stalks were cut and disked on October 14, 1999.

1999 Cotton Field Trial Report  
USDA #99-102-20n      Monsanto #99-278XRAB

November 3, 2000

Monsanto Company

<u>Location</u>	<u>County</u>	<u>State</u>
Monsanto Agronomy Center	Baldwin	AL
CBI DELETED	Pinal	AZ
Monsanto Agronomy Center	Washington	MS
CBI DELETED	Florence	SC
	San Patricio	TX

**Baldwin County, AL**

Planting Date: May 18, 1999

Harvest Date: October 14, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, 50, 50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines when this field trial was monitored on June 15, July 7, August 3 and September 28, 1999. During the observation on August 31, 1999, boll rot was in all the plots.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility when this field trial was monitored on June 15, July 7 and September 28, 1999. During the observations on August 3 and August 31, 1999, insect damage was higher on the non-transgenic plants.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 15, July 7, August 3, August 31 and September 28, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. All plots emerged at the same time. The trial was monitored on June 15, 1999.

**Plant Stand:** The transgenic and the non-transgenic plots have equal plant stands. This observation was made on June 15, 1999.

**Destruct Date and Method:** The remaining stalks were shredded on October 20, 1999.

1999 Cotton Field Trial Report  
USDA #99-102-21n      Monsanto #99-277XRAB

November 3, 2000

Monsanto Company

Location

CBI DELETED

County

State

Perquimans	North Carolina
Washington	North Carolina
Bertie	North Carolina
Onslow	North Carolina
Edgecombe	North Carolina
Edgecombe	North Carolina
Sampson	North Carolina
Perquimans	North Carolina
Barnwell	South Carolina
Lee	South Carolina
Darlington	South Carolina
Barnwell	South Carolina
Hampton	South Carolina
Marlboro	South Carolina
Darlington	South Carolina
Florence	South Carolina
Shelby	Tennessee
Hardeman	Tennessee
Fayette	Tennessee
Madison	Tennessee
Pecos	Texas
Tom Green	Texas
Lubbock	Texas
Hale	Texas
Lubbock	Texas

1999 Cotton Field Trial Report  
USDA #99-102-22n      Monsanto #99-248XRAB

November 3, 2000

Monsanto Company

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED	Limestone	Alabama
	Limestone	Alabama
	Lee	Alabama
	Baldwin	Alabama
	Autauga	Alabama
	Autauga	Alabama
	Macon	Alabama
	Jackson	Arkansas
	Desha	Arkansas
	Jefferson	Arkansas
	Pinal	Arizona
	Pinal	Arizona
	Pinal	Arizona
	Fresno	California

Limestone County, AL

Planting Date: May 19, 1999

Harvest Date: October 8 and October 21, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 2, July 1, July 30, August 30 and October 1, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring on June 2, 1999, no insects observed in any plots; July 1, 1999, no eggs or worms, Lygus damage <10%, aphids light, no differences among transgenics; July 30, 1999, 8% eggs in all plots, 2% worms in non-transgenics, predominantly corn earworm; August 30, 1999, eggs <2%, no worms in any of the plots, western flower thrips throughout; October 1, 1999, few insects in any plot.



1999 Cotton Field Trial Report  
USDA #99-102-22n/Mons #99-248XRAB

[ CBI DELETED ] Pinal County, AZ

This field trial was not conducted.

[ CBI DELETED ] Pinal County, AZ

This field trial was not conducted.

[ CBI DELETED ] Pinal County, AZ

This field trial was not conducted.

[ CBI DELETED ] Fresno County, CA

This field trial was not conducted.

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<u>County</u>	<u>State</u>
Jackson	Florida
Santa Rosa	Florida
Dodge	Georgia
Pulaski	Georgia
Tift	Georgia
Decatur	Georgia
Seminole	Georgia
Terrell	Georgia
Tift	Georgia
Tift	Georgia
Decatur	Georgia
Burke	Georgia
Sumter	Georgia
Mitchell	Georgia
Pemiscot	Missouri
Washington	Mississippi

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n      Monsanto #99-321XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

<u>Location</u>	<u>County</u>	<u>State</u>
[REDACTED] CBI DELETED	Poinsett	Arkansas
	Monroe	Arkansas
	Mississippi	Arkansas
	Burke	Georgia
	Martin	North Carolina
	Johnston	North Carolina
	Edgecombe	North Carolina
	Washington	North Carolina
	Gibson	Tennessee
	Madison	Tennessee

**Poinsett County, AR**

Planting Date: May 27, 1999

Harvest Date: October 6, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 21, July 15, August 7, August 27 and September 22, 1999.

**Field Monitoring for Insect Susceptibility:** Monitoring observation on July 15, 1999, noted that there were aphids across the whole trial. They were treated with 2.5 oz Provado/A. Observations on June 21, August 7, August 27 and September 22, 1999, noted there were no differences between the transgenic lines and their respective non-transgenic lines for insect susceptibility.

**Field Monitoring for Plant Growth Characteristics:** During the monitoring observation on July 15, 1999, one row of DPL50 got Dirextmsma Drift. During the monitoring observations on June 21, August 7, August 27 and September 22, 1999, there were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 21, July 15, August 7, August 27 and September 22, 1999.

1999 Cotton Field Trial Report  
USDA #99-110-22n Monsanto #99-326XRAB

November 3, 2000

Monsanto Company

Location

County

State

CBI DELETED

Washington

MS

Washington County, MS

This site was not planted.

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**1999 Cotton Field Trial Report**  
**USDA #99-061-12n/Mons #99-135XRAB**

**Plant Stand:** There were no differences noted in the transgenic vs. non-transgenic plant stand count. This trait was monitored on This trial was monitored on May 10, June 10 and July 10, 1999.

**Destruct Date and Method:** August 25, 1999.

**Disposition of Seeds:** Buried in the field.

**General Results of Field Trial:** Bollworm tobacco budworms and beet armyworms were all highly susceptible to Bollgard II compared to only tobacco budworm being highly susceptible to Bollgard I and none susceptible to DPL-50.

**Fort Bend County, TX**

This trial was not planted.

**Hidalgo County, TX**

This trial was not planted.

**Willacy County, TX**

**Planting Date:** May 5, 1999  
**Harvest Date:** August 20, 1999  
**Vector Constructs:** PV-GHBK11  
**Lines Planted:** 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the parent lines. This trial was monitored on May 25, June 21, July 13 and August 10, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective parent for insect susceptibility on May 25, 1999. There were differences noted on June 21 1999 (16% transgenic and 12% non-transgenic, boll weevil punctured squares). On July 13, 1999 (75% transgenic and 72% non-transgenic, boll weevil punctured squares). On August 10, 1999 (15% transgenic and 13% non-transgenic, boll weevil punctured bolls).

**1999 Cotton Field Trial Report**  
**USDA #99-061-12n/Mons #99-135XRAB**

**Field Monitoring for Plant Growth Characteristics:** There were differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trial was monitored on May 25, June 21, July 13, and August 10, 1999. In each observation, 100% of the non-transgenic plants were slightly shorter than the transgenic plants. This was probably due to a plant population which was greater than planned in the transgenic plots due to planter problems. The observed height differences were considered to be within the normal variation range for cotton.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trial was monitored on May 25, June 21, July 13 and August 10, 1999.

**Plant Stand:** There were differences noted in the transgenic vs. the non-transgenic plant stand count. This trait was monitored on May 25, 1999 and found that there were 65,000 transgenic plants and 45,500 non-transgenic plants. Double herbicide applications may have caused the differences. Observation on July 13, 1999 noted no change in appearance.

**Destruct Date and Method:** September 14, 1999, buried on the test site.

**Disposition of Seeds:** September 14, 1999, buried on the test site.

**Nueces County, TX**

This trial was not planted.

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**1999 Cotton Field Trial Report**  
**USDA #99-061-13n      Monsanto #99-150XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

**Location**

[ CBI DELETED ]

**County**

Fresno

**State**

CA

**Fresno County, CA**

Planting Date: April 26, 1999

Harvest Date: November 5, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 3, June 30, July 14, July 29 and September 10, 1999.

**Field Monitoring for Insect Susceptibility:** Insect monitoring and controlled evaluations were conducted on a regular basis throughout the season. A beet armyworm bioassay was conducted on cotton leaves sampled on June 24 and July 26. Each evaluation showed high mortality to early instar forms introduced on both Bollgard II plant types, while DP50B, DP50 and an adjacent California Acala cotton standard, Maxxa, showed high worm survival on both dates.

On August 10, 1999 field inspection, beet armyworm hits on leaves were counted and found to be greatest on DP50 while Maxxa and DP50B showed lower but still moderate feeding on plants. Very low worm feeding was observed in 15813 and 15985.

Pink bollworm was not shown to be a significant problem at this field site and is not currently a major California cotton pest. Of the nearly 5,000 boll samples, only one was shown to have bollworm feeding. Nearby pink bollworm traps confirmed the near absence of this pest from these trials.

Overall, no consistent differences were observed in cotton insect pests during the season.

**Cotton Field Trial Report**  
**USDA #99-061-13n/Mons #99-150XRAB**

**Field Monitoring for Plant Growth Characteristics:** During late April, a cone planter was used to plant the four seed varieties including DP50, DP50B, 15813 and 15985 with final plant stands of 19,625, 62,875, 66,125 and 65,750 respectively. Early plant map data showed slight increases in seedling development for DP50 with 5.1 vegetative nodes developed compared to 3.3, 3.8 and 3.9 for 15813, 15985 and DP50B respectively. These differences continued through the June 30 plant mapping when approximately two additional nodes of growth were detected. As the season progressed, these differences in development remained. The lack of shading and improved nutrient and water status in DP50 that resulted from low plant stand numbers may explain these increases in node number for DP50. Low densities may also explain the reduced Height to Node Ratio Index (HNRI) that was observed on most sampling dates. Final plant map data similarly showed a 1.3 to 2.8 node increase in the 95% zone for first position bolls contributing to DP50 yield. Low plant densities were, therefore, thought to be associated with the decreased earliness of this variety.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 3, June 30, July 14, July 29 and September 10, 1999.

**Method of Destruction:** On November 7, 1999, the plot area was disk incorporated and burned.

**Disposition of Seeds:** There was no leftover seed.

**General Results of Field Trial:** Overall, the tests were successful and the value of Bollgard II in controlling a significant beet armyworm population was demonstrated. These trials also found that the genetic material used for gene insertions is at least as good, agronomically as DP50B and DP50. The only significant problem encountered during the field trial was a lack of seedling emergence in DP50 seed.



**1999 Cotton Field Trial Report**  
**USDA #99-061-14n      Monsanto #99-152XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED ]	Austin Fort Bend	Texas Texas

**Austin County, TX**

Planting Date: April 21, 1999

Harvest Date: September 10, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** The trial was monitored during the season. There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines.

**Field Monitoring for Insect Susceptibility:** The trial was monitored during the season. Lines 15813 and 15985 exhibited very good loop control when compared to the DP50B.

**Field Monitoring for Plant Growth Characteristics:** The trial was monitored during the season. Appearance and growth were like the DPL50. However, 15813 matured earlier and 15985 was slower in maturing when compared to DPL50. The maturity difference noted was considered to be within the normal variation range for cotton plants.

**Field Monitoring for Weediness Characteristics:** The trial was monitored during the season. No differences were observed in weediness characteristics of the transgenic plants compared to the non-transgenic plants.

**Plant Stand:** The trial was monitored during the season. The quality of the DPL50 seed provided was of very poor quality.

**Destruct Date and Method:** The material left after harvest was shredded and stalks were pulled.

**Monitoring for Volunteers:** The trial area was monitored on November 9 and December 17, 1999 and on January 12 and February 17, 2000. No volunteers were observed. The harvest as well as the fall and early winter were unseasonably dry. The test site had been shredded and then plowed.

**1999 Cotton Field Trial Report**  
**USDA #99-061-14n/Mons #99-152XRAB**

**Fort Bend County, TX**

Planting Date: May 17, 1999

Harvest Date: September 14, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. A single plant was found in transgenic line 15985 plot which expressed several symptoms of copper top.

**Field Monitoring for Insect Susceptibility:** The trial was monitored during the season. Lines 15813 and 15985 exhibited very good looper control when compared to the DPL 50B.

**Field Monitoring for Plant Growth Characteristics:** Appearance and growth were like the non-transgenic. However, line 15813 matured earlier than the DPL50 while the 15985 could be labeled as a slower maturing variety when compared to DPL50.

**Field Monitoring for Weediness Characteristics:** The quality of the DPL50 seed provided for this trial was of poor quality.

**Monitoring for Volunteers:** The trial area was monitored on November 11 and December 21, 1999 and January 20, 2000. No volunteers were observed. The harvest as well as the fall and early winter were unseasonably dry. The test site had been shredded and then plowed. The site was then disked twice. An early winter application of Atrazine was applied.

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1999 Cotton Field Trial Report  
USDA #99-061-15n      Monsanto #99-151XRAB

[REDACTED]  
November 3, 2000

Monsanto Company

Location

County

State

[ CBI DELETED

Ellis  
Ellis

Texas  
Texas

Ellis County, TX

Planting Date: April 21, 1999

Harvest Date: August 25, 1999

Vector Construct: PV-GHBK11

Lines: 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Plant Growth Characteristics:** During the monitoring observation on April 29, 1999, line number 15985 expressed more vigor in the transgenic line. During the monitoring observations on May 12, May 26 and June 9, 1999, there were no differences in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Plant Stand:** During the monitoring observation on April 29, 1999, the plant stand was 43,500 for the transgenic plants and 44,500 for the non-transgenic plants. Observations on May 12, May 26 and June 9, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On August 30, 1999, the trial area was shredded and plowed.

**Disposition of Seeds:** The seeds were buried 1-1/2 feet in the plot area.

**1999 Cotton Field Trial Report**  
**USDA #99-061-15n/Mons #99-151XRAB**

**General Results of Field Trial:** Mean seasonal fruit injury from bollworm was significantly higher in conventional non-BollGard DP50. No significant fruit injury between BollGard DP50 and BollGard II 15985. The highest yield was observed from BollGard II 15985.

**Stanley Praslicka Farm, Ellis County, TX**

**Planting Date:** April 19, 1999

**Harvest Date:** August 27, 1999

**Vector Construct:** PV-GHBK11

**Lines:** 15813, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Field Monitoring for Plant Growth Characteristics:** During the monitoring observation on April 29, 1999, line number 15813 expressed more vigor in the transgenic line. During the monitoring observations on May 12, May 26 and June 9, 1999, there were no differences in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on April 29, May 12, May 26 and June 9, 1999.

**Plant Stand:** During the monitoring observation on May 12, 1999, the plant stand was 45,500 for the transgenic plants and 47,000 for the non-transgenic plants. Observations on April 29, May 26 and June 9, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On September 7, 1999, the trial area was shredded and plowed.

**Disposition of Seeds:** The seeds were buried 1-1/2 feet in the plot area.

**General Results of Field Trial:** Mean seasonal fruit injury from bollworm was significantly higher in conventional non-BollGard DP50. No significant fruit injury between BollGard DP50 and BollGard II 15813. The highest yield was observed from BollGard II 15813.

1999 Cotton Field Trial Report  
USDA #99-071-15n      Monsanto #99-223XRAB

November 3, 2000

Monsanto Company

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED ]	Pinal	AZ

**Pinal County, AZ**

Planting Date: April 23, 1999

Harvest Date: October 13, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 24, June 25, July 23, August 25 and September 23, 1999.

1999 Cotton Field Trial Report  
USDA #99-095-19n      Monsanto #99-262XR

November 3, 2000

Monsanto Company

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED ]	Pinal	Arizona
	Pima	Arizona
	Bolivar	Mississippi

**Pinal County, AZ**

Planting Date: Mid-June, 1999  
Harvest Date: January 5, 2000  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored in June, July, August, and September, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were observed between the transgenic plants and the non-transgenic plants when the trial was monitored in June, July, and August, 1999. No differences between transgenic plants and non-transgenic plants were observed when monitoring occurred in September, 1999. In June, 1999, the transgenic plants had a moderate amount of fleahoppers. During the July, 1999 monitoring, Lygus was observed. During August, 1999, whitefly, moderate to heavy, was observed in the transgenic plants compared to the non-transgenic plants.

**Field Monitoring for Plant Growth Characteristics:** No differences were observed between the transgenic plants and the non-transgenic plants when the trial was monitored in June, July, August, and September, 1999. The cotton was very largated due to weather and insect pressure and the late planting date. There was heavy square shed through August, 1999.

**Field Monitoring for Weediness Characteristics:** No differences were observed in weediness characteristics when the transgenic plants were compared to the non-transgenic plants. The trial was monitored for weediness characteristics during, June, July, August, and September, 1999.

**Plant Stand:** No differences were observed in plant stand between the transgenic plants and the non-transgenic plants. The trial was monitored for plant stand during June, July, August, and September, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-095-19n/Mons #99-262XRAB**

**Destruct Date and Method:** January 15, 2000. Gin trash returned to field and plowed under.

**Disposition of Seeds:** Seed from trial was provided to Delta Pine Land.

**Pima County, AZ**

**Planting Date:** May 9-May 16, 1999

**Harvest Date:** December 3, 1999

**Vector Construct:** PV-GHBK11

**Lines:** 15813, 15985

**Field Monitoring for Disease Susceptibility:** No differences in disease susceptibility between the transgenic plants and non-transgenic plants were observed. This trait was monitored in June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Field Monitoring for Insect Susceptibility:** No differences in insect susceptibility between transgenic plants and non-transgenic plants were observed. Monitoring occurred on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Field Monitoring for Plant Growth Characteristics:** No differences were observed in plant growth characteristics between the transgenic plants and the non-transgenic plants. Monitoring occurred on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Field Monitoring for Weediness Characteristics:** No differences were observed in weediness characteristics when the transgenic plants were compared to the non-transgenic plants. The trial was monitored for weediness characteristics on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Plant Stand:** No differences were observed in plant stand between the transgenic plants and the non-transgenic plants. The trial was monitored for plant stand on June 17, July 13, August 26, September 24, October 26, and November 27, 1999.

**Destruct Method:** By stalk cutter.

**Disposition of Seeds:** Seed from trial was provided to Delta Pine Land.

**1999 Cotton Field Trial Report**  
**USDA #99-095-19n/Mons #99-262XRAB**

**Bolivar County, MS**

Planting Date: May 13, 1999 (line 15813), May 15, 1999 (line 15985)

Harvest Date: October 1, 2, 3, 1999 (lines 15813 and 15985)

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored from May 24, 1999 through November 1, 1999.

**Field Monitoring for Insect Susceptibility:** No noticeable difference observed between adjoining fields and other varieties for secondary pest. Lepidopteran control was far superior to other fields. This trait was monitored from May 24, 1999 through November 1, 1999.

**Field Monitoring for Plant Growth Characteristics:** Plant emergence, rate of growth, fruiting and retention were normal for both non-irrigated and irrigated. This trait was monitored from May 24, 1999 through November 1, 1999.

**Field Monitoring for Weediness Characteristics:** Plants were plant mapped on a weekly basis from emergence to crop termination with no noticeable irregular characteristics. This trait was monitored from May 24, 1999 through November 1, 1999.

**Plant Stand:** Planted 60,000 seeds/acre with 49,800 plants/acre emerging. Standcount made at same transect locations with an average plant population of 48,00 plants per acre. On May 24, 1999, the standcount for the transgenic plants was 49,800. On June 10, 1999, the standcount for the transgenic plants was 48,100.

**General Results of Field Trial:** Very good. Lepidopteran control was superior to other farm fields which were monitored. Yields were good considering weather problems. No noticeable problems from secondary pest (examples, plant bugs, aphids, white fly). Growth, fruiting, maturity, crop termination, and fiber quality were monitored closely with satisfactory results.



**1999 Cotton Field Trial Report**  
**USDA #99-102-18n      Monsanto #99-249XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

**Location**

**County**

**State**

[ CBI DELETED

Franklin	Louisiana
Franklin	Louisiana
Franklin	Louisiana
Franklin	Louisiana
Bossier	Louisiana
Bossier	Louisiana
Tensas	Louisiana
Rapides	Louisiana
Morehouse	Louisiana
Bolivar	Mississippi
Sharkey	Mississippi
Washington	Mississippi
Washington	Mississippi
Bolivar	Mississippi
Leflore	Mississippi
Tate	Mississippi
Coahoma	Mississippi
Grenada	Mississippi
Washington	Mississippi
Rankin	Mississippi
Oktibbeha	Mississippi
Holmes	Mississippi
Oktibbeha	Mississippi
Jackson	Oklahoma
Jackson	Oklahoma
City of Suffolk	Virginia
City of Suffolk	Virginia
City of Suffolk	Virginia

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Franklin County, LA (1)**

Planting Date: June 2, 1999

Harvest Date: October 28, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 15, July 7, August 12 and September 9, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 15, July 7, August 12 and September 9, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 15, July 7, August 12 and September 9, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 15, July 7, August 12 and September 9, 1999.

**Plant Stand:** There was no significant difference in plant stand between the transgenic and non-transgenic lines.

**Destruct Date and Method:** On October 28, 1999, the trial area was mowed and disked.

**Disposition of Seeds:** Buried in the field at the test site.

**General Results of Field Trial:** Lines 15985 and 15813 provided acceptable control of tobacco budworm equal to that of DP50B. Lines 15985 and 15813 provided significantly greater control of soybean looper and beet armyworm compared to DP50B. Seed cotton yields were equal to or greater for lines 15985 and 15813 compared to DP50B.

**Monitoring for Volunteers:** Observations were made on December 1, 1999 and on January 15, 2000. There were no volunteers observed.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Franklin County, LA (2)**

Planting Date: May 25, 1999

Harvest Date: October 17, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 3, June 26, July 10, July 21, August 7 and September 1, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 3, June 26, July 10, July 21, August 7 and September 1, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 3, June 26, July 10, July 21, August 7 and September 1, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 3, June 26, July 10, July 21, August 7 and September 1, 1999.

**Plant Stand:** Monitoring observations on June 3, June 26, July 10, July 21, August 7, September 1 and October 3, 1999 did not observe any differences in plant stand.

**Monitoring for Volunteers:** Observations were made on November 2, December 16, 1999 and January 2, 2000. There were no volunteers observed.

**Franklin County, LA (3)**

This field trial was not planted.

**Franklin County, LA (4)**

This field trial was not planted.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Bossier County, LA (1)**

Planting Date: June 7, 1999

Harvest Date: October 14, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 27 and August 13, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility in observations on July 27, 1999, 2.5% of the non-transgenics were affected with *Heliothis* spp. , damaged squares; August 3, 1999, 27.5% of the non-transgenics were affected; August 6, 1999 5% of the transgenics and 32.5% of non-transgenics were affected; there were no differences noted on August 9, 1999; and on August 13, 1999, 7.5% of the non-transgenics were affected; observations on August 16, 1999, 12.5% of the non-transgenics were affected with *Heliothis* spp. , damaged squares; August 19, 1999, 7.5% of the non-transgenics were affected; August 23, 1999 5% of the transgenics and 27.5% of non-transgenics were affected; there were no differences noted on August 27, 1999 and on August 31, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on October 27, 1999. Plant height at harvest for transgenic line 15813 is 28.0; for transgenic 15985, it is 28.3; for non-transgenic line DP50B, it is 27.7; and for non-transgenic line DP50 it is 28.2.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on July 27 and August 13, 1999.

**Plant Stand:** Monitoring observation on October 27, 1999 noted that the transgenic plant standcount for line 15813 was 3.5 and for line 15985, it was 3.2. The non-transgenic standcount was 3.4 for DP50B and 3.3 for DP50..

**Destruct Date and Method:** On October 28, 1999, cut stalks and field area disked.

**Disposition of Seeds:** Disked under to allow natural disposition.

**General Results of Field Trial:** The insect pressure in 1999 was extremely low in regard to the bollworm and budworm. Deltapine 50 was treated when a threshold of 3 to 5% bollworm/budworm in terminal was found. On August 13, 1999, all plots were treated with Provado for aphids. Due to the planting date, yields were not what they could have been due to a lack of rain from mid to later in the season. M15813 appeared to be later maturing than M15985.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Bossier County, LA (2)**

Planting Date: May 21, 1999  
Harvest Date: October 12, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 11 (dumping off <2% all plots), July 7, August 5 and September 10, 1999 (small, <2%, amount of boll rot across all plots).

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility, but a slight armyworm/cutworm feeding was noted across the entire field in observations on June 11, 1999. Additional dates of observations were July 7, August 5 and September 10, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 11, July 7, August 5 and September 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 11, July 7, August 5 and September 10, 1999.

**Plant Stand:** Monitoring observation on June 11, July 7, August 5 and September 10, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On October 14, 1999, cut stalks and field area disked.

**Disposition of Seeds:** Left in field and disked.

**General Results of Field Trial:** The field trial was successful. Nothing unusual was noted with either 15813 or 15985. In the sprayed main plots, DP50 yielded significantly more seed cotton on a per acre basis compared to 15813. In the non-sprayed main plots, strain 15985 out-yielded the other three strains by a statistically significant margin.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Tensas County, LA**

Planting Date: June 2, 1999

Harvest Date: October 22, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B

**Field Monitoring for Disease Susceptibility:** There were no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. Observations on June 24, 1999 noted there 1-2% of plants affected in the transgenic plot and 1-2% of plants affected in the non-transgenic plot. There was limited seedling disease and all entries responded similarly. Observations on August 13, 1999 noted there were 5% of plants affected in the transgenic plot and 5% of plants affected in the non-transgenic plot. Fusarium wilt was detected, all entries were similar.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Observations on June 24, 1999 noted thrips, <5 per plant on 100% of the transgenic plants and 100% of the non-transgenic plants. - Observations on July 14, 1999, noted tarnished plant bugs, >25 per 100 sweeps on 90% of the transgenic plants and 90% of the non-transgenic plants. Observations on August 13, 1999 noted mixed bollworm and budworm, few beet armyworms on <20% of transgenic plants and ~50% of the non-transgenic plants. Observations on August 17, 1999 noted the same as the observation on August 13, 1999. Observations on September 27, 1999 noted beet armyworms and soybean loopers on 100% of the transgenic plants and on 100% of the non-transgenic plants.

