DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 02–092–1]

Aventis CropScience; Availability of Petition and Environmental Assessment for Determination of Nonregulated Status for Cotton Genetically Engineered for Glufosinate Herbicide Tolerance

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 Aventis CropScience seeking a determination of nonregulated status for cotton designated as Transformation Event LLCotton25, which has been genetically engineered for tolerance to the herbicide glufosinate. 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 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 that we receive on or before February 14, 2003.

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. 02–092–1, 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. 02–092–1. If you use e-mail, address your comment to *regulations@aphis.usda.gov*. 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. 02–092–1" 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 (202) 690–2817 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. Susan Koehler, Biotechnology Regulatory Services, APHIS, Suite 5B05, 4700 River Road Unit 147, Riverdale, MD 20737–1236; (301) 734–4886. To obtain a copy of the petition or the environmental assessment, contact Ms. Kay Peterson at (301) 734–4885; e-mail: *Kay.Peterson@aphis.usda.gov*.

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 February 12, 2002, APHIS received a petition (APHIS Petition No. 02–042–01p) from Aventis CropScience (Aventis) of Research Triangle Park, NC, requesting a determination of nonregulated status under 7 CFR part 340 for cotton (*Gossypium hirsutum* L.) designated as Transformation Event LLCotton25 (LLCotton25), which has been genetically engineered for tolerance to the herbicide glufosinate. The Aventis petition states that the subject cotton should not be regulated by APHIS because it does not present a plant pest risk.

As described in the petition, LLCotton25 has been genetically engineered to contain a stably integrated *bar* gene isolated from *Streptomyces*

hygroscopicus, strain ATCC21705. The *bar* gene encodes phosphinothricin-N-acetyltransferase (PAT), and the PAT enzyme catalyzes the conversion of L-phosphinothricin, the active ingredient in glufosinate, to an inactive form, thus conferring resistance to the herbicide. Expression of the added genes is controlled in part by gene sequences from the plant pathogens cauliflower mosaic virus and *Agrobacterium tumefaciens*. *Agrobacterium*-mediated gene transfer was used to transfer the added genes into the recipient Coker 312 cotton variety.

LLCotton25 has been considered a regulated article under the regulations in 7 CFR part 340 because it contains gene sequences from plant pathogens. This cotton has been field tested since 1999 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 § 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, Aventis has submitted a pesticide petition to EPA to expand the registration of glufosinate to include use on LLCotton25.

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.

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.

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 (EA) prepared to provide the public with documentation of APHIS' review and analysis of any potential environmental impacts and plant pest risk associated with a proposed determination of nonregulated status for Aventis' LLCotton25.

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). 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 Aventis' herbicide-tolerant LLCotton25 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 10th day of December 2002.

Peter Fernandez,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 02–31567 Filed 12–13–02; 8:45 am]

BILLING CODE 3410–34–P

Recd 8/12/02 SK

Supplemental information 02-042-01p

Bayer CropScience

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August 9, 2002

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Re: Aventis CropScience USA LP Petition for the Determination of Nonregulated Status for Glufosinate-Tolerant Cotton Transformation Event, 02-042-01p

Dear Dr. Pappu:

During our conference call on June 30, 2002, USDA identified additional information needed in order to deem our above referenced petition 'complete.' These items include a more simplified Segregation Analysis of Transformation Event LLCotton25 flow diagram found in the Segregation section and additional information on herbicides commonly used in wheat and peanut fields. Also, in our response letter to USDA dated July 5, 2002, there was in inadvertent error in the section entitled Agronomic Performance. In the first paragraph, Table VI.1 was referenced, when indeed it should have been Table VI.2. The attachment that follows reflects the correct referenced table.

If you have any questions, please feel free to contact me at 919-549-2748, 919-549-3929 (fax) or Luann.Powell@Bayercropscience.com.

Respectfully,

Luann Powell

Luann Powell
Registration Manager – Biotechnology

Attachment

cc: Susan MacIntosh, Director, Regulatory Affairs – Biotechnology
Martine Freyssinet, Global Regulatory Manager

Attachment

Segregation

IV. Molecular Characterization of Transformation Event LLCotton25

A. Mendelian Inheritance of Transformation Event LLCotton25

Primary transformation event LLCotton25 was derived from the transformation of cotton cells as described in Section III. T_1 seed harvested from self-pollinated T_0 plants surviving a Liberty Herbicide greenhouse screen were planted in the greenhouse for multiplication and evaluation. T_1 plants were selected for survival following Liberty Herbicide application. Bolls were harvested from individual plants and T_2 boll rows were planted in June 1999 in Mississippi (USDA Authorization 99-007-08n). Two types of segregation analyses were conducted: 1) census of individual resistant and susceptible plants and 2) the number of fully resistant and partially resistant rows.

Application of Liberty Herbicide was used to score the rows for segregation of the PAT phenotype. All plants in rows containing no sensitive plants were considered to be homozygous for the *bar* gene and therefore derived from homozygous T_1 plants, while plants within the partially resistant rows were considered to be a mixture of homozygous and hemizygous plants and were therefore derived from hemizygous T_1 plants. In this situation, Mendelian inheritance for a single gene locus would predict one fully resistant row for every two partially resistant rows. For each population of LLCotton25 the expected ratio of 1:2 was observed (Table IV. 1). In a total of 145 T_2 boll rows, 89 rows contained no sensitive plants. The fully resistant rows were harvested as independent populations for advanced agronomic and stability evaluation and used for further crosses.

For breeding and further confirmation of inheritance, T_0 and a small number of hemizygous (zygosity confirmed by subsequence T_2 progeny tests) T_1 plants were selected and were crossed with elite germplasm, and the F_1 plants were evaluated in the greenhouse. The BC_1 material was planted in the field and greenhouse in 1999 and segregation ratios were evaluated. The F_2 material was evaluated in the greenhouse during the winter 1999-2000 for segregation of resistance. Mendelian inheritance for a single gene locus would predict one resistant plant for every one susceptible plant within both F1 and BC1 progenies. Furthermore, F2 progeny would be expected to show 3 resistant plants for every one susceptible plant (Table IV.1).

In summary, all data and analyses indicate that the LLCotton25 event behaves genetically as a single allele at one locus.

Table IV.1. Segregation Analysis of Transformation Event LLCotton25*

Generation	Ratio R:S	OBSERVED R	OBSERVED S	EXPECTED R	EXPECTED S	CHI SQUARE VALUES CALC [1]	CHI SQUARE VALUES p =0.05, 1df
Individual T_2 plants [2]	3:1	2959	957	2937	979	0.66	3.84
T_2 boll rows [3]	1:2	89	145	78	156	2.33	3.84
F_1 [4]	1:1	659	597	628	628	3.06	3.84
BC_1 [4]	1:1	166	172	169	169	0.11	3.84
F_2 [4]	3:1	824	270	820	274	0.08	3.84

[1] Assumes one locus model. There was no significant difference (p=0.05) for the Chi Square goodness-of-fit test for the hypothesis of one locus. To reject the null hypothesis, the Chi^2 value must be greater than 3.84, with one degree of freedom;
[2] Every plant counted in every row, data pooled for this analysis;
[3] Segregation of entire versus partially resistant T_2 boll rows derived from resistant T_1 plants. Homozygous boll rows (no segregation for resistance) were the source of the lines that were used in early event agronomic and stability studies; and
[4] Data pooled across genetic backgrounds (no background effect evident).
S=susceptible, R=resistant

*All F_1 material was generated using a hemizyous transgene donor source (these were either T_0 or T_1).

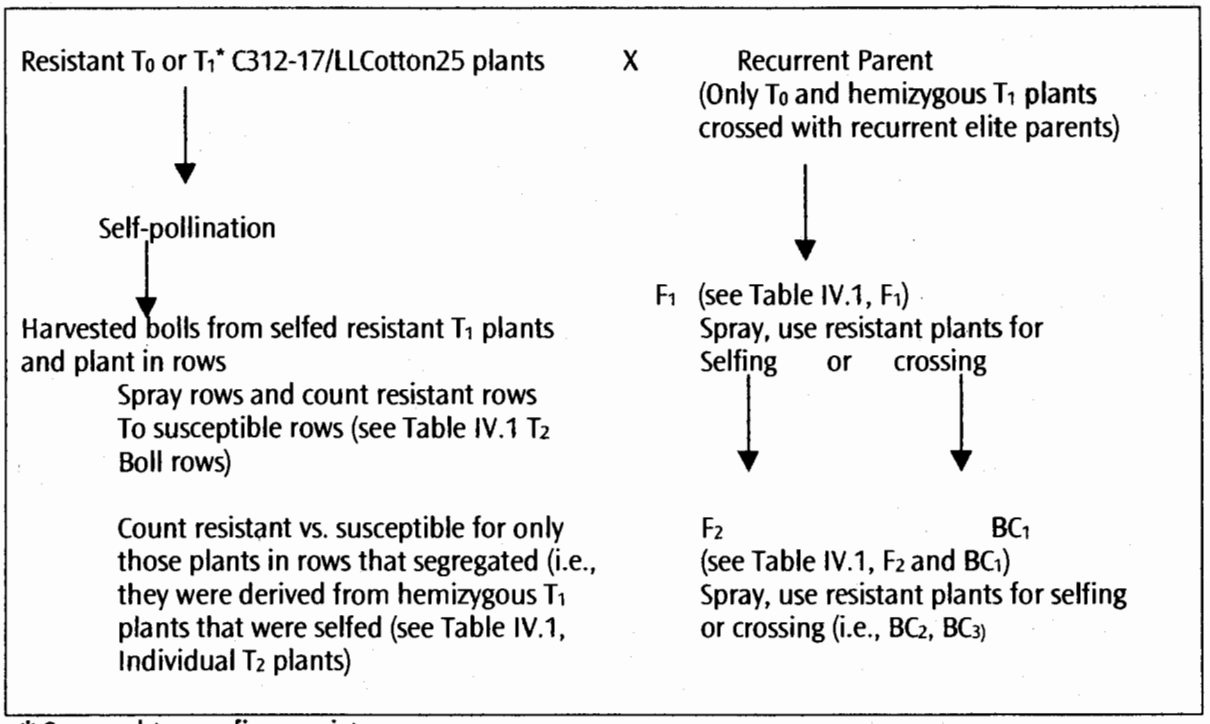
Resistance screening

Resistance screenings were done in the greenhouse for the T_0, T_1, F_1, BC_1, and F_2 generations using a 2% aqueous solution of Liberty applied topically until foliage was completely covered with small droplets at the 2-4-leaf stage followed by an additional treatment as a double check (same rate, method, etc. about 1-2 weeks prior to flowering). The rate was the same for all screening: 2% Liberty with water. F_1 and BC_1 plants were tested again with an additional spray treatment at near flowering stage (about 45 days). F_2 lines (F_3 plant-to-row) treatments were about the same rate but done in the field. Plants were scored as resistant (alive, no damage) or susceptible (damaged severely and dead or dying) 5-7 days post Liberty application. Control plants, that is, plants not resistant to glufosinate, did not survive Liberty treatments and were deemed susceptible. Therefore, no further crosses were made.

T_0, and a small number of T_1, plants were crossed with elite germplasm (recurrent parents), and the resulting F_1 plants were evaluated in the greenhouse. The F_1 for example could result from a cross of the T_0 x the recurrent parent or a cross of a T_1 x recurrent parent (See Figure IV.1). All resistant T_0 plants are hemizygous, however resistant T_1 plants could be either hemizygous or homozygous, thus F_1 prepared from T_1 plants would require additional T_2 progeny testing to determine zygosity of the T_1 parent for the respective F_1 segregation ratio's to be properly interpreted. When T_1 plants were used, the F_1 plants segregated 1:1, indicating that the T_1 plants were hemizygous. The hemizygosity of the T_1 plants was subsequently confirmed by a segregation ratio of 3:1 among the T_2 progeny. The BC_1 material, which is seed from the first backcross, i.e., a cross of the each F_1 x the respective recurrent parent, was planted in the field in 1999 and evaluated. (See Figure IV.1).

The F_2 material was evaluated in the greenhouse during the winter 1999-2000, and the expected segregation ratio of 3:1 was observed.

Segregation Analysis of Transformation Event LLCotton25



* Sprayed to confirm resistance

Agronomic performance

The LibertyLink® Cotton System™ offers two key advantages to cotton growers. First, Liberty® herbicide can be applied season long up to 70 days pre-harvest. The wider application window gives cotton growers more flexibility to use the herbicide based on weed density and size instead of crop stage. Second, Liberty herbicide provides excellent control of morning glory species, as well as additional key weeds including hemp sesbania, pigweed, johnsongrass, barnyardgrass and others. Transformation event LLCotton25, like nontransgenic cotton, can be controlled with commonly used herbicides (Table VI.2).

Today, glyphosate is relied upon for weed control in soybeans, corn and cotton. This wide use creates the potential for weed resistance and weed shifts. LibertyLink fits well in cotton production programs by offering growers an excellent tool to prevent weed shifts and provides for a new weed management system to delay or prevent weed resistance. This system also fits well in production trends toward decreased tillage, fewer applications of pre-emergent herbicides and increased use of broad-spectrum post-emergent herbicides. Currently in the southern cotton region, RoundUp Ready cotton is often grown back to back with Roundup Ready soybeans. The approval of LLCotton25 with a new herbicide, glufosinate ammonium will allow the farmer more diverse

options in technology and chemicals to properly manage weeds. This will also potentially delay or prevent the development of herbicide resistant weeds.

Cotton may also be rotated with peanuts and wheat. There are occasions when volunteer cotton plants may be found in a peanut field. Some of the early season herbicides (Dual II Magnum and Prowl) labeled for peanut are also labeled for use in cotton. None of the post-emergence broadleaf herbicides used in peanuts carry label uses for both peanut and cotton; however, any of the commonly used herbicides used in peanut production, (Basagran, Blazer, Butyrac, Cadre, Classic, Pursuit, Starfire, and Storm) will control volunteer cotton (LLCotton25) plants. Only the grass herbicides such as Poast and Select are labeled for both cotton and peanuts.

Volunteer cotton in wheat fields is rarely a concern for the producer. Geography coupled with irrigation demands of the two crops seldom finds them on the same piece of ground. This market is more segmented as the majority of herbicides utilized are directed at control of broadleaf weeds (i.e., volunteer cotton). However, commonly used herbicides used in wheat production (Amber, Finesse, Aim, Ally, Glean, Harmony, Peak, Stinger, 2,4-D amine, 2,4-D ester) will control volunteer cotton (LLCotton25) plants.

LibertyLink cotton genetics have a distinct advantage over competitive varieties, primarily due to superior lint. FiberMax genetics are highly regarded for their yield potential and quality characteristics. FiberMax genetics continually achieve high premiums for quality. This quality advantage, coupled with top yield performance, gives growers unmatched profit potential in today's tough marketing environment.

The introduction of the LibertyLink gene into FiberMax cotton allows the use of a more effective and flexible herbicide program than can be used in FiberMax cotton today. The combination of superior genetics with a safe, unique and effective herbicide system will provide greater sustainability for cotton growers in the future.

Table VI. 2. Liberty Cotton System Compared to Conventional Herbicide Regime.

Treatment	Conventional Practice		Roundup® Ready Practice		With Liberty® Herbicide	
	Ingredient	Amount g a.i./acre	Ingredient	Amount g a.i./acre	Ingredient	Amount g a.i./acre
Preplant Burndown	Roundup	339	Roundup	339	Roundup	339
Preplant	Trifluralin or Pendamethalin + Fluometuron	375 + 452	Trifluralin or Pendamethalin	375	Trifluralin or Pendamethalin	375
Early Post-emergence over the top or directed	MSMA Pyrithiobac	452 20	Roundup	452	Liberty	189
Mid Post-emergence over-the –top or directed	MSMA + Fluometuron	452 + 339	Roundup	452	Liberty	189
Layby	Prometryn	266	Prometryn	266	Prometryn	266
Total g a.i./acre		2656		1845		1319

02-042-01p

Aventis CropScience

Aventis

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February 8, 2002

Dr. Michael Firko
Assistant Director
Permitting and Risk Assessment
USDA, APHIS, PQ, Unit 133
4700 River Road
Riverdale, MD 20737

Re: Application for the Determination of Nonregulated Status for Glufosinate-Tolerant Cotton Transformation Event LLCotton25

Dear Dr. Firko:

Enclosed please find 2 originals and 4 photocopies of the above referenced petition. Aventis CropScience USA LP requests a determination from APHIS that LibertyLink® Cotton transformation event LLCotton25, and any progeny derived from crosses of event LLCotton25 with traditional cotton varieties, and any progeny derived from crosses of event LLcotton25 with transgenic cotton varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340.

If you have any questions, please feel free to contact me at 919-549-2748, 919-549-3929 (fax) or Luann.Powell@Aventis.com.

Respectfully,

Luann Powell

Luann Powell
Registration Manager – Biotechnology

Enclosures (Contains Confidential Business Information)

cc: Susan MacIntosh, Manager, Regulatory Affairs – Biotechnology
Martine Freyssinet, Global Regulatory Manager

2/11/02

02-042-01p

Petition for Determination of Nonregulated Status:

Glufosinate-Tolerant Cotton Transformation Event LLCotton25

The undersigned submits this petition under 7 CFR 340.6 to request that the Director, Scientific Services, make a determination that the article should not be regulated under 7 CFR 340.

Submitted by:

Luann Powell

Luann Powell
Regulatory Affairs – Biotechnology

Aventis CropScience USA LP
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Contributors:

Martine Freyssinet
Susan MacIntosh
Donna Mitten

Date

February 8, 2002

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2/12/02
MFP

Summary

Aventis CropScience USA LP (Aventis) is submitting a Petition for Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) for LibertyLink® Cotton Transformation Event LLCotton25. Aventis requests a determination from APHIS that LibertyLink Cotton transformation event LLCotton25, and any progeny derived from crosses of event LLCotton25 with traditional cotton varieties, and any progeny derived from crosses of event LLcotton25 with transgenic cotton varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. Transformation event LLCotton25 is considered a regulated article because it contains sequences from the plant pests, cauliflower mosaic virus (CaMV) and *Agrobacterium tumefaciens*.

Glufosinate-ammonium (GA) is in the phosphinothricin class of herbicides. It is a non-systemic, non-selective herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. GA controls weeds through the inhibition of glutamine-synthetase (GS), which leads to the accumulation of phytotoxic levels of ammonia in the plant. GS is responsible for the synthesis of the amino acid glutamine from glutamic acid and ammonia. It is the only enzyme in plants that can detoxify ammonia released by photorespiration, nitrate reduction, and amino acid degradation.

Transformation event LLCotton25 is cotton genetic material that contains a stably integrated gene, *bar*, which encodes phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme catalyzes the conversion of L-phosphinothricin, the active ingredient in GA, to an inactive form, thereby conferring resistance to the herbicide. The *bar* gene in event LLCotton25 was isolated from *Streptomyces hygroscopicus*, strain ATCC21705. (Murakami *et al.*, 1986). The N-terminal codon of the wild type *bar* coding region has been substituted for the codons ATG and GAC, respectively. The gene was introduced by *Agrobacterium*-mediated gene transfer of a fragment of plasmid DNA. Southern blot analyses show event LLCotton25 contains 1 complete copy of the *bar* gene.

LibertyLink Cotton transformation event LLCotton25 has been field tested by Aventis beginning in 1999 in winter nurseries and in adapted growing regions of the United States. These tests have occurred at more than 40 sites under field release authorizations granted by USDA APHIS (USDA authorizations: 99-007-08n, 00-074-14n, 00-108-10n, 00-119-05n, 00-258-02n, 01-075-17n, 01-102-21n, 01-108-05n, 01-271-05n. Data collected from these field trials and laboratory analyses presented herein demonstrate that LibertyLink Cotton transformation event LLCotton25: 1) exhibits no plant pathogenic properties; 2) is no more likely to become a weed than non-modified cotton; 3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species; 4) does not cause damage to processed agricultural commodities; and 5) is unlikely to harm other organisms that are beneficial to agriculture.