**Field Monitoring for Plant Growth Characteristics:** This trait was monitored on July 14 (first fruiting node and height and number of nodes), July 30 (height and number of nodes), August 10 (nodes above white flower) and October 22, 1999 (machine harvested for yield). Comments on general results of the field trial (see below) noted that the transgenic lines were very similar in growth habit to the non-transgenic lines.

**Field Monitoring for Weediness Characteristics:** This trait was not observed for this field trial.

**Plant Stand:** Monitoring observations on June 12 and June 30, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On October 28, 1999, clipped stalks, unpicked border and disked.

**Disposition of Seeds:** Disked under to allow natural disposition.

**General Results of Field Trial:** Two new transgenics evaluated were similar in growth habit to DPL50 and 50B. Entry 15985 appeared to be slightly earlier than 15813. Differences in Looper efficacy were noted with the new transgenics resulting in the greatest control and least damage. Bollworm and budworm populations were extremely low and never reached treatment threshold in the non-transgenic entry.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Rapides County, LA**

Planting Date: May 21, 1999

Harvest Date: October 13, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B

**Field Monitoring for Disease Susceptibility:** There were no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 25, July 22, August 24 and September 20, 1999.

**Field Monitoring for Insect Susceptibility:** There were no differences noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 25 (light aphid infestation in all plots, fungus present); July 22 (2% of the transgenic plants and 2% of the non-transgenic plants had larvae (Hv/Hz) in terminals); August 24 (50% of the transgenic plants and the 58.6% of the non-transgenic plants; August 24 (4[4]% of the transgenic plants and 8[4]% of the non-transgenic plants had damaged bolls (line Hv/Hz); and September 20, 1999 (no insect pressure).

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 25, July 22, August 24 and September 20, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 25, July 22, August 24 and September 20, 1999.

**Plant Stand:** There were no significant differences in plant stand noted between the transgenic and non-transgenic lines.

**Destruct Date and Method:** On October 16, 1999, bush hogged and tilled into the soil.

**Disposition of Seeds:** Dry heat devitalization (500°F for one hour).

**General Results of Field Trial:** Good

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Morehouse County, LA**

Planting Date: May 7, 1999

Harvest Date: October 13, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 17, July 16, August 16, September 14 and October 13, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 17, July 16, August 16, September 14 and October 13, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 17, July 16, August 16, September 14 and October 13, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 17, July 16, August 16, September 14 and October 13, 1999.

**Plant Stand:** Monitoring observation on June 17, July 16, August 16, September 14 and October 13, 1999 did not observe any differences in plant stand.

**Destruction Method:** Stalk cutter.

**Disposition of Seeds:** Put back on the field.



**1999 Cotton Field Trial Report**  
**USDA #99-102-18/nMons #99-249XRAB**

**Bolivar County, MS**

Planting Date: May 19, 1999

Harvest Date: October 15, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on May 25 (approx. 2% of the transgenic plants and approx. 2% of the non-transgenic plants Rhizotonia was noted); June 15, July 13, August 13 and September 10, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on May 25 (no differences noted), June 15 (no differences noted), July 13 (no differences noted), August 13 (2% of the non-transgenic plants had boll/budworm insects) and September 10, 1999 (2% of the non-transgenic plants had boll/budworm insects).

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on May 25, June 15, July 13, August 13 and September 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on May 25, June 15, July 13, and August 13, 1999.

**Plant Stand:** Monitoring observation on May 25 (all varieties emerged uniformly at approximately 5/ft), June 15, July 13, August 13 and September 10, 1999 did not observe any differences in plant stand..

**Destruct Date and Method:** October 25, 1999 - Bush hogged.

**Disposition of Seeds:** Buried at experimental site.

**General Results of Field Trial:** Insect pressure/infestation level too low to adequately evaluate lines 15813 and 15985.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18/nMons #99-249XRAB**

**Sharkey County, MS**

Planting Date: May 25, 1999

Harvest Date: Not Harvested (see note in additional comments)

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 2, June 16, July 19, August 3, August 24 and September 13, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 2, June 16, July 19, August 3, August 24 and September 13, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 2, June 16, July 19, August 3, August 24 and September 13, 1999.

**Field Monitoring for Weediness Characteristics:** This trait was not observed for this field trial.

**Plant Stand:** Monitoring observation on June 2, June 8 and June 16, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** October 22, 1999 - stalk shredder.

**Disposition of Seeds:** In field destruction.

**Additional Comments:** This trial was not harvested - red-vine and morning glory weed too thick for harvest.

**Washington County, MS (1)**

This field trial was not planted.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Washington County, MS (2)**

Planting Date: May 21, 1999

Harvest Date: October 20, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 4, June 9, June 25, July 15, August 17 and September 7, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 28, 1999 (Hizea, Hyisesceus (very low infestation level); July 22, 1999 (2% of transgenic plants and 2% of non-transgenic shows Lygus lineolaris (light infestation); and August 11, 1999 (insect population negligible).

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 4, June 9, June 25, July 15, August 17 and September 7, 1999.

**Field Monitoring for Weediness Characteristics:** This trait was not observed for this field trial.

**Plant Stand:** Monitoring observation on May 28 and June 4, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** October 20, 1999 - shredded in the field.

**Disposition of Seeds:** The seed was autoclaved on January 27, 2000.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Bolivar County, MS**

Planting Date: May 28, 1999

Harvest Date: October 15, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 1, July 29, August 25, September 16 and October 14, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 1, July 29, August 25, September 16 and October 14, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 1, July 29, August 25, September 16 and October 14, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 1, July 29, August 25, September 16 and October 14, 1999.

**Plant Stand:** Monitoring observations on July 1, July 29, August 25, September 16 and October 14, 1999 did not observe any differences in plant stand.

**Destruction Method:** Stalk Cutter.

**Disposition of Seeds:** Put back on the field.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Leflore County, MS**

Planting Date: May 19, 1999

Harvest Date: September 27, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B, DPL428B

**Field Monitoring for Disease Susceptibility:** There appeared to be no differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 1, July 1, August 11, and September 10, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 1 (some thrips), July 1 (no significant insects), August 11 (bollworms were low in both the transgenic and non-transgenic plants), and September 10, 1999 (no differences noted).

**Field Monitoring for Plant Growth Characteristics:** A difference in the color of the transgenic plants compared to the non-transgenic plants was noted. Plant growth was monitored on June 1 (cotyledon stage), July 1 (8-9 nodes for the transgenic and non-transgenic plants – line 15985 plots were lighter yellow in color than any other lines in the trial), August 11 (2-3 nodes above P1 white bloom in the transgenic and non-transgenic plants), and September 10, 1999 (boll opening stage). The color difference noted was attributed to herbicide injury.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 1, July 1, August 11, and September 10, 1999.

**Plant Stand:** Monitoring observations on June 1, 1999 observed that the plant stand for the transgenic plants was 78-93000/A and the plant stand for non-transgenic plants was 48-52000/A – the transgenic plots were hand thinned; and, July 7, 1999 (the standcount for the transgenic plants was 50-52000/A and the standcount for the non-transgenic plants was 47-55000/A). Differences in stand count observed on June 1 were likely due to use of hand-pushed seeder which was difficult to calibrate. Plots were thinned after emergence to consistent stand.

**Destruction Method and Date:** The plot area was destroyed by shredding and disking on November 17, 1999.

**Disposition of Seeds:** Buried in plot area.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**General Results of Field Trial:** Heliothine populations were extremely low in all plots throughout the season, making detection of differences in efficacy among lines extremely difficult. Heliothine populations were slightly higher in the DPL50 (Non-Bt) variety, but there are no apparent differences between any of the other lines. Lint yield of the two Bollgard II lines was similar to that of the DPL50 and 50B lines, but notably less than that of DPL428B. Gin turnout of the 15985 line was similar to that of the 50 and 50B lines.

During the growing season it was noted that the 15985 line had a lighter green (yellow) foliage color than any of the other lines in the trial. This appeared to be a characteristic of the line, rather than an indication of poor vigor or health. However, on June 7, 1999 it was noted that the 15985 line exhibited symptoms of herbicide injury that were not apparent on the other lines. Consultation with the producer revealed that the plots had been inadvertently over sprayed with an application of MSMA that was being post directed to the surrounding older cotton. The 15985 line clearly showed greater damage as a result of this over spray, suggesting that this line may be more susceptible to this type of injury.

**Monitoring for Volunteers:** The trial was monitored on March 31 and April 27, 2000. No volunteers were found. The trial area was monitored again on May 18, 2000. It was plowed and re-planted to cotton.

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**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Tate County, MS**

Planting Date: May 20, 1999

Harvest Date: October 6, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50, DP428B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 8, July 13 and August 10, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 8, July 13 August 10 and August 25, 1999. Insect pressure for season was very low.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 8, July 13 and August 10, 1999. No unusual or atypical patterns observed.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 8, July 13 and August 10, 1999. Weeds controlled by normal cultural practices of cultivation and herbicides.

**Plant Stand:** Monitoring observations on May 27 and June 3, 1999 observed differences in plant stand. Any differences observed were primarily due to planting. All stands obtained were considered to be commercially acceptable and any plant stand differences were within the normal variation range for cotton plants.

**Destruct Date and Method:** Mowed down on October 15, 1999.

**Disposition of Seeds:** The remaining 15813 and 15985 seeds were buried into the plot area 12" deep on June 18, 1999.

**General Results of Field Trial:** Insect pressure during the entire 1999 season was too low to get any insect data from these plots.

**Monitoring for Volunteers:** Dates of observations were November 8, December 2 (several nights <32°F), December 22, 1999, January 11, February 1, March 4, March 10, April 12, April 29, 2000. On May 17, 2000, we planted the 2000 cotton field in the same area. No volunteers were observed during any of the observations.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18/nMons #99-249XRAB**

**Coahoma County, MS**

Planting Date: May 24, 1999

Harvest Date: October 6, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 7, June 21, July 7, July 29, August 23 and September 9, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 7, June 21, July 7, July 29, August 23 and September 9, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 7, June 21 (no difference in the transgenic or the non-transgenic, but one plant was stunted), July 7, July 29, August 23 and September 9, 1999.

**Field Monitoring for Weediness Characteristics:** This trait was not observed for this field trial.

**Plant Stand:** Monitoring observation on June 7, and June 21, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** October 6, 1999 - on site, shred.

**Disposition of Seeds:** Autoclaved on January 27, 2000.



**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Grenada Country, MS**

Planting Date: May 20, 1999

Harvest Date: October 1, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B, DPL428B

**Field Monitoring for Disease Susceptibility:** There appeared to be no difference in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on May 25 (some seedling disease in all plots), June 8 (some seedling disease in all plots), July 7 (no observable disease problems), and August 11, 1999 (no observable disease problems).

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on May 25 and June 8 (thrips noted in 100% of the transgenic plants and in 100% of the non-transgenic plants), July 7 and August 11, 1999 (bollworms across the plot area).

**Field Monitoring for Plant Growth Characteristics:** No differences were noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on May 25 (70% emerged), June 8 (2.5 nodes), July 7 (10 to 12 nodes), and August 11, 1999 (2 to 3 nodes above white bloom).

**Field Monitoring for Weediness Characteristics:** This trait was monitored on May 25, June 8, July 7 and August 11, 1999. No observations were noted on these dates.

**Plant Stand:** Monitoring observations on May 25, 1999 observed that 70% of the plants had emerged – and observations on June 8, 1999 noted the standcount for the transgenic plants was 40-42000/A and the standcount for the non-transgenic plants was 31-41000/A. Any differences noted in stand count were likely due to use of hand-pushed seeder which was difficult to calibrate. Plots were thinned after emergence to consistent stand.

**Disposition of Seeds:** Buried in plot area.

**Destruct Date and Method:** October 20, 1999 - Mowed to destroy plots.

**General Results of Field Trial:** Heliothine populations were extremely low in all plots throughout the season, making detection of differences in efficacy among lines extremely difficult. Levels of caterpillar induced boll damage were slightly higher in the DPL50 (non-Bt) variety. Low levels of caterpillar induced boll damage (1% to 2%) were detected in the Bollgard II lines, which indicates that these lines are not immune to caterpillar damage. Lint yield of the 15985 Bollgard II line was similar to that of the DPL50 and 50B lines, but notably less than that of DPL428B and DPL NC33B. Gin turnout of the 15985 line was similar to that of the 50 and 50B lines, while gin turnout of the 15813 line was lower than that of 15985.

## 1999 Cotton Field Trial Report

### USDA #99-102-18n/Mons #99-249XRAB

During the growing season it was noted that the 15985 line had a lighter green (yellow) foliage color than any of the other lines in the trial. This appeared to be a characteristic of the line, rather than an indication of poor vigor or health. The color difference was less obvious at this location than at the Leflore County location where a similar trial was conducted.

**Monitoring for Volunteers:** Observations for volunteers were made on March 31, 2000. On May 17, 2000, the plot area received a burn down herbicide and was re-planted with BollGard Plus Cotton.

#### Washington County, MS

Planting Date: May 19, 1999

Harvest Date: September 20, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 18, July 14, August 16 and September 8, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 18, July 14, August 16 and September 8, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 18, July 14, August 16 and September 8, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 18, July 14, August 16 and September 8, 1999.

**Plant Stand:** Monitoring observations on June 18, July 14, August 16 and September 8, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On September 20, 1999, destruction was by a harvester and clipper.

**Disposition of Seeds:** All seed cotton returned to release site after weighing.

**General Results of Field Trial:** CryX lines grew comparably to DP50. Insect pressure was light. Plots were planted late and cut out fairly early limiting growth/yield potential.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Rankin County, MS**

Planting Date: May 25, 1999  
Harvest Date: October 5, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DPL50, DPL50B, DPL428

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 15, June 30, July 15, July 30 and August 15, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 15, June 30, July 15 (15% of the non-transgenic plants showed Heliothine insects were present), July 30 (22% of the non-transgenic plants showed Heliothine insects were present) and August 15, 1999 (42% of the non-transgenic plants showed Heliothine insects were present).

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 15, June 30, July 15, July 30 and August 15, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 15, June 30, July 15, July 30 and August 15, 1999.

**Plant Stand:** Monitoring observations on June 15, June 30, July 15, July 30 and August 15, 1999 did not observe any differences in plant stand for the transgenic or non-transgenic plants.

**Destruct Date and Method:** Bush hogged.

**Disposition of Seeds:** Buried.

**General Results of Field Trial:** Good insect control in CryX. Also good Fall Armyworm control. Good trial, but needs to be planted earlier for optimum yields.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Oktibbeha County, MS**

Planting Date: May 20, 1999

Harvest Date: October 7, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B, DPL428B

**Field Monitoring for Disease Susceptibility:** There appeared to be no disease noted in any plots throughout duration of the study.

**Field Monitoring for Insect Susceptibility:** Seasonal observations - high soybean looper numbers in DP50 and DP50B, DP428B varieties (few in 15985 and 15813). More bollworm damage in plots of DP50 and to lesser extent, DP50B and DP428B.

**Field Monitoring for Plant Growth Characteristics:** Seasonal observations - similar growth of all varieties.

**Field Monitoring for Weediness Characteristics:** The trial was monitored on June 1, 1999. The germination is similar in all varieties.

**Plant Stand:** Monitoring observations on June 1, 1999 noted there were no differences among varieties.

**Destruct Date and Method:** October 11, 1999 - Disking.

**Disposition of Seeds:** Autoclaved.

**General Results of Field Trial:** Lines 15985 and 15813 gave superior control of soybean looper and bollworms than other varieties. No other pests were observed in significant numbers. Yields of seed cotton were higher in lines 15985 and 15813 than DP50.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Holmes County, MS**

Planting Date: May 19, 1999 (Re-plant June 17, 1999)

Harvest Date: October 25, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B, DPL428B

**Field Monitoring for Disease Susceptibility:** Monitoring observation for this trait on July 22, 1999 that 1% of the transgenic plants and 1% of the non-transgenic plants displayed *Rhizoctonia Solani*. There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines during monitoring observations on August 25, September 23 and October 25, 1999.

**Field Monitoring for Insect Susceptibility:** Monitoring observations on July 22, 1999 noted that <1.0% of the transgenic plants and 2% of the non-transgenic plants display *Heliothine* sp., very small (neo nate). Observations on August 25, 1999 noted that 2% of the non-transgenic plants displayed *Heliothine* sp., and Fall Armyworm. Observations on September 23 and October 25, 1999, noted there were no differences between the transgenic lines and their respective non-transgenic lines for insect susceptibility.

**Field Monitoring for Plant Growth Characteristics:** This trait was monitored on July 22, August 25 and September 23, 1999. Observations on October 25, 1999 noted that the DPL50B plot had part of plot in drought soil and was shorter than all other. There was very little plant height difference among other varieties throughout the trial. Any observed height difference was considered to be within the normal variation range for cotton.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on July 22, August 25, September 23 and October 25, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Plant Stand:**

Plant Stand for first planting taken on May 28, 1999.

	<u>Transgenic</u>	<u>Non-Transgenic</u>
DP428B		49
15985	41	
DP50		42
15813	47	
DP50B		50

Plant Stand for re-planting taken on June 24, 1999.

	<u>Transgenic</u>	<u>Non-Transgenic</u>
DP428B		48
15985	45	
DP50		44
15813	47	
DP50B		49

**Destruct Date and Method:** June 26, 1999 (of excess seed) after planting.

**Disposition of Seeds:** Seeds destroyed by placing in hole in soil of plot area, after harvesting.

**General Results of Field Trial:** Trial was first planted on May 19, 1999 and emerged on May 25, 1999 for unknown reasons plants began to die. This left plots with less than 50% stand by June 12, 1999. The decision was reached, after consultation, to destroy and re-plant. This planting was done on June 19, 1999. Cotton emerged normally with very little seedling disease, as before, and all varieties grew normally except rain fall was scarce and plants were stunted by lack of moisture after the sixth internode. Pest pressure was very light.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Oktibbeha County, MS**

Planting Date: May 18, 1999  
Harvest Date: October 13, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 24, July 22, August 19 and September 23, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 24, July 22, August 19 and September 23, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 24, July 22, August 19 and September 23, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 24, July 22, August 19 and September 23, 1999.

**Plant Stand:** Monitoring observations on June 24, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** On October 13, 1999, put cotton back on field, shredded stalks and disked.

**Disposition of Seeds:** Put back on field as seed cotton.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Jackson County, OK (1)**

Planting Date: June 7, 1999

Harvest Date: October 21, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 14, June 21, June 28, July 6 and July 26, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on July 26, August 9, August 16, August 23 and August 30, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 14, June 21, June 28, July 6 and August 30, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 14, June 21, June 28, July 6 and August 30, 1999.

**Plant Stand:** No significant differences in plant stand were noted between the transgenic and non-transgenic lines.

**Destruct Date and Method:** October 22, 1999 - Shredded.

**Disposition of Seeds:** Buried in plot.

**General Results of Field Trial:** No difference was observed.



**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**Jackson County, OK (2)**

Planting Date: May 25, 1999

Harvest Date: October 21, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 25, July 23, August 25, and September 24, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 25, July 23, August 25, and September 24, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 25, July 23, August 25, and September 24, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 25, July 23, August 25, and September 24, 1999.

**Plant Stand:** No differences in plant stand were noted between the transgenic and non-transgenic lines.

**Destruct Date and Method:** November 18, 1999. Shredded, disked, buried by tillage.

**Disposition of Seeds:** Buried in plot.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**City of Suffolk, Virginia (1)**

Planting Date: May 20, 1999

Harvest Date: December 10, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 3, June 25, July 23, August 19, August 26 and September 16, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 3, June 25, and July 23, 1999 no differences were noted. Observation on August 19, 1999 noted that 20-30% of the non-transgenic plants had damaged squares and 10-20% damaged bolls. Observation on August 26, 1999 noted that <10% of the non-transgenic plants had damaged squares and 30-50% damaged bolls. Observation on September 16, 1999 did not observe any differences.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 3, June 25, July 23, August 19, August 26 and September 16, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 3, June 25, July 23, August 19, August 26 and September 16, 1999.

**Plant Stand:** No significant differences in plant stand were noted between the transgenic and non-transgenic lines.

**Destruct Date and Method:** December 10, 1999 - Bush hog (mowed).

**Disposition of Seeds:** Remaining seeds were burned.

**General Results of Field Trial:** The CryX varieties performed well in our location. Insect pressure was heavy this year as noted in the DP50 non-Bt check plots having up to 50% boll damage. The CryX plots had no damaged bolls.

**1999 Cotton Field Trial Report**  
**USDA #99-102-18n/Mons #99-249XRAB**

**City of Suffolk County, VA (2)**

This field trial was not planted.

**City of Suffolk, Virginia (3)**

Planting Date: June 1, 1999

Harvest Date: November 11, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 29, July 22, August 2, August 17, August 25 and August 31, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 29 and noted that thrips injury scattered in all the plot. Observations on July 22 and August 2, 1999 noted there were no differences. Observations on August 17, August 25 and August 31, 1999 noted that there was low bollworm pressure.

**Field Monitoring for Plant Growth Characteristics:** There were differences in the general appearance and growth of the transgenic and the non-transgenic plants. Observation on June 29, 1999, no differences noted. Observation on July 22, 1999 noted in the transgenic plants there was a distortion in discoloration. No differences were noted in observations on August 2, August 17, August 25 and August 31, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 29, July 22, August 2, August 17, August 25 and August 31, 1999.

**Plant Stand:** Monitoring observations on June 10 and June 21, 1999, but the dates are meaningless due to drought.

**Destruction Method:** Disked into the field

**General Results of Field Trial:** Late planting resulted in poor yields.

**Monitoring for Volunteers:** No volunteers were observed in May 2000. A peanut crop was planted into the field.

**1999 Cotton Field Trial Report**  
**USDA #99-102-19n      Monsanto #99-279XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED ]	Bossier	Louisiana

**Bossier County, LA**

Planting Date: May 21, 1999

Harvest Date: October 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. In observations on June 4, 1999, noted that less than 3% of the transgenic and the non-transgenic plants damping off occurred field wide, the percentage is small. Observations on July 7 and August 5 noted no differences. In observations on September 10, 1999 there were no differences noted, but a small amount of boll rot, less than 2% appeared fairly uniform in all plots.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. In observations on June 4, 1999, noted that less than 3% of the transgenic and the non-transgenic plants had cutworm/armyworm damage field wide. Observations on July 7, August 5 and September 10, 1999 noted no differences in the plants.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 4, July 7, August 5 and September 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 4, July 7, August 5 and September 10, 1999.

**Plant Stand:** Observations on June 4, 1999 noted that line 15813 7-DAP plants stand low but 14-DAP plant stands similar to 15985 and control. The transgenic and the non-transgenic plots observed on July 7, August 5 and September 10, 1999 noted no differences in plant stand.

**Destruct Date and Method:** The remaining stalks were cut and disked on October 14, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-102-19n/Mons #99-279XRAB**

**Disposition of Seeds:** Seed cotton to be ginned and seed (all) and lint sample shipped to Monsanto, St. Louis.

**General Results of Field Trial:** This field trial was successful. No unusual abnormalities noted with either 15813 or 15985.

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1999 Cotton Field Trial Report  
USDA #99-102-20n      Monsanto #99-278XRAB

[REDACTED]  
November 3, 2000

Monsanto Company

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED ]	Baldwin	AL
	Pinal	AZ
	Washington	MS
	Florence	SC
	San Patricio	TX

**Baldwin County, AL**

Planting Date: May 18, 1999  
Harvest Date: October 14, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, 50, 50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines when this field trial was monitored on June 15, July 7, August 3 and September 28, 1999. During the observation on August 31, 1999, boll rot was in all the plots.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility when this field trial was monitored on June 15, July 7 and September 28, 1999. During the observations on August 3 and August 31, 1999, insect damage was higher on the non-transgenic plants.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 15, July 7, August 3, August 31 and September 28, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. All plots emerged at the same time. The trial was monitored on June 15, 1999.

**Plant Stand:** The transgenic and the non-transgenic plots have equal plant stands. This observation was made on June 15, 1999.

**Destruct Date and Method:** The remaining stalks were shredded on October 20, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-102-20n/Mons #99-278XRAB**

**Disposition of Seeds:** All seed was shipped to the Production Supervisor.

**General Results of Field Trial:** This trial was completed with no complications.

**Monitoring for Volunteers:**

January 21, 2000	No volunteers present	
February 18, 2000	No volunteers present	Weather will not allow germination.
March 17, 2000	No volunteers present	Winter cover sprayed with Roundup
April 17, 2000	No volunteers present	Ground recently disked and bedded.
May 26, 2000	Entire area planted back to same transgenic crop for 2000 growing season. Monitoring for volunteers will stop and in-season monitoring for 2000 will begin.	

**Pinal County, AZ (1)**

Planting Date: May 14, 1999

Harvest Date: October 18, 1999

Project Study #:99-01-36-03

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 28, June 25, July 23, August 23 and September 15, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on May 28, June 25, July 23, August 23 and September 15, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on May 28, June 25, July 23, August 23 and September 15, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on May 28, June 25, July 23, August 23 and September 15, 1999.

**Plant Stand:** There was no difference in plant stand count between the transgenic and the non-transgenic. The trial was monitored on May 28, June 25, July 23, August 23 and September 15, 1999.

**General Results of Field Trial:** All four lines performed similarly. In the early season, although 15813 seemed less robust than the other lines/events. Toward mid-season this line caught up to the others eventually becoming indistinguishable from the others.

**1999 Cotton Field Trial Report**  
**USDA #99-102-20n/Mons #99-278XRAB**

**Pinal County, AZ (2)**

Planting Date: May 19, 1999

Harvest Date: November 24, 1999

Project Study #: 99-01-36-07

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 3, June 24, July 27, August 25 and September 8, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 3, June 24, July 27, August 25 and September 8, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 3, June 24, July 27, August 25 and September 8, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored for this trait on June 3, June 24, July 27, August 25 and September 8, 1999.

**Plant Stand:** There was no difference in plant stand count between the transgenic and the non-transgenic. The trial was monitored for this trait on May 27 and June 3, 1999.

**Destruct Date and Method:** On December 3, 1999, the stacks were cut down, roots pulled and debris disked under.

**General Results of Field Trial:** All four lines performed similarly. In the early season, the "visual" quality of the transgenic events "813" and "985" was somewhat questionable when compared to the conventional DP50 and commercial DP50B. The quality of seed was questioned originally (i.e., by our visual assessment), but unjustified by late-early season to mid-season. Again, early-season presumed discrepancies were unfounded, and all four lines/events performed similarly throughout the season, and most certainly by season's end.



**1999 Cotton Field Trial Report**  
**USDA #99-102-20n/Mons #99-278XRAB**

**Washington County, MS (1)**

Planting Date: May 20, 1999  
Harvest Date: October 12, 1999  
Project Study #: 99-01-36-03  
Vector Construct: PV-GHBK11  
Lines: Cry X 15813, Cry X 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 10, June 17, July 15, August 16, September 8 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility when this trial was monitored on June 10, June 17, July 15, August 16 and October 5, 1999. Approximately 5% boll damage was observed in DP50 on September 8, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 10, June 17, July 15, August 16, September 8 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 10, June 17, July 15, August 16, September 8 and October 5, 1999.

**Plant Stand:** There was no difference in plant stand count between the transgenic and the non-transgenic. The trial was monitored on June 10, June 17, July 15, August 16, September 8 and October 5, 1999.

**Destruction Method:** Harvested and clipped with bush hog.

**Disposition of Seeds:** Shipped to St. Louis on October 13, 1999.

**General Results of Field Trial:** The transgenic lines 15985 and 15813 grew comparably to the nontransgenics, DP50 and DP50B cotton. There were no differences in agronomics or disease susceptibility were observed. Insect pressure was light but a few more damaged bolls from tobacco budworm/cotton bollworm were observed in the DP50 and DP50B than in the Cry X 15813 and Cry X 15985.

**1999 Cotton Field Trial Report**  
**USDA #99-102-20n/Mons #99-278XRAB**

**Washington County, MS (2)**

Planting Date: May 20, 1999  
Harvest Date: October 13, 1999  
Project Study #: 99-01-36-07  
Vector Construct: PV-GHBK11  
Lines: Cry X 15813, Cry X 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 18, June 14, August 17, September 8 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility when this trial was monitored on June 18, July 14, August 17, and September 8, 1999. Five to 10% boll damage was observed in the non-transgenic control vs. the insect protect lines on October 5, 1999.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 18, June 14, August 17, September 8 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 18, June 14, August 17, September 8 and October 5, 1999.

**Plant Stand:** There was no difference in plant stand count between the transgenic and the non-transgenic. The trial was monitored on June 18, June 14, August 17, September 8 and October 5, 1999.

**Destruction Method:** All of the border and buffer rows were clipped and tilled.

**Disposition of Seeds:** The seed was segregated, labeled and stored in a locked cabinet. Some of the seeds were returned to the release field and some seeds were shipped to St. Louis, MO.

**1999 Cotton Field Trial Report  
USDA #99-102-20n/Mons #99-278XRAB**

**Florence County, SC**

Planting Date: May 26, 1999  
Harvest Date: November 8, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 21, July 6, July 20, August 2, August 16, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility when this trial was monitored on July 6, July 20 and August 16, 1999. Yellow striped army worms were feeding on leaves on 3.3% of the transgenic plants and 7.7% of the non-transgenic plants on June 21, 1999. On August 2, 1999, 2.2% were on the transgenic and 6.1% of the non-transgenic plants had stink bug damage.

**Field Monitoring for Plant Growth Characteristics:** Observations were made on June 21, July 6 and July 20, 1999. No differences were noted in growth habits of transgenic lines compared to non-transgenic lines when field notebook data from this site were analyzed.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants.

**Plant Stand:** See attached forms. Observations were made on June 2, June 9 and June 25.

**Destruction Method:** Mowed and then disked into the field on November 10, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-102-20n/Mons #99-278XRAB**

**San Patricio County, TX**

Planting Date: May 23, 1999  
Harvest Date: September 23, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on May 30, June 13, July 3, July 10, July 21, August 4 and September 1, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility when this trial was monitored on May 30, June 13, July 3, July 21, August 4 and September 1, 1999. During the monitoring for insect susceptibility on July 10, 1999, it was noted that approximately 10% of the nontransgenic plants were infected with bollworms/budworms.

**Field Monitoring for Plant Growth Characteristics:** Observations were made on June 21, July 6 and July 20, 1999. Monitoring on May 30, 1999 indicated that 33% of line 15813 appears to be slower emerging. Monitoring on June 13, August 3, July 10, and July 21, 1999 showed no differences. Observation on August 4, 1999, showed that 100% of the transgenic plants had more white blooms. Monitoring on September 1, 1999, showed no differences.

**Field Monitoring for Weediness Characteristics:** It was noted that the germination of transgenic plants was no different from the non-transgenic plants. The trial was monitored on May 30, June 13, July 3, July 10, July 21, August 4 and September 1, 1999.

**Plant Stand:** Some differences were noted in stand count between the transgenic and the non-transgenic plants. Observation on May 30, 1999 noted that line 15813 was slower emerging. The standcount for transgenic plant line 15813 was 26.5 plants, and the stand count for transgenic plant line 15985 was 53.0 plants. The nontransgenic line #DP50 had 56.8 plants in comparison. These differences were considered to be within normal variation expected for cotton plants. The other dates of monitoring showed no differences in plant stand count. The dates of monitoring were June 13, July 3, July 10, July 21, August 4 and September 1, 1999.

**Destruction Method:** On September 1999, the plot area was shredded and the stalks were pulled with a stalk puller.

**Disposition of Seeds:** The seeds were all planted. The harvested seeds were sent to Monsanto.

**General Results of Field Trial:** The lines all grew as expected.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n      Monsanto #99-277XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

**Location**

**County**

**State**

[ CBI DELETED ]

Perquimans	North Carolina
Washington	North Carolina
Bertie	North Carolina
Onslow	North Carolina
Edgecombe	North Carolina
Edgecombe	North Carolina
Sampson	North Carolina
Perquimans	North Carolina
Barnwell	South Carolina
Lee	South Carolina
Darlington	South Carolina
Barnwell	South Carolina
Hampton	South Carolina
Marlboro	South Carolina
Darlington	South Carolina
Florence	South Carolina
Shelby	Tennessee
Hardeman	Tennessee
Fayette	Tennessee
Madison	Tennessee
Pecos	Texas
Tom Green	Texas
Lubbock	Texas
Hale	Texas
Lubbock	Texas

1999 Cotton Field Trial Report  
USDA #99-102-21n/Mons #99-277XRAB

Perquimans County, NC

This field trial was not planted.

Washington County, NC

This field trial was not planted.

Bertie County, NC

This field trial was not planted.

Onslow County, NC

This field trial was not planted.

Edgecombe County, NC (1)

This field trial was not planted.

Edgecombe County, NC (2)

This field trial was not planted.

Sampson County, NC

This field trial was not planted.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Perquimans County, NC**

Planting Date: May 25, 1999

Harvest Date: October 26, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 7, June 21 (1-2% of the transgenic and the non-transgenic plants shows Phoma/Aschochoptes disease), July 15, July 29, August 15 and August 30, 1999.

**Field Monitoring for Insect Susceptibility:** Monitoring observation on June 7, 1999 noted that 100% of the transgenic plants and 100% of the non-transgenic had Thrips. They were treated from June 9 to June 17 with 802/A Orthone. Observations on June 21 and July 15, 1999 noted there were no differences between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Observations on July 29 noted that 12% eggs in terminals of both the transgenic plants and the non-transgenic plants. Observations on August 15 and August 30, 1999 noted Bollworms present in the standard cotton (DP50).

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 7, June 21, July 15, July 29, August 15 and August 30, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 7, June 21, July 15, July 29, August 15 and August 30, 1999.

**Plant Stand:** Monitoring observations on June 7, 1999 noted that both the transgenic and the non-transgenic plant standcount was 6-8/foot and they needed thinning. On June 21, 1999 the plants were thinned by hoeing to 3-4 plants/foot. Observation on July 15, 1999 noted that both the transgenic and the non-transgenic plant standcount was 3-4/foot.

**Destruct Method:** Mowing followed by disking.

**Disposition of Seeds:** Buried on site.

**General Results of Field Trial:** This trial endured 35+ inches of rain between August 29 through October 16, 1999 from Hurricanes Dennis, Floyd and Brene. No abnormal growth was observed. Fruit size, shape and load appeared normal in all varieties. Yields ranged from 331 lbs. line/acre to 569 lbs. lint/acre.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Barnwell County, SC**

Planting Date: May 21, 1999

Harvest Date: November 24, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 1, August 2, September 3, October 2 and November 1, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 1, August 2, September 3, October 2 and November 1, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 1, August 2, September 3, October 2 and November 1, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 1, August 2, September 3, October 2 and November 1, 1999.

**Plant Stand:** Monitoring observations on July 1, August 2, September 3, October 2 and November 1, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 29, 1999 - Seed cotton harvested, dumped in fallow field and disked under. The test site was moved and then disked under.

**Disposition of Seeds:** Picked up by a Monsanto Representative.

**General Results of Field Trial:** Lines 15813, 15985 and DP50B, all Bt lines, had less insect activity and more yield than the non-transgenic variety. Lines DP50, 15813 and 15985 had slightly less insect activity than DP50B but yields were equivalent among all the Bt lines. Very little differences in insect activity and yield between lines 15813 and 15985. Overall, most pressure unusually light this year.



**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Lee County, South Carolina**

Planting Date: May 19, 1999

Harvest Date: November 3, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 17, July 26, August 16, and November 3, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 17, July 26, August 16, and November 3, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 17, July 26, August 16, and November 3, 1999.

**Field Monitoring for Weediness Characteristics:** No differences were noted between the transgenic lines and their respective non-transgenic lines for weediness characteristics. This trait was monitored on June 17, July 26, August 16, and November 3, 1999.

**Plant Stand:** Differences in plant stand were noted between the transgenic lines and their respective non-transgenic lines. Observations on June 9, 1999 indicated average stand count was 18.2 for transgenic lines and 23.0 for non-transgenic lines. On June 17, 1999, average stand count was 11.4 for transgenic lines and 12.6 for non-transgenic lines. Differences in plant stand were considered to be within the normal variation for cotton plants.

**Destruct Date and Method:** November 9, 1999 - by rotary mower.

**Disposition of Seeds:** Buried in plot.

**Monitoring for Volunteers:** Dates of observations were December 9, 1999 and January 4, February 3, March 3, April 2, and May 12, 2000. No volunteers were observed.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Darlington County, South Carolina**

Planting Date: May 26, 1999

Harvest Date: November 4, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 20, July 20, August 20, September 20 and October 20, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 20, 1999 (no differences noted), July 20, 1999 (aphids were noted in both the transgenic plants and the non-transgenic plants), August 20, 1999 (some differences noted), no differences noted for observations on September 20 and October 20, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 20, July 20, August 20, September 20 and October 20, 1999.

**Field Monitoring for Weediness Characteristics:** The emergence of transgenic plants was different from non-transgenic plants, but differences were considered to be field related and were within the normal variation range for cotton plants. This trait was monitored on June 20, July 20, August 20, September 20 and October 20, 1999.

**Plant Stand:** Monitoring observations on June 10, 1999 (dry weather and poor standcount for the transgenic and for the non-transgenic plants); July 7, 1999 (rain came and had better standcount for both the transgenic and the non-transgenic).

**Destruct Date and Method:** November 12, 1999 - Burned and disked.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Barnwell County, SC (2)**

Planting Date: May 27, 1999

Harvest Date: November 27, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 15, July 20 and August 13, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was July 21, July 28, August 2, August 11 and August 20, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 15, July 20 and August 13, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 15 and July 20, 1999.

**Plant Stand:** Monitoring observations on June 13 and June 26, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 18, 1999 - Burned harvested cotton and mowed remainder.

**Disposition of Seeds:** Buried in plot area in July 1999.

**General Results of Field Trial:** Overall, there was no apparent differences between the transgenic and non-transgenic lines. There was more susceptibility to Bollworm in the non-transgenic than in the transgenic.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Hampton County, SC**

Planting Date: May 27, 1999, Re-planted June 15, 1999

Harvest Date: November 19, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 28, August 19, September 17, October 8, and November 19, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 28, August 19, September 17, October 8, and November 19, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 28, August 19, September 17, October 8, and November 19, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 28, August 19, September 17, October 8, and November 19, 1999.

**Plant Stand:** Monitoring observations on June 25, 1999 noted that the re-plant standcount was near 98% germination.

**Destruct Date and Method:** November 19, 1999 - Rotary mower.

**Disposition of Seeds:** Buried in plot.

**General Results of Field Trial:** Agronomic characteristics good. Insufficient insect pressure to separate treatments. The excess planting seed was buried in the plot area. The disposal method for the seed bag was burning.

**Monitoring for Volunteers:** Dates of observations were December 27, 1999, no volunteers were observed; on January 24, 2000, it was noted that the plot area was frozen.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Marlboro County, SC**

Planting Date: May 27, 1999, Re-planted June 15, 1999

Harvest Date: November 19, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DPL50, DPL50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on May 27, June 17, July 18, August 5 and September 3, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Observations on May 27, June 17, July 18, August 5 did not note any differences. Observation on September 3, 1999 noted that 20% of the transgenic and 20% of the non-transgenic had stink bugs damaging small bolls.

**Field Monitoring for Plant Growth Characteristics:** No differences were noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait when monitored on May 27, June 17 and July 18, 1999 did not note any differences. The observation on August 5, 1999 noted that 90% of the transgenic and 90% of the non-transgenic plants throwing off small bolls and drought. The observation on September 3, 1999 noted that 100% of the transgenic and 100% of the non-transgenic plants wilted and were devoid of bolls.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on May 27, June 17, July 18, August 5 and September 3, 1999.

**Plant Stand:** Monitoring observations on May 27, 1999 noted standcount for the transgenic plants at 3.7/ft. and for the non-transgenic plants at 3.9/ft. Monitoring observations on June 17, 1999 noted standcount for the transgenic plants at 3.1/ft. and for the non-transgenic plants at 3.2/ft.

**Destruct Date and Method:** November 9, 1999 - Bush-hogged and disked.

**Disposition of Seeds:** Landfill.

**General Results of Field Trial:** Bollworm pressure was too light to properly evaluate these varieties. The non-Bt variety looked as good as those with the Bt gene, since bollworm populations were sub-economic. There was a late-season infestation of stink bugs, but no differences were noted between varieties.

**Darlington County, SC**

This field trial was not planted.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Florence County, SC**

Planting Date: May 26, 1999

Harvest Date: November 8, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored throughout the season.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored throughout the season.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored throughout the season.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored throughout the season.

**Plant Stand:** This trait was not monitored.

**Destruction and Method:** Mowing was followed by disk incorporation.

**Disposition of Seeds:** Returned to trial site after weighing plot yields in the field followed by disc incorporation of seed cotton.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Shelby County, TN**

Planting Date: May 21, 1999

Harvest Date: November 5, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on May 25, June 15, July 6, July 27, August 17 and September 7, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on May 25, June 15, July 6, July 27, August 17 and September 7, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on May 25, June 15, July 6, July 27, August 17 and September 7, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on May 25, June 15, July 6, July 27, August 17 and September 7, 1999.

**Plant Stand:** Monitoring observations on May 25, June 15, July 6, July 27, August 17 and September 7, 1999 noted for the transgenic line 15985 that there was a higher standcount, due to small seed size.

**Destruct Date and Method:** October 8, 1999 - bushhog, disk.

**Disposition of Seeds:** Destroyed in plot area (buried).

**General Results of Field Trial:** Bollgard plus cotton project went very well during the growing season. Plots were planted in good soil moisture in May. Emergence was rapid. The months of June and the first and second week of July were excellent for crop production. The weather then turned off dry for the next three months up to harvest. The dry weather was the limiting factor to our low yields this year. Insect lepidopteran pest pressure was also down compared to previous years.

**Monitoring for Volunteers:** Dates of observations were December 27, 1999, no volunteers were observed; on January 24, 2000, it was noted that the plot area was frozen.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Hardeman County, TN**

Planting Date: May 24, 1999

Harvest Date: September 27 and October 7, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 24, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 25 (all plots were treated for aphids); June 30, July 13 and July 28 (no differences noted); August 4 (DP50 showing damage; no difference in Bollgard); August 16, 1999 (collected larvae from DP50; 70% TBW).

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 25, June 30, July 13, July 28, August 4 and August 16, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants.

**Plant Stand:** There were no significant differences in standcount among lines.

**Destruction Method:** Left in plot area and bush hogged.

**Disposition of Seeds:** Burned.

**General Results of Field Trial:** In the unsprayed test, yields among Bollgard lines (2) and DP50B not significantly different and all three different from conventional DP50.

**Monitoring for Volunteers:** Three seedling plants were found in the border area on May 9, 2000. The plants were pulled up.



**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Fayette County, TN**

Planting Date: May 20, 1999

Harvest Date: October 6, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 13, July 20, July 27, August 5 and August 11, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 13, July 20, July 27, August 5 and August 11, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 13, July 20, July 27, August 5 and August 11, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 13, July 20, July 27, August 5 and August 11, 1999.

**Plant Stand:** Monitoring observations on June 1, 1999 noted there were more differences between transgenics than between the transgenics vs. the non-transgenic plants.

**Destruct Date and Method:** October 6, 1999 - mechanical shredder.

**General Results of Field Trial:** No significant difference between treatments. Insect infestations were low.

**Monitoring for Volunteers:** Volunteer dates of observations were November 5, 1999, March 10 and April 14, 2000. No volunteers were observed.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Madison County, TN**

Planting Date: May 21, 1999

Harvest Date: September 24 and October 4, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 30, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 13, July 20, July 27, August 5 and August 11, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 30 and July 19, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 30, 1999.

**Plant Stand:** The trial was monitored for plant stand on May 28 and June 4, 1999. Observations indicated differences in final plant stand between transgenics and non-transgenics. Any differences observed were considered to be within the normal variation range for cotton plants.

**Destruct Date and Method:** ~October 20, 1999 - Bush Hog.

**General Results of Field Trial:** Burned planting seed

**Monitoring for Volunteers:** Experienced drought July 15 through October 9, 1999. Single bollworm/budworm spray did not increase first harvest yields in IPC but all Bollgard lines (II and 50B) had more cotton than unsprayed DP50.

**Pecos County, TX**

This field trial was not planted.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Tom Green County, TX**

Planting Date: June 2, 1999

Harvest Date: November 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 9, July 5, August 4, August 31, September 24 and October 24, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 9, July 5, August 4, August 31, September 24 and October 24, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 9, July 5, August 4, August 31, September 24 and October 24, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 9, July 5, August 4, August 31, September 24 and October 24, 1999.

**Plant Stand:** Monitoring observations on June 9, July 5, August 4, August 31, September 24 and October 24, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 13 through November 16, 1999. - burned and disked.

**Disposition of Seeds:** Buried in the field.

**General Results of Field Trial:** The trial went well. Plots were initially hand harvested and then machine harvested. Cotton stripper was cleaned prior to and after harvesting plots. All seed cotton was dumped in site and burned and then disked under.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Lubbock County, TX**

Planting Date: May 21, 1999

Harvest Date: November 8, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on May 28, June 15, July 8, August 23 and September 24, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on May 28, June 15, July 8, August 23 and September 24, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on May 28, June 15, July 8, August 23 and September 24, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on May 28, June 15, July 8, August 23 and September 24, 1999.

**Plant Stand:** Monitoring observations on May 28, June 15, July 8, August 23 and September 24, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 8, 1999 - crop destruct on site, not harvested.

**Disposition of Seeds:** Incorporated into soil where plots were planted.

**General Results of Field Trial:** The CryX Seeds were planted eight days after rest of field was planted. CryX seeds had good to excellent vigor and emergence was within five days. Ninety percent or more of the seeds were planed, germinated and emerged. The CryX plants grew and developed normally. The squares blooms and bolls produced by the CryX plants were normal and similar to the non-transgenic plants planted next to them. The estimated yield of the CryX plants was equal to or exceeded the yield of the surrounding varieties. Insect pressure (lepidopteran) was virtually non-existent.

**1999 Cotton Field Trial Report**  
**USDA #99-102-21n/Mons #99-277XRAB**

**Hale County, TX**

Planting Date: May 24, 1999  
Harvest Date: October 13, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 8, June 15, June 24, July 6, July 13, 1999 and throughout the remainder of the season.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 8, June 22, July 6, July 20, July 27 1999, and throughout the remainder of the season.

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 8, June 22, July 6, July 20, July 27 1999, and throughout the remainder of the season.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 8, June 22, July 6, July 20, July 27 1999, and throughout the remainder of the season.

**Plant Stand:** Monitoring observations on June 8, June 22, July 6, July 20 and July 27, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** October 13, 1999 - Shredded and plowed under.

**General Results of Field Trial:** Overall, the trial was established in late May under good growing conditions with no differences in emergence of each line. Insect populations, very low. Trial lacked moisture in late season (didn't want to have this variety too far North). Lines harvested and yields taken on October 13, 1999 (hand harvested). No apparent differences in yield.

**Lubbock County, TX (2)**

This field trial was not planted.

1999 Cotton Field Trial Report  
USDA #99-102-22n      Monsanto #99-248XRAB

November 3, 2000

Monsanto Company

<u>Location</u>	<u>County</u>	<u>State</u>
[ CBI DELETED	Limestone	Alabama
	Limestone	Alabama
	Lee	Alabama
	Baldwin	Alabama
	Autauga	Alabama
	Autauga	Alabama
	Macon	Alabama
	Jackson	Arkansas
	Desha	Arkansas
	Jefferson	Arkansas
	Pinal	Arizona
	Pinal	Arizona
	Pinal	Arizona
	Fresno	California

Limestone County, AL

Planting Date: May 19, 1999

Harvest Date: October 8 and October 21, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 2, July 1, July 30, August 30 and October 1, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring on June 2, 1999, no insects observed in any plots; July 1, 1999, no eggs or worms, Lygus damage <10%, aphids light, no differences among transgenics; July 30, 1999, 8% eggs in all plots, 2% worms in non-transgenics, predominantly corn earworm; August 30, 1999, eggs <2%, no worms in any of the plots, western flower thrips throughout; October 1, 1999, few insects in any plot.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Field Monitoring for Plant Growth Characteristics:** Observations on June 2, 1999 noted that all plots were at 90% stand and no differences noted; July 1, 1999, cotton late, but okay, no striking differences; July 30, 1999 ca. 2<sup>nd</sup> week of bloom, all plots about equal; August 30, 1999 few white blooms left and no open bolls, center 2 reps yellow due to some excess moisture (no loss); October 1, 1999, close to harvest.

**Field Monitoring for Weediness Characteristics:** This characteristic was not measured for this trial.

**Plant Stand:** This trait was monitored on June 2, 1999. There were no differences noted.

**Destruct Date and Method:** Plants were shredded right after harvest on October 8, 1999 and on October 21, 1999.

**Disposition of Seeds:** Harvested cotton was spread in plots before shredding. Note: It has been too dry to break land here. Tilling will be noted in the "monitoring for volunteers" form.

**General Results of Field Trial:** All transgenic lines controlled Heliothines. No differences were noted in sprayed and unsprayed plots. Line 15985 slightly out-performed line 15813 and appeared competitive with the comparison varieties.

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**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Limestone County, AL**

Planting Date: May 19, 1999

Harvest Date: October 8 and October 21, 1999

Vector Construct: PV-GHBK11

Lines: Cryx/15813 and Cryx/15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This characteristic was monitored on June 4, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring on June 4, 1999, no insects observed in any plots; June 11, 1999 checked for plant bugs, but cotton too young to be damaged; June 22, 1999, plant bugs noted, sprayed Bidoin (402/A); July 9, 1999, plant bugs, sprayed Karate (3.2 oz/A); July 28, 1999 plant bugs in some blooms, about 8% eggs, but no damage, did not spray; August 2, 1999, sprayed Karate (3.2 oz/A) for worms; August 9, 1999, sprayed Karate (3.2 oz/A) for worms.

**Field Monitoring for Plant Growth Characteristics:** Observations on June 4, 1999 noted that cotton up to a good stand; July 1, 1999, cotton at 7-8 nodes, looks good; July 9, 1999, 8oz Pix applied, cotton growing rapidly; July 23, 1999, cotton getting tall, 10oz. Pix applied; August 9, 1999, counted nodes above white blooms; September 27, 1999, noted 15813 and 15985 slightly later than 50B; 15985 seems shorter and not as vigorous growth as 15813.

**Field Monitoring for Weediness Characteristics:** Observations on June 4, 1999 noted that weeds were controlled; observation on July 6, 1999 noted weeds, cultivated.

**Plant Stand:** This trait was observed on June 4, 1999 and there were no problems, the stand was excellent in all the plots. On June 10, 1999 made stand counts by plot.

**Destruct Method:** Used flail mower to cut stalks in plots and border areas.

**Disposition of Seeds:** Returned to the field and buried in alleys.

**General Results of Field Trial:** The trial was planted later than normal for area, but with irrigation made good yields. Insect pressure was low, only had some worm pressure in early August. The Cryx/15813 was a littler taller than Cryx/15985 and seemed to be a little earlier.