Therefore, Aventis requests a determination from USDA APHIS that LibertyLink Cotton transformation event LLCotton25, and any progeny derived from crosses of event

LLCotton25 with traditional cotton varieties, and any progeny derived from crosses of event LLCotton25 with transgenic cotton varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 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 relevant data and information known to the petitioner which are unfavorable to the petition.

Luann Powell

Luann Powell
Regulatory Affairs – Biotechnology

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2/12/02
MKP

ACRONYMS AND SCIENTIFIC TERMS

APHIS	Animal and Plant Health Inspection Service
AVSxxx	Aventis cotton line
bar	phosphinothricin acetyltransferase gene (origin *Streptomyces hygroscopicus*)
C312	Coker 312 cotton line
CaMV	Cauliflower Mosaic Virus
D &PL	Delta & Pine Land
ELISA	enzyme linked immunosorbent assay
ELS Cotton	Extra-long staple
FMxxx	FiberMax® cotton line
GA	glufosinate-ammonium
GS	glutamine synthetase
HSxxx	Paymaster cotton variety
LL	LibertyLink®
PAT	phosphinothricin acetyltransferase (enzyme)
USDA	United States Department of Agriculture

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Statement of Grounds for Nonregulated Status

I. Rationale for Development of Glufosinate-Tolerant Cotton

Cotton is the United States and the worlds leading fiber crop. In the US for the 1999/2000 production year, cotton was grown on 5,433,000 hectares, the major producing states being Texas (2.6 million ha), Georgia (0.6 million ha), Mississippi (0.5 million ha), Arkansas, North Carolina and California (0.4 million ha each). The world total planted area was 32 million ha, for a production of 87 million bales (18.9 million tons). (Source: USDA Foreign Agriculture Service, 2001).

Cotton is grown in the United States using mechanized practices for planting and harvesting. Cultural practices, including irrigation and crop rotation, and herbicides are employed to control weeds. Weed management is critical to maximum cotton yield and herbicides are used on most cotton acreage grown in the United States. The grower is typically interested in applying a herbicide for weed control that has a broad weed spectrum, does not injure the crop, is cost effective, and has positive environmental attributes. Several classes of herbicides have effective broad spectrum weed control, however they may injure or kill the cotton crop when used at the application rates suggested for weed control.

The phosphinothricin herbicide glufosinate-ammonium (GA, chemical name: ammonium-DL-homoalanin-4-yl(methyl)phosphinate) is registered for nonselective weed control on both non-food use (Finale ®) and food use plants (Rely ®, Liberty®) in the United States. Outside of North America the herbicide is generally sold as Basta ®. Glufosinate is a contact, non-selective herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. It is highly biodegradable, has no residual activity, and has very low toxicity for humans and wild fauna (Anonymous, 1991). Resistance to the herbicide has now been achieved, through the insertion of a resistance gene, in over 20 commercially important plant species including cotton. Preliminary studies suggest that glufosinate treatments to LibertyLink® cotton may be less injurious to the reproductive development of the cotton plant compared to glyphosate treatments to Roundup Ready cotton plants (see Appendix E). Genetically engineered LibertyLink Cotton will provide a selective use for glufosinate, a valuable new weed management tool to cotton producers and potentially a superior quality crop that may lead to higher yields.

Commercialization of Cotton with LibertyLink, specifically transformation event LLCotton25, following the receipt of the required authorizations, including this Determination of Nonregulated Status, will offer a unique, efficacious and environmentally friendly option to growers for weed control in cotton.

II. The Cotton Family

Cotton, *Gossypium hirsutum*, has been cultivated for millennia in many parts of the world. About 90 percent of the production of cotton is *G. hirsutum*. Cotton is primarily used worldwide for its lint. Lint is produced on the seed coat, and is spun into fine strong threads. Only the United States and a few other countries have developed major

commercial uses for the seed. Raw unprocessed cottonseed may be fed to ruminants in the form of cottonseed meal and hulls or the seed can be processed for oil, the primary component consumed by humans. Linters, the short fibers that remain on the hulls after the removal of the lint have both edible and non-edible use.

Cotton belongs to the genus *Gossypium*, which is in the Malvaceae or Mallow family. Other members of this family include okra, hollyhock, rose of sharon, and even such plants as teaweed, spurred anoda, and velvetleaf that are weed pests in cotton. Only the genus *Gossypium*, and a few isolated species of the other genera, is characterized by the seed hairs, or trichomes, which are outgrowths of the epidermis of the seed coat. The 39 species in the genus *Gossypium* are quite diverse, but only four of them produce commercial-type lint (Brown, 1986).

The tribe Gossypieae has two specific characters: the form of the embryo (which is more complex than in the balance of the Malvaceae) and the presence of distinctive punctae in various parts of the plant but especially in the cotyledons. These punctae are now known as "gossypol glands" and are distinctive in morphology and chemical contents. They are believed to be unique to the tribe (Fryxell, 1976).

A. History and Uses of Cotton

Cotton, *Gossypium* spp. has been grown for its fiber for several thousand years. Its cultivation and manufacture into cloth developed independently in both the Eastern and Western Hemispheres. One of the oldest records of cotton textiles, dating back about 5,000 years, was found in the Indus River Valley in what is now Pakistan. Excavations in Peru and Mexico have uncovered cotton cloth identified as being 4,500 to 7,000 years old. Cotton fabrics have also been found in the remains of some of the ancient civilizations of Egypt and in the ruins of Indian pueblos of the Southwestern United States, dating back hundreds of years before Christ. Other products, such as cottonseed oil, cake, and cotton linters are by-products of fiber production.

Cottonseed, a raw agricultural product which was once largely wasted, is now converted into food for people, feed for livestock, fertilizer and mulch for plants, fiber for furniture padding and cellulose for a wide range of products from explosives to computer chip boards. Cotton is indeed nature's food and fiber plant. Although lint is the most valuable product from a field of cotton, it is very important to keep in mind that this versatile plant is also an important vegetable oil source. From this point of view, cotton is a food crop.

Cotton, *Gossypium hirsutum* L., is mainly produced in China, the United States, India, Pakistan and Uzbekistan, with these five countries contributing to 70% of world production. See Table II.1.

In the United States for the 1999/2000 production year, cotton was grown on 5,433,000 hectares, the major producing states being Texas (2.6 million ha), Georgia (0.6 million ha), Mississippi (0.5 million ha), Arkansas, North Carolina and California (0.4 million ha each).

The world total planted area was 32 million ha, for a production of 87 million bales (18.9 million tons). (Source: USDA Foreign Agriculture Service, 2001).

The total production of cotton as an oilseed was 30.6 million tons in 1999-2000 out of a world total of 309 million tons. Cottonseed oil, with a production estimated at 4.3 million tons in 2000/01, accounts only for 3% of total world oil production. With 1.2 million tons in 2000, China is by far the most important producer (Source: FAOStat).

Table II.1. Cotton: Production in Specified Countries and the World (Millions 480- Pound Bales)

Country	1996	1997	1998	1999
China	19.3	21.1	20.7	17.6
U.S.A.	18.9	18.8	13.9	17.0
India	13.9	12.3	12.9	12.3
Pakistan	7.3	7.2	6.3	8.6
Uzbekistan	4.8	5.2	4.6	5.2
Australia	2.8	3.1	3.3	3.3
World	89.6	91.6	84.5	87.0

Source: USDA-Foreign Agriculture Service.

B. Taxonomy of the Genus

Scientific name:	*Gossypium hirsutum* L.
Family:	Malvaceae
Genus:	*Gossypium*
Species:	*hirsutum* (2n=52, Upland cotton), *barbadense* (2n=52, Pima cotton), *arboreum* (2n=26), *herbaceum* (2n=26)
Cultivar/breeding line:	numerous varieties and breeding lines
Common name:	Cotton

The predominant type of cotton grown in the United States is *Gossypium hirsutum*, known as American Upland. The Upland type, which usually has a staple length of 1 to 1 1/4 inches, accounts for about 97 percent of the annual U.S. cotton crop. Upland cotton is grown throughout the U.S. Cotton Belt as well as in most major cotton-producing countries. The balance of U.S.-grown cotton is *Gossypium barbadense*, commonly referred to as American Pima or extra-long staple (ELS). ELS cotton, which has a staple length of 1 1/2 inches or longer, is produced predominantly in California, Arizona, New Mexico, and southwest Texas, where it is particularly well adapted to the arid environmental conditions. The markets for ELS cotton are mainly high-value products such as sewing thread and expensive apparel.

C. Pollination of Cotton

Gossypium hirsutum is generally considered to be a self-pollinating crop (Niles and Feaster, 1984). The morphology of cotton pollen, i.e., it is heavy and somewhat sticky, does not lend itself to wind pollination. Cotton can, however, be pollinated by insects. Honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) are the primary insect pollinators.

McGregor (1976) traced the movement of pollen from a cotton field surrounded by a large number of honeybee colonies. Movement of the pollen was traced by means of fluorescent particles. McGregor found that at 150 to 200 feet away from the source plant, only 1.6 percent showed the presence of the fluorescent particles. By comparison, the isolation distances for Foundation, Registered and Certified seeds in 7 CFR Part 201 are 1320, 1320 and 660 feet, respectively.

D. Genetics of Cotton

The genus *Gossypium* consists of 39 species, of which 4 to 5 are generally cultivated (Fryxell, 1984). The cultivated species are *G. hirsutum*, *G. barbadense*, *G. arboreum* L., *G. herbaceum* and *G. lanceolatum* Todaro.

At least seven genomes, designated A, B, C, D, E, F, and G, are found in the genus (Endrizzi, 1984). Diploid species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum* and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*.

By far, the most important agricultural cottons are *G. hirsutum* and *G. barbadense*. These are both allotetraploids (2n=4x=52) of New World origin, and presumably of ancient cross between Old World A genomes and New World D genomes. How and when the original crosses occurred has been subject to much speculation. Euploids of these plants have 52 somatic chromosomes, and are frequently designated as AADD (they behave as disomic polyploids). Four additional New World allotetraploids occur in the genus, including *G. tomentosum*, the native of Hawaii. Due to the difference in ploidy level, *G. hirsutum* cannot cross with wild diploid cottons. *G. hirsutum* is readily cross-compatible only with other tetraploid members of the tribe *Gossypium*, which includes *G. tomentosum* in Hawaii, *G. darwinii* in the Galapagos, *G. mustelinum* in northeastern Brazil, *G. hirsutum* and *G. lanceolatum* in tropical/subtropical America, and *G. barbadense* in South America, as well as cultivated forms of *G. hirsutum* and *G. barbadense* (Fryxell, 1976). *Gossypium tomentosum* has been crossed with *G. hirsutum* in breeding programs; however, no commercial cotton is produced in Hawaii (Jenkins, 1993).

The New World allotetraploids are peculiar in the genus, because the species, at least in their wild forms, grow near the ocean, as invaders in the constantly disturbed habitats of strand and associated environs. It is from these "weedy" or invader species that the cultivated cottons developed (Fryxell, 1976).

E. Weediness Potential of Cotton

In the United States, cotton (*G. hirsutum*) is not a weed pest and has no sexually compatible weedy relatives except perhaps *G. tomentosum* in Hawaii, which will be discussed in the next section. A number of references confirm the lack of weediness of cotton: Crockett, 1977, Holm *et al.*, 1977, Muenscher, 1980. Some feral cotton populations do exist in the US, but they are rare and found in areas hundreds of miles from commercial cotton production areas.

Cotton is a domesticated crop that requires human intervention to survive in non-cotton production area. Since cotton is an exotic species in the US and has not become a weed pest over many centuries, there is no expectation that a new cotton variety with a single gene introduction would enhance that risk by becoming weedy in non-cotton production areas.

Within cotton production areas, the addition of the LibertyLink® (LL) trait (PAT protein) into domesticated cotton will not cause it to become weedy. Traditional cotton breeding has provided new cotton varieties with resistance to disease, insects and herbicides, tolerance to various environmental conditions (heat, cold, drought, etc.) and enhanced phenotypic traits, such as faster germination and rapid seedling growth. Despite the many enhanced cotton varieties, none have shown any evidence of weediness. Crops modified by molecular or cellular methods, which are highly specific, should present no different risks than those introduced by traditional, less controlled methods. Of specific concern may be the addition of herbicide tolerance to produce LL cotton, but experience with many other herbicide-tolerant crops demonstrates no change in weediness potential. For example, rapeseed, cotton, corn, soybean, tobacco, tomato and other crops have been transformed to resist herbicides such as glyphosate, glufosinate, bromoxynil, and sulfonylurea without any evidence of weediness. Perhaps the largest concern is with volunteer plants that could become weedy in subsequent years. Yet these plants can easily be controlled by pre- or post-emergent herbicides. For example, LibertyLink cotton volunteers could easily be controlled by using glyphosate.

F. Potential for Outcrossing

The potential for outcrossing can be defined as the ability of gene escape to wild cotton relatives. While gene flow could occur vegetatively, by seed or pollen, only pollen flow has any potential risk for cotton. Vegetative propagation is uncommon for cotton and seed dispersal (wind, birds, and animals) is rarely successful due to the properties of the boll structure. Cotton pollen is not transferred by wind due to its large, heavy and sticky nature (Niles and Feaster, 1984). Natural cross-pollination results from pollen being carried by insects, bees being the most important cotton pollinators (McGregor, 1976).

In Upland cotton, outcrossing studies suggest that pollen carryover decreases very rapidly as the distance to the closest marker pollen row increases, and that very little pollen is transferred beyond 12 meters. Vaissière (1990) prepared a report containing a literature review on cotton pollination and a summary of his study, "Pollen Dispersal and Carryover in Upland Cotton," conducted in Texas in 1983. The Texas study was conducted using a

male sterile line surrounded by male fertile plants. Sixty honeybee colonies were supplied. Results showed that the pollen carryover in upland cotton decreased in proportion to the inverse of the distance to the closest pollinator row, and there was no significant pollen carryover past 12 meters.

Meredith and Bridge (1973) detected no outcrossing between adjacent plants in a study conducted in Stoneville, MS; the approximate limit of detection for the sample size and methods was approximately 0.046%.

Outcrossing data using bromoxynil-tolerant cotton is reported for seven locations in Figure II.1 (Kareiva *et al.*, 1994). Seed samples were collected in the border rows of Calgene's winter nursery sites in Catamarca, Argentina and Pongola, Republic of South Africa, as well as in Stoneville, MS, U.S.A. Sampling distance was one to 20 meters away from the bromoxynil-tolerant cotton. The frequency of outcrossing is determined by the crop and the pollinator. It is interesting to note that although the rate is higher for Argentina and South Africa (most likely due to the behavioral differences between European and African honeybees) the pattern of decline with distance is the same.

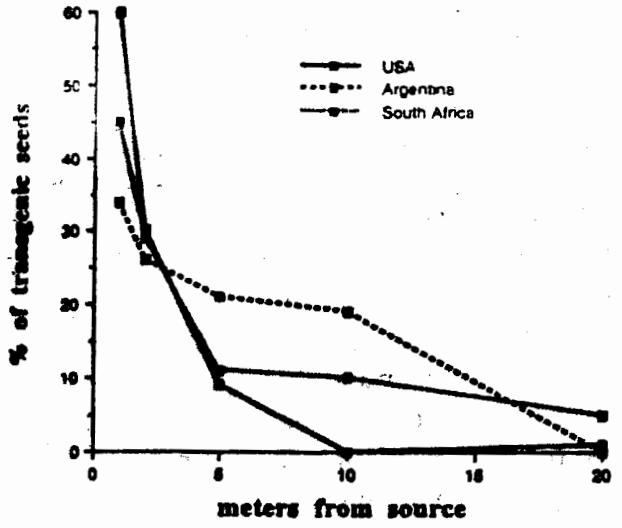


Figure II.1. Outcrossing Studies with Bromoxynil-Tolerant Cotton: The Decline in Transgenic Cotton Seeds as a Function of Distance Away from a Source for USA (Five Different States Lumped Together), Argentina and South Africa (Figure 1 in Kareiva *et al.*, 1994).

The percentage is out of the total transgenic seeds recovered at five distances (1, 2, 5 10 and 20 m away), with that total being 78 in USA, 179 in Argentina, and 728 in South Africa. The total number of seeds scored in order to obtain these transgenic dispersal events was 15024 in USA, 7632 in Argentina and 28097 in South Africa. By standardizing to a percentage the graphs are more easily compared, even though different numbers of seeds were collected at each field trial.

Recently, Sundstrom (2001) studied pollen flow from a commercial seed field of Roundup® Ready (RR) cotton in California, at various distances from non-transgenic cotton fields (see Figure II.2). The results obtained confirm - and refine - those of Kareiva *et al.* (1994), as

larger distances were studied. In spite of variations due to the respective cardinal positions of the fields, the same decline with distance is observed.

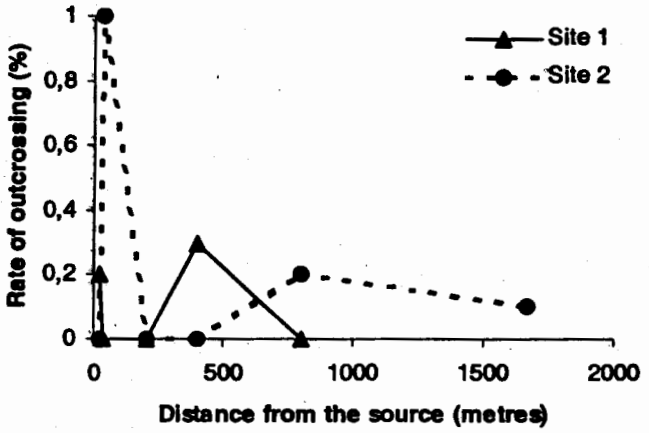


Figure II.2. Outcrossing Studies with Roundup Ready® Cotton: The Decline in Transgenic Cotton Seeds as a Function of Distance Away from a Source for California (page 33 in Sundstrom, 2001).

The percentage is out of the total transgenic seeds recovered at six distances from the RR cotton field: 24 m (1 site), 33 m (2 sites), 201 m (2 sites) 402 m (2 sites), 805 m (2 sites) and 1668 m (1 site).

In the US, there are four cotton species, two that are cultivated commercially – *G. hirsutum* L. and *G. barbadense* L. and two wild relatives – *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seemann (Fryxell 1979). Of these four species, only three *Gossypium* species could be recipients for *G. hirsutum* - *G. hirsutum* itself, *G. barbadense* and *G. tomentosum*. *G. hirsutum* grows feral only in the southern tip of Florida and in Hawaii, which is hundreds of miles from any commercial cotton fields. *G. barbadense* is only found in very small commercial plots and is not found in wild environments in the US. Thus outcrossing to wild *G. hirsutum* or commercial plots of *G. barbadense* is unlikely.

Outcrossing of the tetraploid *G. hirsutum* to the wild diploid *G. thurberi*, which occurs in Arizona, is extremely unlikely. Crosses between these species in breeding programs have been done, but the vigor of the hybrid seed is much reduced and the plants are usually infertile. In addition, native populations of G. thurberi reside in the higher altitudes and are thus isolated from commercial cotton production (Fryxell, 1979). Therefore, outcrossing of commercial LL cotton to *G. thurberi* is not a concern.

G. tomentosum is only found in the Hawaiian archipelago, occurring in dry coastal areas far removed from agricultural areas. The flowers of *G. tomentosum* are only receptive at night, rather than in the day as for *G. hirsutum* and moths, rather than bees generally pollinate them. Finally, outcrossing is unlikely since there are no commercial cotton production areas on the islands and there would be no selective advantage since glufosinate is not used in natural non-agricultural areas.