On October 8, 1999 at harvest, approximately a 1-lb. Sample of seed cotton was taken per plot to determine line percentage. The sample was ginned on a table top gin and all seed and lint returned to the field after weighing. The gin was cleaned after the samples were run.



**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Lee County, AL**

Planting Date: May 18, 1999

Harvest Date: October 15, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 4, June 29, July 21, and August 20, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring on June 4, 1999, noted there was no thrip damage; June 29, 1999, no worms, some plant bugs but too wet to test; July 21, 1999, 100% of the transgenic and 100% of the non-transgenic had plant bugs, applied Curacron at 5oz/A; August 2, 1999 (see scout report ).

**Field Monitoring for Plant Growth Characteristics:** No significant differences were noted in the general appearance, growth, flowering, and/or seed production of the transgenic and non-transgenic plants. Monitoring dates were June 4, June 29, July 21, August 20, and October 4, 1999.

**Field Monitoring for Weediness Characteristics:** Monitoring noted there were no differences in the germination of the transgenic plant from the non-transgenic plant. Observations on June 4, June 29, July 21 and August 2, 1999 noted good weed control in all of the plants.

**Plant Stand:** Differences in the plant stand count were noted on June 4, 1999. The transgenic was at 5/foot and the non-transgenic was at 4/foot. There were no differences noted during monitoring on June 29, July 21, August 21 and September 21, 1999.

**Destruct Date:** October 22, 1999.

**Disposition of Seeds:** None kept or collected – harvested areas of plots was weighed, then seed cotton emptied on ground next to test area and left to rot after disking area. Test area was mowed, then disked.

**General Results of Field Trial:** Dryland cotton suffered during July and August. Light insect pressure during the season.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Baldwin County, AL**

Planting Date: May 20, 1999  
Harvest Date: October 18, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 15, July 7, August 3, August 31 and September 28, 1999. It was noted that on August 31, 1999, 5% of the transgenic and 5% of the non-transgenic had boll rot that was equal across lines.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring on June 15, 1999, no insect pressure at this time; July 7, 1999, no significant insect pressure; August 3, 1999, insect damage higher in non-transgenics; August 31, 1999, insect damage higher in non-transgenics; and observation on September 28, 1999 observed no significant insect pressure.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. This trial was monitored on June 15, July 7, August 3, August 31 and September 28, 1999. All plots appear similar.

**Field Monitoring for Weediness Characteristics:** Observations on June 15, 1999 and July 7, 1999 noted that all plots emerged at the same time.

**Plant Stand:** This trait was monitored on June 15, 1999. Plant stands are equal for transgenic and conventional lines.

**Destruct Date and Method:** On October 26, 1999 - bush hog.

**Disposition of Seeds:** Excess seed buried in the test area.

**General Results of Field Trial:** Trials completed with no problems and stalks bush hogged on October 26, 1999.

**Monitoring for Volunteers:**

January 21, 2000	No volunteers present	
February 18, 2000	No volunteers present	Weather will not allow germination.
March 17, 2000	No volunteers present	Winter cover sprayed with Roundup
April 17, 2000	No volunteers present	Ground recently disked and bedded.
May 26, 2000	Entire area planted back to same transgenic crop for 2000 growing season. Monitoring for volunteers will stop and in-season monitoring for 2000 will begin.	

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Autauga County, AL**

Planting Date: May 21, 1999, re-planted June 7, 1999

Harvest Date: November 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 14, July 8, August 2, August 23 and September 3, 1999.

**Field Monitoring for Insect Susceptibility:** No significant differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring occurred on July 15, August 10, August 17, August 23, and August 31, 1999. On August 10, 1999, 20% of the non-transgenic and 0% of the transgenics had Bollworms/T. Budworms damaging DPL-50; August 17, 1999, 15% of the non-transgenics and none of the transgenics had Bollworms/T. Budworms damaging DPL-50; August 23, 1999, 18% of the non-transgenics and none of the transgenics had Bollworms/T. Budworms damaging DPL-50; and August 31, 1999, 10% of the non-transgenics and none of the transgenics had Bollworms/T. Budworms damaging DPL-50.

**Field Monitoring for Plant Growth Characteristics:** There were no significant differences in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. Monitoring on June 14, July 8, and August 2, 1999 observed no differences. Monitoring on August 23 and on September 3, 1999, observed that DPL50 was slightly shorter than other varieties.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants is no different from non-transgenic plants. This trait was monitored on June 14, July 8, August 2, August 23 and September 3, 1999.

**Plant Stand:** This trait was monitored on June 29, 1999. It was noted that the stand count for the transgenic plant was 11.5/3ft. and the stand count for the non-transgenic was 12/3 ft. This was an indication of no differences in the plant stand.

**Destruct Date and Method:** On November 17, 1999 the stalks were shredded with a bush hog.

**Disposition of Seeds:** Excess seed buried in the test area.

**General Results of Field Trial:** Successful trial but low insect pressure.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Autauga County, AL**

Planting Date: May 21, 1999, re-planted June 8, 1999

Harvest Date: November 15, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on July 7, July 15, August 12, August 31, September 22 and October 6, 1999.

**Field Monitoring for Insect Susceptibility:** This trait was not evaluated for this trial.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. The trial was monitored on July 7, July 15, August 12, August 31, September 22 and October 6, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants is no different from non-transgenic plants. This trait was monitored on July 7, July 15, August 12, August 31, September 22 and October 6, 1999.

**Plant Stand:** This trait was monitored on July 15, 1999. The plants were thinned to 3-4 plants/foot.

**Destruct Date and Method:** On November 17, 1999, the plants were mowed; burned seed cotton in plot area on November 19, 1999.

**Disposition of Seeds:** Seed was soaked for 26 hours then buried in plot.

**General Results of Field Trial:** Due to a stand loss, the first planting experiment was destroyed and re-planted on June 8, 1999. Gramoxone was used to destroy the old stand. Plants grew normally but the crop was very late due to re-planting. The final yield results are not yet available but are being processed.

**Monitoring for Volunteers:** Monitoring will take place once each month and volunteers removed via mechanical, chemical and/or hand-weeding.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Macon County, AL**

Planting Date: May 21, 1999

Harvest Date: November 11, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 21, July 16, August 12 and September 10, 1999.

**Field Monitoring for Insect Susceptibility:** There was not a higher incidence of non-target insect species in the transgenic plants than in the non-transgenic plants. Monitoring occurred on June 21, July 16, August 12 and September 10, 1999. It was noted that during the monitoring on June 21, 1999, that 100% of the transgenic and 100% of the non-transgenic had light thrips infestation.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. The trial was monitored on June 21, July 16, August 12 and September 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plans is no different from non-transgenic plants. The trial was monitored on June 21, July 16, August 12 and September 10, 1999.

**Plant Stand:** The trial was monitored on May 28 and June 4, 1999. There were no differences noted in the plant stand count.

**Destruct Date and Method:** On November 12, 1999, the plants were rotary mowed and disked.

**Disposition of Seeds:** Buried in the plot. Planted 0.15 acres of unregistered transgenic cottonseed using approximately 1 lb. of each and burying 4 lbs. of each in the plots. Box and bags that the seed came in were burned.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Jackson County, AR**

Planting Date: May 22, 1999  
Harvest Date: October 7, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 25, July 23, August 20 and October 7, 1999.

**Field Monitoring for Insect Susceptibility:** There were no differences noted that the transgenic plants were more susceptible to insect feeding than the non-transgenic plants. The trial was monitored on June 25, July 23, August 20 and October 7, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. The trial was monitored on June 25, July 23, August 20 and October 7, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plans is no different from non-transgenic plants. The trial was monitored on June 25, July 23, August 20 and October 7, 1999.

**Plant Stand:** There were no differences noted in the plant stand count. The trial was monitored on June 25, July 23, August 20 and October 7, 1999.

**Destruct Date and Method:** On October 22, 1999, the plot was destroyed by brush hogging and disking.

**Disposition of Seeds:** Left over planting seeds were incinerated.

**General Results of Field Trial:** The regulated lines both performed very well in growth characteristics and in yield.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Desha County, AR**

Planting Date: May 21, 1999

Harvest Date: October 21, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. Plots examined weekly and no diseases noted. This trait was monitored Mondays or Tuesdays to the end of August 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. Monitoring on July 26, August 3, August 9 and August 16, 1999 noted low pressure Heliothines. Monitoring on September 15, 1999, noted that 100% of the non-transgenics and none of the transgenics had high pressure Loopers. Monitoring on September 24, 1999, noted that 10% of the non-transgenics and none of the transgenics had moderate pressure Hi virescens.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. Plots examined weekly on Mondays or Tuesdays and no abnormal growth characteristics noted.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plans is no different from non-transgenic plants. Plots examined weekly and no abnormal germination noted..

**Plant Stand:** There were no differences noted in the plant stand count. The trial was monitored on June 3 and June 9, 1999. Not much difference was observed.

**Destruct Date and Method:** On October 21, 1999, the plot was shredded. 1-1/2 inch rain on October 31, 1999.

**Disposition of Seeds:** Left on site.

**General Results of Field Trial:** Poor in-season data on bollworm and tobacco budworm because of light worm pressure. Good late season data on introduced beet armyworm, soybean looper, cabbage looper and late season tobacco budworm.

**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

**Jefferson County, AR**

Planting Date: May 20, 1999  
Harvest Date: October 12, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50B, DP50

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 28, July 26 and August 16, 1999.

**Field Monitoring for Insect Susceptibility:** There were no differences noted that the transgenic plants were more susceptible to insect feeding than the non-transgenic plants. The trial was monitored on June 28, July 26 and August 16, 1999. It was noted that during the monitoring on August 16, 1999, there was good beet armyworm control in Cryx.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance, growth, flowering and/or seed production of the transgenic and non-transgenic plants. The trial was monitored on June 28, July 26 and August 16, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plans is no different from non-transgenic plants. The trial was monitored on June 28, July 26 and August 16, 1999.

**Plant Stand:** The trial was monitored on June 28 and July 26, 1999. There were no differences noted in the plant stand count. At each observation the stand count was 3-4/ft.

**Destruct Date and Method:** October 12, 1999, bush-hog plot area.

**Disposition of Seeds:** Buried inside test area.

**General Results of Field Trial:**

June 28, 1999	Excellent crop
July 26, 1999	Excellent crop condition, appears ahead of former crop.
August 16, 1999	Excellent crop, good fruit set, appears to have excellent yield potential.



**1999 Cotton Field Trial Report**  
**USDA #99-102-22n/Mons #99-248XRAB**

[ CBI DELETED ] Pinal County, AZ

This field trial was not conducted.

[ CBI DELETED ] Pinal County, AZ

This field trial was not conducted.

[ CBI DELETED ] Pinal County, AZ

This field trial was not conducted.

[ CBI DELETED ] Fresno County, CA

This field trial was not conducted.

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**1999 Cotton Field Trial Report**  
**USDA #99-102-23n      Monsanto #99-276XRAB**

[REDACTED]  
**November 3, 2000**

**Monsanto Company**

**Location**

**County**

**State**

[ CBI DELETED ]

Jackson  
 Santa Rosa  
 Dodge  
 Pulaski  
 Tift  
 Decatur  
 Seminole  
 Terrell  
 Tift  
 Tift  
 Decatur  
 Burke  
 Sumter  
 Mitchell  
 Pemiscot  
 Washington

Florida  
 Florida  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Georgia  
 Missouri  
 Mississippi

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**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Jackson County, FL**

Planting Date: June 9, 1999

Harvest Date: December 21, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** No differences were observed in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored during the season. Monitoring observation on July 7, 1999, noted that the transgenic and the non-transgenic plants produced Rhizoctonia.

**Field Monitoring for Insect Susceptibility:** No differences were observed between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored during the season. Monitoring observation on July 7, 1999 noted that 100% of the transgenic plants and 100% of the non-transgenic plants were infested with Thrip.

**Field Monitoring for Plant Growth Characteristics:** No differences in plant growth characteristics were observed between the transgenic lines and their respective non-transgenic lines. The trial was monitored during the season. Monitoring observation on July 7, 1999 noted that 100% of the transgenic plants and 100% of the non-transgenic plants noted cadre carryover across the plot area.

**Field Monitoring for Weediness Characteristics:** The trial was monitored during the season. The germination of transgenic plants was no different from non-transgenic plants.

**Plant Stand:** No differences in plant stand between the transgenic lines and their respective non-transgenic lines. Monitoring observations on June 25, 1999 (4 per foot for the transgenic plants and 4 per foot for the non-transgenic plants), July 16, August 16, September 16 and October 18, 1999 indicated no differences in plant stand.

**Destruct Date and Method:** December 21, 1999 - Mowed and plowed.

**Disposition of Seeds:** Bags were burned - .63 lbs. of each line were planted and the remainder of seed buried within the plot area.

**General Results of Field Trial:** Cotton struggled in early growth stages due to seedling diseases and some cadre carryover. Later yields may also be due to some late season stink bugs which caused bolls not to open in the top. No worm pressure throughout the year.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Santa Rosa County, FL**

Planting Date: June 21, 1999

Destruct Date: November 17, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 1, July 29, August 26, September 23 and October 21, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 1, July 29, August 26, September 23 and October 21, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 1, July 29, August 26, September 23 and October 21, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 1, July 29, August 26, September 23 and October 21, 1999.

**Plant Stand:** Monitoring observations on July 19, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 17, 1999 - mowed and disked under.

**Disposition of Seeds:** Buried in plot.

**General Results of Field Trial:** The test was planted late. Dry conditions persisted resulting in poor plant growth, very late maturity and little yield. Therefore, the plots were not harvested for yield data.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Dodge County, GA**

Planting Date: May 27, 1999  
Harvest Date: November 19, 1999  
Destruct Date: November 20, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** The trial was monitored during the season. There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines.

**Field Monitoring for Insect Susceptibility:** The trial was monitored during the season. No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility.

**Field Monitoring for Plant Growth Characteristics:** The trial was monitored during the season. There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The trial was monitored during the season. The germination of transgenic plants was no different from non-transgenic plants.

**Plant Stand:** Monitoring observations on June 15, 1999 (2.2 for the transgenic plants and 2.2 for the non-transgenic plants) did not observe any differences in plant stand.

**Destruct Date and Method:** November 20, 1999 - mowed and disked.

**Disposition of Seeds:** Spread where grown and disked in.

**General Results of Field Trial:** Performed as good or better than other varieties. No problems.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Pulaski County, GA**

Planting Date: June 8, 1999

Harvest Date: November 5, 1999

Destruct Date: November 11, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 2, August 6, September 3 and October 4, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 2, August 6, September 3 and October 4, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 2, August 6, September 3 and October 4, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 2, August 6, September 3 and October 4, 1999.

**Plant Stand:** Monitoring observations were completed on June 15 and June 22, 1999. While there were differences noted, these were considered to be within the normal variation range for cotton plants.

**Destruct Date and Method:** November 11, 1999 -- rotary cut and harrowed.

**Disposition of Seeds:** Burned.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Tift County, GA (1)**

Planting Date: May 31, 1999

Harvest/Destruct Date: November 4, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 1, August 9, September 21 and October 29, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 1, August 9, September 21 and October 29, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 1, August 9, September 21 and October 29, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 1, August 9, September 21 and October 29, 1999.

**Plant Stand:** Monitoring observations were completed on July 1, August 9, September 21 and October 29, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 4, 1999 - burned on site.

**Disposition of Seeds:** Buried on site.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Decatur County, GA (1)**

Planting Date: June 2, 1999

Harvest Date: November 29, 1999

Destruct Date: November 30, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 3, August 5, September 4 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 3, 1999 (no differences were observed); August 5, 1999 (budworm noted on 50% of the non-transgenic plants); September 4, 1999 (loopers noted on 50% of the non-transgenic plants); and monitoring on October 5, 1999 did not note any differences.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 3, August 5, September 4 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 3, August 5, September 4 and October 5, 1999.

**Plant Stand:** Monitoring observations were completed on July 3, August 5, September 4 and October 5, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 30, 1999 - plot was mowed and then harrowed.

**Disposition of Seeds:** Buried in plot.

**General Results of Field Trial:** Overall insect pressure from Tobacco Budworm and Soybean Loopers was much heavier on DP50 than the other varieties. There was little to no pressure from other larval insects to evaluate in this trial.



**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Seminole County, GA**

Planting Date: June 2, 1999

Harvaest Date: November 10, 1999 and November 12, 1999

Destruct Date: November 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 23, July 18, August 21, September 17 and October 20, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 23 (90% of the transgenic plants and 90% of the non-transgenic plants were infested with heavy Thrip pressure); observation on July 18, 1999 (25% of the non-transgenic plants were infested with budworm and corn earworm); August 21, 1999 (30% defoliation of the non-transgenic plants were infested with-looper); September 17, 1999 (10% of the transgenic plants and 10% of the non-transgenic plants were infested with Stinkbugs); and monitoring on October 20, 1999 did not note any differences.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 23, July 18, August 21, September 17 and October 20, 1999.

**Field Monitoring for Weediness Characteristics:** No significant differences were noted in the germination of transgenic plants compared to the non-transgenic plants. This trait was monitored on June 23 (50% of the transgenic plants and 50% of the non-transgenic plants observed sicklepod, Tx. Panium). Observations on July 18, August 21, September 17 and October 20, 1999 did not note any differences.

**Plant Stand:** Monitoring observations completed on June 23, 1999 noted some Rhizoctonia on 5% of the transgenic plants and 5% on the non-transgenic plants. Monitoring observations completed July 18, August 21, September 17 and October 20, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 12, 1999 - mowed stalks and disked.

**Disposition of Seeds:** Buried in plot.

**General Results of Field Trial:** Growth characteristics were same as parent variety. Did not notice any difference in fruiting. Both the 158713 and 15985 lines looked pretty good and seemed to well adapted to my area.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Terrell County, GA**

Planting Date: May 26, 1999  
Harvest Date: November 11, 1999  
Destruct Date: November 12, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 23, July 21, August 23, September 20, October 13 and November 11, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 23 (80% of the transgenic plants and 80% of the non-transgenic plants were infested with thrips and aphids); July 21 (80% of the transgenic plants and 80% of the non-transgenic plants were infested with Thrips); August 23, 1999 (5% of the transgenic plants and 80% of the non-transgenic plants were infested with budworm, bollworm, fall armyworm); September 20, 1999 (50% of the transgenic plants and 50% of the non-transgenic plants were infested with stinkbugs); October 13, 1999 (70% of the transgenic plants and 70% of the non-transgenic plants were infested with stinkbugs); and November 11, 1999 (70% of the transgenic and 70% of the non-transgenic plants were infested with stinkbugs).

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 23, July 21, August 23, September 20, October 13 and November 11, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 23, July 21, August 23, September 20, October 13 and November 11, 1999.

**Plant Stand:** No significant differences were noted in plant stand of the transgenic and the non-transgenic plants. Observations were made on June 23, July 21, August 23, September 20, October 13, and November 11, 1999.

**Destruct Date and Method:** November 12, 1999 - Disc harrowed.

**Disposition of Seeds:** Buried 12" in ground.

**General Results of Field Trial:** Low populations of bollworm/budworm, therefore, no differences in yield noticed.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Tift County, GA (2)**

Planting Date: May 27, 1999

Harvest Date: November 5, 1999

Destruct Date: November 8, 1999; November 11, 1999; November 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 3, 1999 (<5% of transgenic plants and <5% of non-transgenic had slight Rhizoctonia and this is nothing unusual); June 11, and June 16, 1999 (~5% of the transgenic plants and ~5% of the non-transgenic had slight Rhizoctonia, and this is nothing unusual).

**Field Monitoring for Insect Susceptibility:** The trial was monitored throughout the season. The experimental lines appeared less susceptible to damage caused by *Heliothis/Helicoverpa* spp. than was the non-transgenic DPL50 variety. Heavy infestation of stink bugs was noted on August 31, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 3, June 11, July 7, 1999 (no differences were noted). Monitoring observations were made weekly from July 13 through August 31, 1999 (DP50 became greener and slightly taller late in the season due to insect damage). Observation on October 5, 1999 noted that DP50 was still less mature. Others will be defoliated.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 3, 1999.

**Plant Stand :** The trial was monitored during the season. No significant differences were noted in plant stand of the transgenic and the non-transgenic plants.

**Destruct Date and Method:** Harvested material burned on 8 November 1999. Non-harvested area mowed by 11 November and 12 November 1999.

**Disposition of Seeds:** Burned.

**General Results of Field Trial:** The Cry-X varieties performed as well as DP50B and better than the non-transgenic. No problems were seen with the Cry-X varieties and yield was very good.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Tift County, GA (3)**

Planting Date: May 28, 1999

Harvest Date: October 28, 1999

Destruct Date: November 2, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 29, July 27, August 24 and September 21, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 29, 1999 (in-furrow insecticide reduced populations), July 27, August 24 and September 21, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 29, July 27, August 24 and September 21, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 29, July 27, August 24 and September 21, 1999.

**Destruct Date and Method:** November 2, 1999.

**General Results of Field Trial:** Low insect pressure provided no separation between transgenic and non-transgenic lines. No yield differences, but a good yield.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Decatur County, GA (2)**

Planting Date: June 2, 1999

Harvest/Destruct Date: November 18, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 2, July 27, August 22, September 20 and October 19, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 2, 1999 (10% of the non-transgenic plants were infested with bollworms, 10% and beet armyworms, 1%); July 27, 1999 (15% of the non-transgenic plants were infested with bollworms); August 22, 1999 (17.5% of the non-transgenic plants were infested with bollworms); monitoring on September 20 and October 19, 1999 did not note any differences.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 2, July 27, August 22, September 20 and October 19, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 2, July 27, August 22, September 20 and October 19, 1999.

**Plant Stand:** Monitoring observations on July 2, July 27, August 22, September 20 and October 19, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** Harvested cotton and put in sacks. Mowed cotton stalks and disked field after harvest.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Burke County, GA**

Planting Date: May 27, 1999

Harvest Date: November 9, 1999

Destruct Date: November 10, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 7 and July 12, 1999.

**Field Monitoring for Insect Susceptibility:** Four experimental cotton varieties were planted at the test site. The varieties were DPL50, DPL50B, 15813 and 15985. Ten terminals, squares and/or bolls were examined for insects and damage beginning on July 21, 1999 and continuing more or less weekly until September 8, 1999. During this period the variety 15985 showed outstanding resistance to terminal, square and boll infestation by bollworms. The variety DPL50B also had low damage but not as good as 15985. DPL50 and 15813 had variable but sustained infestations by bollworms during the test period.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 7 and July 12, 1999.

**Field Monitoring for Weediness Characteristics:** No significant differences were noted in the germination of transgenic plants compared to the non-transgenic plants. This trait was monitored on July 7 (very weedy area, 100% of plants infected) and July 12 and August 15, 1999 (herbicide control was effective).

**Plant Stand:** Monitoring observations on July 21, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 10, 1999 - burning of harvested cotton.

**Disposition of Seeds:** Buried at test site.

**General Results of Field Trial:** A moderate infestation occurred at the location during the season. DP50 had terminal and fruit damage during the season and DP50 had a small amount of injury. Lines 15813 and 15985 had outstanding resistance during the season.

**Monitoring for Volunteers:** The trial area was monitored on November 10 and December 7, 1999. No volunteers were observed.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Sumter County, GA**

Planting Date: June 1, 1999

Harvest/Destruct Date: November 10, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on June 17, July 1, July 21, July 28, September 3, September 9, September 17, September 26, October 5 and November 10, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on June 17, July 1, July 21, July 28, September 3, September 9, September 17, September 26, October 5 and November 10, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on June 17, July 1, July 21, July 28, September 3, September 9, September 17, September 26, October 5 and November 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on June 17, July 1, July 21, July 28, September 3, September 9, September 17, September 26, October 5 and November 10, 1999.

**Plant Stand:** Monitoring observations on June 17 and July 1, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** November 10, 1999 - mowed entire area.

**Disposition of Seeds:** Unplanted seed buried in plot area. Harvested seed cotton was burned within the plot area.

**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Mitchell County, GA**

Planting Date: June 2, 1999

Harvest Date: November 5, 1999 and November 8, 1999

Destruct Date: November 8, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** The trial was monitored on June 20, July 15, August 17, September 20, and October 22, 1999. No significant differences were noted between transgenic and non-transgenic plants. Rhizoctonia was observed in all plants on June 20, 1999.

**Field Monitoring for Insect Susceptibility:** The trial was monitored on June 20, July 15, August 17, September 20, and October 22, 1999. No significant differences were noted between transgenic and non-transgenic plants. Thrip was observed in all plants on June 20, 1999.

**Field Monitoring for Plant Growth Characteristics:** The trial was monitored on June 20, July 15, August 17, September 20, and October 22, 1999. No significant differences were noted between transgenic and non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The trial was monitored on June 20, July 15, August 17, September 20, and October 22, 1999. No significant differences were noted between transgenic and non-transgenic plants.

**Plant Stand:** The trial was monitored on June 20, July 15, August 17, September 20, and October 22, 1999. No significant differences were noted between transgenic and non-transgenic plants.

**Destruct Date and Method:** On November 8, 1999, the trial area was mowed and disked.

**Disposition of Seeds:** Buried in plot area.



**1999 Cotton Field Trial Report**  
**USDA #99-102-23n/Mons #99-276XRAB**

**Pemiscot County, MO**

Planting Date: June 1, 1999  
Harvest Date: October 28, 1999  
Destruct Date: October 29, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on July 2, July 9, July 16, July 23 and July 30, 1999.

**Field Monitoring for Insect Susceptibility:** Differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on July 2, 1999 (no differences noted); July 9, 1999 (8.5 of the transgenic plants and 9.5% of the non-transgenic plants were infested with aphids); July 16, 1999 (no differences noted); July 23, 1999 (60/20 of the transgenic plants and 60/20 of the non-transgenic plants were infested with aphids and red spider mites); and July 30, 1999 (30/20 of the transgenic plants and 30/20 of the non-transgenic plants were infested with aphids and red spider mites).

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on July 2, July 9, July 16, July 23 and July 30, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 2, July 9, July 16, July 23 and July 30, 1999.

**Plant Stand:** Monitoring observations on July 2, July 9, July 16, July 23 and July 30, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** October 29, 1999 - Bush hog mowed and disked into the soil.

**Disposition of Seeds:** Incinerated.

**General Results of Field Trial:** Extremely dry conditions. There was virtually no rainfall in July and August that greatly reduced plant development. The fruit load aborted due to lack of moisture. Yields were very low.

**Washington County, MS**

This field trial was not planted.

1999 Cotton Field Trial Report  
USDA #99-110-19n      Monsanto #99-321XRAB



November 3, 2000

Monsanto Company

Location

County

State

[ CBI DELETED

Poinsett	Arkansas
Monroe	Arkansas
Mississippi	Arkansas
Burke	Georgia
Martin	North Carolina
Johnston	North Carolina
Edgecombe	North Carolina
Washington	North Carolina
Gibson	Tennessee
Madison	Tennessee

Poinsett County, AR

Planting Date: May 27, 1999

Harvest Date: October 6, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 21, July 15, August 7, August 27 and September 22, 1999.

**Field Monitoring for Insect Susceptibility:** Monitoring observation on July 15, 1999, noted that there were aphids across the whole trial. They were treated with 2.5 oz Provado/A. Observations on June 21, August 7, August 27 and September 22, 1999, noted there were no differences between the transgenic lines and their respective non-transgenic lines for insect susceptibility.

**Field Monitoring for Plant Growth Characteristics:** During the monitoring observation on July 15, 1999, one row of DPL50 got Dirextmsma Drift. During the monitoring observations on June 21, August 7, August 27 and September 22, 1999, there were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 21, July 15, August 7, August 27 and September 22, 1999.

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Plant Stand:** Monitoring observations on June 21, July 15, August 7, August 27 and September 22, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** Border rows were mowed on September 29, 1999 to prevent accidental harvest (test conducted w/n 200 acre block of cotton). Test area was flagged and test plots were hand picked on October 6, 1999.

**Disposition of Seeds:** The harvested material went back into the plot area for destruction. It was mowed and disked under.

**General Results of Field Trial:** Target insect pest populations were low (Bollworm and Budworm). Insect injury by target pests was not detectable on any sample data. The study was treated for aphids (Provado) and mites (Curacron). Boll weevil was the primary source of boll injury. The two experimental lines performed well and yielded well as did the DPL50B. Though the cotton was planted late, plants matured rapidly and set a high percentage of harvestable bolls despite hot weather. The test was irrigated and did not suffer drought conditions which aided crop development.

Yields:	<u>DPL50</u>	<u>DPL50B</u>	<u>15985</u>	<u>15813</u>
lbs seed cotton/acre	1513.2 lbs	3851.7 lbs	3356.5 lbs	2998.8 lbs

**Monitoring for Volunteers:** Cotton stalks were mowed on October 17, 1999. Crop debris within trial area was exposed to winter weather until March 27, 2000. On April 27, 2000, all crop debris in the trial was cultivated, i.e., buried under the soil. A 2000 field trial will be located at the same location.

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Monroe County, AR**

Planting Date: May 24, 1999

Harvest Date: October 26, 1999

Vector Construct: PV-GHBK11

Lines: 15813, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 8, July 6, August 10, September 3 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility, but light insect pressure was noted. The trial was monitored on June 8, July 6, August 10, September 3 and October 5, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 8, July 6, August 10, September 3 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 8, July 6, August 10, September 3 and October 5, 1999.

**Destruction Method:** Tilled under.

**Disposition of Seeds:** The seed was left in the field and tilled in the soil.

**Monitoring for Volunteers:** The trial area was monitored on November 15, 1999 and April 10, 2000. No volunteers were observed.

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Mississippi County, AR**

Planting Date: June 2, 1999

Harvest Date: October 26, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 14, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. The trial was monitored on June 14, 1999 (looked for thrips and none were found) and weekly thereafter (standard insect scout – all cotton plots on farm sprayed for bollworms on August 4).

**Field Monitoring for Plant Growth Characteristics:** There were no differences noted in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on August 4 (NAWF counted – nodes above white flower), August 10 (NAWF counted – nodes above white flower) and October 18, 1999 (end-of-season plant map using COTMAP).

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 4 (smart weed and dock present – treated with pre-emerging herbicide) and June 9, 1999 (cocklebur and morning glory plowed and post dir. herbicide).

**Plant Stand:** Monitoring observations on June 14, 1999 did not observe any differences in plant stand. There was a good stand on all plots.

**Destruct Date and Method:** On November 10, 1999, plants were shredded with frail mower than tilled and re-bedded on November 12, 1999.

**Disposition of Seeds:** Buried in the plot area.

**Additional Comments:** Fifty boll samples were hand sampled from three replications. Samples were weighed and ginned in the plot area using a small plot gin and generator. After weighing and removing a small sample of lint, all seeds were buried in the plot area.

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Burke County, GA**

Planting Date: June 7, 1999

Harvest Date: November 5, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on July 7, July 28, August 4, August 10 and August 19, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility, but light insect pressure was noted. The trial was monitored on July 7, July 28, August 4, August 10, and August 19, 1999.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on July 7, July 28, August 4, August 10, and August 19, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on July 7, July 28, August 4, August 10, and August 19, 1999.

**Plant Stand:** Differences were noted in the plant stand. On June 15, 1999, the difference in plant stand between the transgenic and the non-transgenic plants was 2.6. On June 28, 1999, the difference in plant stand between the transgenic and the non-transgenic plants was 2.4. Differences observed were considered to be within the normal variation for cotton plants.

**Destruction Date:** November 6, 1999.

**Disposition of Seeds:** Buried in the plot.

**General Results of the Field Trial:** Agronomic performance was good and insect pressure was minimal.

**Monitoring for Volunteers:** Dates of observation for volunteers were December 13, 1999, January 10, February 14, March 20 and April 17, 2000. There were no volunteers observed.

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Martin County, North Carolina**

Planting Date: May 20, 1999

Harvest Date: November 12, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 12, July 7, July 28, August 10, and September 8, 1999.

**Field Monitoring for Insect Susceptibility:** Observations on July 28, 1999 indicated that in 5% of the transgenic and 12% non-transgenic plants, and in observations on August 4, 1999, 10.5% of the transgenic and 15% of the non-transgenic plants, noted there were no differences in incidence of non-target species between transgenic and non-transgenic. Observations on August 10, 0.5% of the transgenic and 12.5% of the non-transgenic; and in observations on August 17, 1999, 1.0% of the transgenic and 22% of the non-transgenic noted that the transgenic plants were less susceptible to bollworm feeding than the non-transgenic plants. On August 25, 1999 2.2% of the transgenic and 12.5% of the non-transgenic were observed in the field trial. On the dates of July 28 and August 4, 1999 are terminal damage; the rest are boll damage.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on July 7, July 28, August 4, August 10, and August 19, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 3 and June 12, 1999.

**Plant Stand:** Monitoring observations on June 3 and June 12, 1999 did not observe any differences in plant stand.

**Destruction Method:** Incinerated.

**Disposition of Seeds:** Incinerated.

**Johnston County, North Carolina**

This field trial was not planted.

**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Edgecombe County, NC**

Planting Date: May 24, 1999

Harvest Date: November 1, 1999

Vector Construct: PV-GHBK11

Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 11, July 6, July 28, August 13, and September 10, 1999.

**Field Monitoring for Insect Susceptibility:** Observations on July 28, 1999 indicated that in 6% of the transgenic and 20% non-transgenic plants, and in observations on August 3, 1999, 4.6% of the transgenic and 32% of the non-transgenic plants, noted there were no differences in incidence of non-target species between transgenic and non-transgenic. Observations on August 5, 3% of the transgenic and 24.5% of the non-transgenic; and in observations on August 13, 1999, 1.3% of the transgenic and 45% of the non-transgenic noted that the transgenic plants were less susceptible to bollworm feeding than the non-transgenic plants. On August 20, 1999 3.3% of the transgenic and 32.5% of the non-transgenic were observed in the field trial. On the dates of July 28 and August 3, 1999, are terminal damage; the rest are boll damage.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 11, July 6, July 28, August 13, and September 10, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 2 and June 11, 1999.

**Plant Stand:** Monitoring observations on June 2 and June 11, 1999 did not observe any differences in plant stand.

**Destruction Method:** On November 1, 1999, the harvested seed cotton was burned, then disked.

**Disposition of Seeds:** Burned and then disked under residue.



**1999 Cotton Field Trial Report**  
**USDA #99-110-19n/Mons #99-321XRAB**

**Washington County, NC**

Planting Date: May 21, 1999  
Harvest Date: November 5, 1999  
Vector Construct: PV-GHBK11  
Lines: 15813, 15985, DP50, DP50B

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. The trial was monitored on June 12, July 7, July 28, August 10, and September 8, 1999.

**Field Monitoring for Insect Susceptibility:** Observations on July 28, 1999 indicated that in 6% of the transgenic and 20% non-transgenic plants, and in observations on August 4, 1999, 10% of the transgenic and 28% of the non-transgenic plants, noted there were no differences in incidence of non-target species between transgenic and non-transgenic. Observations on August 10, 1.5% of the transgenic and 10% of the non-transgenic; and in observations on August 17, 1999, 2.3% of the transgenic and 28% of the non-transgenic noted that the transgenic plants were less susceptible to bollworm feeding than the non-transgenic plants. On August 25, 1999 1.5% of the transgenic and 29.5% of the non-transgenic were observed in the field trial. On the dates of July 28 and August 4, 1999 are terminal damage; the rest are boll damage.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. The trial was monitored on June 12, July 7, July 28, August 10, and September 8, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. The trial was monitored on June 3 and June 12, 1999.

**Plant Stand:** Monitoring observations on June 3 and June 12, 1999 did not observe any differences in plant stand.

**Destruct Date and Method:** Incinerated - November 5, 1999.

**Disposition of Seeds:** Incinerated.

**Gibson County, TN**

This field trial was not planted.

**Madison County, TN**

This field trial was not planted.

1999 Cotton Field Trial Report  
USDA #99-110-22n      Monsanto #99-326XRAB



November 3, 2000

Monsanto Company

Location

County

State

[ CBI DELETED ]

Washington

MS

Washington County, MS

This site was not planted.

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1999 Cotton Field Trial Report  
USDA #99-110-24n      Monsanto #99-329XRAB

[REDACTED]  
November 3, 2000

Monsanto Company

Location

Monsanto Agronomy Center

County

Washington

State

MS

Washington County, MS

Planting Date: June 9, 1999

Harvest Date: September 29, 1999

Project Study #: 99-TD-IPC-04

Vector Constructs/Lines: PV-GHBK11 (Lines 16509, 17180, 16456-2, 16871-5, 17168-1, 15985, 15813  
PV-GHBK12 (Lines 17079, 17133, 17159, 17206, 15830-5)  
PV-GHBK13 (Lines 16169, 17282)  
PV-GHBK14 (Lines 16221, 16960, 17117, 17377, 17405, 16380-11)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility July 5, August 2 and September 3, 1999. In observances on October 5, 1999, it was noted that there was 10% - 20% boll damage in the non-transgenic controls vs. the IP cotton with essentially no boll damage.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Plant Stand:** There were no differences noted in the transgenic vs. non-transgenic plant stand count. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Destruct Date and Method:** Destroyed on September 29, 1999 -- clipped with bush hog clippers.

**Disposition of Seeds:** Remained in the field in the release site. It was devitalized by burying 2' deep.

**1999 Cotton Field Trial Report**  
**USDA #99-110-24n/Mons #99-329XRAB**

**Washington County, MS**

Planting Date: June 9, 1999

Harvest Date: September 29, 1999

Project Study #: 99-TD-IPC-05

Vector Constructs/Lines: PV-GHBK11 (Lines 16509, 17180, 16456-2, 16871-5, 17168-1, 15985, 15813

PV-GHBK12 (Lines 17079, 17133, 17159, 17206, 15830-5)

PV-GHBK13 (Lines 16169, 17282)

PV-GHBK14 (Lines 16221, 16960, 17117, 17377, 17405, 16380-11)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility July 5, August 2 and September 3, 1999. In observances on October 5, 1999, it was noted that there was 10% - 20% boll damage in the non-transgenic controls vs. the IP cotton with essentially no boll damage.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Plant Stand:** There were no differences noted in the transgenic vs. non-transgenic plant stand count. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Destruct Date and Method:** Destroyed on September 29, 1999 -- clipped with bush hog clippers.

**Disposition of Seeds:** Remained in the field in the release site. It was devitalized by burying 2' deep.

**1999 Cotton Field Trial Report**  
**USDA #99-110-24n/Mons #99-329XRAB**

**Washington County, MS**

Planting Date: June 9, 1999

Harvest Date: October 25, 1999

Project Study #: 99-TD-IPC-06

Vector Constructs/Lines: PV-GHBK11 (Lines 16509, 17180, 16456-2, 16871-5, 17168-1, 15985, 15813  
PV-GHBK12 (Lines 17079, 17133, 17159, 17206, 15830-5)  
PV-GHBK13 (Lines 16169, 17282)  
PV-GHBK14 (Lines 16221, 16960, 17117, 17377, 17405, 16380-11)

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility July 5, August 2 and September 3, 1999. In observations on October 5, 1999, it was noted that there was 10% - 20% boll damage in the non-transgenic controls vs. the IP cotton with essentially no boll damage.

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Plant Stand:** There were no differences noted in the transgenic vs. non-transgenic plant stand count. This trial was monitored on July 5, August 2, September 3 and October 5, 1999.

**Destruct Date and Method:** Destroyed on October 25, 1999 -- clipped with bush hog clippers.

**Disposition of Seeds:** Remained in the field in the release site. It was devitalized by burying 2' deep.

1999 Cotton Field Trial Report  
USDA #99-110-23n      Monsanto #99-331XRAB

[REDACTED]  
November 3, 2000

Monsanto Company

Location

Monsanto Agronomy Center

County

Baldwin

State

AL

Baldwin County, AL

Planting Date: June 7, 1999

Harvest Date: November 9, 1999

Project Study #: 99-TD-IPC-01

Vector Constructs: PV-GHBK11

Lines Planted: DP50, DP50B, 15813, 15985, 16221, 17180

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trial was monitored on July 7, August 3, August 31 and September 28, 1999.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trial was monitored on July 7 (no significant insect damage), August 3 (insect damage greater in non-transgenic), August 31 (insect damage greater in non-transgenic) and September 28, 1999 (no significant insect damage).

**Field Monitoring for Plant Growth Characteristics:** There was no difference in the general appearance and growth of the transgenic and the non-transgenic plants. This trial was monitored on July 7, August 3, August 31 and September 28, 1999.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on July 7, 1999. All plots emerged at the same time.

**Plant Stand:** There were no differences noted in the transgenic vs. non-transgenic plant stand count. This trait was monitored on July, 1999. Plant stands are equal.

**Destruct Date and Method:** On November 9, 1999, shredded all remaining stalks.

**Disposition of Seeds:** All of the seed was planted. After harvest all seed cotton was destroyed in the field.

**General Results of Field Trial:** The trial was completed with no complications.

**1999 Cotton Field Trial Report**  
**USDA #99-110-23n/Mons #99-331XRAB**

**Monitoring for Volunteers:**

January 21, 2000	No volunteers present	
February 18, 2000	No volunteers present	Weather will not allow germination.
March 17, 2000	No volunteers present	Winter cover sprayed with Roundup
April 17, 2000	No volunteers present	Ground recently disked and bedded.
May 26, 2000	Entire area planted back to same transgenic crop for 2000 growing season. Monitoring for volunteers will stop and in-season monitoring for 2000 will begin.	

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1999 Cotton Field Trial Report  
USDA #99-252-07n      Monsanto #99-700XRAB

November 3, 2000

Monsanto Company

Location

Monsanto Research Farm

County

Susua Baja

State

Puerto Rico

Susua Baja County, PR

Planting Date: December 6, 1999

Harvest Date: May 25, 2000

Vector Construct: PV-GHBK11

Lines: 15985

**Field Monitoring for Disease Susceptibility:** There appeared to be no significant differences in disease susceptibility of the transgenic lines compared with the non-transgenic lines. This trait was monitored on January 18, March 2 and April 27, 2000.

**Field Monitoring for Insect Susceptibility:** No differences were noted between the transgenic lines and their respective non-transgenic lines for insect susceptibility. This trait was monitored on January 18, March 2 and April 27, 2000.

**Field Monitoring for Plant Growth Characteristics:** There were no differences in the general appearance and growth of the transgenic and the non-transgenic plants. This trait was monitored on January 18, March 2 and April 27, 2000.

**Field Monitoring for Weediness Characteristics:** The germination of transgenic plants was no different from non-transgenic plants. This trait was monitored on January 18, March 2 and April 27, 2000.

**Plant Stand:** Monitoring observations on January 18, March 2 and April 27, 2000 did not observe any differences in plant stand.

**General Results of Field Trial:** No differences were measured between lines because of a lack of insect pressure.



**Request for Determination of Non-Regulated Status for the Regulated  
Article: Bollgard® II Cotton Event 15985 (*Gossypium hirsutum* L.) Producing  
the Cry2Ab Insect Control Protein derived from  
*Bacillus thuringiensis* subsp. *kurstaki***

**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

**[CBI Deleted]**

**Section 1. Amended Report for Molecular Characterization of  
Cotton Event 15985. (49 pages)**

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the Cry2Ab Insect Control Protein derived from  
*Bacillus thuringiensis* subsp. *kurstaki***

**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

**[CBI Deleted]**      Section 2. Molecular Analysis of the Stability of Cotton Event  
15985. (30 pages)

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*Bacillus thuringiensis* subsp. *kurstaki***

**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

**[CBI Deleted]**

Section 3. Field Report: Production of Tissue Samples from  
Insect Protected Cotton Events Grown in 1998 U.S. Field Trials  
(41 pages)

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MONSANTO

Food • Health • Hope™



00-342-01p  
CBI-deleted  
amendment

MONSANTO COMPANY  
600 13TH STREET, N.W.  
SUITE 660  
WASHINGTON, D.C. 20005  
TEL: [REDACTED]  
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May 16, 2001

[REDACTED]  
Staff Biotechnologist  
U.S. Department of Agriculture, APHIS, PPQ  
Permits and Risk Analysis Branch  
4700 River Road, Unit 133  
Riverdale, MD 20737-1236

Subject: Response to USDA Questions Received on March 16, 2001; Petition 00-342-01p

Dear [REDACTED]

The following additional information is provided by Monsanto to the Permits and Risk Analysis Branch of USDA APHIS PPQ as an amendment to our request for a determination of nonregulated status for Bollgard II cotton event 15985 (Petition Number 00-342-01p). Some of the attachments contain confidential business information and therefore a CBI deleted version is also being supplied, along with a CBI justification statement.

The Agency noted that the following issues in bold text needed to be addressed prior to declaration of the petition as technically complete. The responses are listed following each question.

**P.2 para 2. Declare whether Monsanto intends to separate the cry2Ab2 and cry1Ac genes at a later date so that only cry2Ab2 would be expressed without cry1Ac.**

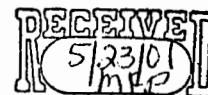
One of the significant benefits of Bollgard II cotton is a combination of insect-protection genes to reduce the likelihood of resistance development in target insects. Therefore it is not Monsanto's intention to commercialize progeny derived from this event that contains only a single insect-protection gene.

**P.4 para 2. For your future reference, the FPPA and PQA are replaced by the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772).**

We will provide the appropriate reference in future requests.

**P. 12 Describe the origin of the line DP50 for the Abbreviation list.**

DP50 is a non-transgenic, traditionally-bred, commercial cotton variety produced by the Delta and Pine Land Company. It has background genetics representative of Bollgard II cotton event 15985.



***For you future petitions, you may delete sections that refer to general material in previous petitions.***

We acknowledge that such sections are optional.

***P. 19 para 1 (G. Characteristics...) Give a complete description of the parental type for 15985, mentioning date of deregulation, and also the origin of this parental line DP50B.***

The cotton cultivar used as the parental variety for transformation was Delta and Pine Land Company variety 50B (DP50B), derived from Bollgard cotton event 531 by traditional breeding with DP50 non-transgenic cotton. Bollgard cotton event 531 was deregulated by USDA in June, 1995 and was commercialized in the United States in 1996.

***P. 21 Describe whether the two intervening sequences between the terminal transit peptide and the cry2Ab2 gene (at 3960-3965 bp) and the one between the cry2Ab2 gene (at 5874-5896 bp) and the NOS terminator are expressed as amino acids in the Cry2Ab2 protein.***

DNA encoding the *Arabidopsis thaliana* EPSPS chloroplast transit peptide (bp 3729-3959) was coupled via a synthetic linker (bp 3960-3965) to the DNA encoding the Cry2Ab protein (bp 3966-5873). This entire coding region is predicted to encode a single 713 amino acid protein. Post-translational cleavage of the chloroplast transit peptide from the Cry2Ab protein is predicted to occur between amino acids 76 and 77 of this protein (Keegstra *et al.*, 1989). The resulting Cry2Ab protein is predicted to contain an additional three amino acids at the N-terminus, two of which were encoded by the synthetic linker (bp 3960-3965). A diagram of the anticipated amino acid sequence of the Cry2Ab protein is shown in Figure 3 of the petition. The underlined portion of the figure defines the predicted three additional amino acids, the latter two derived from the polylinker sequence.

No sequence was derived from the Edman degradation conducted to verify the amino acid structure of the N-terminus, suggesting the protein is N-terminally blocked. Mass spectrometry experiments have been conducted on cotton plant-produced Cry2Ab protein to verify the primary structure of the protein and clarify whether or not the predicted additional amino acids are present. Mass spectrometry techniques have included MALDI and LC/MS/MS, which are commonly used in the assessment of pharmacological protein sequencing. The MALDI time-of-flight experiments were described in Appendix 6, Section 5 of the petition. No confirming sequence of the Cry2Ab N-terminus has been obtained from these experiments.

A second synthetic linker (bp 5874-5896) was used to couple the DNA encoding the Cry2Ab protein (bp 3966-5873) and the genetic element, NOS 3' (bp 5897-6152). Two translational stop codons are present at the 3' end of the *cry2Ab* coding region (bp 5868-5873). Thus DNA downstream of this position (bp 5868-5873) is not predicted to encode protein. Therefore, the synthetic linker sequence is not expected to be translated.

**Reference**

Keegstra, K., L.J. Olsen, and S.M. Theg. 1989. Chloroplastic precursors and their transport across the envelope membranes. *Ann Rev Plant Physiol Plant Mol Biol* 40:471-501.

**P. 27 para 1&2. Rearrange the contents of the first two paragraphs which summarize the findings of the molecular analysis. Follow the format presented in Directive 99-1. The findings and conclusions are to be reported first, and then the experiments that were accomplished along with the data that were obtained are to be reported second.**

The analysis demonstrates that Bollgard II cotton event 15985 contains one DNA insertion from the linear fragment of PV-GHBK11 (Table 2). The insert contains one copy each of the *cry2Ab* and *uidA* cassettes. The *cry2Ab* gene cassette is comprised of an enhanced CaMV 35s promoter, a chloroplast transit peptide from *Arabidopsis thaliana* and has a polyadenylation sequence derived from *Agrobacterium tumefaciens*. The *cry2Ab* coding region and cassette are complete; however, the restriction site following the NOS 3' polyadenylation sequence in the cassette is no longer present. The *uidA* gene cassette consists of an enhanced CaMV 35s promoter and has a polyadenylation sequence derived from *Agrobacterium tumefaciens*. The *uidA* coding region and its NOS 3' polyadenylation sequence are also complete; however, 260 bp of the 5' end of the enhanced CaMV 35S promoter of the *uidA* cassette is not present in the inserted *uidA* gene cassette. The e35S promoter is still functional despite this truncation, as demonstrated by production of the GUS protein. This event does not contain any detectable backbone sequence derived from plasmid PV-GHBK11. It is therefore concluded that full-length Cry2Ab and GUS proteins should be produced in event 15985 as a result of integration of the DNA segment derived from plasmid PV-GHBK11. Production of the full-length Cry2Ab and GUS proteins in cotton event 15985 has been confirmed (Appendix 6, Section 5).

**P. 33 Cite the location in the submitted volumes where the experimental methods for Southern and PCRs (such as construction of probes) can be found.**

The experimental methods for Southern blots and PCRs are located in Appendix 6, Section 1. The methods described contain all of the information from the referenced Standard Operating Procedure.

The base pair numbers corresponding to the primers used to generate the probes for Southern analyses are:

[CBI Deleted]

**P. 30 Figure 6 and all similar figure legends for Southern blots: Describe the volume of solution in which the genomic DNA was applied to each lane.**

Thirty microliters of solution were applied to each lane for all the Southern blots presented in the petition.

**P. 51 and following pages on PCR analysis of flanking sequences: Cite the volume or appendix where the primers and the methodology for PCRs are described. For PCR**

***data, how was the plant genome sequenced at both ends of the insert (for construction of the pairs of probes)?***

Experimental methods for the PCRs and a graphical description of the primers are located in Appendix 6, Section 1. Genome Walker technology was used to determine the DNA sequence flanking both ends of the insert. In order to verify those sequences, PCR products of the expected sizes containing the sequences flanking the 5' and 3' ends of the cry2Ab insert in Bollgard II cotton event 15985 generated with two primer pairs were isolated by gel electrophoresis of 10 µl of the PCR products on 1% agarose gels. PCR products representing the sequences flanking the 5' or 3' ends of the insert were excised from the gel and purified using the QIAquick Gel Extraction Kit (Qiagen) following the procedure supplied by the manufacturer. For both analyses, the purified PCR products were then sequenced with the PCR primers using dye-terminator chemistry by the Monsanto Genomics Sequencing Center. Due to the length of the PCR products, sequencing was performed with both the primers used to generate the products, as well as primers designed internal to the amplified sequence.