III. The Transformation System and Plasmid Used

The LibertyLink Cotton transformation event LLCotton25 contains the *bar* gene derived from *Streptomyces hygroscopicus*, strain ATCC21705 (Murakami *et al.*, 1986). Since the native *bar* gene has a GTG initiation codon, the N-terminal end of the *bar* coding region was substituted for two complementary synthetic oligonucleotides in order to obtain an ATG initiation codon, to guarantee correct translation initiation in plants (De Block *et al.*, 1987). The *bar* gene encodes the enzyme phosphinothricin acetyltransferase (PAT), which confers tolerance to the herbicide GA. The chimeric *bar* gene construct contains the 35S promoter of the Cauliflower Mosaic Virus (Odell *et al.*, 1985). The *bar* coding sequence (Thompson *et al.*, 1987) is followed by the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (Depicker *et al.*, 1982). This chimeric gene of pGSV71 that can be transferred to plants is denoted as P35S-*bar*-3'nos. *Agrobacterium*-mediated gene transfer of pGSV71 results in transfer to the plant genome of the DNA fragment between the T-DNA border repeats. Even though some of the genes used in the transformation process were derived from *A. tumefaciens*, a known plant pathogen, the genes that cause crown gall disease were removed, and therefore, not incorporated into the recipient plant (Deblaere *et al.*, 1985).

A. Transformation System

Gossypium hirsutum tissue from variety Coker 312 was transformed with the vector pGSV71 (Reynaerts, 1999) using *A. tumefaciens* transformation. The explants were regenerated to whole plants using the appropriate regeneration media with antibiotics to eliminate residual Agrobacterium and selected with glufosinate.

The best lines derived from transformation event LLCotton25 have been evaluated for agronomic characteristics since their first greenhouse screens of the T_0 generation. T_0 plantlets were transitioned from tissue culture, transferred to greenhouse soil, and allowed to flower and set seed. Plantlets were evaluated for fertility, fecundity and tolerance to GA. Seed (T_1 generation) collected from plants that passed the greenhouse screen was planted in winter nursery for the primary field evaluations. Cotton bolls were selected from T_1 plants that survived an increased herbicide pressure and continued to express acceptable fertility and fecundity. Selected cotton bolls were harvested and advanced to a secondary field evaluation of the T_2 generation. Each row was planted with the seed of a single cotton boll. The cotton boll rows were evaluated for Mendelian inheritance (Section IV.A) and seed from homozygous rows was harvested for further evaluation.

B. Parent line

Transformation event LLCotton25 has its origin in the variety Coker 312. The variety Coker 312 (PVP 7200100) is an U.S. Protected Variety of SEEDCO Corporation, Texas. Coker 312 was developed from a cross of Coker 100 X D&PL-15 and selected through successive generations of line selection. This variety is well suited for both dryland and irrigated production south of Lubbock, Texas (Metzer and Supak)

C. Construction of the Plasmid Used for Transformation

The plasmid pGSV71 has been derived from plasmid pGSV1, which is essentially derived from pGSC1700 (Cornelissen and Vandewiele, 1989). Plasmid pGSV1 comprises the following structural elements :

- The plasmid core comprising the origin of replication from the plasmid pBR322 (Bolivar *et al.*, 1977) for replication in *Escherichia coli* (pBR*ori*) and a restriction fragment comprising the origin of replication from the *Pseudomonas* plasmid pVS1 (Itoh *et al.*, 1984) for replication in *Agrobacterium tumefaciens* (pVS1*ori*);
- A selectable marker gene conferring resistance to streptomycin and spectinomycin (*Sm/Sp*) for selection of the plasmid in *Escherichia coli* and *Agrobacterium tumefaciens*; and
- An artificial T-DNA region consisting of the left and right border sequences of the TL-DNA from pTiB6S3 and multilinker cloning sites allowing the insertion of chimeric genes between the T-DNA border repeats. There are no residual T-DNA sequences present between the border repeats.

Plasmid pGSV71 has essentially been derived from pGSV1, by inserting the gene of interest, P35S-*bar*-3'nos, between the T-DNA border repeats of pGSV1. Plasmid pGSV71 is constructed in *Escherichia coli*, and thereafter transferred to a suitable *Agrobacterium tumefaciens* strain, which is used in plant transformation.

A map of the plasmid pGSV71 is shown in Figure III.1. A description of the DNA elements in P35S-*bar*-3'nos is shown in Table III.1. The amino acid sequence of the protein is provided in Figure III.2; the nucleotide sequence is published in Thompson *et al.*, 1987.

D. Open Reading Frames and Associated Regulatory Regions in P35S-*bar*-3'nos

Transformation was performed through *Agrobacterium*-mediated gene transfer of the T-DNA from pGSV71. This T-DNA contains one open reading frame, *bar*, which is intact and functional in transformation event LLCotton25, as will be shown in Section IV. The LibertyLink Cotton transformation event LLCotton25 has been considered a regulated article because it contains DNA sequences from Cauliflower Mosaic Virus and *Agrobacterium*, which are plant pests. This section contains a more thorough description of the inserted genetic material responsible for expression of the glufosinate resistance trait. Refer to Table III.1 for a description of all introduced genetic sequences.

Table III.1. Genetic Elements of the Plasmid Vector pGSV71

Position in Vector	Genetic element and Function
0198 – 0222	Right border repeat from the TL-DNA from pTiB6S3, Gielen *et al.* (1984).
0223 – 0249	Synthetic polylinker sequence
0250 – 1634	P35S: promoter region from the Cauliflower Mosaic Virus 35S transcript, Odell *et al.* (1985); 1384 bp.
1635 – 2186	The coding sequence of the bialaphos resistance gene (*bar*) of *Streptomyces hygroscopicus*, Thompson *et al.* (1987). The N-terminal two codons of the wild type *bar* coding region have been substituted for the codons ATG and GAC, respectively; 551 bp.
2187 – 2205	Synthetic polylinker sequence
2206 – 2465	A 260bp *TaqI* fragment from the 3' untranslated end of the nopaline synthase gene (3' nos) from the T-DNA of pTiT37, Depicker *et al.* (1982); 259 bp.
2466 – 2519	Synthetic polylinker sequence
2520 – 2544	Left border repeat from the TL-DNA from pTiB6S3, Gielen *et al.* (1984).

1. CaMV 35S promoter

The *35S* promoter sequence is derived from CaMV and controls expression of the *bar* gene. CaMV is a double-stranded DNA caulimovirus with a host range restricted primarily to cruciferous plants (Odell, 1985). The *35S* promoter directs high level constitutive expression and is widely used as a promoter for high expression of genes (Harpster, *et al.*, 1988). The CaMV sequences, as used in the LibertyLink Cotton, do not cause the cotton to become a plant pest.

2. *bar*

The *bar* gene was isolated from *Streptomyces hygroscopicus*, strain ATCC21705 (Murakami *et al.*, 1986). It encodes the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of GA. Genes encoding PAT enzymes have been isolated from *S. viridochromogenes* (Hara, *et al.*, 1991) and *S. hygroscopicus* (Thompson, *et al.*, 1987).

Members of the genus *Streptomyces* are gram-positive sporulating soil bacteria. These organisms synthesize numerous unique compounds, secondary metabolites, that often possess antibacterial, antitumor, or antiparasitic activity (Demain, *et al.*, 1983). Both *S. viridochromogenes* and *S. hygroscopicus* produce one such compound, the antibiotic bialaphos. Bialaphos (syn. L-phosphinothricyl-L-alany-L-alanine) is a herbicidally active tripeptide consisting of two L-alanine molecules and an analog of L-glutamic acid called phosphinothricin. When it is released by peptidases, the L-phosphinothricin moiety, is a potent inhibitor of glutamine synthetase (GS) (Bayer, *et al.*, 1972). L-phosphinothricin is the active component of the commercial herbicides, Herbiace® (Meiji Seika Ltd.) and Liberty®, Basta®, Ignite®, Rely®, Remove® and Finale® (AgrEvo GmbH). Herbiace is

bialaphos that is commercially produced using *S. hygroscopicus*. The other herbicides are the ammonium salts of phosphinothricin, common name GA, and are chemically synthesized.

L-phosphinothricin is a potent inhibitor of the enzyme GS in both bacteria and plants, where it apparently binds competitively to the enzyme by displacing L-glutamate from the active site. Evidently GS binds L-phosphinothricin better than the substrate. GS plays a central role in nitrogen metabolism of higher plants where it is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration (Miflin and Lea, 1976). Ammonia, although a plant nutrient and metabolite, is toxic in excess and leads to death of plant cells (Tachibana, *et al.*, 1986).

Although the GS from both *S. viridochromogenes* and *S. hygroscopicus* are sensitive to L-phosphinothricin, the bacteria produce an inactivating enzyme, PAT. PAT catalyzes the conversion of L-phosphinothricin to N-acetyl-L-phosphinothricin in the presence of acetyl CoA as a co-substrate. N-acetyl-L-phosphinothricin does not inactivate GS, and, thus, has no herbicidal activity. Therefore, plants expressing the PAT enzyme are resistant to the phosphinothricin class of herbicides. The PAT enzyme is encoded by the *bar* (bialaphos-resistance) gene in *S. hygroscopicus*, and by the *pat* gene in *S. viridochromogenes*. These genes function both as an integral part of the biosynthetic pathway of bialaphos and as an enzyme which confers resistance (Kumada, 1988).

3. 3' nos terminator

A 260bp *TaqI* fragment from the 3' nontranslated region of the nopaline synthase gene (3' nos) from the T-DNA of pTiT37 was isolated from *Agrobacterium tumefaciens* (Depicker *et al.*, 1982). The 3' nos terminator controls the expression of the *bar* gene due to its role in transcription termination and polyadenylation (Depicker *et al.*, 1982). The *Agrobacterium* sequences, as used in the LibertyLink Cotton, do not cause the cotton to become a plant pest.

Figure III.1. Vector Map of Plasmid pGSV71

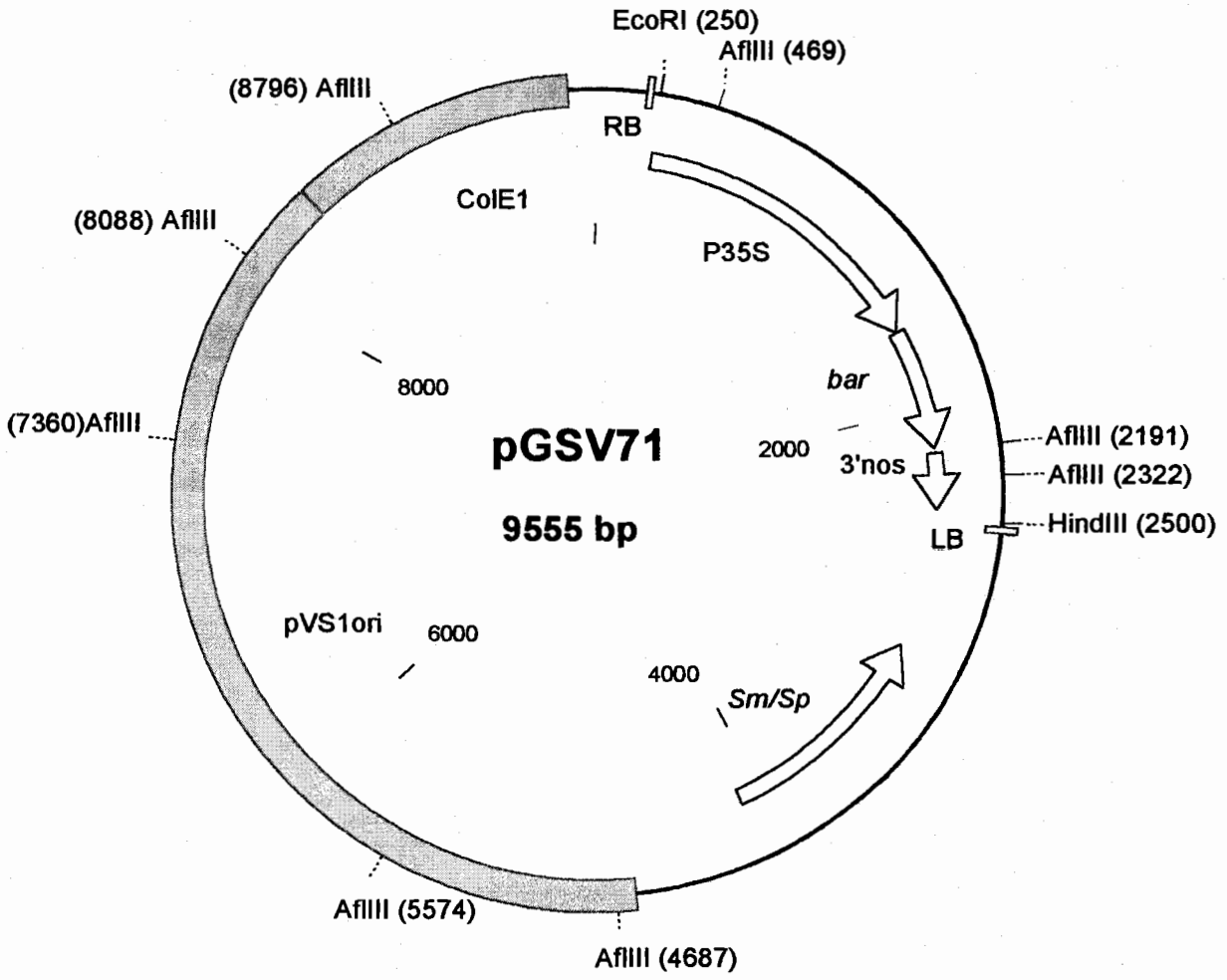


Figure III.2. Protein Sequence of the PAT Protein as Produced in Transformation Event

LLCotton25

MSPERRPADI RRATEADMPA VCTIVNHYIE TSTVNFRTEP QEPQEWTDDL
VRLRERYPWL VAEVDGEVAG IAYAGPWKAR NAYDWTAEST VYVSPRHQRT
GLGSTLYTHL LKSLEAQGFK SVVAVIGLPN DPSVRMHEAL GYAPRGMLRA
AGFKHGNWHD VGFWQLDFSL PVPPRPVLPV TEI

IV. Molecular Characterization of Transformation Event LLCotton25

A. Mendelian Inheritance of Transformation Event LLCotton25

Primary transformation event LLCotton25 was derived from the transformation of cotton cells as described in Section III. T_1 seed harvested from self-pollinated T_0 plants surviving a Liberty Herbicide greenhouse screen were planted in the greenhouse for multiplication and evaluation. T_1 plants were selected for survival following Liberty Herbicide application. Bolls were harvested from individual plants and T_2 boll rows were planted in June 1999 in Mississippi (USDA Authorization 99-007-08n). Two types of segregation analyses were conducted: 1) census of individual resistant and susceptible plants and 2) the number of fully resistant and partially resistant rows.

Application of Liberty Herbicide was used to score the rows for segregation of the PAT phenotype. Rows containing no sensitive plants were considered to be homozygous for the *bar* gene, while the partially resistant rows were considered hemizygous. In this situation, Mendelian inheritance for a single gene locus would predict one fully resistant row for every two partially resistant rows. For each population of LLCotton25 the expected ratio of 1:2 was observed (Table IV. 1). In a total of 145 T_2 boll rows, 89 rows contained no sensitive plants. The fully resistant rows were harvested as independent populations for advanced variety evaluation and used for crosses.

At the same time, the selected T_1 plants were crossed with elite germplasm, and the F_1 plants were evaluated in the greenhouse. The BC_1 material was planted in the field in 1999 and evaluated. The F_2 material was evaluated in the greenhouse during the winter 1999-2000.

Table IV.1. Segregation Analysis of Transformation Event LLCotton25

Generation	Ratio R:S	OBSERVED R	OBSERVED S	EXPECTED R	EXPECTED S	CHI SQUARE VALUE CALC	CHI SQUARE VALUE D=0.05, 1DF
Individual T_2 plants [2]	3:1	2959	957	2937	979	0.33	3.85
T_2 boll rows [3]	2:1	145	89	156	78	2.33	3.85
F_1 [4]	1:1	659	597	628	628	3.06	3.85
BC_1 [4]	1:1	166	172	169	169	0.11	3.85
F_2 [4]	3:1	824	270	820	274	0.08	3.85

[1] Assumes one locus model. There was no significant difference (p=0.05) for the Chi Square goodness-of-fit test for the hypothesis of one locus. To reject the null hypothesis, the Chi^2 value must be greater than 3.84, with one degree of freedom;
[2] Every plant counted in every row, data pooled for this analysis;
[3] Segregation of entire versus partially resistant T_2 boll rows derived from resistant T_1 plants. Homozygous boll rows (no segregation for resistance) were the source of the lines that were used in the subsequent crossing program; and
[4] Data pooled across genetic backgrounds (no background effect evident).
S=susceptible; R=resistant

B. DNA Analysis of Transformation Event LLCotton25

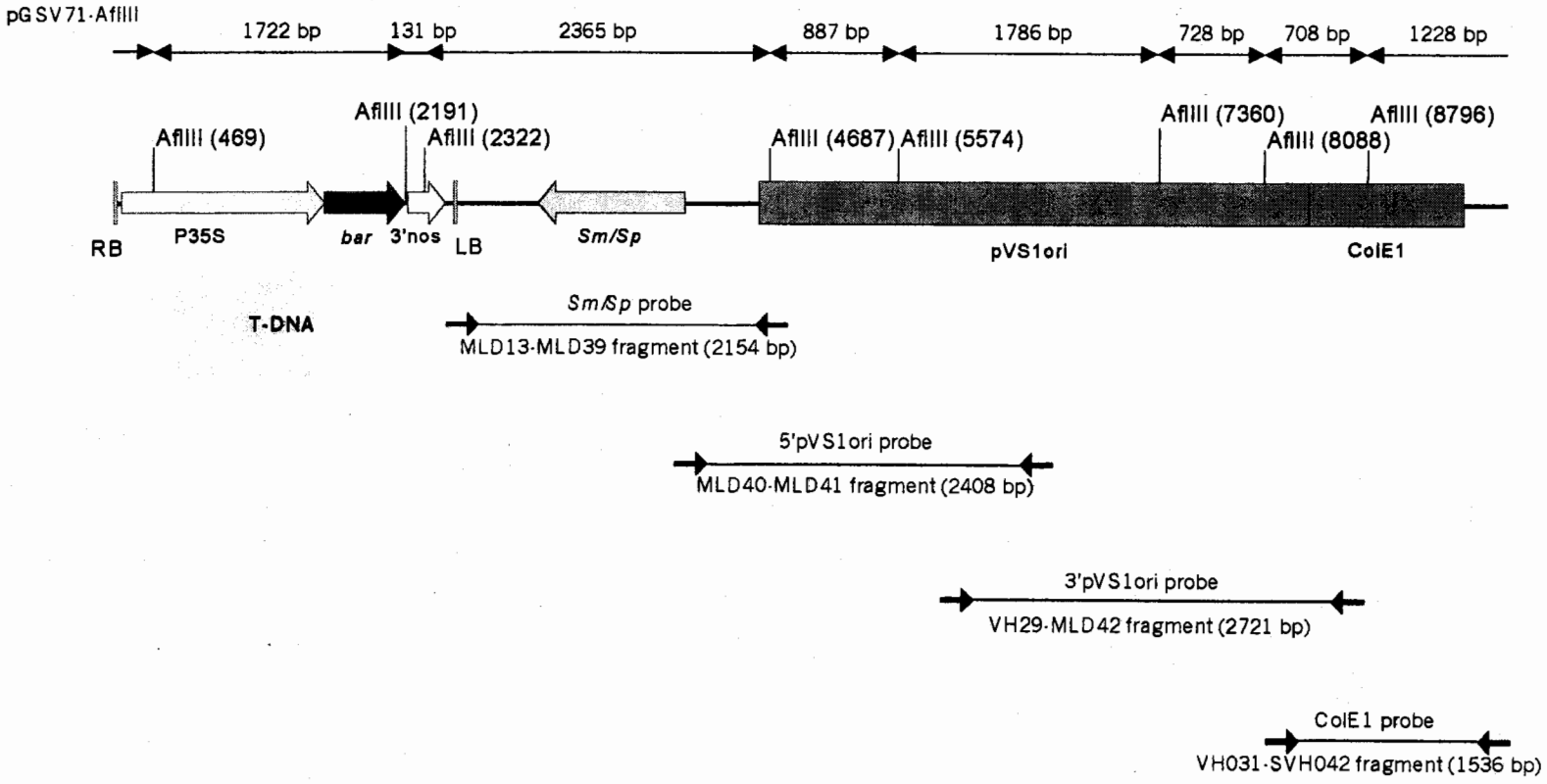
To determine the nature, number, integrity and stability of insertions in transformation event LLCotton25, Southern hybridization was used. In these experiments, restriction digested genomic DNA from transgenic plants homozygous for the integrated DNA were run in parallel with digested genomic DNA from a nontransgenic plant, and with digested genomic DNA from a nontransgenic plant supplemented with approximately 1 copy of digested transforming plasmid. The determination of the number of integrated copies was deduced from analyzing all obtained Southern blot data.

Several aliquots of LLCotton25 DNA were digested with the restriction enzyme *AflIII*. See Figure III.1 and Figure IV.1 to locate restriction sites in pGSV71. After separation of the DNA by electrophoresis, the DNA was transferred to a nylon membrane and hybridized with four overlapping gel purified ^{32}P-labeled probes, covering the complete vector backbone (Figure IV.1 and Table IV.2). A fifth ^{32}P-labeled P35S-*bar*-3'*nos* (*bar* cassette) probe was also utilized. Lanes contain approximately 5 or 10 µg of restricted DNA (as indicated in each gel). The amount of restricted pGVS71 in positive control lanes is equivalent to 1.0 copy of the plasmid integrated in 10 µg of cotton DNA. The probed membranes were visualized by autoradiography. Electronic scans of the autoradiographs are presented in this document. Standard molecular biology methods were used (Sambrook, *et al.*, 1989).

Table IV.2. Probes used in Southern Hybridization of Transformation Event LLCotton25

Probe	Features	Position in Vector	Length
pGSV71 MLD013 – MLD039	Vector Backbone (*Sm/Sp*)	2570 → 4724	2154
pGSV71 MLD040 - MLD041	Vector Backbone (5'pVS1*ori*)	4136 → 6544	2408
pGSV71 VH029 – MLD042	Vector Backbone (3'pVS1*ori*)	5964 → 8685	2721
pGSV71 VH031 – SVH042	Vector Backbone (*ColE1*)	8184 → 165	1536
pGSV71 *EcoRI – HindIII*	P35S-*bar*-3'*nos* (*bar* cassette)	250 → 2500	2250

Figure IV.1. Schematic Drawing of the Hybridization Strategy



For the molecular verification of absence of pGSV71 vector backbone sequences in transformation event LLCotton25, Southern blot analysis was performed using four overlapping probes (Table IV.2. and Figure IV.1). The sizes of some hybridizing fragments can be predicted by the location of restriction enzyme cleavage sites internal to the inserted DNA. The results of these analyses are provided below for each probe and are summarized in Table IV.3.

Sm/Sp probe:
In the DNA positive controls the expected 2365bp *AflIII* fragment was observed (see Figure IV.2). No hybridization signals were observed in the transgenic LLCotton25 sample nor in the Coker312 wild type DNA (negative control)(Figure IV.2 and Table IV.3).

The blot was also hybridized with the P35S-*bar*-3'*nos* (*bar* cassette) probe to demonstrate that there was ample DNA loaded on the gel. In the positive controls, the expected fragments of 2365bp, 1722bp and 1228bp could be observed. The 131bp fragment could not be observed due to its small size. Genomic LLCotton25 DNA, digested with *AflIII*, shows the 1722bp internal fragment. Both integration fragments, resulting from restriction enzyme cleavage in the integrated *bar* cassette and in the adjacent plant DNA, are not visible due to the limited overlap between the probe and the integration fragments (178bp and 219bp, respectively)(see Figure IV.2). The hybridization intensity of the 1722bp internal fragment of the LLCotton25 DNA sample is higher as compared to that of the 1 copy pGSV71 control DNA sample. This demonstrates that either the amount of plasmid DNA used for the positive controls is below the amount needed to represent 1 copy, or the amount of genomic LLCotton25 DNA, loaded on the gel, was underestimated.

5'pVS1*ori* probe
In the DNA positive controls the expected 2365bp, 1786bp and 877bp *AflIII* fragments were observed. No hybridization signals were observed in the transgenic LLCotton25 sample nor in the Coker312 wild type DNA (negative control)(Figure IV.3. and Table IV.3.).

3'pVS1*ori* probe
In the DNA positive controls the expected 1786bp, 728bp and 708bp *AflIII* fragments were observed. No hybridization signals were observed in the transgenic LLCotton25 sample nor in the Coker312 wild type DNA (negative control)(Figure IV.4. and Table IV.3.). Due to the similarity in size, the 728bp and 708bp fragments are overlapping and appear as one band on the Southern blot.

ColE1 probe
In the DNA positive controls the expected 1228bp and 708bp *AflIII* fragments were observed. No hybridization signals were observed in the transgenic LLCotton25 sample nor in the Coker312 wild type DNA (negative control)(Figure IV.5. and Table IV.3.).

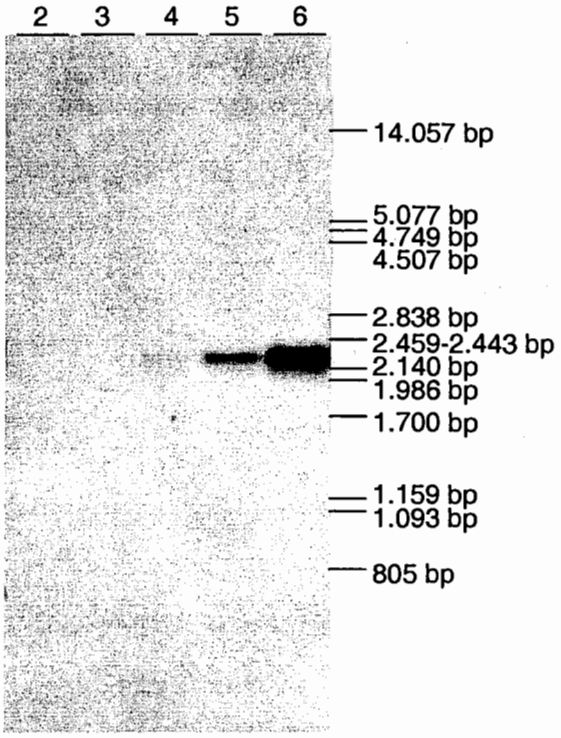
Table IV.3. Summary of Hybridization Results – Demonstration of the Absence of Vector Sequences in pGSV71

Target Sequences	LLCotton25	Negative Control (Coker 312)	Coker 312 wild type pGSV71
Sm/Sp	No hybridization	No hybridization	2365bp
5'pVS1*ori*	No hybridization	No hybridization	2365bp, 1786bp, 887bp
3'pVS1*ori*	No hybridization	No hybridization	1786bp, 728bp, 708bp
ColE1	No hybridization	No hybridization	1228bp, 708bp
bar cassette	1722bp	No hybridization	2365bp, 1722bp, 1228bp

In summary, the performed Southern blot hybridization data obtained with LLCotton25 transformation event demonstrate that 1 intact copy of the gene cassette is integrated into the plant genome. Transformation event LLCotton25 contains no vector backbone sequences evidenced by using overlapping probes covering the complete pGSV71 vector backbone sequences (including *Sm/Sp*, pVS1*ori* and *ColE1*).

To demonstrate the stability of transformation event LLCotton25 over multiple generations and in different genetic backgrounds, Southern blot analysis was performed using LLCotton25-C312-T_4, LLCotton25-C312-T_5, LLCotton25-FM966-BC_3/F_3, LLCotton25-FM832-BC_3/F_3, LLCotton25-AVS9023-BC_3/F_3, LLCotton25-HS26-BC_3/F_3 and LLCotton25-FM832. Isolated DNA from leaf tissue was digested with the restriction enzyme *NcoI*. There are two recognition sites in the transforming DNA. The digested genomic DNA from transformation event LLCotton25 was probed with the complete T-DNA and showed the expected internal T-DNA fragment and the expected Right Border integration fragment in all test samples thus showing the stability of the transformation event LLCotton25 at the genomic level (see Figures IV.6 though IV.13). Segregation data (Section IV.A) further confirm the stability of the insert, and show that it segregates as one dominant Mendelian locus.

Figure IV.2: Southern blot analysis – Demonstration of the absence of vector backbone sequences in *Gossypium hirsutum* Transformation event LLCotton25 – *Sm/Sp* probe

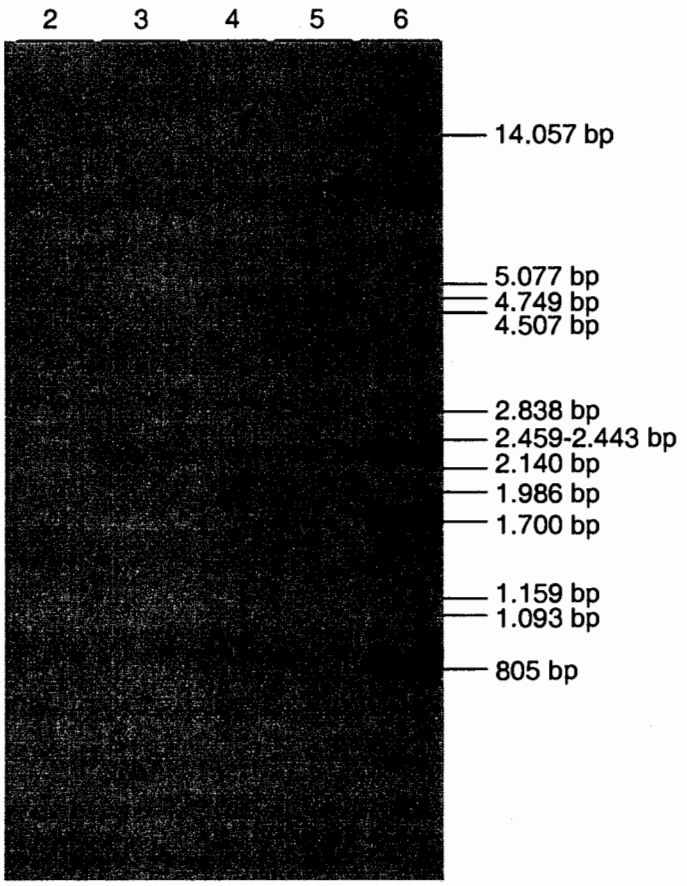


Genomic DNA was isolated from *Gossypium hirsutum* LLCotton25 plants and from non-transgenic counterpart Coker312 plants. DNA's (5 µg) were digested with *AflIII* and probed with the *Sm/Sp* probe (2154 bp MLD013 – MLD039 fragment of pGSV71).

Blot: MAE55B

Lane 1: λ - *PstI* digested
Lane 2: *Gossypium hirsutum* event LLCotton25 – *AflIII* digested
Lane 3: Coker312 WT – *AflIII* digested
Lane 4: Coker312 WT + 0.1 copy pGSV71 – *AflIII* digested
Lane 5: Coker312 WT + 1 copy pGSV71 – *AflIII* digested
Lane 6: Coker312 WT + 10 copies pGSV71 – *AflIII* digested
Lane 7: λ - *PstI* digested

Figure IV.3. Southern blot analysis – Demonstration of the absence of vector backbone sequences in *Gossypium hirsutum* transformation event LLCotton25 – 5'pVS1*ori* probe

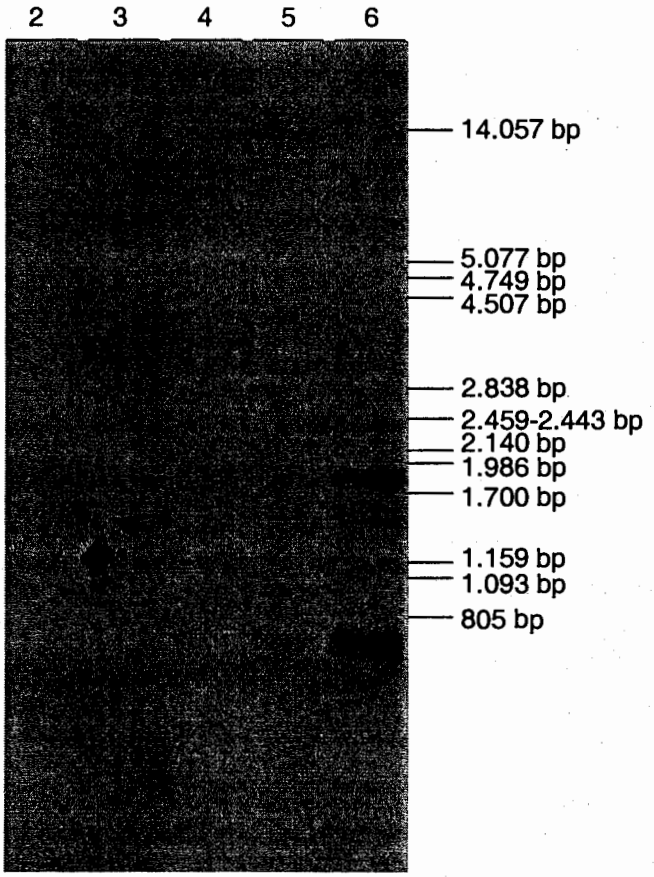


Genomic DNA was isolated from *Gossypium hirsutum* LLCotton25 plants and from non-transgenic counterpart Coker312 plants. DNA's (10 µg) were digested with *AflIII* and probed with the 5'pVS1*ori* probe (2408 bp MLD040 – MLD041 fragment of pGSV71).

Blot: MAE68

Lane 1: λ - *PstI* digested
Lane 2: *Gossypium hirsutum* event LLCotton25 – *AflIII* digested
Lane 3: Coker312 WT – *AflIII* digested
Lane 4: Coker312 WT + 0.1 copy pGSV71 – *AflIII* digested
Lane 5: Coker312 WT + 1 copy pGSV71 – *AflIII* digested
Lane 6: Coker312 WT + 10 copies pGSV71 – *AflIII* digested
Lane 7: λ - *PstI* digested

Figure IV.4. Southern blot analysis – Demonstration of the absence of vector backbone sequences in *Gossypium hirsutum* transformation event LLCotton25 – 3'pVS1*ori* probe

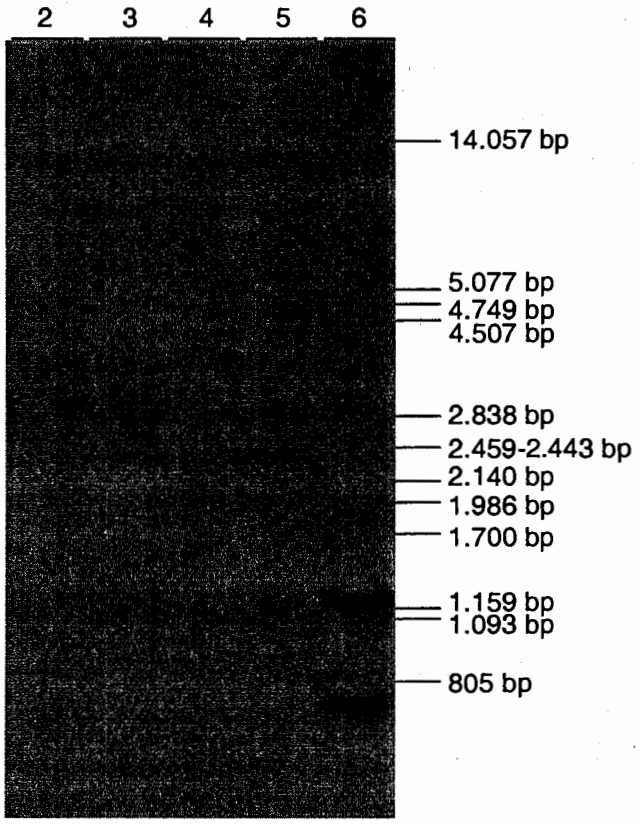


Genomic DNA was isolated from *Gossypium hirsutum* LLCotton25 plants and from non-transgenic counterpart Coker312 plants. DNA's (10 µg) were digested with *AflIII* and probed with the 3'pVS1*ori* probe (2721 bp VH029 – MLD042 fragment of pGSV71).

Blot: MAE69

Lane 1: λ - *PstI* digested
Lane 2: *Gossypium hirsutum* event LLCotton25 – *AflIII* digested
Lane 3: Coker312 WT – *AflIII* digested
Lane 4: Coker312 WT + 0.1 copy pGSV71 – *AflIII* digested
Lane 5: Coker312 WT + 1 copy pGSV71 – *AflIII* digested
Lane 6: Coker312 WT + 10 copies pGSV71 – *AflIII* digested
Lane 7: λ - *PstI* digested

Figure IV.5. Southern blot analysis – Demonstration of the absence of vector backbone sequences in *Gossypium hirsutum* transformation event LLCotton25 – *ColE1* probe



Genomic DNA was isolated from *Gossypium hirsutum* LLCotton25 plants and from non-transgenic counterpart Coker312 plants. DNA's (10 µg) were digested with *AflIII* and probed with the *ColE1* probe (1536 bp VH031 – SVH042 fragment of pGSV71).