**[CBI Deleted]**

***P. 54 Table 3. Cite the volume or appendix where the details of the ELISAs are described, including validation, sensitivity, etc. See the Appendix I requirements noted previously.***

The methods described in Appendix 6, Section 4 contain references to proprietary Standard Operating Procedures developed by Monsanto Company. Copies of the appropriate Cry2Ab methods are located in Attachments 1 and 2. Two ELISA methods are provided for Cry2Ab. The first was used in the analysis of protein production levels located in Tables 4-8 and Appendix 6, Section 4 of the petition. The second was used in the generation of data on segregation, located in Table 3. A description of the ELISA method for the GUS protein is located in Appendix 6.4.

***P. 57-58. Table 4, 5 and 6. A. Describe the source of the leaf - is it overseason leaf or young leaf (description on P. 55)? Indicate the notification number under which the leaves were grown for this field test.***

The cotton leaf tissue in Tables 4 and 5 is young leaf, as described on page 55 of the petition. In Table 6, the first column represents the young leaf sample, whereas the second, third and fourth columns represent the overseason leaf samples obtained at approximately monthly intervals of 55, 85, 108 days after planting, respectively. The plants from which the leaves were obtained were grown under notifications 98-084-23n, 98-084-22n and 98-085-19n.

***B. Emphasize that data for the root content of cry protein is subsumed within the whole plant data, although no measurements of Cry2Ab2 were made of roots, no root exudation of Cry2Ab2 protein into soil. Submit the data on Cry protein content from Monsanto's study of degradation of plant residues in the soil. If protein exudation data is not submitted, discuss why this data is not relevant to the petition.***

The whole plant samples analyzed for Cry2Ab protein content as described in Appendix 6, Section 4 of the petition consisted of leaves, roots and stem, but not bolls. Plants were removed from the ground by digging up the roots. Any bolls were removed and then the plant was chopped into 2-3 inch pieces and shipped to the Monsanto research facility on dry ice. Further processing occurred at Monsanto to refine and homogenize the samples prior to protein extraction. Because the whole plant sample contains the root, protein levels observed for whole plant could be considered to be a conservative estimate of levels expressed in root.

The Cry2Ab soil degradation report amended to the petition on February 21, 2001 demonstrates that the Cry2Ab protein degrades rapidly in soil, similar to other Cry proteins. Lyophilized cotton leaf tissue from Bollgard II cotton event 15985, containing both insecticidal proteins Cry2Ab and Cry1Ac, was mixed with sandy loam soil typical of that found in cotton growing areas of the U.S. Samples were incubated at approximately 25°C for up to 56 days. The amount of insecticidal activity in the soil was assessed by diet incorporation insect bioassay. Estimated half-life of the insecticidal activity in the soil was 2.3 days and a dissipation time for a 90% decrease in original concentration (DT90) estimate was 15 days. These dissipation rates are within the published range for Cry1Ac dissipation from cotton tissue (2.2-46 days as cited in the report). Although the insect bioassay method cannot distinguish between the biological activity of the two proteins, there was virtually no detectable insecticidal activity in the final three incubation samples at 42, 49 and 56 days.

Separate experiments were not provided to estimate the contribution of protein to the soil from root exudation for several reasons:

1. We are not aware of any evidence for root exudation of Cry proteins from cotton, although it has been reported for corn containing the *cry1Ab* gene (Saxena and Stotzky, 2000).
2. Dosing of the soil in the Bollgard II cotton event 15985 soil study described above was performed at a much higher rate of leaf tissue than would be expected if above-ground biomass were the only contributor. Based on the highest expression rate found in the 1998 field studies of 49.4 µg Cry2Ab protein per gram of dry Bollgard II cotton event 15985 leaf (Appendix 6, Section 4 of the petition), a conservative estimate of the level of Cry2Ab protein added to the top three inches of soil in a typical cotton field with 60K plants per acre and an average dry weight of 238 g per plant would be 2.29 µg Cry2Ab protein per gram of dry soil. The soil concentrations used in the study discussed above were two times greater than this worst-case estimate of the amount of Cry2Ab protein that would be expected in soil. This high soil concentration provided a sufficiently high initial protein concentration such that detectable quantitative measurements by insect bioassay could be obtained for several weeks after initial soil application to allow appropriate calculation of dissipation rates (DT50 and DT90). In addition, Bollgard II cotton leaf tissue was mixed as if the entire potential load reached the soil at once as a worst-case estimate.



3. Previous soil degradation experiments with the Cry1Ac protein in soil compared the rate of degradation of pure protein versus protein produced in cotton tissue. Results demonstrated that soil degradation of the protein produced in cotton tissue was somewhat slower than that of pure protein. Based on these results, Bollgard II cotton event 15985 experiments were conducted only with lyophilized leaf tissue, which was anticipated to provide the most conservative estimate of degradation in soil. The exaggerated rate of soil application was designed to account for all potential routes of protein incorporation in soil, including exudation if it were to occur. The referenced Cry1Ac study was conducted using similar insect bioassay methodology to measure insecticidal activity present in the soil. Purified Cry1Ac protein or lyophilized cotton leaf tissue powder containing the Cry1Ac protein was added to a silt loam soil typical of cotton growing regions of the U.S. Samples were incubated at approximately 24°C for up to 54 days. Purified Cry1Ac protein dissipated with an estimated half-life of 9.3 to 20.2 days. Cry1Ac protein added to soil as a component of cotton leaf tissue at a 0.01g dwt/g soil dissipated with an estimated half-life of 41 days. This study is presented in Attachment 3 and was previously submitted to EPA.
4. A field soil study was conducted in 1998 at six locations where Bollgard cotton had been continuously grown for three to six consecutive years. Levels of insecticidal activity in the soil of these fields was determined three months post-harvest using diet incorporation insect bioassay methodology. Soil samples from these fields represented all sources of protein load, including cotton plant tissues and potential root exudation. Results from each location showed no detectable levels of insecticidal activity at a detection limit of <20 ng/g. This study is presented in Attachment 4. This study is currently in preparation for publication.

**Reference:**

D. Saxena and G. Stotzky. 2000. Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn in vitro and in situ. FEMS Microbiology Ecology 33, 35-39.

**P. 56 top para. The reference to Appendix 5 should be corrected to read Appendix 6.4 (volume 6 of 22).**

Monsanto acknowledges this correction.

**P. 56 para. 5. The data reports that Cry2Ab2 from pollen was below the limits of detection in this assay. However, in Volume 14, Appendix 6.12, the summary says that the dose for the honeybee adult was chosen to be "the maximum concentration found in insect protected corn or cotton pollen." From this, 3.4 and 68 ug/ml Cry2Ab2 were selected as appropriate for the assay. Explain the discrepancy: what is known about the quantity of Cry2Ab2 protein expressed in the pollen?**

In the 1998 field season, Monsanto evaluated two Cry2Ab-containing cotton events, 15985 and 15813, transformed with the same linear fragment of vector PV-GHBK11 by particle gun techniques. Pollen was obtained from both events for analysis by Enzyme Linked ImmunoSorbent Assay (ELISA). Cry2Ab protein levels in Bollgard II cotton event 15985 were below the limit of detection in the assay (<0.25µg/ g fwt); however,

protein levels in pollen from cotton event 15813 ranged from 1.12-1.22  $\mu\text{g/g}$  fwt, with a mean of 1.17  $\mu\text{g/g}$  fwt and standard deviation of 0.07 (Appendix 6, Section 4). Therefore, the value of 1.22  $\mu\text{g/g}$  fwt was chosen as the maximum concentration observed in pollen for the purposes of conducting a study of Cry2Ab protein toxicity in adult honey bee. This study is necessary to conduct an environmental safety assessment of Cry2Ab2-containing crops such as corn and cotton and therefore the highest potential protein production value was used to establish a dose with appropriate safety margins higher than anticipated field exposure.

Subsequent to the initiation of the honey bee adult toxicity study, a decision was made to advance only the Bollgard II cotton event 15985 as a commercial candidate.

**P. 59-62 Tables 7, 8, 9, 10. Indicate the notification number for the leaves used in these field tests.**

The plants from which the leaves were obtained were grown under notifications 98-084-23n, 98-084-22n and 98-085-19n.

**P. 61 top para. The statement is made, "In summary, the levels of the Cry2Ab2 and GUS proteins expressed in tissues for Bollgard II cotton event 15985 are low", but are low compared to what?**

The levels of the Cry2Ab and GUS proteins produced in tissues of Bollgard II cotton event 15985 are extremely low compared to the total protein content. Cry2Ab in leaf represents 0.014% of total protein and GUS represents 0.072%.

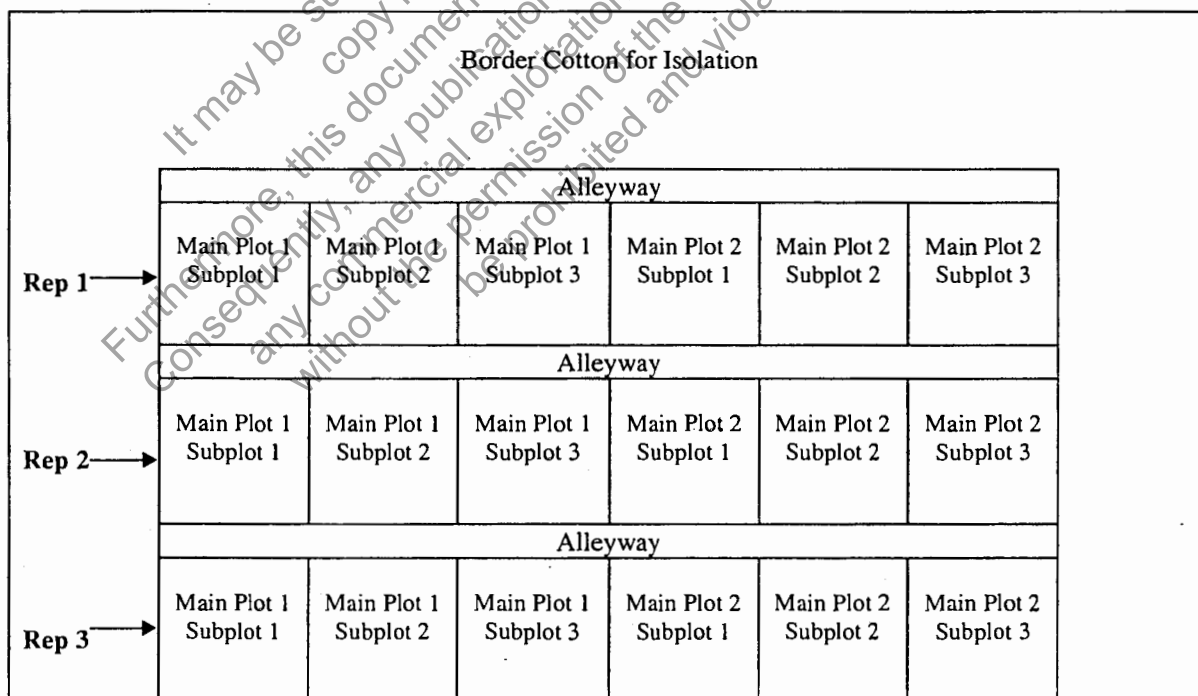
**P. 62 first para. A. Describe in the initial paragraph what cultivars were used as the controls for observations in the field trials. From reading the legend of Table 12 (P. 67), it appears that the controls for insect observations were DP50. From the text on P. 64 it appears that the controls for the pathogen observations were DP50B.**

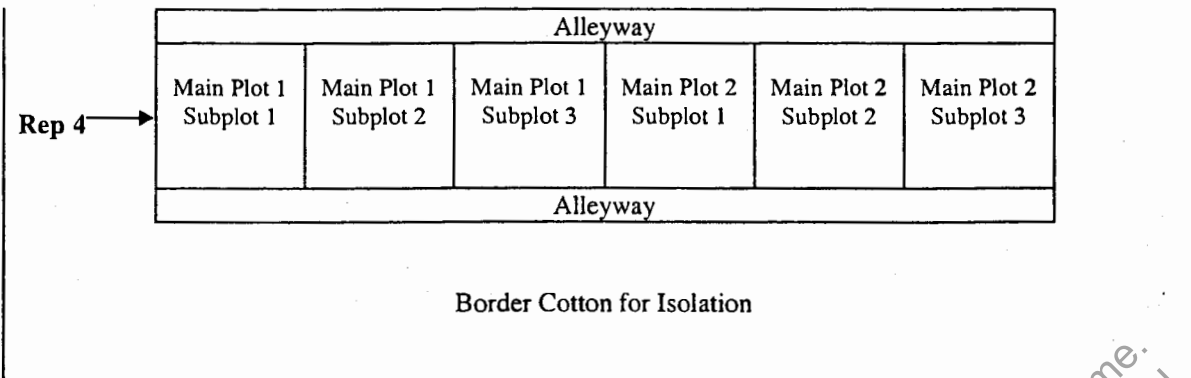
In agronomic and efficacy trials with a transgenic variety, the non-transgenic parent variety is typically chosen as the experimental control. In the case of Bollgard II cotton event 15985, however, the parental variety was also transgenic. Therefore, the field trials included both DP50 non-transgenic cotton and DP50B Bollgard as controls in the evaluation of Bollgard II cotton event 15985. Observations of disease and insect susceptibility were made on the new transgenic event compared to each control cotton variety. Reporting of results in Table 12 was based on the recorded observations of field researchers compared to the non-transgenic control, DP50, since the insect efficacy and effect on non-target organisms could best be assessed compared to plants without an insect-protection trait. Disease observations were similarly reported. Documented results of the Bollgard II cotton event 15985 plants relative to the parental control, DP50B, supported the same results listed in Table 12. The data specific to each trial was submitted in the field reports in Appendix 5 of the submission.

**B. The field trial protocol describes a layout of four rows of cotton in randomized complete blocks (RCB). Does that imply that there is a fourth cotton variety in this trial (in addition to 15985, DP50B and DP50)? If so, describe it.**

The following diagram, not necessarily to scale, shows the typical randomized complete block split-plot design. The trial is blocked into four replications, designated as rep 1, rep 2 rep 3 and rep 4. Main plots were either sprayed or unsprayed and these treatments are randomly assigned to main plot 1 or main plot 2 within each rep. For example, main plot 1 may get the sprayed treatment in rep 1 and 3 whereas main plot 2 may be the sprayed treatment in reps 2 and 4. Furthermore, the three treatments (Bollgard II, Bollgard and conventional) are randomized across the subplots within each main plot. Each subplot is 4 rows of cotton from 30-60 feet long oriented up and down in this diagram. Each block (replication) is separated by a small alleyway to facilitate access to the plots and to separate treatments. The entire study area is surrounded by the prescribed border cotton to isolate the regulated cotton from surrounding cotton. The alleyways do not cross the border rows of cotton used for containment.

A second cotton event known as 15813 was also being tested in the 1998 and 1999 field trials. Data collected from this cotton event was not presented in the petition summary because it is not relevant to the request for determination of non-regulated status of Bollgard II cotton event 15985. In 1999, the majority of the field trials also included a second commercial Bollgard cotton variety in addition to the DP50 and DP50B controls. Additional commercial Bollgard varieties were also occasionally used as additional controls. This additional control variety was selected for each trial based on its agronomic performance in the trial area. The purpose of this additional control variety was to assess the economic value of the Bollgard II cotton event 15985 performance relative to multiple commercial varieties of Bollgard cotton. These controls were not for the purpose of detailed agronomic comparisons.





***C. Damage ratings were said to have been taken in the protocol for field trials, but are not reported in Table 12. Egg and larval counts were said to have been taken as well, but these are not reported in the field trial data. Summarize this data for the petition.***

The field trial protocols did specify the collection of damage rating information for most trials with Bollgard II cotton event 15985. The protocols for the trials allowed damage ratings to be made in any of a number of ways, but did not specify that all of the methods had to be used, since all are not appropriate for all insects. Insect pressure was light in many of the locations, which did not allow for the collection of any damage rating data. For instance, a Tennessee location in 1999, observed no heliothine larvae at any of seven different timepoints throughout the season among 50 plants sampled of each line at each timepoint.

A summary of the damage was implied in the efficacy results in the petition on page 68, in the paragraph above Figure 19. This efficacy data is not specifically requested, but was included per previous USDA/APHIS requests for this information in both herbicide-tolerant and insect-protected crop determinations. Of the trials with sufficient insect pressure, damage ratings showed higher populations of larvae and more damage on the non-transgenic control DP50 plants compared to Bollgard II cotton event 15985 and the parental control DP50B. Further, about half of these locations reported less damage from cotton bollworm, armyworm and loopers in the Bollgard II cotton event 15985 plants than the parental DP50B control. Representative data from each of the three key pests, as well as armyworm and loopers was provided in Figures 19-23 to support the conclusions. The conclusions of the insect damage were included on the field reports submitted in Appendix 5 of the petition.

Even though insect pressure was light in 1998 and 1999, some insect efficacy data was generated. Cotton damage ratings were collected at three locations in 1998 and 30 trials in 1999. In general, egg counts did not differ between the cotton varieties in the tests, which was used to verify pests were present. Larval counts and damage on bolls and squares were statistically significantly less on Bollgard II cotton event 15985 compared to DP50 non-transgenic control. Representative data from [REDACTED] 2001, is presented in Attachment 5.

*Reference:*

2001. Efficacy of Bollgard and Bollgard II Cottons against Bollworm, *Helicoverpa Zea* (Boddie), in Field and Greenhouse Studies.

***D. Summarize from the field trials the number of times a difference was observed for damage by various insects. Target insects for CryI-protected DP50B plants (BW, TBW, PBW) could be summarized together, as could additional targets for Cry2Ab2-protected 15985 plants (armyworms, loopers) and additional pests not targeted by either Bt cry gene (stinkbugs, thrips, aphids).***

Approximately 45 of the trials planted in 1998 and 1999 reported no differences between insect infestations between Bollgard II cotton event 15985 and DP50 control plants due to extremely light insect pressure. Increased control of target insects relative to the non-transgenic control DP50 were noted in 36 trials. Increased control of armyworms and loopers were observed at 14 trials over the same period. These pests are significantly more sporadic than the key target insect pests of cotton: cotton bollworm, tobacco budworm and pink bollworm. Finally, only one observation of a difference in non-targeted pests was observed throughout all of the trials. In Pinal County, AZ in 1999 (99-095-19n), fleahoppers, lygus and whitefly were observed to have higher populations in the Bollgard II cotton event 15985 plots. This result confirms the well-established specificity of the Cry proteins to a narrow range of insect pests.

***E. Ten plants were selected from "the center row" for damage ratings. If an RCB protocol was followed with the four rows indicated in the first paragraph, and there were multiple cultivars growing in each plot, describe how sampling could take place from "the center row" in each plot, because there would be many times when none of the 15985 cultivar would be sited in the center row.***

Each treatment was in its own four-row block within the plot. The four-row blocks were then replicated in an randomized complete block (RCB) fashion within the plot area. Samples were taken from the center two rows of each block.

***68 ff. Figures 19-22 are offered in support of efficacy and are ascribed to academic cooperators.***

***A. Critical information about these assays is lacking. Bar graphs show no data on the number of plants sampled, and no details are given of how many observations were made. Provide the missing details of the experiments.***

Studies conducted by [REDACTED] (Figure 19 of the petition) used from tissue from a minimum of 100 plants per treatment infested with one or two small larvae in laboratory containers. Mortality was visually assessed at 72 hours post-infestation.

Studies conducted by [REDACTED] (Figures 20 and 22 of the petition) involved sampling one leaf or square from 64 individual plants per treatment. The cotton tissue was then placed in laboratory containers for two tests of 4 replicates each, with 8 squares or leaf discs per replicate. One larvae of the appropriate type was added per square or per leaf disc. Visual observations of mortality were assessed at 6 days after infestation for tobacco budworms on cotton squares (Figure 20). In the fall armyworm assay using leaf

tissue, a second leaf disc was added 3 days post infestation and visual observations of mortality taken at 7 days post infestation.

For Figure 21, a total of 20 plants were sampled on a weekly basis beginning when infestation in the non-transgenic control blocks reached the five to ten percent level. Sampling consisted of ten randomly selected plants in each of the two center rows of each block.

***B. Most of these graphs have no analysis for significance, or in the few that do, there is no citation of the statistical test that has been accomplished. Provide the statistical analysis that was done to establish the validity of any conclusions that are drawn from these observations.***

The data in Figure 19 represents the mean of four replications tested with an ANOVA at  $P=0.05$ . Means were separated using Duncan's New MRT. Statistical differences were seen between 15985 and DP50, however there was no statistical difference between DP50B and 15985 or DP50.

The data in Figures 20 and 22 represent the mean of four replications with eight subsamples per replication tested with an ANOVA at  $P=0.05$ . Means were separated using a Least Square Determination (LSD).

The data in Figure 21 represents the mean of 40 bolls randomly picked per treatment. No statistical analysis was conducted.

***C. List notification numbers in the legends for all those observations that are made in the field or on leaves from field collected plants.***

The applicable notifications are 99-102-18n and 99-057-05n.

***P. 69 bottom para. Correct the reference to Figures 9 and 10 which presumably should be figures 22 and 23.***

Monsanto acknowledges this correction.

***P. 72 fig 24, 25. List the notification numbers that are associated with these assays.***

The notifications associated with the data in Figures 24 and 25 are:

98-084-22n, 98-084-23n, 98-085-19n, 99-057-05n, 99-061-11n, 99-061-12n, 99-061-13n, 99-061-14n, 99-061-15n, 99-071-15n, 99-095-19n, 99-102-18n, 99-102-19n, 99-102-20n, 99-102-21n, 99-102-22n, 99-102-23n, 99-110-19n, 99-110-22n, 99-110-23n, and 99-110-24n.

***P. 72 para 2. The text indicates that there were no significant differences between Bollgard II cotton and nontransgenic cotton, but Figure 25 has left off the significance form the bars. Add the statistical significance levels to the bar graphs or to a legend for the figure.***

All of the bars in Figure 25 could be marked with "A", as there was no statistical significance at the  $p < 0.05$  level. This information was provided in the text rather than on the figure itself.

***P. 73 para. 2. Describe the details of the categories measured which have been listed in Table 13 and 14, such as what height:node ratios were measured. What is the stage that is termed "cut out"? What is the significance of "cracked bolls" as a measurement?***

Cut-out is a growth stage commonly referred to by cotton breeders. It is not a clearly defined event, but is a gradual change over one to two weeks during which vegetative growth ceases. It is the time when row closure is often achieved and when blooms currently on the plant have a small chance of surviving to maturity. The best method of estimating cut-out is to monitor the number of nodes above the highest first position white flower. When this value is between four and five, cut-out has been reached.

The height-to-node ratio is a determination of the plants' vigor or growth potential. This measure reflects the degree of stress that plants experience throughout the season and is numerically equivalent to the average distance between nodes. This measurement uses the number of main stem nodes (a measure of plant age) and plant height (a measure of plant stress) to determine if the plant is the proper size for its age. The height: node ratios listed in Table 14 were measured at maximum plant height at approximately the time of cut-out.

Counting the number of cracked bolls is one of several methods used to estimate yield. Bolls that are cracked open with the white lint exposed will contribute to the final yield and are a more appropriate estimate than the total boll load or number of bolls on a plant.

Reference:

Kerby, T.A. and K.D. Hake, 1996. Monitoring Cotton's Growth. *In* Cotton Production Manual. eds. S.J. Hake, T.A. Kerby and K.D. Hake, University of California Division of Agriculture and Natural Resources.

***P. 73 para 1. Criteria for the evaluation of morphology were mentioned but data was not given in detail. Please give numbers of observations (and significance if possible) for data on days to 50% open bolls, fruit retention, plant mapping and days to harvest that are listed here.***

Plant mapping was conducted on approximately 14-day intervals beginning 66 days after planting (August 7, 1998) by a team of cotton breeders at a single location in AZ (98-085-19n). A summary of the results was included in Appendix 6, Section 3 and is also published (Sieglaff *et al.*, 1998). The report noted no differences in the test and control cotton plants in the position of the first fruiting branch, number of aborted or missing fruit positions, length of top five nodes and number of nodes above white flower.

Reference:



Sieglaff, D.H., P.C. Ellsworth, J.C. Sivertooth and K. Hamilton. 1998. Preliminary Evaluation of the "Next Generation" of Bt Cotton. In J.C. Sivertooth [ed.], Cotton, A College of Agriculture Report. Series P-112. University of Arizona, College of Agriculture, Tucson, AZ.

Plant mapping was collected at 10 locations in October, 1999 by Delta and Pine Land. Data was collected in AL, AR, GA, LA, MS, OK, SC and VA under notifications 99-095-19n, 99-102-18n, 99-102-21n, 99-102-22n and 99-102-23n. Typical cotton breeding data was collected on height, total nodes, vegetative nodes, fruiting branches, height-to-node ratio, bolls per plant, percent boll retention at four fruiting positions, nodes with a first position cracked boll and date of 50% open bolls. Statistically significant differences between DP50, DP50B and 15985 at  $p < 0.05$  across the ten locations were only found in bolls per plant and bolls retained, where retention was significantly higher in the transgenic cotton plants. This was expected, based on the additional protection provided by Bollgard II cotton event 15985 relative to Bollgard DP50B or the non-transgenic control DP50.

[CBI Deleted]

***P. 74, 75. Table 13, 14. Describe sample size.***

Table 13. The mean for percent seedlings emerged is based on 120 seeds planted per plot, for a total of 480 seeds/line/site or 3840 seeds/line across all eight sites. Cooperators were asked to record when the first white flower and the first cracked boll were observed in any of the plots, which contained approximately 480 plants/line/site.

Table 14. The mean results are presented across all eight locations where the regulatory trials were grown, representing a maximum of 3840 plants per line.