Blot: MAE70

Lane 1: λ - *PstI* digested
Lane 2: *Gossypium hirsutum* event LLCotton25 – *AflIII* digested
Lane 3: Coker312 WT – *AflIII* digested
Lane 4: Blank
Lane 5: Coker312 WT + 1 copy pGSV71 – *AflIII* digested
Lane 6: Coker312 WT + 10 copies pGSV71 – *AflIII* digested
Lane 7: λ - *PstI* digested

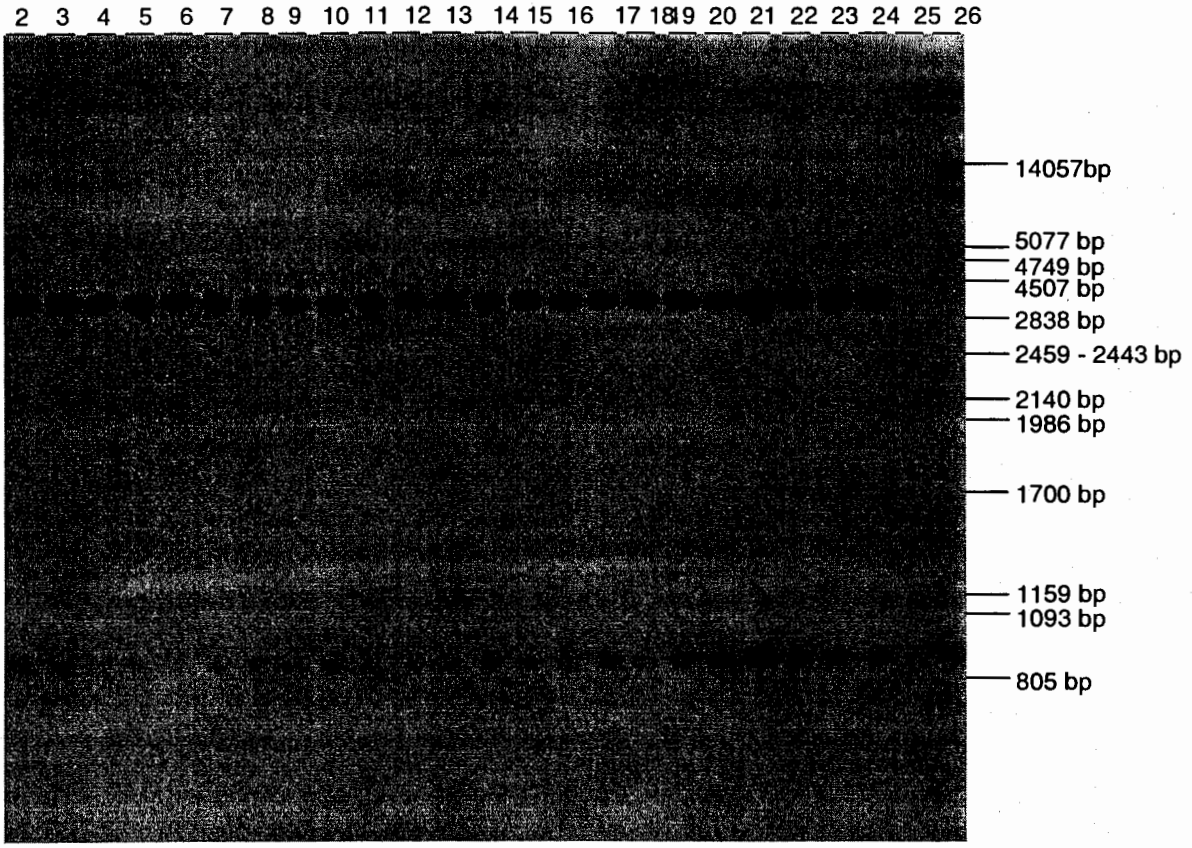


Figure IV.6: Demonstration of the stability of LL25 (Background: Coker312 - Generation: T4)

Gel: MAE65
Probe: Complete T-DNA (2250 bp EcoRI- HindIII fragment of pGSV71)

Genomic DNA was prepared from *Gossypium hirsutum* elite event LL25 (Background Coker312; Generation: T4) and the notransgenic counterpart (Coker312). 10 μg genomic DNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - C312 - T4 plant 1
3. LL25 - C312 - T4 plant 2
4. LL25 - C312 - T4 plant 3
5. LL25 - C312 - T4 plant 4
6. LL25 - C312 - T4 plant 5
7. LL25 - C312 - T4 plant 6
8. LL25 - C312 - T4 plant 7
9. LL25 - C312 - T4 plant 8
10. LL25 - C312 - T4 plant 9
11. LL25 - C312 - T4 plant 10
12. LL25 - C312 - T4 plant 11
13. LL25 - C312 - T4 plant 12
14. LL25 - C312 - T4 plant 13
15. LL25 - C312 - T4 plant 14
16. LL25 - C312 - T4 plant 15
17. LL25 - C312 - T4 plant 16
18. LL25 - C312 - T4 plant 17
19. LL25 - C312 - T4 plant 18
20. LL25 - C312 - T4 plant 19
21. LL25 - C312 - T4 plant 20
22. LL25 - C312 - T4 plant 21
23. LL25 - C312 - T4 plant 22
24. LL25 - C312 - T4 plant 23
25. Wild type C312-17
26. Wild type C312-17 + 1 copy pGSV71 -NcoI

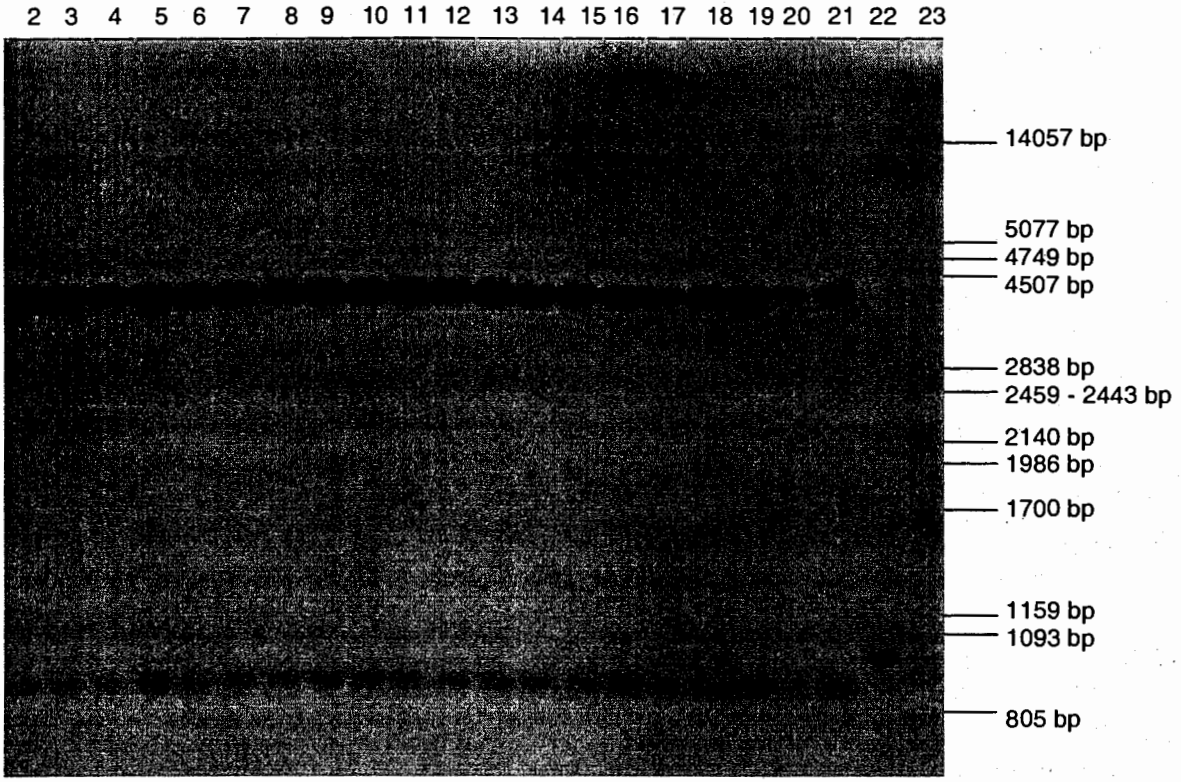


Figure IV.7: Demonstration of the stability of LL25 (Background: Coker312 - Generation: T5)

Gel: MAE51B
Probe: Complete T-DNA (2250 bp EcoRI HindIII fragment of pGSV71)

GenomicDNA was prepared from *Gossypium hirsutum* elite event LL25 (Background: Coker312; Generation: T5) and the non-transgenic counterpart (Coker312). 10 µg genomicDNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - C312 - T5 plant 1
3. LL25 - C312 - T5 plant 2
4. LL25 - C312 - T5 plant 3
5. LL25 - C312 - T5 plant 4
6. LL25 - C312 - T5 plant 5
7. LL25 - C312 - T5 plant 6
8. LL25 - C312 - T5 plant 7
9. LL25 - C312 - T5 plant 8
10. LL25 - C312 - T5 plant 9
11. LL25 - C312 - T5 plant 10
12. LL25 - C312 - T5 plant 11
13. LL25 - C312 - T5 plant 12
14. LL25 - C312 - T5 plant 13
15. LL25 - C312 - T5 plant 14
16. LL25 - C312 - T5 plant 15
17. LL25 - C312 - T5 plant 16
18. LL25 - C312 - T5 plant 17
19. LL25 - C312 - T5 plant 18
20. LL25 - C312 - T5 plant 19
21. LL25 - C312 - T5 plant 20
22. Wild type C312-17 + 0.25 copy pGSV71- BglII
23. Wild type C312-17

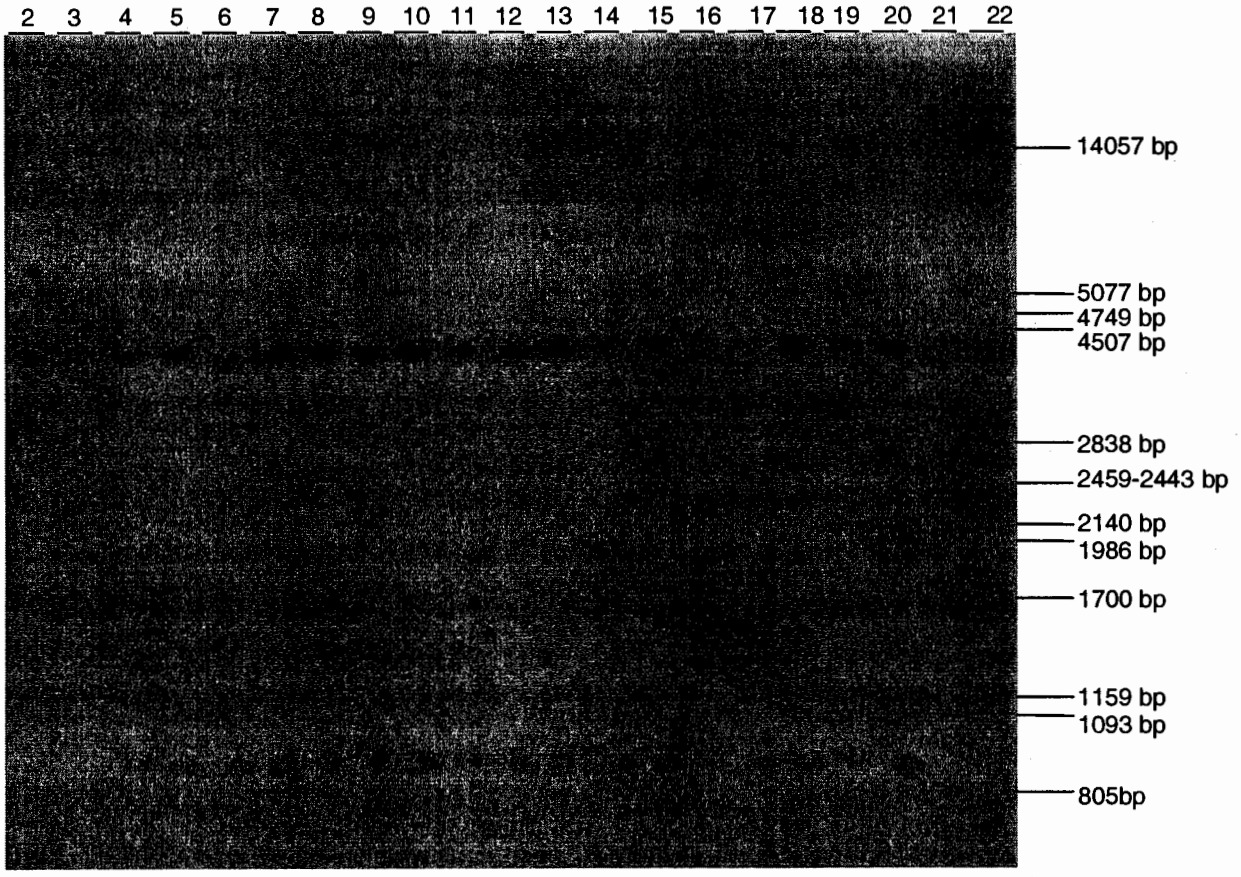


Figure IV.8: Demonstration of the stability of LL25 (Background: FM966 - Generation: BC3/F3)

Gel: MAE76
Probe: Complete T-DNA (2250 bp EcoRI HindIII fragment of pGSV71)

Genomic DNA was prepared from *Gossypium hirsutum* elite event LL25 (Background: FM966; Generation: BC3/F3) and the notransgenic counterpart (FM832). 10 μg genomic DNA were digested with Ncolenzyme and probed with the complete T-DNA.

2. LL25 - FM966 - BC3/F3 plant 1
3. LL25 - FM966 - BC3/F3 plant 2
4. LL25 - FM966 - BC3/F3 plant 3
5. LL25 - FM966 - BC3/F3 plant 4
6. LL25 - FM966 - BC3/F3 plant 5
7. LL25 - FM966 - BC3/F3 plant 6
8. LL25 - FM966 - BC3/F3 plant 7
9. LL25 - FM966 - BC3/F3 plant 8
10. LL25 - FM966 - BC3/F3 plant 9
11. LL25 - FM966 - BC3/F3 plant 10
12. LL25 - FM966 - BC3/F3 plant 11
13. LL25 - FM966 - BC3/F3 plant 12
14. LL25 - FM966 - BC3/F3 plant 13
15. LL25 - FM966 - BC3/F3 plant 14
16. LL25 - FM966 - BC3/F3 plant 15
17. LL25 - FM966 - BC3/F3 plant 16
18. LL25 - FM966 - BC3/F3 plant 17
19. LL25 - FM966 - BC3/F3 plant 18
20. LL25 - FM966 - BC3/F3 plant 19
21. Wild type FM832
22. Wild type FM832 + 1 copy pGSV71- NcoI

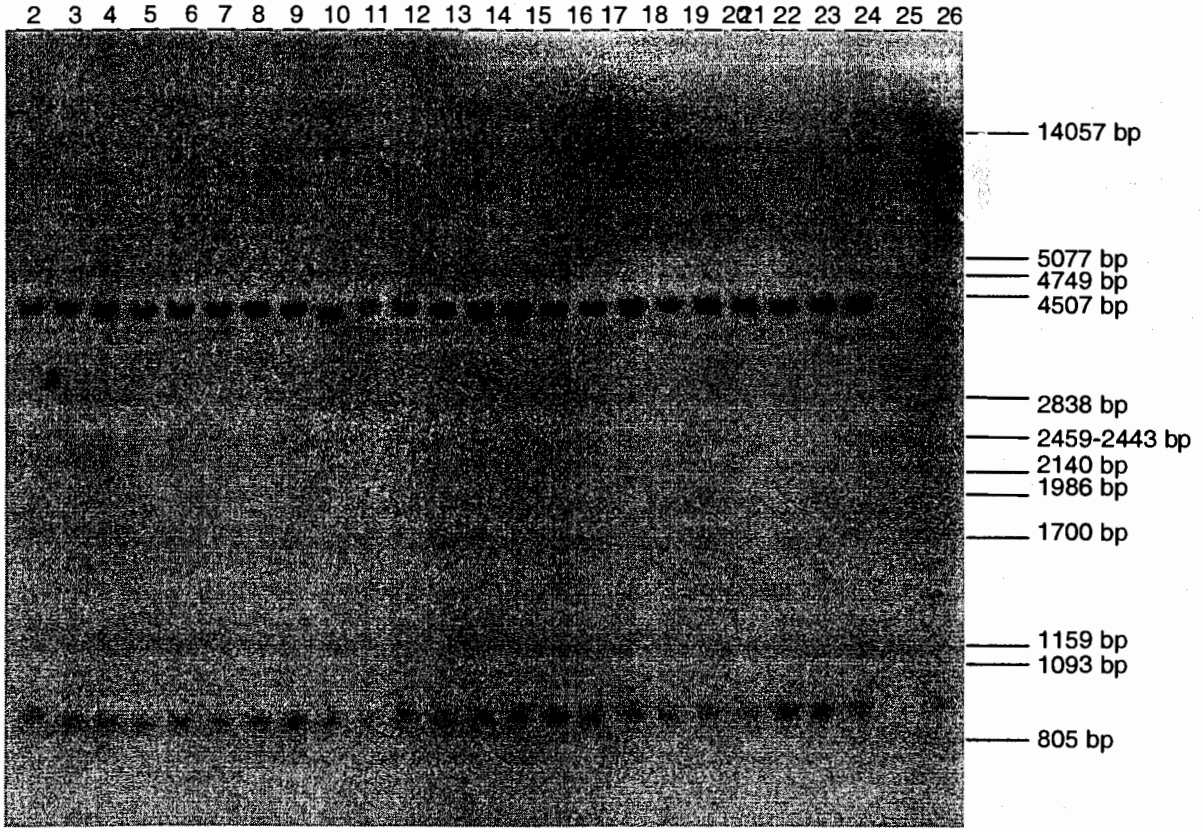


Figure IV.9: Demonstration of the stability of LL25 (Background: FM832 - Generation: BC3/F3 (seed lot A))

Gel: MAE78

Probe: Complete T-DNA (2250 bp EcoRI - HindIII fragment of pGSV71)

Genomic DNA was prepared from *Gossypium hirsutum* elite event LL25 (Background: FM832; Generation: BC3/F3) and the non transgenic counterpart (FM832). 10 µg genomic DNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - FM832 - BC3/F3 (seed lot A) plant 1
3. LL25 - FM832 - BC3/F3 (seed lot A) plant 2
4. LL25 - FM832 - BC3/F3 (seed lot A) plant 3
5. LL25 - FM832 - BC3/F3 (seed lot A) plant 4
6. LL25 - FM832 - BC3/F3 (seed lot A) plant 5
7. LL25 - FM832 - BC3/F3 (seed lot A) plant 6
8. LL25 - FM832 - BC3/F3 (seed lot A) plant 7
9. LL25 - FM832 - BC3/F3 (seed lot A) plant 8
10. LL25 - FM832 - BC3/F3 (seed lot A) plant 9
11. LL25 - FM832 - BC3/F3 (seed lot A) plant 10
12. LL25 - FM832 - BC3/F3 (seed lot A) plant 11
13. LL25 - FM832 - BC3/F3 (seed lot A) plant 12
14. LL25 - FM832 - BC3/F3 (seed lot A) plant 13
15. LL25 - FM832 - BC3/F3 (seed lot A) plant 14
16. LL25 - FM832 - BC3/F3 (seed lot A) plant 15
17. LL25 - FM832 - BC3/F3 (seed lot A) plant 16
18. LL25 - FM832 - BC3/F3 (seed lot A) plant 17
19. LL25 - FM832 - BC3/F3 (seed lot A) plant 18
20. LL25 - FM832 - BC3/F3 (seed lot A) plant 19
21. LL25 - FM832 - BC3/F3 (seed lot A) plant 20
22. LL25 - FM832 - BC3/F3 (seed lot A) plant 21
23. LL25 - FM832 - BC3/F3 (seed lot A) plant 22
24. LL25 - FM832 - BC3/F3 (seed lot A) plant 23
25. Wild type FM832
26. Wild type FM832 + 1 copy pGSV71-NcoI

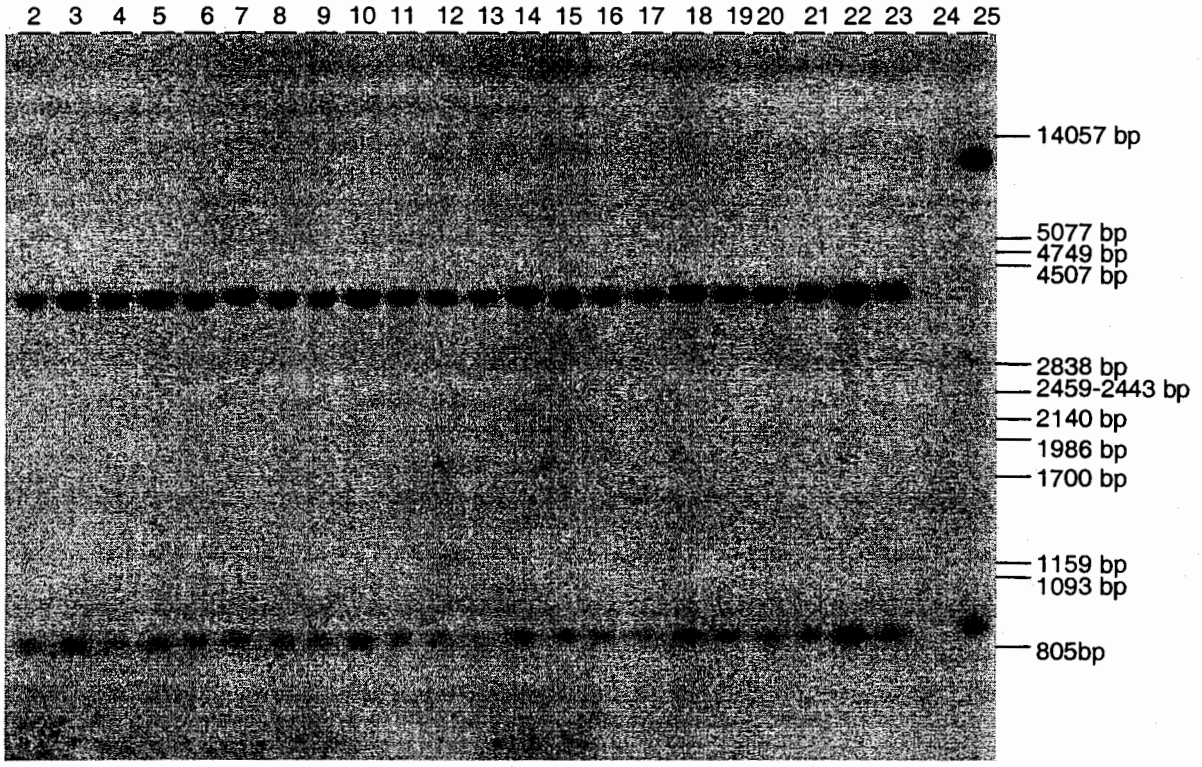


Figure IV.10: Demonstration of the stability of LL25 (Background: FM832 - Generation: BC3/F3 (seed lot B)

Gel: MAE62

Probe: Complete T-DNA (2250 bp EcoRI - HindIII fragment of pGSV71)

GenomicDNA was prepared from *Gossypium hirsutum* elite event LL25 (Background FM832) and the non transgeniccounterpart (FM832). 10 μg genomic DNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - FM832 - BC3/F3 (seed lot B) plant 1
3. LL25 - FM832 - BC3/F3 (seed lot B) plant 2
4. LL25 - FM832 - BC3/F3 (seed lot B) plant 3
5. LL25 - FM832 - BC3/F3 (seed lot B) plant 4
6. LL25 - FM832 - BC3/F3 (seed lot B) plant 5
7. LL25 - FM832 - BC3/F3 (seed lot B) plant 6
8. LL25 - FM832 - BC3/F3 (seed lot B) plant 7
9. LL25 - FM832 - BC3/F3 (seed lot B) plant 8
10. LL25 - FM832 - BC3/F3 (seed lot B) plant 9
11. LL25 - FM832 - BC3/F3 (seed lot B) plant 10
12. LL25 - FM832 - BC3/F3 (seed lot B) plant 11
13. LL25 - FM832 - BC3/F3 (seed lot B) plant 12
14. LL25 - FM832 - BC3/F3 (seed lot B) plant 13
15. LL25 - FM832 - BC3/F3 (seed lot B) plant 14
16. LL25 - FM832 - BC3/F3 (seed lot B) plant 15
17. LL25 - FM832 - BC3/F3 (seed lot B) plant 16
18. LL25 - FM832 - BC3/F3 (seed lot B) plant 17
19. LL25 - FM832 - BC3/F3 (seed lot B) plant 18
20. LL25 - FM832 - BC3/F3 (seed lot B) plant 19
21. LL25 - FM832 - BC3/F3 (seed lot B) plant 20
22. LL25 - FM832 - BC3/F3 (seed lot B) plant 21
23. LL25 - FM832 - BC3/F3 (seed lot B) plant 22
24. Wild type FM832
25. Wild type FM832 + 1 copy pGSV71- NcoI

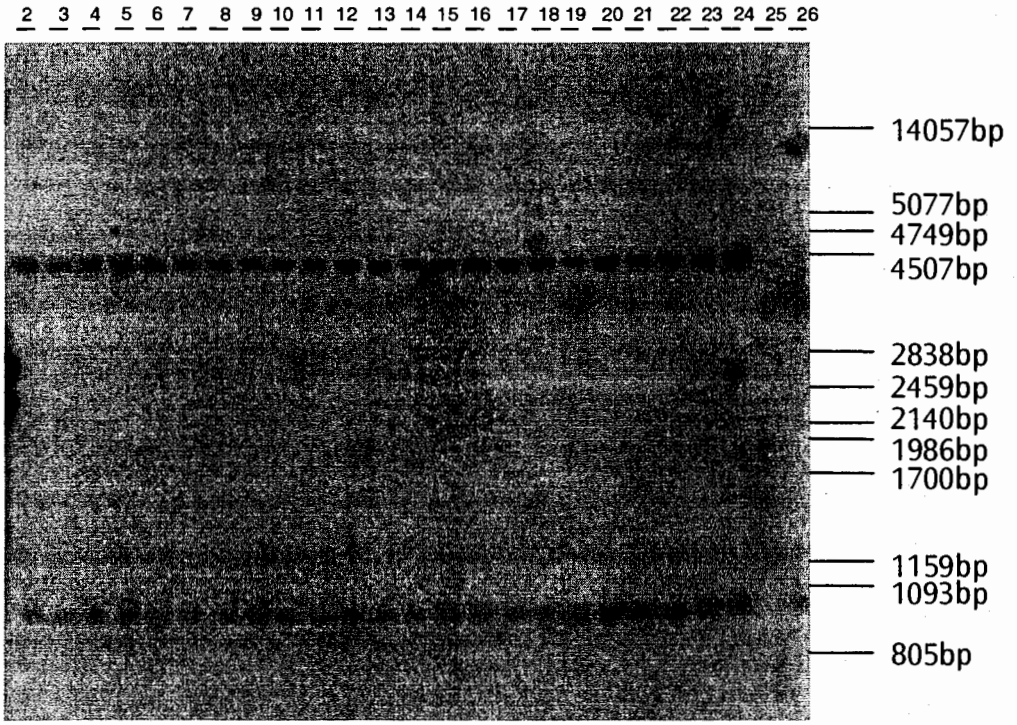


Figure IV.11: Demonstration of the stability of LL25 (Background: FM989 - Generation: BC3/F3)

Gel: MAE83B

Probe: Complete T-DNA (2250bp EcoRI - HindIII fragment of pGSV71)

GenomicDNA was prepared from *Gossypium hirsutum* elite event LL25 (Background: FM989; Generation: BC3/F3) and a non-transgenic plant (FM832). 10 µg genomic DNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - FM989 - BC3/F3 plant 1
3. LL25 - FM989 - BC3/F3 plant 2
4. LL25 - FM989 - BC3/F3 plant 3
5. LL25 - FM989 - BC3/F3 plant 4
6. LL25 - FM989 - BC3/F3 plant 5
7. LL25 - FM989 - BC3/F3 plant 6
8. LL25 - FM989 - BC3/F3 plant 7
9. LL25 - FM989 - BC3/F3 plant 8
10. LL25 - FM989 - BC3/F3 plant 9
11. LL25 - FM989 - BC3/F3 plant 10
12. LL25 - FM989 - BC3/F3 plant 11
13. LL25 - FM989 - BC3/F3 plant 12
14. LL25 - FM989 - BC3/F3 plant 13
15. LL25 - FM989 - BC3/F3 plant 14
16. LL25 - FM989 - BC3/F3 plant 15
17. LL25 - FM989 - BC3/F3 plant 16
18. LL25 - FM989 - BC3/F3 plant 17
19. LL25 - FM989 - BC3/F3 plant 18
20. LL25 - FM989 - BC3/F3 plant 19
21. LL25 - FM989 - BC3/F3 plant 20
22. LL25 - FM989 - BC3/F3 plant 21
23 .LL25 - FM989 - BC3/F3 plant 22
24. LL25 - FM989 - BC3/F3 plant 23
25. Wild type FM832
26. Wild type FM832 + 1 copy pGSV71- NcoI

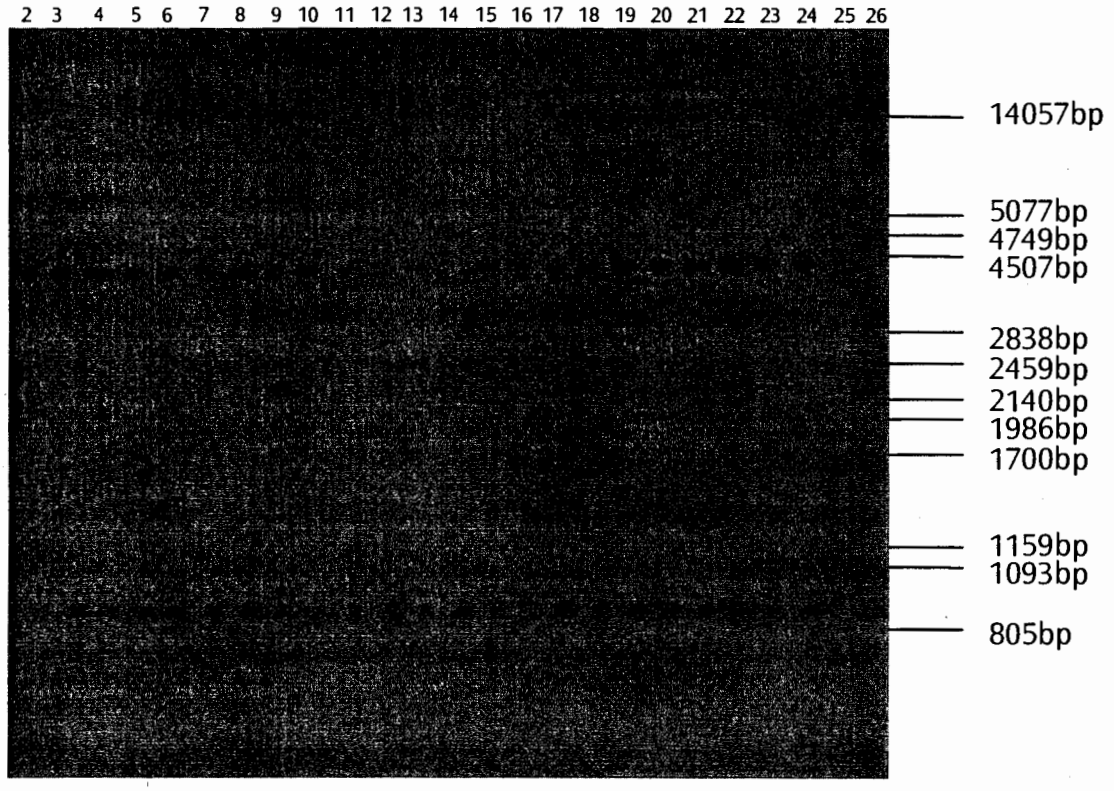


Figure IV.12: Demonstration of the stability of LL25 (Background: HS26 - Generation: BC3/F3)

Gel: MAE79B

Probe: Complete T-DNA (2250 bp EcoRI - HindIII fragment of pGSV71)

Genomic DNA was prepared from *Gossypium hirsutum* elite event LL25 (Background: HS26; Generation: BC3/F3) and a non-transgenic plant (FM832). 10 μg genomic DNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - HS26 - BC3/F3 plant 1
3. LL25 - HS26 - BC3/F3 plant 2
4. LL25 - HS26 - BC3/F3 plant 3
5. LL25 - HS26 - BC3/F3 plant 4
6. LL25 - HS26 - BC3/F3 plant 5
7. LL25 - HS26 - BC3/F3 plant 6
8. LL25 - HS26 - BC3/F3 plant 7
9. LL25 - HS26 - BC3/F3 plant 8
10. LL25 - HS26 - BC3/F3 plant 9
11. LL25 - HS26 - BC3/F3 plant 10
12. LL25 - HS26 - BC3/F3 plant 11
13. LL25 - HS26 - BC3/F3 plant 12
14. LL25 - HS26 - BC3/F3 plant 13
15. LL25 - HS26 - BC3/F3 plant 14
16. LL25 - HS26 - BC3/F3 plant 15
17. LL25 - HS26 - BC3/F3 plant 16
18. LL25 - HS26 - BC3/F3 plant 17
19. LL25 - HS26 - BC3/F3 plant 18
20. LL25 - HS26 - BC3/F3 plant 19
21. LL25 - HS26 - BC3/F3 plant 20
22. LL25 - HS26 - BC3/F3 plant 21
23 .LL25 - HS26 - BC3/F3 plant 22
24. LL25 - HS26 - BC3/F3 plant 23
25. Wild type FM832
26. Wild type FM832 + 1 copy pGSV71- NcoI

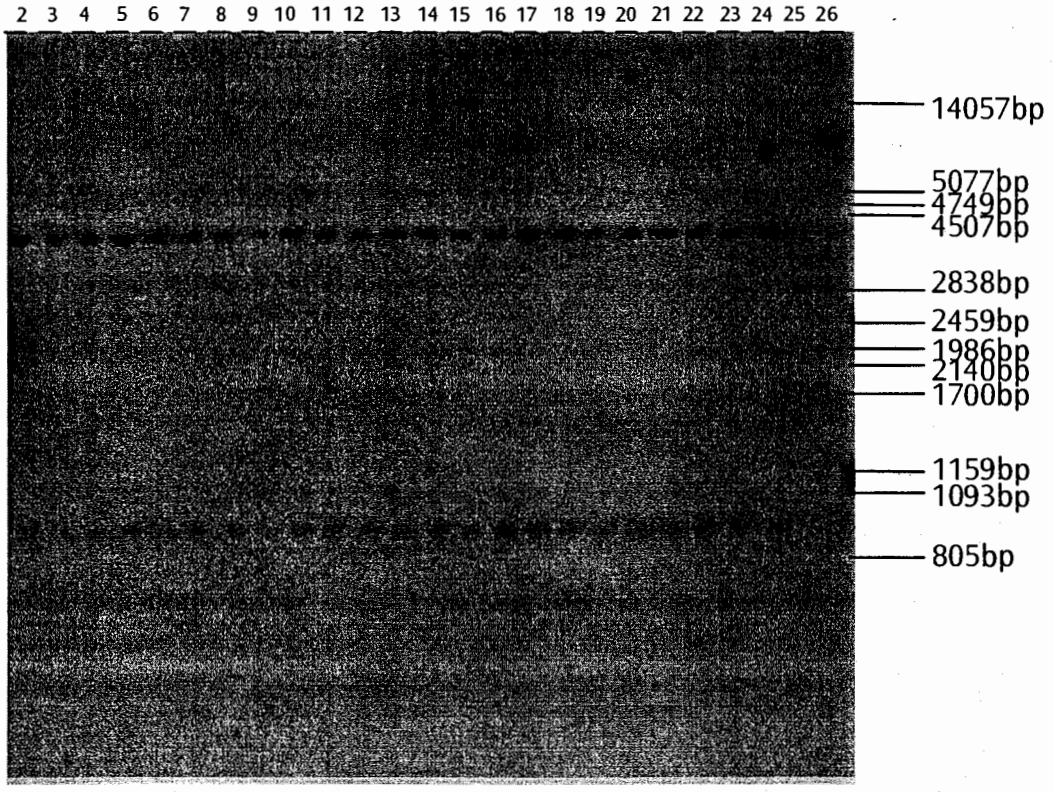


Figure IV.13: Demonstration of the stability of LL25 (Background: AVS9023 - Generation: BC3/F3)

Gel: MAE80B

Probe: Complete T-DNA (2250 bp EcoRI - HindIII fragment of pGSV71)

Genomic DNA was prepared from *Gossypium hirsutum* elite event LL25 (Background: AVS9023; Generation: BC3/F3 and a non-transgenic plant (FM832). 10 µg genomic DNA were digested with NcoI enzyme and probed with the complete T-DNA.

2. LL25 - AVS9023 - BC3/F3 plant 1
3. LL25 - AVS9023 - BC3/F3 plant 2
4. LL25 - AVS9023 - BC3/F3 plant 3
5. LL25 - AVS9023 - BC3/F3 plant 4
6. LL25 - AVS9023 - BC3/F3 plant 5
7. LL25 - AVS9023 - BC3/F3 plant 6
8. LL25 - AVS9023 - BC3/F3 plant 7
9. LL25 - AVS9023 - BC3/F3 plant 8
10. LL25 - AVS9023 - BC3/F3 plant 9
11. LL25 - AVS9023 - BC3/F3 plant 10
12. LL25 - AVS9023 - BC3/F3 plant 11
13. LL25 - AVS9023 - BC3/F3 plant 12
14. LL25 - AVS9023 - BC3/F3 plant 13
15. LL25 - AVS9023 - BC3/F3 plant 14
16. LL25 - AVS9023 - BC3/F3 plant 15
17. LL25 - AVS9023 - BC3/F3 plant 16
18. LL25 - AVS9023 - BC3/F3 plant 17
19. LL25 - AVS9023 - BC3/F3 plant 18
20. LL25 - AVS9023 - BC3/F3 plant 19
21. LL25 - AVS9023 - BC3/F3 plant 20
22. LL25 - AVS9023 - BC3/F3 plant 21
23. LL25 - AVS9023 - BC3/F3 plant 22
24. LL25 - AVS9023 - BC3/F3 plant 23
25. Wild type FM832
26. Wild type FM832 + 1 copy pGSV71-NcoI

C. Gene Expression in Transformation Event LLCotton25

The content of phosphinothricin acetyltransferase (PAT) protein, a *bar* gene product, in the transformation event LLCotton25 was determined in cottonseed by an Enzyme Linked Immunosorbent Assay (ELISA). Polyclonal antibodies recognizing PAT protein were used in the ELISA. PAT ELISA detects both inactive and intact PAT enzyme. Therefore, the enzyme detected may not be functional.

The PAT ELISA is a sandwich immunoassay in which PAT specific antibodies are used to coat the wells and serve as capture antibodies for PAT protein. Samples consisting of transformant extracts, non-transformant extracts as controls, and pure PAT protein as a standard are added to the wells. Following incubation, during which time the PAT in the sample is captured by the coated antibodies, the unbound material is removed by rinsing with a wash solution. The plate was subsequently incubated with the second antibody, which recognizes another epitope of PAT protein. The binding of the second PAT antibody to the PAT protein was detected by the incubation of a third antibody labeled with horseradish peroxidase (HRP). A peroxidase substrate, Tetramethylbenzidine (TMB), is then added and converted by the peroxidase to a blue product in proportion to the amount of PAT protein present in the sample. The reaction is stopped with 0.5 M H_2SO_4 which changes the color to a yellow product. The resultant color development is proportional to the concentration of PAT protein in each microwell. Three dilutions of each extract are tested and the value nearest to the midpoint of the standard curve is used to determine the PAT content.

ELISA assays were performed on cottonseed and lint for glufosinate-tolerant cotton transgenic event LLCotton25 collected from four different sites in the US. Because the cottonseed (fuzzy seed) had been ginned but not delinted, it could not be ground into a homogeneous material. A procedure was found to effectively remove the lint and the associated seed coat. This created two fractions, which are designated Cleaned Seed and Lint Coat. The Cleaned Seed could be easily ground to homogeneity and the Lint Coat fraction was a relatively homogeneous matrix of intertwined cotton fibers and broken fragments of seed coat. These fractions were analyzed separately for PAT protein and the respective values added to give values for the fuzzy seed as received from the field.

Detection and quantitation limits were determined for each matrix tested. The Limit of Detection (LOD) was 2.08, 1.66 and 4.25 ng/g sample for Cleaned Seed, Lint Coat and Lint, respectively. The Limit of Quantitation (LOQ) was the same for all three samples, 18.75 ng/g sample (18.75ppb).

Results from the quantification of PAT protein are shown in Table IV.4. PAT protein was found in all fractions of transgenic fuzzy seed and lint. More than 98.5% of the PAT protein was found in the Cleaned Seed fraction and thus also in the fuzzy seed fraction (Cleaned Seed + Lint Coat). The Lint Coat and lint fractions contained less than 1.5% of the PAT protein. PAT protein content varied between different trial sites and between treatments with Liberty. The values ranged from 61.3 µg/g to 74.5 µg/g fresh weight for LLCotton25 cotton sprayed with Liberty herbicide regime (159 g a.i./acre) and from 48.2 µg/g to 70.7 µg/g fresh weight in LLCotton25 cotton sprayed with conventional herbicide regime. PAT

protein was approximately 0.029% and 0.003% of crude protein for fuzzy seed and lint, respectively.