***P. 75 Table 15. List the numbers of seeds that were assessed, transferring the information recorded in appendix 6.20 to the petition document.***

Table 15 lists the results for 200 seeds per plot from two locations for a total of 1600 seeds/line. This is the seed sample size used routinely by Delta and Pine Land. A separate study was conducted at BioDiagnostics (Appendix 6, Section 20) using 1200 seeds per line in each of eight temperature regimes.

***P.75 para 1. Briefly describe the applicable AOSCA standards, or supply a reference citation to where they may be found.***

The appropriate reference is AOSA Rules for Testing Seeds, revised November 2000, Association of Official Seed Analysts, Lincoln, Nebraska.

***P. 77 para. 4. A. The statement was made that for the cyclopropanoid fatty acids, malvalic, dihydrosterculic and sterculic acids, "none of the four replicated field locations showed statistically significant differences between 15985 and the control when the data is compared on a site-by-site basis." However, the column labeled***



***“Number of sites with significant differences” lists 3 of the 4 sites in which dihydrosterculic acid was measured and found to be different. The overall conclusion made was that site-by-site findings were more biologically meaningful than the overall summarized data and that therefore there were no differences between control and transgenic lines for the analyzed chemicals. The finding for dihydrosterculic cannot be subsumed under this explanation. How was the data consistent with the summarizing statement?***

The data presented in Table 17 for dihydrosterculic acid correctly indicate statistically significant differences ( $p < 0.05$ ) at three of the four sites were observed when test event 15985 was compared to the non-transgenic control. The previous statement cited in the question above was in error. However, these differences are not considered to be biologically relevant since the mean of the test line 15895 (Table 17 of petition), as well as all the test values (Appendix 2 of petition) for dihydrosterculic acid, were within the ranges found for the commercial varieties grown in the same field trials, analyzed and reported in this study (Table 17). In addition, the magnitude of the difference between the test and control for the combined sites expressed as a percentage of the control ( $0.03/0.15 \times 100 = 20\%$ ) was found to be small.

***B. Likewise, stearic acid shows a difference between controls and 15985 in both the overall average concentration over four trials, and also individually in three of the four trials. Discuss this observation in detail and make a conclusion appropriate to the data in the text.***

The comparisons of stearic acid levels found in test event 15985 and the control, DP50B, were found to be statistically different ( $p < 0.05$ ) for three of four individual sites and also for the combined site analysis as indicated in Table 17. The magnitude of the difference for the combined site analysis was found to be small when expressed as a percentage of the control ( $0.25/2.38 \times 100 = 10.5\%$ ). In addition, the mean of the test event values for the combined analysis (Table 17 of petition) as well as the range of the values for each individual site (Appendix 2 of petition) were found to fall within the range of the non-transgenic commercial varieties grown, analyzed and reported in this study. Therefore, it is concluded that the small differences between test event 15985 and the control line are not biologically relevant and the cottonseed from test event 15985 is considered to be compositionally equivalent to that of conventional cotton seed.

***C. List the notification number for the sites at which sampling was accomplished for Table 17.***

The plants from which the seeds were obtained for compositional analyses were grown under notifications 98-084-23n, 98-084-22n, 98-085-19n and 98-106-02n.

***P. 78 Table 17. Footnote 1. A. Explain how the data was collected at “four replicated sites” and yet includes data from “eight regulatory field locations.”***

There were a total of eight field sites in the regulatory trials in 1998. These locations were all used to collect samples of plant material for analysis. Four of these sites were randomized complete blocks with four replicates and the other four of the sites were single blocks. The data from all eight sites were analyzed and presented in the summary table. Individual site analysis could only be performed on the replicated sites.

**B. What are the units for the values in the table?**

The units for the fatty acids are percent of total fatty acids.

**P. 80 Table 18. A. Describe the (bacterial) source of the Cry2Ab protein for Table 18. The appendices (whose data on nontarget effects are summarized here) do not present the source of the protein, or may specify only that it was from a B.t. strain. This designation could refer to either the original source of the coding sequence or to the expression vehicle for the production of the protein. Only appendix volume 6.6 adequately describes the source.**

Due to the extremely low levels of Cry2Ab protein produced in cotton, it was necessary to produce sufficient quantities of Cry2Ab protein by bacterial fermentation for the development of analytical methods (e.g., ELISA) and to conduct protein safety studies. Cry2Ab protein was produced in and purified from *Bacillus thuringiensis* strain EG7699. To create B.t. strain EG7699, the *cry2Ab* gene for the wild-type Cry2Ab protein was cloned into a bacterial expression vector to improve production of this protein in B.t. The *cry2Ab* gene was subsequently recloned, introduced into a crystal-negative strain of B.t. and designated B.t. strain EG7699. The Cry2Ab protein produced by *Bacillus thuringiensis* strain EG7699 was shown to have equivalent molecular weight and immunoreactivity to the protein expressed in cotton, to lack detectable post-translational modification (glycosylation), to have equivalent electrophoretic mobility and detection with specific antibodies, and to have similar functional activity (Appendices 6.5 and 6.6 of the petition). Thus, the Cry2Ab proteins derived from both bacterial fermentation and plant sources were established to be physicochemically and functionally equivalent. This equivalence validated the use of the B.t.-produced protein as the test substance in the protein safety studies described in the petition.

**P. 81 Section B. GUS Protein. Cite a reference for the assertion that GUS has no insecticidal properties. Cite the previous summary in this petition for the lack of effects on other organisms (p. 26).**

The statement of no insecticidal properties of the GUS protein is found in the following citation:

Gilissen, L.J. W., P. L. J. Metz, W. J. Stiekema and J.-P. Nap. 1998. Biosafety of *E. coli*  $\beta$ -glucuronidase (GUS) in plants. Trans. Res. 7:157-163.

We acknowledge that the reference to Section IV.B. of the petition is appropriate to support the statement on page 81 that there is "no evidence of the protein producing environmental harm."

**P. 81. Para.2. A. Provide a reference for the assertion that "deposition of pollen on host plants is unlikely" and if that reference is to a previous Monsanto cotton petition, cite that specifically. The effects on nontarget insects such as Lepidoptera can only be estimated based on an adequate analysis of this statement.**

None of the non-target lepidopterans in the cotton growing regions of the U.S. deliberately feed on any tissues of cotton plants (University of Georgia, personal communication). Consequently, the only possible route of exposure to

Cry2Ab for these species is through cotton pollen drifting onto their host plants and being inadvertently consumed by the larvae. This requires that a species be sensitive to the Cry2Ab protein, be in the larval stages during the short period of pollen shed of cotton, and that the larval host plants be close enough to cotton fields for pollen to be deposited on that plant. Various studies of pollen dispersal have shown negligible wind dispersal of cotton pollen and therefore pollen movement limited to about three meters (Appendix 3 of the Monsanto Bollgard Petition, 94-041-01p). Common practices of herbicide use in cotton fields will mean that suitable host plants will only very rarely be found in cotton fields and the risk of exposure to cotton pollen will therefore be minimal. In addition, data provided in the Bollgard II cotton event 15985 petition demonstrate that the levels of Cry2Ab protein in cotton pollen is below the limit of detection of the ELISA assay. Therefore, only substantial pollen deposition could cause any adverse effects to even an extremely sensitive species. In fact, the risks to lepidoptera present in the cotton field will be greatly reduced relative to traditional insecticide use for the control of cotton pests.

***B. Provide references for the toxicity of Cry2Ab2 proteins to Diptera, since other Cry2 proteins are known to kill Diptera as well as Lepidoptera (Hofte and Whitely, 1989). What studies have been done to analyze the effects of Cry2Ab2 on Dipteran species that visit cotton plots? If toxicity to cotton-associated Diptera has not been analyzed, give a reference that summarizes the diversity of nontarget insects (especially Dipterans) that are known to visit or use cotton as a host.***

The Cry2Aa protein produced by *B.t.k.* is toxic to both dipteran and lepidopteran larvae, whereas the Cry2Ab protein is toxic only to lepidopteran larvae. This statement comes from page 569 of Liang and Dean, 1994, which states "while CryIIA is highly toxic to both dipteran and lepidopteran larvae, CryIIB is toxic only to lepidopteran larvae (Widner and Whiteley, 1989)." The citations for the two referenced papers were included in the reference section of the petition for deregulation.

***P. 81. Summarize the findings of potential allergenicity from Appendix 6.9 here in the petition. Potential allergenicity of both Cry2Ab2 and GUS should be discussed. This is relevant to possible effects on workers, and to the potential presence of the protein in the textiles made from these plants.***

Neither the *cry2Ab* or the *uidA* sequences inserted in Bollgard II cotton event 15985 was obtained from sources considered allergenic (*B.t.k.* and *E. coli*, respectively). A database of protein sequences associated with allergy and coeliac disease was assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt) and from current literature. The keyword "allergen" was used to retrieve allergen sequences from the public domain databases. Additional unique allergens found in only current literature were appended creating a database containing 567 unique protein sequences. The amino acid sequence of the Cry2Ab and GUS proteins were compared to these sequences using the sequence alignment tool FASTA. The test protein sequences, Cry2Ab and GUS, share no structurally significant sequence similarity to sequences within the allergen database.

***P. 85 top para. The statement is made that "Bollgard II cotton will provide an additional tool to delay the development of lepidopteran resistance to Bt Proteins in cotton." Justify the statement, documenting the changes in selection pressure brought by Bollgard II compared to Bollgard I and using reference to appropriate models show that resistance may indeed be delayed.***

Monsanto withdraws this statement as it is not relevant to the USDA decision. It was provided only as information for the reader.

***P. 86 para 1. The statement is made that "monitoring of plots during and after harvest for the past two years of Bollgard II field trials has not revealed any differences in survivability and competitiveness relative to other varieties of cotton." Present data with numbers of plants and numbers of locations from which this conclusion was drawn.***

Throughout all the field trials of Bollgard II cotton event 15985 conducted in the 1998 and 1999 seasons, there were no reports by cooperators of unusual survivability or competitiveness characteristics. The conclusion, above, was based on the lack of concerns raised, the data in the field reports provided in Appendix 5 of the petition and specific compliance data on volunteers reported from 212 observations at 40 field locations in 12 states. Of the 212 observations, only nine documented the presence of volunteer cotton plants and all of these were within two months of harvest. These volunteers were readily controlled with cultivation or herbicide application and did not persist in the environment. These volunteers were not atypical of cotton production.

Should you have any further questions regarding the petition or these responses, please contact me at [REDACTED] or [REDACTED] at [REDACTED]

Sincerely,

[REDACTED]

Monsanto Company  
Regulatory Affairs Manager, Cotton

cc: 00-CT-017U  
[REDACTED]

***Attachments***

## CONFIDENTIAL BUSINESS INFORMATION JUSTIFICATION

### LEGAL BACKGROUND

The Freedom of Information Act ("FOIA"), 5 U.S.C. § 552, specifically exempts from release "trade secrets and commercial or financial information obtained from a person and privileged or confidential" ("Exemption 4") 5 U.S.C. § 552(b)(4). Exemption 4 applies where the disclosure of information would be likely to cause substantial harm to the competitive position of the owner, or where, in the case of voluntarily submitted information, the submitter would be less likely in the future to share data with the agency voluntarily. National Parks & Conservation Association v. Morton, 498 F.2d 765, 770 (D.C. Cir. 1974); Gulf & Western Industries, Inc. v. U.S., 615 F.2d 527, 530 (D.C. Cir. 1979).

A party seeking to demonstrate "substantial competitive harm" need not show actual competitive harm, but must only demonstrate the presence of competition and the likelihood of substantial competitive injury. Id. at 530; National Parks & Conservation Association v. Kleppe, 547 F.2d 673, 679 (D.C. Cir. 1976); Miami Herald Pub. Co. v. U.S. Small Business Administration, 670 F.2d 610, 614 (5th Cir. Unit B 1982).

For the purposes of FOIA, courts have defined the term "trade secret" to mean a "secret, commercially valuable plan, formula, process, or device that is used for the making, preparing, compounding, or processing of trade commodities and that can be said to be the end product of either innovation or substantial effort." Public Citizen Health Research Group v. FDA, 704 F.2d 1280, 1288 (D.C. Cir. 1983); Anderson v. Dept. of Health & Human Services, 907 F.2d 936, 943-44 (10th Cir. 1990).

Where, as in the case of the Monsanto products subject to this FOIA request, the development time and costs of the products have been substantial and the information can only be obtained by competitors at considerable cost, disclosure is prohibited. Greenberg v. Food and Drug Administration, 803 F.2d at 1213, 1216-1218 (D.C. Cir. 1986); Worthington Compressors, Inc. v. Costle, 622 F.2d 45, 51-52 (D.C. Cir. 1981).

The U.S. Department of Agriculture's Animal and Plant Health Inspections Service (APHIS) has defined "Confidential Business Information" for the purposes of biotechnology submissions within the boundaries of these statutory and court interpretations of Exemption 4. "Policy Statement on the Protection of Privileged or Confidential Business Information," (the CBI Policy Statement), 50 Fed. Reg. 38561 (Sept. 23, 1985). The CBI Policy Statement defines CBI to consist of "Trade Secrets" and "Commercial or Financial Information." "Trade Secrets" are, in turn, defined as: "information relating to the production process. This includes production data, formulas, and processes, and quality control tests and data, as well as research methodology and data generated in the development of the production process. Such information must be (1) commercially valuable, (2) used in one's business and (3) maintained in secrecy."

The CBI Policy Statement states that "Commercial or Financial Information" will also be deemed confidential if review establishes that substantial competitive harm would result from

disclosure. Information such as safety data, efficacy or potency data, and environmental data may be such confidential information. Persons desiring protection for confidential information must submit a detailed statement containing facts to show that the person faces active competition in the area to which the information relates, and that substantial competitive harm would result from disclosure."

Disclosure of these types of materials is also prohibited under another exemption from FOIA's disclosure provisions. This exemption prohibits the disclosure of materials specifically exempted from disclosure by another federal statute ("Exemption 3"), 5 U.S.C. § 552(b)(3). Here, APHIS is seeking information required and protected by the U.S. Environmental Protection Agency (EPA) for purposes of pesticide registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Exemption 3 provides additional authority for APHIS to protect the materials at issue here from disclosure.

### JUSTIFICATION

The study reports, analytical methods and proprietary plant mapping data fall squarely within the well-established boundaries of Confidential Business Information as recognized by the federal courts and by APHIS. All of the information at issue here either constitute Monsanto trade secrets or commercial or financial information, as APHIS and the courts have defined those terms. As discussed more fully below, the Study Reports comprise the results of extensive research and intellectual property required both for the commercial viability and regulatory authorization of this product. This information would be worth millions of dollars to one of Monsanto's competitors in this field, and should be accorded the protections due such confidential, and valuable information.

Monsanto is at the leading edge in the development of biotechnology products in a rapidly growing and highly competitive industry. Monsanto faces a number of strong, multinational competitors in this field, including Aventis, Dow AgroSciences, Syngenta and Dupont. Monsanto's competitors, both domestic and international, have the expertise not only to replicate Monsanto's products, but also to use Monsanto's technology to develop other, competing products, thereby saving millions of dollars and years of development efforts.

Monsanto has been working on the development of transgenic crops since the early 1980s, and has become a leader in the field through the expenditure of several million dollars in research and testing costs. Monsanto can document the development and testing costs by means of monthly summaries of the worker hours devoted to these projects, budgetary documents, field test agreements and project documents.

Presently, Monsanto's competitors cannot duplicate Monsanto's commercially valuable products from information in the public domain without going through the same painstaking trial and error development and testing that Monsanto has undertaken. Although certain information regarding Monsanto products has been made available, e.g., in the context of patent applications, this information is voluminous and general in nature, and does not identify information Monsanto has



found most effective for a particular product. A competitor cannot determine from the patent applications which particular combination of genes and transgenic products will prove to be commercially valuable.

Access to the Study Reports and other information marked as confidential could be used by competitors to create essentially "copy-cat" products (avoiding any technical patent infringement) that would result in a market share loss for Monsanto of millions of dollars. By performing simple copy work, these competitors would avoid the millions of dollars and many years of research and development effort expended by Monsanto to develop its commercial products. The risk of this type of intellectual property usurpation is even more heightened in the international arena, where patent protections are not as fully developed and strictly enforced as they are domestically.

The release of the Study Reports and other information marked as confidential could provide competitors with commercially valuable knowledge regarding the characteristics of particular products Monsanto is planning to commercialize and the likely time frame for commercialization. This information would be extremely helpful to these companies in developing their own marketing strategies and development plans in a highly competitive market.

The commercial value of the type of information contained in the Study Reports has been recognized by Congress in its enactment of FIFRA and the FFDCA. Section 3 of FIFRA sets up an elaborate system of protections for these types of data, protecting them from any use by other manufacturers for a period of ten years, and requiring compensation for the use of these data by competitors after that initial ten year period. In 1996, Congress amended the FFDCA to provide both disclosure protections and compensation equivalent to that provided by FIFRA for health and safety data submitted to support pesticide residue tolerance and tolerance exemption applications. FFDCA § 408(i). APHIS should recognize the Congressional action to protect the commercial nature of these types of documents. APHIS's failure to do so could result in the loss of millions of dollars to Monsanto in data use and compensation rights.

In addition to the compensation provisions for these type of data set forth by FIFRA and FFDCA, each statute contains independent provisions for the protection from disclosure of this information. FIFRA § 10(g); FFDCA § 408(i). FOIA prohibits the disclosure of information specifically protected by statutes such as these. 5 U.S.C. § 552(b)(3). This prohibition provides additional justification for the protection of these data.

In summary, the Study Reports and other information marked as confidential are required in order for Monsanto to obtain regulatory authorization, and thereby, commercial approval, for this product. The information regarding study design, detailed research methodology, contract laboratory and report construction information available from this information could save such competitors millions of dollars in research. Monsanto has demonstrated, and Congress has recognized, the commercial value and confidential nature of these data. The Study Reports and other information marked as confidential are an integral part of Monsanto's business and should be protected as such.

## **Attachment 1**

### **Validated Method for Extraction and Direct ELISA Analysis of Cry2Ab2 in Cottonseed**

**[CBI Deleted]**

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## Attachment 2

### Procedure for Quantitative Cry2Ab ELISA for Cotton

[CBI Deleted]

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### Attachment 3

#### **Aerobic Soil Degradation of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein Bioactivity**

**[CBI Deleted]**

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#### Attachment 4

### **Cry1Ac Protein Levels in Soil after Multiple Years of Transgenic Cotton (Bollgard) Use: Implications for Environmental Risk to Soil-Dwelling Organisms**

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## Attachment 5

### **Efficacy of Bollgard and Bollgard II Cottons Against Bollworm, *Helicoverpa Zea* (Boddie), in Field and Greenhouse Studies**

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EFFICACY OF BOLLGARD AND BOLLGARD II COTTONS  
AGAINST BOLLWORM, *HELICOVERPA ZEA* (BODDIE), IN  
FIELD AND GREENHOUSE STUDIES

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Department of Entomology  
Raleigh, NC

Abstract

Bollgard™ and Bollgard II™ cottons, along with the conventional sister line, were evaluated in field and greenhouse experiments with respect to efficacy against bollworm in North Carolina in 2000. Field studies were proposed to resolve the question of increased efficacy of Bollgard II over Bollgard on bollworm, as well as to determine survivorship parameters of bollworm on each cotton genotype. The impact of supplemental bollworm control on yield was evaluated at field sites. A greenhouse study was designed to determine the efficacy of three *Bacillus thuringiensis* Berliner (*B. t.*) cotton genotypes on a field-collected bollworm strain and a Cry1Ac-tolerant bollworm strain that had been selected for tolerance to the Cry1Ac toxin in the laboratory for two generations. Results from field studies indicated that although DP50BX (Bollgard II) illustrated no significant effects on larval survival beyond that of DP50B (Bollgard), a significant reduction in the level of fruit damage sustained by DP50 and DP50B was observed for the stacked-gene line. Yield response to supplemental pyrethroid oversprays varied among test sites due to differing bollworm infestation levels and moderate levels of boll rot. Greenhouse study results suggested that DP50BX was equally effective in reducing numbers of both the Cry1Ac-tolerant and susceptible bollworm larvae, as well as surface-damaged and penetrated fruit below that of DP50B.

Introduction

The commercialization of Bollgard™ (Monsanto Agric. Co., St. Louis, MO) cottons has provided growers with an excellent alternative for controlling lepidopteran pests. However, reports from Stone and Sims (1993) indicated that *B. t.* endotoxins were much less effective in controlling bollworm, *Helicoverpa zea* (Boddie), than the tobacco budworm, *Heliothis virescens* (Fab.). Results from field trials conducted in North Carolina verify that Bollgard cottons could sustain significant fruit damage and yield losses, thus creating the need for supplemental insecticide oversprays for bollworm (Burd et al. 1999; Lambert et al. 1996, 1997; Mahaffey et al. 1994, 1995). This is most likely caused by the observed drop in average levels of Cry1Ac endotoxin (Greenplate 1999; Greenplate et al. 1998) which coincides with the major bollworm flight in North Carolina. With the bollworm population receiving a sublethal dose of Cry1Ac, the "high dose strategy" for insect resistance evolution is violated by Bollgard cottons, which institutes a need for new technological developments.

Bollgard II™ cottons produce two proteins, Cry1Ac and Cry2Ab, that are active against bollworm. The novel dual-gene line illustrates approximately the same level of expression of the Cry1Ac endotoxin as the Bollgard varieties (Jackson et al. 2000). Jackson et al. (2000) also reported that the Cry2Ab endotoxin is expressed at a much higher level than Cry1Ac in the Bollgard II line. The dual-gene construct, therefore, would more appropriately fit the "high dose strategy" for insect resistance evolution than would the currently utilized single-gene construct. Reports from field and greenhouse trials conducted in North Carolina demonstrated that Bollgard II genotypes significantly reduced numbers of susceptible and Cry1Ac-tolerant bollworm larvae below that of the Bollgard cottons (Jackson et al. 2000). This suggests that the implementation of Bollgard II cottons could possibly extend the time frame for bollworm resistance evolution beyond that of current Bollgard varieties.

Results from field and greenhouse trials evaluating the efficacy of Bollgard and Bollgard II cottons and the conventional sister line by measuring bollworm numbers, fruit damage, and yield under pyrethroid-treated and untreated systems are reported herein.

## Materials and Methods

### Field Studies

Experiments were conducted at the Central Crops Research Station, Johnston Co., NC, the Upper Coastal Plain Research Station, Edgecombe Co., NC, and the Tidewater Research Station, Washington Co., NC, in 2000. Each test site consisted of a randomized complete split-plot design with four replicates. Whole plots were 16, 20, and 24 rows by 60 feet for DP50, DP50B, and DP50BX, respectively, at each location. Different plot sizes were chosen for determination of the survival of bollworm in each genotype through mass sampling procedures in which large numbers of bolls were examined for each genotype to estimate bollworm survival on a per acre basis for making resistance management decisions and estimating refuge requirements. Subplots consisted of 12, 16, and 20 untreated rows for DP50, DP50B, and DP50BX, respectively, and 4 rows that were treated with a pyrethroid as required for supplemental bollworm control. Yield differences between pyrethroid-treated and untreated subplots were determined for each site.

Cotton genotypes DP50, DP50B, and DP50BX were planted on 15 May in Edgecombe Co., 17 May in Johnston Co., and 18 May in Washington Co. Aldicarb (Temik 15G, Aventis CropScience, Research Triangle Park, NC) was applied in-furrow at planting at 5.0 lb. a.i./acre for control of early season insect pests. Acephate (Orthene 97, Valent USA Corp., Walnut Creek, CA) was applied at 0.75 lb. a.i./acre as a mid-season overspray for stink bugs and plant bugs, as well as to eliminate arthropod predators. Two applications of lambda cyhalothrin (Karate Z 2.08 CS, Zeneca Inc., Wilmington, DE) were made to appropriate subplots for supplemental bollworm control at Johnston and Edgecombe counties on 19 July and 7 August, as well as at Washington Co. on 27 July and 9 August. Fertilization, plant growth regulation, weed control, and defoliation were accomplished as recommended by North Carolina State University.

Terminal region assessments of bollworm eggs, live larvae, and damage were made the weeks of 30 July and 6 August by the random sampling of 25 terminals per plot. Square evaluations for live larvae and damage were made the weeks of 6 and 13 August by examination of 50 squares per treatment per replicate. Boll assessments for live larvae and damage were made the weeks of 13, 20, and 27 August, as well as 3 September by examination of 50 bolls per treatment per replicate. Bollworm egg, live larvae, and damage numbers per treatment per replicate were converted to percentages prior to analysis. Yields were determined by harvesting two center rows of each subplot with a mechanical cotton picker on 30 October at Edgecombe Co. and 7 November at Johnston and Washington counties. Yields were converted to lb. seed cotton per acre prior to analysis.

The total numbers of fourth to fifth instar larvae, harvestable bolls, and damaged harvestable bolls were counted in a randomly selected area of five row feet per treatment per replicate at the Edgecombe and Washington county sites on three sample dates (24 and 31 August and 7 September). Large larvae, fourth and fifth instars, were transported to the laboratory and reared on bolls from the respective genotypes until the prepupal stage. Prepupae were then placed into 30 ml plastic cups containing non-*B. t.* artificial diet which served as a substrate to tunnel into for pupation. Numbers of successfully emerged adults from each genotype were counted and converted to a per acre basis prior to analysis along with total numbers of harvestable bolls, damaged bolls, and live larvae. These numbers provide an estimation of survival parameters of the bollworm, which is important in resistance management.

### Greenhouse Study

An experiment was conducted in a greenhouse chamber at the Tidewater Research Station, Washington Co., NC. The test was a randomized complete block design with seven replicates. Each replicate consisted of 12 plants, two plants per treatment combination of cotton genotype and bollworm strain. Plants within blocks were separated by a distance of two feet, whereas, blocks were separated by a 3 foot space on tables. Cotton genotypes DP50B, DP50X, and DP50BX were planted in three gallon pots at one plant per plot on 27 June. Arthropod natural enemies, as well as aphids and whiteflies, were eliminated by side-dressing cotton plants two weeks prior to bollworm infestation with Aldicarb to achieve a rate of 0.75 lb. a. i./acre. Fertilization and plant growth regulation were provided as recommended for greenhouse cotton plants.