Table IV.4. Quantities of PAT Protein in Raw Agricultural Commodities of Transgenic Cotton Event LLCotton25 as Detected by ELISA

Sample	Trial Number	[a]Average PAT Content (µg/g Sample) ± standard deviation				Average PAT Content (as % of crude protein)	
		Liberty® Herbicide 159 g a.i./acre		Conventional Herbicide Regime		Liberty® Herbicide 159 g a.i./acre	Conventional Herbicide Regime
Cleaned Seed	02-01	135	± 8.3	128	± 15	NA[c]	NA[c]
	04-02	130	± 15	123	± 15	NA[c]	NA[c]
	04-05	108	± 12	84	± 19	NA[c]	NA[c]
	06-06	136	± 20	117	± 18	NA[c]	NA[c]
Average		**127**	**± 18**	**113**	**± 24**	NA[c]	NA[c]
Lint Coat	02-01	1.05	± 0.16	1.02	± 0.09	NA[c]	NA[c]
	04-02	1.11	± 0.18	1.13	± 0.42	NA[c]	NA[c]
	04-05	0.85	± 0.36	0.30	± 0.06	NA[c]	NA[c]
	06-06	1.6	± 0.59	1.22	± 0.56	NA[c]	NA[c]
Average		**1.15**	**± 0.45**	**0.92**	**± 0.50**	NA[c]	NA[c]
Fuzzy seed	02-01	70.7	NA[b]	70.7	NA	0.034	0.035
	04-02	74.8	NA[b]	68.9	NA	0.033	0.031
	04-05	61.3	NA[b]	48.2	NA	0.025	0.019
	06-06	72.8	NA[b]	64.2	NA	0.028	0.024
Average		**69.9**	**± 6.0**	**63.0**	**± 10.3**	**0.030**	**0.027**
Lint	02-01	0.13	± 0.07	0.13	± 0.14	0.001	0.001
	04-02	0.25	± 0.06	0.29	± 0.06	0.002	0.001
	04-05	1.40	± 0.25	0.50	± 0.17	0.003	0.002
	06-06	1.33	± 0.32	1.06	± 0.41	0.006	0.006
Average		**0.78**	**± 0.63**	**0.50**	**± 0.42**	**0.003**	**0.003**

[a] Mean and Standard Deviation of PAT content in samples derived from LLCotton25 grown with the recommended Liberty herbicide regime or under conventional herbicide regime.

[b] Standard Deviation is not applicable (NA) for fuzzy seed data because the value is the numerical sum of the Cleaned Seed and Lint Coat data. A standard deviation is calculated for the average PAT value of fuzzy seed. This is based only on the calculated average values (Cleaned Seed + Lint Coat) obtained at the four sites. Standard deviations for all other matrices are calculated from all measurements taken at all sites. Thus they are based on 16 actual measurements (4 measurements at 4 sites) of the PAT protein in the matrix. The data for the fuzzy seed were calculated from the amount of PAT protein present in Cleaned Seed and Lint Coat fractions taking into account their respective weights.

[c] Average PAT as % of crude protein is not applicable (NA) because protein determinations were not made on these samples.

V. Agronomic Performance and Compositional Analysis of Glufosinate-Tolerant Cotton Event LLCotton25

A.' Field Tests of Transformation Event LLCotton25

Transformation event LLCotton25 has been field tested by Aventis CropScience USA Company in winter nurseries and all the regions of adaptation in the United States. These tests have occurred at more than 40 sites under field release authorizations granted by USDA APHIS (USDA authorizations: 99-007-08n, 00-074-14n, 00-108-10n, 00-119-05N, 00-258-02n, 01-075-17n, 01-102-21n and 01-108-05n). Table V.1 lists the USDA field trial authorizations issued for LLCotton25 and provides a chronology of the Aventis field activities. During the 1999-2000 season, lines based upon transformation event LLCotton25 were field tested on a limited basis in Mississippi, and grown counter-season in Puerto-Rico and Guatemala for seed increase and breeding purposes. In the 2000 growing season, advanced lines were placed in replicated yield trials, breeding lines were evaluated in nursery plots and herbicide efficacy and registration trails were undertaken. Seed of advanced breeding lines were increased in the 2000-2001 winter season nursery in Puerto Rico. A second season of advanced line evaluation across the cotton belt was undertaken in the 2001 growing season. A field release is currently in progress under notification 01-271-05n in Puerto Rico. Outside the USA, LibertyLink Cotton event LLCotton25 has been field tested in a breeding nursery in Guatemala and is currently being field tested in a breeding nursery in South Africa.

Cotton plants were observed from emergence through maturity in all the field release sites (Table V.1). No differences were observed between transgenic and nontransgenic cotton in emergence, seedling vigor, stand establishment and maturity. Observations were conducted at least four times in the growing season; 1) emergence, 2) pre-bloom, 4) peak bloom and 4) boll stage. At all field sites, data which may provide indications of weediness, disease susceptibility, insect susceptibility, differences in plant morphology, growth and plant development, and occurrence of volunteers in the subsequent season were collected. Appendix A contains termination reports submitted to the USDA for the environmental releases that have been completed in the United States. Final reports for the 2001-growing season will be completed in early 2002, however interim termination reports are also provided in Appendix A.

Table V.1. Summary of Field Release Authorizations Granted USDA APHIS for Transformation Event LLCotton25

USDA Authorization	Planting dates	Number of locations	Type of trial	Location (county/state)
99-007-08n	6/6/99	1	Efficacy, Breeding, and Nutritional Composition Studies	Washington, MS
00-074-14n	5/15/00 6/1/00	5	Efficacy, Breeding, Residue Analysis and Nutritional Composition Studies	Crittenden, AR Wayne, NC Washington (2), MS Wharton, TX
00-108-10n	5/15/00 6/1/00	4	Efficacy, Breeding, Residue Analysis and Nutritional Composition Studies	Shelby, TN Washington (2), Issequana, MS
00-119-05n	5/15/00 6/1/00	3	Efficacy, Breeding, Residue Analysis and Nutritional Composition Studies	Stoddard, MO Washington, MS Lubbock, TX
00-258-02n	10/20/00	1	Breeding	Juana Diaz, PR
01-075-17n		26	Efficacy, Breeding, Residue Analysis and Nutritional Composition Studies	Macon, AL Crittenden, Drew, Jackson, AR Colquitt, Mitchell, Tift, GA Tensas Parish, LA Noxubee, Washington (4), MS Wayne (2), NC Barnwell, Horry, Marion, SC Shelby, TN Burleson, Lubbock (3), Uvalde, Waller, Wharton, TX
01-102-21n	6/9/01	1	Breeding	Scurry, TX
01-108-05n	5/17/01	1	Efficacy	Worth, GA
01-271-05n	10/30/01	1	Breeding	Juana Diaz, PR

B. Agronomic Characteristics

Observations were collected from the plant breeding and herbicide efficacy field trials in the United States. In the breeding trials, detailed plant habit, performance, maturity and fiber quality data were collected to facilitate the selection of the best lines. In the efficacy trials, different rates of Liberty® herbicide (GA) were applied to evaluate weed control and tolerance of the crop to the herbicide. Observations were recorded for agronomic characteristics and disease/pest susceptibility under the conditions of conventional and Liberty herbicide regimes. Mature bolls were harvested for yield, fiber quality and nutritional composition analyses of cottonseed.

In the growing seasons of 2000 and 2001, advanced selections of transformation event LLCotton25 in the Coker 312 genetic background were evaluated in replicated yield trials. In 2000, T_5 generation seed were planted in isolated blocks for yield evaluation at four locations (00-108-10n), in randomized, complete block replicated trials. Plant mapping measurements were taken from ten plants per plot at three life stages; prebloom, bloom and mature boll in the 2000 season trials and at mature boll stage in the 2001 trials. Agronomic and yield parameters were measured based upon 40 ft. plots, three replications per location. Statistical analysis of the agronomic parameters and ranking statistics of the plant conformation and other non-parametric data used ANOVA and two-tailed T test to compare the Coker312-LLCotton25 to the non-transgenic counterpart variety, Coker 312 (Appendix B, Season 2000). The T_6 generation was evaluated in a similar way at 10 locations (01-075017n) in 2001 (Appendix C, COKER312-LL25 Performance Across 10 Locations). An overview of the findings is provided in Table V.2. In general, no differences were noted that could be attributed to the pleitrophic effects of the insertion. Some differences in plant maturity were noted related to the impact of crop tolerance to the herbicide regime, in that earliness was noted when the conventional herbicide regime was replaced by the recommended Liberty herbicide regime.

Agronomic parameters which may indicate a fitness advantage outside an agricultural setting or may provide some indication of unintended or pleiotropic effects will be especially considered in this environmental safety assessment. Important agronomic parameters to consider measure 1) plant growth and morphology at different life stages, 2) reproduction and fecundity, 3) agricultural productivity, and 4) quality of the fiber. A common comparison of cotton varieties is the assessment tool known as plant mapping. Aventis can report that plant mapping at eight locations (01-075-17n) and with the transformation event, LLCotton25 in six different genetic backgrounds, found no changes in growth pattern when comparing the traditional cotton variety to its transgenic counterpart (Table V.3). As expected differences are observed between the genetic backgrounds for some yield components (lint yield, seed per boll, seed index, and sympodia length), some maturity components (node of first fruiting branch and percent open bolls) and some aspects of fiber quality. However, differences between the pairs of similar genetic backgrounds were only observed for stand counts, three LLCotton25-derived lines were lower and fiber quality. One LLCotton25-derived line had improved uniformity and one line had lower fiber strength than its recurrent parent. Protocols and statistical tables may be found in Appendix C (Six LLCotton25 –derived lines compared to

recurrent parents and Crop tolerance to Glufosinate in LLCotton25 –derived lines representing six genetic backgrounds). For a description of plant mapping of the parameters measured, see Appendix D.

Observations of reproductive traits related to maturity (days to flower), fertility (pollen abundance) and fecundity (seed index) were collected at field trials (Table V.2 and V.3). Two studies were undertaken to compare the Coker312-LLCotton25 line to the Coker 312 variety for floral and pollination characteristics. Observations of flower morphology and pollen viability record the similarity of the variety and its derived transgenic line (Appendix F). A pollen dissemination study conducted in Mississippi in 2001 recorded no incidence of cross pollination from a central 15 x 20 meter plot of Coker312-LLCotton25 into a 12 meter perimeter of Coker 312 (Pollen Dissemination Study, Appendix C). Aventis observed no impact of transformation event LLCotton25 on the reproductive biology of cotton.

Table V.2. Findings of the comparison of LLCotton25, T_5 and T_6 generations to the Non-transgenic Coker 312 Counterpart

Characteristic	Different (Statistical differences indicated by 2 stars compared to CS 320 ANOV across herbicide treatments)	No Different
Plant Growth and Morphology		
Stand Count		-
Seedling Vigor (1-4, 1 best)		-
Plant Height (inches)		-
Height to Node Ratio		-
Sympodia Length		-
Leaf morphology		-
Overall plant morphology		-
Disease Susceptibility		-
Herbicide Tolerance[1]	+	
Reproductive traits		
Days to first bloom[2]	+	-
Flower morphology		-
Boll retention		-
Days to 50% open bolls		-
Fertility		-
Seed Index		-
Number of seed per boll		-
Productivity		
Yield seed cotton		-
Lint percent		-
Lint yield (lbs/acre)		-
Fiber Quality		
Micronaire		-
Fiber Elongation %		-
Fiber Strength (g/tex)		-
Fiber Length (inches)		-
Fiber length uniformity %		-

1 Crop tolerance is excellent, no plant damage was observed following Liberty herbicide applications. In one location, fiber elongation was lower at the extreme (4X of label recommended) Liberty rates.

2 Some variation is noted for days to first bloom, especially at the Shelby TN site in 2000.

Table V.3. Findings of the comparison of transformation event LLCotton25 in six different genetic background to the non-transgenic Coker 312 counterpart

Characteristic	Different (Statistical differences indicated by + compared to the respective recurrent parent)	No Difference
Plant Growth and Morphology		
Stand Count [1]	+	-
Seedling Vigor (1-4, 1 best)		-
Plant Height (inches)		-
Height to Node Ratio		-
Sympodia Length		-
Leaf morphology		-
Overall plant morphology		-
Disease Susceptibility		-
Herbicide Tolerance	+	
Reproductive traits		
Days to first bloom		-
Flower morphology		-
Boll retention		-
Days to 50% open bolls		-
Fertility		-
Seed Index		-
Number of seed per boll		-
Productivity		
Yield seed cotton		-
Lint percent[3]		-
Lint yield (lbs/acre)		-
Fiber Quality [2]		
Micronaire		-
Fiber Elongation %		-
Fiber Strength (g/tex)		-
Fiber Length (inches)		-
Fiber length uniformity %		-

1 Three of the six LLCotton25-derived lines had lower stand counts.

2 One LLCotton25-derived line had improved uniformity and one line had lower strength than its recurrent parent..

C. Seed Characteristics

Measurements of seedling vigor, stand counts (germination under field conditions) and seed index (weight of 100 seed) were collected at every field test site. In all cases, no

differences were observed in any of the seed characteristics (Appendix B and C). Seed germination (rolled paper towel test) compared room temperature to a cold treatment (overnight at 0°C). Breaking dormancy is not an absolute requirement for cotton seeds, and a cold treatment is sometimes used to increase the number of germinating seeds. Analysis of the data show that the cold treatment of seed from two of the five locations resulted in reduced germination of the transgenic Coker312-LLCotton25 compared to that of the non transgenic C312 (Seed Germination Study, Appendix C).

D. Disease and Pest Characteristics

There are many viral, bacterial, fungal, and insect pests that can damage cotton and cause disease. In any given year one such pest infestation could result in severe damage and yield reduction to the cotton crop. However, high disease pressure is rare in cotton. Company researchers and cooperators made visual observations for plant pathogenic organisms in trials containing transformation event LLCotton25 during the 1999-2001 growing seasons. Such observations revealed some minor pathogen infections but no infestations (see Appendix A).

Infestations with green stinkbug (*Nezara viridula*), boll weevil (*Anthonomus grandis*) and cotton bollworm (*Helicoverpa zea*) were noted in 1999, and with fall armyworm (*Spodoptera frugiperda*), beet armyworm (*Spodoptera exigua*), green stinkbug, boll weevil, thrips, cotton aphids, whiteflies, plant bugs (*Lygus lineolaris*) cutworms (*Agrotis upsilon*), and cotton bollworm in 2000. Whenever pests were observed there were no differences in damage or populations found between transformation event LLCotton25 and the nontransgenic counterpart Coker 312. In addition, no differences in pest damage or insect populations were observed between plots of event LLCotton25 treated conventional herbicide or Liberty herbicide regimens (Appendix B and C). Transformation event LLCotton25 did not influence susceptibility to disease or pest organisms in diverse genetic backgrounds.

In conclusion, transformation event LLCotton25 is no more susceptible to disease or insect infestation or severity than its non-transgenic counterpart, Coker312. The genetic background in which the *bar* locus was placed does not appear to influence susceptibility to disease and insect pests.

E. Compositional Analysis

To provide an indication of potential pleiotropic effects resulting from the gene insertion, proximate analyses (lint and cottonseed), antinutrient levels (cottonseed only) and a check of key minerals (cottonseed only) were conducted using transgenic, glufosinate-tolerant cotton (Event LLCotton25) and its non-transgenic, non-tolerant counterpart (variety Coker 312). Six field trials were conducted in EPA Regions II, IV and VI in North Carolina, Arkansas, Mississippi and Texas, all important cotton growing regions of the Southern United States. The plants in this study were grown under conditions typical of production practices. There were six transgenic plots and three non-transgenic plots at each test site. Three of the transgenic plots were sprayed twice with glufosinate-ammonium (Liberty®

Herbicide) at 0.52 lb ai/A (target application rate), and three transgenic received conventional herbicide regime.

Proximates include: total protein, total fat, moisture, fiber, carbohydrate and ash. The key minerals include: calcium, phosphorus, potassium, iron, magnesium and zinc, and Vitamin E (alpha-tocopherol). The values reported are corrected for moisture. In addition, the three known antinutrients found in cotton, phytic acid, cyclopropenoid fatty acids and gossypol (total and free) were also analyzed in the cottonseed samples.

The results of the proximate analyses in cottonseed and lint are provided in Tables V.4 and V.5, respectively. The means of the proximates are expressed on a dry matter basis, except for % moisture, of the transgenic cottonseed (sprayed and unsprayed cotton) and the non-transgenic counterpart. No significant differences were observed between these sample sets for either cottonseed or lint.

The results of the mineral analyses for cottonseed are provided in Tables V.6. The means of the mineral values are expressed on a dry matter basis of the transgenic cottonseed (sprayed and unsprayed cotton) and the non-transgenic counterpart. No significant differences were observed between these sample sets for cottonseed.

The analyses for the antinutrients are provided in Table V.7 for cottonseed. No differences were found between the transformation event, LLCotton25 (sprayed or unsprayed) and the non-transgenic control.

Further analysis is underway of processed fractions. This data will be provided to the FDA in support of Aventis' food and feed safety assessment of transformation event LLCotton25. However, there are no apparent differences between the transgenic and non-transgenic counterparts. All the results clearly demonstrate that LibertyLink Cotton is substantially equivalent to non-transgenic counterparts.

Table V.4. Mean Proximate Composition of Whole Cottonseed of Transgenic Cotton Event LLCotton25 and Its Non-transgenic Counterpart

Parameter*	Non-transgenic, Coker 312, Conventional Herbicide Regime		Transgenic, LLCotton25 Liberty Herbicide Regime		Transgenic, LLCotton25 Conventional Herbicide Regime		Literature Range [a, b, c, d, e, f, g, h, i]
N =	18		18		18		
Moisture (%)**	8.66	± 1.17	9.69	± 1.16	9.62	± 1.26	5.5-16.4
	Expressed as % Dry Matter						
Crude Fat/Oil (%)	19.1	± 3.2	18.3	± 2.5	18.9	± 4.1	11.8-26.7
Protein (%)	24.4	± 2.6	25.6	± 2.3	26.4	± 4.0	20.7-31.9
Crude Fiber (%)	28.2	± 2.5	27.4	± 2.3	26.7	± 5.3	20.8-33.0
Ash (%)	4.27	± 0.54	4.46	± 0.36	4.54	± 0.66	3.34-4.80
ADF (%)	35.5	± 2.5	35.5	± 1.9	34.2	± 6.4	29.0-49.6
NDF (%)	42.1	± 2.5	41.4	± 2.1	40.1	± 7.3	39.2-63.4
CHO Calc In Feed	52.3	± 3.3	51.6	± 2.9	50.2	± 7.2	38.0-51.0

Data represent an average of three replicate samples at six field test sites.

* Fat = Crude Fat; Protein = Crude protein; ADF = Acid Detergent Fiber; NDF = Neutral Detergent Fiber; CHO = Carbohydrates (calculated).

** Moisture is expressed as % fresh weight

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c Cotton Incorporated. 2000a. Cottonseed terminology can be confusing *In* 1999-2000 Cottonseed Sourcebook Page 5, Published on <www.cottoninc.com>. Cotton Incorporated, Cary, NC27513.

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Table V.5. Mean Proximate Composition of Cotton Lint of Transgenic Cotton Event LLCotton25 and Its Non-transgenic Counterpart

Parameter*	Non-transgenic, Coker 312, Conventional Herbicide Regime		Transgenic, LLCotton25 Liberty Herbicide Regime		Transgenic, LLCotton25 Conventional Herbicide Regime	
N =	18		18		18	
Moisture (%)**	7.50	± 0.78	8.24	± 1.72	8.34	± 2.20
	Expressed as % Dry Matter					
Crude Fat/Oil (%)	1.34	± 0.83	1.38	± 0.97	1.33	± 0.89
Protein (%)	2.02	± 0.58	2.63	± 1.41	2.56	± 1.36
Crude Fiber (%)	86.5	± 6.1	81.7	± 8.4	80.9	± 11.4
Ash (%)	2.82	± 1.51	2.95	± 1.60	3.10	± 1.91
ADF (%)	94.7	± 3.8	91.6	± 6.3	91.0	± 8.4
NDF (%)	99.0	± 5.0	97.6	± 5.4	97.1	± 7.6
CHO Calc In Feed	93.8	± 2.7	93.1	± 3.7	93.0	± 3.8

Data represent an average of three replicate samples at six field test sites.