Local bollworm adults were collected from light traps and held in the laboratory for egg collection. Neonate larvae from the eggs were used as the control strain in the experiment. A Cry1Ac-tolerant strain of bollworm was originally collected from light traps and selected for tolerance to the Cry1Ac toxin (MVP) in artificial diet for two generations. Infestation of five neonate larvae onto fruiting structures on respective plants from each genotype using a fine-haired artist paint brush occurred when cotton plants reached approximately 100 days. Assessments of surviving larvae, superficial fruit damage, and fruit penetration were made by whole plant examination at 14 and 21 days after infestation. Surviving larvae from the last evaluation were taken to the laboratory and placed into 30 ml plastic cups containing non-*B. t.* artificial diet which served as a medium for larvae to tunnel into for pupation. Numbers of bollworm that successfully pupated and those that successfully emerged as adults were recorded and converted to percentages.

### Data Analysis

All data were subjected to ANOVA using PROC GLM (SAS Institute 1990), and means for each treatment were separated ( $P < 0.05$ ) using Fisher's Least Significant Difference test or LSMEANS in SAS.

### Results

#### Field Studies

No differences were found among the three cotton genotypes with distribution of bollworm eggs in cotton terminals being consistent across field sites (Table 1). No significant differences were evident between DP50BX and DP50B with respect to percent live bollworm larvae in cotton terminals, and both Bollgard genotypes reduced percent bollworm larvae below that of the conventional variety (Table 1). Percent terminal damage was significantly lessened by both Bollgard genotypes compared to the conventional variety with DP50BX further reducing percent terminal damage below that of DP50B (Table 1). Square evaluations revealed that Bollgard cottons did not differ with respect to the percentage of squares containing live bollworm larvae, but both contained a significantly lower percentage of live larvae than the conventional variety (Table 2). Data in Table 2 also show a significant reduction in percent bollworm damaged squares by both Bollgard genotypes compared to the conventional variety with DP50BX further reducing square damage below that of DP50B. Boll evaluations indicated that Bollgard cottons did not differ with respect to percent live bollworm larvae in bolls but significantly lessened percent live bollworm larvae compared to the conventional variety (Table 2). However, Table 3 indicates that DP50BX sustained less boll damage than DP50B at each test site with both Bollgard cottons having less boll damage than the conventional variety.

Field evaluations for determination of total harvestable fruit on a per acre basis revealed that the conventional variety had a significantly lower number of bolls than the Bollgard cottons (Table 4). Results in Table 4 also indicate that the Bollgard cottons had significantly lower numbers of damaged bolls than the conventional variety in which greater than half the bolls were bollworm damaged. Table 5 illustrates that no differences were evident between Bollgard genotypes with respect to the number of surviving fourth to fifth instar bollworm larvae per acre. However, only

DP50BX contained a significantly lower number of larvae per acre compared to the conventional variety. The number of large larvae per acre closely relates to the number of successfully emerged bollworm adults on a per acre basis which was much lower in the Bollgard genotypes than in the conventional variety (Table 5). Differences in number of successfully emerged bollworm adults were not evident among Bollgard cottons even though successful emergence was much lower numerically in DP50BX than in DP50B.

Yield differences measured in pounds of seed cotton per acre between pyrethroid-treated and untreated subplots in three cotton genotypes varied among test sites. Pyrethroid-treated and untreated subplots did not differ significantly with respect to yield in lb. seed cotton per acre at the Central Crops Research Station (Table 6). Cotton genotype also had no impact on yield at this site. Results in Table 7 reveal a genotype\*insecticide interaction at the Upper Coastal Plain Research Station in which all treatment combinations were compared. Yield benefits from pyrethroid applications were only evident in the conventional variety. Both untreated Bollgard genotypes illustrated significantly improved yields compared to the untreated conventional variety. Among pyrethroid-treated subplots, DP50B produced more seed cotton than DP50BX. Neither pyrethroid-treated Bollgard variety differed from the treated conventional variety. At the Tidewater Research Station, pyrethroid-treated subplots averaged across cotton genotypes produced significantly higher yields than untreated subplots (Table 8). Yields in lb. seed cotton per acre averaged across subplots were higher in DP50BX than DP50B, which also out-yielded the conventional variety.

#### Greenhouse Study

The Cry1Ac-tolerant bollworm strain displayed a significantly higher percent larval survival than the susceptible bollworm strain on DP50B, but no differences among larval strains were evident for the DP50X and DP50BX genotypes (Table 9). As with larval survival, Table 10 indicates that the Cry1Ac-tolerant bollworm strain caused a significantly higher percent surface-damaged fruit than did the susceptible bollworm strain in DP50B only. Bollworm strains did not differ with respect to percent surface damaged fruit within genotypes DP50X and DP50BX. Data in Table 11 indicate that the percentage of penetrated fruit averaged across cotton genotypes was significantly higher for the Cry1Ac-tolerant strain compared to the susceptible strain. Although no differences in percent penetrated fruit existed between DP50X and DP50BX, only DP50BX significantly reduced the percentage of penetrated fruit below that of DP50B. The percentage of larvae from the Cry1Ac-tolerant bollworm strain that developed on DP50B and successfully pupated was 4.29%. No other larvae on any genotype survived until pupation. Of those that pupated, 66.7% successfully emerged as adults.

#### Discussion

##### Field Studies

Bollworm populations varied spatially in 2000, with an uncharacteristically low population at the Central Crops Research Station and moderate to high infestations at the Upper Coastal Plain and Tidewater Research Stations. In field studies, both DP50B and DP50BX provided similar levels of bollworm control with respect to percent live bollworm larvae in terminals, squares, and bolls, with both transgenic cultivars reducing larval numbers below that of the conventional variety. The DP50BX line, however, was more effective in sustaining less fruit damage than DP50B in these plant regions. Significantly less fruit damage should have equated to higher yields, but moderate levels of boll rot penalized the better treatments, rendering some possible yield differences inseparable. Both transgenic cottons out-performed DP50 with respect to production of total and damaged harvestable bolls per acre, as well as successfully emerged bollworm adults per acre. Only DP50BX contained significantly lower numbers of large live larvae than the conventional variety. Cotton genotypes DP50B and DP50BX also performed similarly with respect to production of total and damaged harvestable bolls per acre, as well as numbers of large live larvae and



successfully emerged adults per acre. However, numerical reductions made by DP50BX in numbers of bollworm that successfully completed development may extend the time frame for bollworm resistance evolution but possibly for a shorter time period than originally expected.

#### Greenhouse Study

The Cry1Ac-tolerant bollworm strain out-performed the susceptible strain with respect to larval survival and surface damage inflicted onto fruiting structures, which was largely due to the increased performance of the Cry1Ac-tolerant strain compared to the susceptible strain on DP50B. Averaged across genotypes, increased fruit penetration by the Cry1Ac-tolerant bollworm strain over the susceptible strain was caused by the increased success of the Cry1Ac-tolerant strain on genotypes DP50B and DP50X. Fruit penetration by the Cry1Ac-tolerant strain on DP50BX was approximately 10X less than that of DP50X and DP50B, which is likely due to some additional effect of the two-gene construct versus that of the single-gene. Averaged across bollworm strains, DP50BX sustained less fruit penetration than DP50B. These results support reports from Jackson et al. (2000) indicating the increased bollworm control provided by Bollgard II lines over the commercial Bollgard variety with respect to larval survival and fruit penetration. In addition to larval survival and fruit penetration, the percentage of the Cry1Ac-tolerant bollworm strain that successfully pupated and emerged as adults was higher in DP50B (4.29 and 2.86%, respectively) than in DP50BX (0.00%) and DP50X (0.00%). These greenhouse data and those from field studies suggest that the commercialization of the dual-gene construct (DP50BX) would reduce bollworm damage over that experienced by Bollgard varieties, as well as eliminate the need for supplemental insecticide application for bollworm control. With the incidence of field resistance to Bollgard cottons in North Carolina (Jackson et al. 2000; Burd et al. 1999; Lambert et al. 1997, 1998; Mahaffey et al. 1994, 1995), the implementation of Bollgard II lines may also be necessary to provide control for that portion of the bollworm population already expressing resistance traits to Bollgard cottons.

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Table 1. Mean (SE) percent bollworm egg, live larvae, and damage in the terminal region of three cotton genotypes averaged across three locations and two sample dates in North Carolina in 2000.

Genotype	Percent Egg	Percent Larvae	Percent Damage
DP50	8.00 (.018) a	4.25 (.006) a	15.92 (.019) a
DP50B	11.33 (.020) a	0.67 (.002) b	6.42 (.012) b
DP50BX	7.50 (.017) a	0.25 (.002) b	3.33 (.010) c

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 2. Mean (SE) percent live bollworm larvae in squares, square damage, and live bollworm larvae in bolls averaged across three locations and respective sample dates in North Carolina in 2000.

Genotype	Square Evaluations		Boll Evaluations
	Percent Larvae	Percent Damage	Percent Larvae
DP50	4.92 (.005) a	16.25 (.005) a	4.92 (.009) a
DP50B	0.42 (.002) b	3.33 (.004) b	1.00 (.003) b
DP50BX	0.00 (.000) b	0.25 (.001) c	0.17 (.001) b

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 3. Mean (SE) percent boll damage for each cotton genotype averaged across four sample dates for each test site in North Carolina in 2000.

Genotype	Central Crops Research Station	Upper Coastal Plain Research Station	Tidewater Research Station
DP50	12.50 (.008) a	49.25 (.030) a	44.25 (.037) a
DP50B	0.86 (.003) b	10.25 (.010) b	7.75 (.013) b
DP50BX	0.00 (.000) c	1.38 (.005) c	0.75 (.003) c

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 4. Mean (SE) number of total bolls and damaged bolls per acre for each cotton genotype averaged across three sample dates and two locations in North Carolina in 2000.

Genotype	Number of Bolls Per Acre	Number of Damaged Bolls Per Acre
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DP50	311,817 (23,319) b	156,695 (24,328) a
DP50B	458,832 (12,637) a	27,830 (3,185) b
DP50BX	448,668 (18,047) a	7,502 (5,008) b

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 5. Mean (SE) number of live large bollworm larvae and successfully emerged adults per acre for each cotton genotype averaged across three sample dates and two locations in North Carolina in 2000.

Genotype	Number of Larvae Per Acre	Number of Emerged Adults Per Acre
DP50	6893 (1689) a	6893 (2,165) a
DP50B	3155 (816) ab	528 (197) b
DP50BX	1990 (1601) b	72 (72) b

Means within the same column or row followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 6. Mean (SE) weight of seed cotton in pounds per acre for pyrethroid-treated and untreated subplots of each genotype at the Central Crops Research Station in North Carolina in 2000.

Genotype	Untreated	Treated
DP50	3,722 (193) a	3,618 (165) a
DP50B	4,107 (90) a	4,076 (84) a
DP50BX	4,055 (110) a	3,846 (135) a

Means within the same column or row followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 7. Mean (SE) weight of seed cotton in pounds per acre for pyrethroid-treated and untreated subplots of each cotton genotype at the Upper Coastal Plain Research Station in North Carolina in 2000.

Genotype	Insecticide	Pounds of Seed Cotton Per Acre
DP50B	Untreated	3,902 (240) a
DP50B	Treated	3,808 (108) ab
DP50BX	Untreated	3,624 (90) abc
DP50	Treated	3,452 (258) bc
DP50BX	Treated	3,230 (166) c
DP50	Untreated	1,440 (229) d

Means within the same column followed by the same letter are not significantly different, LSMEANS ( $P \leq 0.05$ ).

Table 8. Mean (SE) weight of seed cotton in pounds per acre for pyrethroid-treated and untreated subplots of each genotype at the Tidewater Research Station in North Carolina in 2000.

Genotype	Untreated	Treated	Mean
DP50	1,399 (27)	2,083 (193)	1,741 (157) c
DP50B	2,455 (199)	2,474 (100)	2,465 (103) b
DP50BX	2,846 (131)	3,057 (90)	2,952 (84) a
Mean	2,233 (198) b	2,537 (140) a	

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 9. Mean (SE) percent surviving larvae for each bollworm strain within each cotton genotype averaged across two evaluation dates in the greenhouse in North Carolina in 2000.

Strain	DP50B	DP50X	DP50BX
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Cry1Ac-tolerant Strain	5.00 (.022) a	0.71 (.007) a	0.00 (.000) a
Susceptible Strain	0.00 (.000) b	0.00 (.000) a	0.00 (.000) a

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 10. Mean (SE) percent surface-damaged fruit for each bollworm strain within each cotton genotype averaged across two evaluation dates in the greenhouse in North Carolina in 2000.

Strain	DP50B	DP50X	DP50BX
Cry1Ac-tolerant Strain	4.36 (.009) a	0.65 (.005) a	1.36 (.006) a
Susceptible Strain	1.19 (.006) b	0.00 (.000) a	1.36 (.006) a

Means within the same column followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

Table 11. Mean (SE) percent penetrated fruit for each cotton genotype and each bollworm strain averaged across two evaluation dates in the greenhouse in North Carolina in 2000.

Genotype	Cry1Ac-tolerant Strain	Susceptible Strain	Mean
DP50B	4.93 (.017)	0.85 (.005)	2.89 (.009) a
DP50X	4.10 (.020)	0.00 (.000)	2.05 (.010) ab
DP50BX	0.45 (.004)	0.00 (.000)	0.22 (.002) b
Mean	3.16 (.009) a	0.28 (.002) b	

Means within the same column or row followed by the same letter are not significantly different, Fisher's LSD ( $P \leq 0.05$ ).

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**Appendix 6.**

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Section 4. Amended Report for MSL-16081: Protein Levels in  
Insect Protected Cotton Samples Produced in the 1998 U.S. Field  
Trials. (53 pages)

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**Section 5. Assessment of the Equivalence of Proteins Expressed  
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**Section 6. Characterization of Insect Protection Protein 2 (IPP2)  
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Section 7. Acute Oral Toxicity Study of Insect Protection Protein  
2 (IPP2) in Mice. (209 pages)

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Section 8. Assessment of the *in vitro* Digestibility of Insect  
Protection Protein 2 (IPP2) Utilizing Mammalian Digestive Fate  
Models.(34 pages)

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Section 9. Bioinformatics Analysis of Insect Protection Protein 2  
(IPP2) Sequence Utilizing an Allergen Database. (36 pages)

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Section 10. Bioinformatics Analysis of Insect Protection Protein  
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**Section 11. Evaluation of the Dietary Effect(s) of Insect  
Protection Protein 2 on Honey Bee Larvae. (29 pages)**

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Section 12. Evaluation of the Dietary Effect(s) of Insect  
Protection Protein 2 on Adult Honey Bees (*Apis mellifera* L.).  
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**Request for Determination of Non-Regulated Status for the Regulated  
Article: Bollgard® II Cotton Event 15985 (*Gossypium hirsutum* L.) Producing  
the Cry2Ab Insect Control Protein derived from  
*Bacillus thuringiensis* subsp. *kurstaki***

**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

**[CBI Deleted]**

**Section 13. Insect Protection Protein 2: A Dietary Toxicity Study  
with Green Lacewing Larvae (*Chrysoperla carnea*). (33 pages)**

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**Section 14. Insect Protection Protein 2: A Dietary Toxicity Study  
with Parasitic Hymenoptera (*Nasonia vitripennis*). (33 pages)**

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**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

**[CBI Deleted]**

**Section 15. Insect Protection Protein 2: A Dietary Toxicity Study  
with the Ladybird Beetle (*Hippodamia convergens*). (40 pages)**

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**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

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**Section 16. Insect Protection Protein 2: An Acute Toxicity Study  
with the Earthworm in an Artificial Soil Substrate. (38 pages)**

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**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

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Section 17. Assessment of Chronic Toxicity of Cotton Tissue  
Containing Insect Protection Protein 2 to *Collembola* (*Folsomia*  
*candida*). (49 pages)

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**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

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**Section 18. Insect Protection Protein 2 in Cottonseed Meal: A  
Dietary Toxicity Study with the Northern Bobwhite. (40 pages)**

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**Appendix 6.**

**Study Reports Supporting Regulatory Approval of Bollgard II Cotton Event 15985**

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Section 20. Germination and Dormancy Evaluation of Insect-  
Protected Cotton Event 15985 for Ecological Assessment of Plant  
Weediness. (29 pages)

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00-342-01p  
addendum

MONSANTO COMPANY  
700 CHESTERFIELD PARKWAY NORTH  
ST. LOUIS, MISSOURI 63198  
PHONE [REDACTED]  
<http://www.monsanto.com>

February 4, 2002

[REDACTED] Ph.D.  
Senior Operations Officer, Biotechnology  
U.S. Department of Agriculture / APHIS  
4700 River Road  
Riverdale, MD 20737

Re: Bollgard II® Cotton Event 15985, USDA Petition 00-342-01p

Dear Dr. [REDACTED]

Per the information on Bollgard II cotton event 15985 recently verbally communicated to you by Dr. [REDACTED] the enclosed documents are being provided to update the file on Bollgard II cotton event 15985.

Monsanto requests that the enclosed information be inserted as an addendum to file for Petition 00-342-01p for Bollgard II cotton event 15985. If you have any questions regarding this information, please contact me at [REDACTED]

Sincerely,

[REDACTED]

[REDACTED] Ph.D.  
Regulatory Affairs Manager

Enclosure

cc: Dr. [REDACTED]  
00-CT-017U

Received  
3/4/02  
[REDACTED]

## Executive Summary: Safety Assessment of $\beta$ -Glucuronidase E377K in Bollgard II<sup>®</sup> cotton

[REDACTED]

Monsanto Company, Product Safety Center, Biotech Regulatory Sciences, 700  
Chesterfield Parkway N.,  
St. Louis, MO, 63198

Bollgard II cotton event 15985 was produced by insertion of the *cry2Ab* insect control gene and the  $\beta$ -glucuronidase (*uidA*) scorable marker gene into the genome of insect-protected Bollgard cotton. The DNA insertion results in gene expression and production of the Cry2Ab and  $\beta$ -glucuronidase (GUS) proteins respectively, as expected ([REDACTED] et al., 1999; [REDACTED] et al., 2000; Gustafson, 2000a). Bollgard II cotton event 15985 was evaluated extensively from 1998 through 2001 in numerous food, feed and environmental safety studies. The purpose of this review is to briefly summarize results of recent sequence analysis of the DNA inserted in the genome of Bollgard II cotton event 15985, and to discuss these results within the context of the established food, feed and environmental safety of this product.

Food and feed safety assessments of Bollgard II cotton event 15985 included safety studies with the GUS protein. The GUS protein used in safety assessments was produced in gram quantities by fermentation and purification from *E. coli*, since the protein is produced at insufficient levels in Bollgard II cottonseed to conduct these studies. The GUS protein produced in *E. coli* was shown to be equivalent to the GUS protein produced in Bollgard II cotton with respect to molecular weight and immunoreactivity and to have similar functional activity. Thus, the GUS proteins derived from both bacterial fermentation and plant sources were established to be physicochemically and functionally equivalent ([REDACTED] 1992; [REDACTED] et al., 1996; [REDACTED] et al., 1999). Demonstration of protein equivalence justified substitution of the bacterially-produced GUS protein for the plant-produced protein in protein safety assessment studies.

Recent DNA sequence analyses of the *uidA* gene in Bollgard II cotton event 15985 have shown that the plant-expressed version of the inserted *uidA* gene encodes a single amino acid substitution at position 377 in the GUS protein relative to the GUS protein produced by *E. coli* fermentation. This substitution predicts the replacement of a glutamic acid (E) with a lysine (K) at this position, hereafter referred to as GUS E377K.

It is concluded from this review that the amino acid substitution in GUS E377K does not confer any significant structural or functional changes in the GUS protein produced in cotton. This conclusion is based on amino acid sequence and protein structure comparisons among GUS proteins, as well as modeling studies with the GUS E377K

<sup>®</sup> Bollgard II is a registered trademark of Monsanto Co., St. Louis, MO

protein. There are over 250 nucleotide sequences and over 100 protein sequences deposited in the NCBI's (National Center for Biotechnology Information) data bases that are classified as  $\beta$ -glucuronidases. Amino acid sequence identity ranges from 33% to 99.8% depending on which GUS sequences are compared, illustrating the high level of amino acid sequence divergence exhibited by this functionally conserved enzyme family. In addition, the three dimensional structure of the *E. coli* GUS protein has been homology modeled to the 2.4 Å 3-D X-ray crystal coordinates of the human GUS protein (Jain et al., 1996; Matsumura et al., 1999). When the single amino acid change in the GUS E377K protein is introduced there is no effect on the active site and no significant impact on overall 3-D structure of the protein.

The GUS protein is ubiquitous in nature, and is commonly found in a wide range of organisms in the environment. GUS activity has been detected in over 50 plant species in various tissues including embryo, fruit, seed coat and endosperm (Hu *et al.*, 1990). These species include a number of human food sources, including potato, apple, almond, rye, rhubarb, and sugar beet (Schulz and Weissenböck, 1987; Hodal *et al.*, 1992; Wozniak and Owens, 1994). GUS protein is also present in cattle and in a number of invertebrate species, including nematodes, mollusks, snails and insects (Gilissen et al., 1998). Human exposure to the GUS protein is also commonplace through intestinal epithelial cells and intestinal microflora, bacterial exposure and numerous foods containing the GUS protein, with no known harmful effects (Gilissen, et al., 1998).

Given the wide divergence in primary structure of GUS proteins, the minimal impact of amino acid substitution in GUS E377K on 3-D structure of *E. coli*-produced GUS, and the history of consumption of GUS proteins in the food supply, it is unlikely that the single amino acid substitution observed in the GUS E377K protein produced in Bollgard II cotton event 15985 would change the conclusion that Bollgard II cotton event 15985 is as safe and as nutritious as seed of conventional cotton varieties and does not pose an increased risk to the environment relative to conventional cotton varieties (██████████ et al., 2002).

Furthermore, the GUS E377K protein was a constituent of Bollgard II cotton event 15985 plants and materials that were tested in extensive regulatory field trials, composition, nutrition, and safety studies considered during the safety assessment. Therefore, the safety of the GUS E377K protein has already been addressed. Bollgard II cotton event 15985 was previously established as safe as conventional cotton varieties based on: the safety of the genetic elements contained on the transformation vector used to produce Bollgard II cotton event 15985 (Gustafson, 2000a, b); the history of safe use, specificity, mode-of-action and toxicological studies conducted on Cry2 proteins (Gustafson, 2000a, b); history of safe use and toxicological studies with the GUS protein (Gilissen et al., 1998); the functionality and safety assessment of the Cry2Ab and GUS proteins as assessed by mouse acute gavage and digestibility studies (██████████ 2000; Leach et al., 2000; Naylor, 1992; ██████████, 1996); the assessment of compositional and nutritional equivalence of event 15985 (comparing the key nutrients and anti-nutrients to the parental event and conventional cotton) (██████████ 2000; Pyla et al., 2001; Gustafson, 2000b); a

comparison of crop agronomic characteristics of event 15985 to the parental and conventional cotton varieties (Gustafson, 2000a); and a comparison of the safety and nutritional properties of event 15985 to parental and conventional cotton varieties in animal feeding studies with cows, quail, and catfish (██████ et al., 2001; ██████ et al., 1999; Li and Robinson, 2000). These studies establish that whole cottonseed and cottonseed meal from Bollgard II cotton, including all the constituent components, cause no untoward effects on any of the animal species tested. In addition, human consumption of any cottonseed protein is extremely low since humans only consume cottonseed oil and products containing highly purified cellulose products derived from cotton linters (*Cottonseed and Its Products*, 1989). Because of the methods of extraction and purification of the oil, protein is not detected in the oil fraction at a limit of detection of 1.3 micrograms per gram of oil (██████ et al., 1993). Similarly, linters are highly processed through stringent conditions that protein would not be expected to survive (*Cottonseed and Its Products*, 1989; Sims et al., 1996). Therefore, there is negligible human exposure to proteins in cottonseed through normal consumption of cottonseed products.

The safety studies described above establish that the GUS E377K protein is not expected to have allergenic or toxic properties. These conclusions were further confirmed by bioinformatics analyses. Bioinformatics analyses were performed to assess potential structural similarity of the GUS E377K protein amino acid sequence to known allergens, toxins or other pharmacologically active proteins relevant to human and animal health (██████ et al., 2002). From these analyses it was concluded that the GUS E377K protein sequence does not share any biologically relevant structural similarities to known allergens, toxins or other pharmacologically active proteins.

Extensive agronomic and environmental safety studies were conducted with Bollgard II cotton event 15985. All of these studies were conducted with plants that contained the GUS E377K protein. Comparisons of Bollgard II cotton event 15985 were made to conventional cotton plants with regard to disease and pest susceptibilities, yield, morphology, weediness, effect on non-target organisms and other relevant characteristics (Gustafson, 2000a; Queensland Dept. of Primary Industries, 2000 and 2001). Based on results of these studies it was concluded that Bollgard II cotton event 15985 does not pose a plant pest risk or any increased risk to other plants or the environment compared to conventional cotton varieties. These conclusions are not altered by the expression of the GUS E377K protein in Bollgard II cotton.

This review of the safety assessment of Bollgard II cotton event 15985, which accounts for the expression of the GUS E377K protein, confirms the conclusions reached previously that: 1) seed of Bollgard II cotton event 15985 is as safe and as nutritious as seed of conventional cotton varieties and; 2) Bollgard II cotton event 15985 does not pose a plant pest risk or otherwise pose an increased risk to the environment relative to conventional cotton varieties. Any risks to human health or the environment associated with the production and consumption of Bollgard II cotton event 15985 are no different from those associated with conventional cotton varieties.



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