* Fat = Crude Fat; Protein = Crude protein; ADF = Acid Detergent Fiber; NDF = Neutral Detergent Fiber; CHO = Carbohydrates (calculated).

** Moisture is expressed as % fresh weight. .

Table V.6. Mean Mineral and Vitamin E Composition of Cottonseed of Transgenic Cotton Event LLCotton25 and Its Non-transgenic Counterpart

	% Dry Matter			
Parameter*	**Non-transgenic, Coker 312, Conventional Herbicide Regime**	**Transgenic, LLCotton25 Liberty Herbicide Regime**	**Transgenic, LLCotton25 Conventional Herbicide Regime**	**Literature Range [a,b,c,d]**
N =	18	18	18	
Ca (%)**	0.12 ± 0.03	0.13 ± 0.05	0.15 ± 0.08	0.108-0.210
P (%)	0.67 ± 0.15	0.71 ± 0.10	0.68 ± 0.16	0.447-0.750
Mg (%)	0.39 ± 0.05	0.41 ± 0.03	0.41 ± 0.05	0.305-0.460
K (%)	1.17 ± 0.07	1.19 ± 0.07	1.18 ± 0.08	0.99-1.28
Fe (ppm)	78 ± 42	73 ± 33	79 ± 47	9-151
Zn (ppm)	33.5 ± 3.6	38.2 ± 3.2	36.5 ± 5.8	24.9-42.0
Vitamin E (IU/kg)	167 ± 52	171 ± 41	180 ± 48	NS

Data represent an average of three replicate samples from six field test sites.

[a] Calhoun M.C., Kuhlmann S.W., Baldwin B.C. 1995. Cotton feed composition and gossypol availability and toxicity. *In* Proc. 2nd National Alternative Feeds Symp., Wooster, OH, Sept. 24-26, 1995. 125-145.

[b] Cotton Incorporated. 2000a. Cottonseed terminology can be confusing *In* 1999-2000 Cottonseed Sourcebook Page 5, Published on <www.cottoninc.com>. Cotton Incorporated, Cary, NC27513.

[c] Forster L.A. Jr., Calhoun, M.C. 1995. Nutrient values for cottonseed products deserve new look. Feedstuffs 67 (44). 1-5.

[d] National Cottonseed Products Association. 2000b. Cottonseed Feed Products Guide. 2000 Digital Edition. Published on www.cottonseed.com. National Cottonseed Products Association, Inc. P.O. Box 172267. Memphis, TN 38187-2267.

NS not specified.

Table V.7. Mean Antinutrient Composition of Whole Cottonseed of Transgenic Cotton Event LLCotton25 and Its Non-transgenic Counterpart

Parameter*	Non-transgenic, Coker 312, Conventional Herbicide Regime		Transgenic, LLCotton25 Liberty Herbicide Regime		Transgenic, LLCotton25 Conventional Herbicide Regime		Literature Range [a, b, c, d, e, f, g, h, i, j]
N =	18 (15)*		18		18		
	% Dry Matter						
Gossypol – Free	0.89	± 0.23	0.73	± 0.23	0.78	± 0.39	0.44-1.01
Gossypol – Total	1.32	± 0.28	1.17	± 0.28	1.30	± 0.24	0.67-1.63
Phytic Acid (%)	1.93	± 0.54	2.02	± 0.31	2.12	± 0.58	2.57**
	% Total Fatty Acids						
Sterculic***	0.25	± 0.055	0.20	± 0.060	0.21	± 0.060	0.005-0.7
Malvalic***	0.36	± 0.14	0.35	± 0.12	0.34	± 0.11	0.015-1.9
Dyhydrosterculic***	0.17	± 0.031	0.15	± 0.002	0.15	± 0.003	0.16-0.8

* Data represent an average of three replicate samples from six field test sites. However, due to insufficient size sample, the three non-transgenic samples of one site were not analyzed for cyclopropenoid fatty acids.

** Glandless cottonseed kernels (Wozenski and Woodburn, 1975)

*** Cyclopropenoid fatty acids

a Berberich S.A., Ream J.E, Jackson T.L., Wood R., Stipanovic R., Harvey P., Patzer S., Fuchs, R.L. 1996. The composition of insect-protected cottonseed is equivalent to that of conventional cottonseed. J. Agric. Food Chem. 44. 365-371.

b Calhoun M.C., Kuhlmann S.W., Baldwin B.C. 1995. Cotton feed composition and gossypol availability and toxicity. *In* Proc. 2nd National Alternative Feeds Symp., Wooster, OH, Sept. 24-26, 1995. 125-145.

c Cherry J.P., Leffler H.R. 1984. Seed. *In* Cotton (Kohel, R.J. and Lewis, C.F., eds.) Amer. Soc. Agron. Madison, WI. 511-558.

d Jones L.A. 1987. Recent advances in using cottonseed products. *In* Proc. Florida Nutrition Conference, March 12-13, 1987. Daytona Beach, Florida. 119-138

e Jones L.A., King C.C. 1993. Cottonseed Oil. National Cottonseed Products Association, Inc. and The Cotton Foundation. Memphis, TN.

f National Cottonseed Products Association. 2000b. Cottonseed Feed Products Guide. 2000 Digital Edition. Published on www.cottonseed.com. National Cottonseed Products Association, Inc. P.O. Box 172267. Memphis, TN 38187-2267.

g Nida D.L., Patzer S., Harvey P., Stipanovic R., Wood R., Fuchs, R.L. 1996. Glyphosate-tolerant cotton: the composition of the cottonseed is equivalent to that of conventional cottonseed. J. Agric. Food Chem. 44. 1967-1974

h Phelps R.A., Shenstone F.S., Kemmerer R.J., Evans, R.J. 1965. A review of cyclopropenoid compounds: biological effects of some derivatives. Poult. Sci. 44. 358-394.

i Wood R. 1986. Comparison of the cyclopropene fatty acid content of cottonseed varieties, glanded and glandless seeds and various seed structures. Biochemical Archives 2. 73-80

j Wozenski J., Woodburn, M. 1975. Phytic acid (myoinositol hexaphosphate) and phytase activity in four cottonseed protein products. Cereal Chemistry 52. 665-669

Whole cottonseed contains an average of 0.68% free gossypol, and is currently fed to ruminants up to 8 lbs per animal per day (15% of the ration) (Cotton Incorporated, 2000).

VI. Potential for Environmental Impact from Noncontained Use of Glufosinate-Tolerant Cotton Event LLCotton25

A. Potential for Gene Transfer from Glufosinate-Tolerant Cotton to Other Organisms

1. Outcrossing with wild and weedy relatives

The potential for outcrossing to occur is covered in Section II.E. For effective outcrossing to occur, certain criteria must be met. First and foremost, pollination must occur. Due to characteristics of cotton pollen, wind pollination is unlikely; therefore, insect pollination must occur. Secondly, the two parents must be sexually compatible with the ability to produce viable, fertile progeny. Transformation event LLCotton25 is not expected to outcross with other cotton species since no other genera in the Gossypieae tribe is endemic to the United States. Wild *G. thurberi* does occur in the US (Arizona); however, *G. hirsutum* is not grown in its vicinity. *G. thurberi* is a diploid and not sexually compatible with *G. hirsutum* which is a tetraploid. The resulting progeny would be infertile. As for *G. tomentosum* grown in Hawaii, pollination is not expected to occur since cultivated cotton is not grown commercially in Hawaii.

2. Outcrossing to cultivated cotton

Cultivated cotton is primarily self-pollinating, however when transformation event LLCotton25 is grown for commercial grain production it may participate in unconfined outcrossing with other cultivated cotton. The opportunity for outcrossing depends upon the proximity of the growing range and overlapping flowering period. However, certain biological factors act to limit gene flow. Cotton pollen is large, heavy and sticky and, thus, is not transferred by wind. The self-pollinating nature of cotton results in stigma with a preponderance of self-pollen, thus it is not likely that pollen from another source can compete and result in outcrossed seed (see Section II.E - Potential for Outcrossing). Should seed result from outcrossing, volunteer cotton can be controlled in rotational crops (see Section VI.E - Effects on Agricultural Practices of Cotton).

3. Transfer of genetic information to organisms with which it cannot interbreed

Movement of transgenes from genetically engineered plants to microorganisms has been suggested as a risk if such plants are released into the environment. As initially stated in the USDA's Interpretive Ruling on Calgene, Inc. Petition for Determination of Regulatory Status of FLAVR SAVR™ Tomato (USDA-APHIS, 1992), and subsequently repeated in other USDA Determination documents, "There is no published evidence for the existence of any mechanism, other than sexual crossing" by which genes can be transferred from a plant to other organisms. As summarized in these Determination documents, evidence suggests that, based on limited DNA homologies, transfer from plants to microorganisms may have occurred in evolutionary time over many millennia. Even if such transfer were to take place, transfer of the *bar* gene to a microbe would not pose a plant pest risk. Genes encoding both PAT enzymes and acetyl transferases are found in microbes in nature. Indeed, as described earlier in this document, the *bar* gene present in LibertyLink Cotton event LLCotton25 was isolated from a naturally occurring soil microbe.

4. Likelihood of Appearance of Glufosinate-Resistant Weeds

Herbicide resistance may be achieved by either a) gene flow to sexually compatible species and subsequent introgression of the trait into weed populations or b) through intensive use of the herbicide, which can select for naturally occurring resistant mutants in weed populations. In the case of Liberty cotton grown in the United States, there are no weedy relatives for which gene flow might occur. Naturally occurring mutations for Liberty tolerance in plants have not been documented and are not expected due to the mode of action of Liberty herbicide (Donn 1998, OECD 1999a). Glufosinate inhibits the enzyme, glutamine synthetase, thereby causing phytotoxic level of ammonium to accumulate in the plant. Work done to characterize plants with variation in susceptibility to glufosinate-ammonium has found that absorption at the leaf surface and poor translocation of the herbicide explain the differences. For example, glufosinate-ammonium is not readily translocated, thus some perennial weeds maybe able to reemerge following damage to foliar growth. There has been no evidence of differential inhibition among the isoenzymes of glutamine synthetase, and thus, no evidence of a genetic mutation to protect glutamine synthetase from the herbicidal activity of glufosinate-ammonium (Pline, Wu, and Harzios, 1999; Ridley and McNally, 1985). GA has no residual activity and thus would not provide a prolonged selective advantage for weed populations (see Section VI.E).

B. Weediness Potential of Glufosinate-Tolerant Cotton

Cotton is generally not regarded as a weed. Cotton is not considered to be a noxious weed in the United States and is not included on the noxious weed list (http://www.aphis.usda.gov/ppq/weeds/nwpolicy2001.html), nor is it listed as a weed anywhere else in the world (Holm, *et al.*, 1979). Cottonseed is not dormant and is not able to remain viable in the soil for extended periods. Only in the southern regions of Florida does the potential exist for the seed to over-winter; however, cultivated cotton is not grown in this area. Monitoring of field plots have not revealed any differences in transformation event LLCotton25 as compared to non-modified cotton with respect to competitiveness and survivability.

C. Effects of Glufosinate-Tolerant Cotton on Non-target Organisms

LibertyLink Cotton transformation event LLCotton25 has been field tested at numerous sites across the United States and no toxicity or alteration of population levels have been observed for beneficial insects, birds or other species that frequent cotton fields (see Termination Reports, Appendix A). There were no qualitative differences between beneficial species and populations present on transgenic and non-transgenic cotton plants. This observation was expected since LibertyLink Cotton contains a gene which encodes a protein that shares no homology with proteins that are known to be toxic or allergenic (see Section VI.E). The two known antinutrients found in cotton, phytic acid and gossypol were measured in seed of transformation event LLCotton25 and its non-transgenic counterparts. The levels were found in the expected range for cottonseed (Section V.E, and Table V.6.

Glufosinate-ammonium is an ecologically sound herbicide that degrades rapidly in microbially active soils and also readily binds to soil particles. Glufosinate-ammonium poses less risk of adverse effects of drift to non-target areas than current market standards. Glufosinate-ammonium and its short-lived metabolites have not been found to accumulate in the environment. Aventis conducted an acute intravenous mouse study and a 14-day acute oral toxicity study the PAT protein and no adverse effects were reported. Soil microorganisms, bees, earthworms, birds and mammals are unaffected by glufosinate-ammonium.

As indicated in Section II.A, cotton is grown foremost for fiber, but it is also a food source for both humans and livestock. Aventis (formally AgrEvo GmbH) conducted studies on purified, synthetic PAT enzyme which show that the enzyme is both heat and acid labile. The enzyme loses 100% of its activity upon incubation at 75°C (103°F) or greater for 30 minutes. At pH values of 4 or less it is inactive after exposure for 30 minutes. Both the heat treatments used for the processing should eliminate most PAT activity.

Should there be any PAT protein remaining after processing, the only route of exposure for humans and livestock to PAT in glufosinate-tolerant cotton would be via oral ingestion. In addition, animals would be exposed orally to PAT present in unprocessed seed, cottonseed meal and forage (grazing). Aventis confirmed experimentally that PAT protein in a plant matrix is rapidly degraded *in vitro* by the gastric juices from swine, chicken, and cattle. These animals represent the three primary types of gastric systems among livestock. It has also been experimentally confirmed that PAT is readily degraded in simulated human gastric fluids within minutes.

The PAT enzyme does not have the characteristics of an allergen or a toxin. It is acid and heat labile and contains no potential glycosylation sites. The protein has no homology to proteins other than PAT proteins from other organisms. The substrate specificity for the PAT enzyme is very strict in that the only substrate is L-PPT. Neither any protein amino acid nor D-PPT is acetylated by PAT. Acetyl transferases are abundant and ubiquitous in nature where they share the common function of transferring an acetyl group from acetyl CoA to a substrate. Acetyl transferases differ in substrates and the metabolic pathways in which they function (Webb, 1992).

Based on 1) the substrate specificity of PAT; 2) the physicochemical properties of PAT; 3) its rapid degradation upon ingestion; 4) the low levels of PAT in whole tissues (Table IV.5.); and 5) the ubiquitous presence of acetyl transferases in nature, adverse effects to nontarget organisms and wildlife from exposure is not likely.

D. Effects on Agricultural Practices of Cotton

1. Current Practices

Cotton has been grown across 13-15 million acres over the past 5 years. Greater than 97% of these acres receive a herbicide application for weed control. Many acres are treated multiple times resulting in a total of 37 to 44 million treated acres. Standard treatments of

the past included several herbicides and several applications timing along with cultivation. In more recent years the introduction of Roundup Ready cotton and BXN cotton have reduced the number of herbicides used and the number of applications across a cotton acre. A typical herbicide program today includes the use of a pre-emergent or pre-plant incorporated herbicide followed by a post-emergent herbicide application. Trifluralin, pendamethalin and fluometuron are the most common preplant herbicides used. These 3 herbicides make up 30% of the herbicide treatment in Cotton. Glyphosate is the most popular post-emergent treatment making up 37% of the total herbicide market. Pyrithiobac sodium, MSMA and Bromoxynil are used postemergence and make up 11% of the herbicide market.

The main weed species across all cotton include redroot pigweed (*Amaranthus retroflexus)* and other amaranth's, morning glories (*Ipomoea spp*), cocklebur (*Xanthium strrumarium)*, Johnsongrass (*Sorghum halepense*), crabgrass (*Digitaria spp*), barnyardgrass and watergrass (*Echinochloa spp*), sicklepod (*Cassia obtusifolia*), and Texas panicum (*Panicum texanum*). Cotton is grown across the southern United States in 5 distinct regions (southeast, mid-atlantic, midsouth, southwest and west). Weed species infestations change across these regions and weed control methods are adjusted accordingly.

Control of these diverse species requires the use of multiple herbicide families and multiple applications. The innovations in weed control methods resulting from the introduction of genetically enhanced cotton has shifted the concerns growers have for weed control programs. One of the biggest concerns is the reliance on glyphosate for weed control in soybeans, corn and cotton and the potential for changes in weed species or selection of resistant weed populations. Morning glory is one of the more tolerant weeds to glyphosate and it is a key weed in all five growing regions of cotton. Multiple herbicide families are still required in cotton to give growers the opportunity to prevent weed population shifts and resistance. New herbicide families for cotton use must be environmentally sound and improve upon the high use rates of many of the cotton herbicides. Crop tolerance has always been a concern for cotton growers. To get satisfactory weed control cotton growers had to typically accept crop injury. This is especially true for postemergent herbicides, in which directed sprays were often used to help minimize crop injury. Crop injury is still a concern today due to the limited application window of Roundup Ready Cotton. Preliminary studies suggest that glufosinate treatments to LibertyLink cotton may be less injurious to the reproductive development of the cotton plant compared to glyphosate treatments to Roundup Ready cotton plants (see Appendix E). No single weed management strategy will eliminate grower concerns for weed control, however continual improvement in weed control methods that provide greater flexibility for cotton growers and improve upon environmental exposure are warranted.

2. Possible Effect of LibertyLink® Cotton on Current Practices

a. The Herbicide Glufosinate-ammonium and Current Uses

Glufosinate-ammonium the active ingredient in Liberty Herbicide, is a potent inhibitor of the enzyme glutamine synthetase in plants. The inhibition of this enzyme stops the

conversion of glutamate to glutamine. The lack of glutamine leads to a rapid disruption of photosynthesis. Glufosinate-ammonium is a nonselective herbicide for both non-crop and crop uses. It is highly biodegradable, has no residual activity, and has very low toxicity for humans. Glufosinate-ammonium is an ecologically sound herbicide that degrades rapidly in microbially active soils and also readily binds to soil particles. Glufosinate-ammonium poses less risk of adverse effects of drift to non-target areas than current market standards. Glufosinate-ammonium and its short-lived metabolites have not been found to accumulate in the environment. Soil microorganisms, bees, earthworms, birds and mammals are unaffected by glufosinate-ammonium.

There are presently no registered uses for glufosinate-ammonium in cotton, but an EPA registration is pending and expected prior to the 2003-growing season. However, glufosinate-ammonium is registered for use as a non-selective herbicide on turf (trade name Finale™) and apples, grapes, and tree nuts and as a desiccant for potatoes (trade name Rely®) in the United States. Glufosinate-ammonium has been successfully introduced as Liberty® Herbicide for use on LibertyLink corn hybrids and canola varieties. Liberty has been used in LibertyLink crops for over 4 years and has achieved over 4 million acres of use. Outside the United States, GA is registered for use on plantation crops, tree nuts, and vines, and for industrial/non-agricultural weed control under a variety of trade names including Basta® and Ignite®.

b. Possible Effects as Indicated by Results from Agronomic Practice and Weed Control Efficacy Trials

Studies across the cotton growing regions of the United States have tested Liberty Herbicide against all of the aforementioned important weeds in cotton. Study results have shown acceptable control of every important weed in cotton. The use of Liberty Herbicide fits well with the new common agronomic practices for weed control in cotton. These include less tillage, less herbicide combinations used preemergent and the increase use of broadspectrum postemergence herbicides. The option to wait for crop establishment to assess weed infestations and the need for weed control, allows the grower flexibility and avoids blind application of pre-plant and pre-emergence herbicides. Compared to glyphosate, Liberty has two key advantages that growers desire: 1) Liberty Herbicide provides excellent control of morning glory species and 2) Liberty Herbicide can be applied 4-6 weeks longer than glyphosate. The wider application window gives the farmer more flexibility to use the herbicide on an "as-needed" basis. The rush to control weeds in a narrow window of application can lead to misapplication, potential drift, and increased use of cultivation. Liberty Herbicide allows the grower the option to delay herbicide application until the level of weed infestation is known. Liberty Herbicide's unique mode of action lends itself as an excellent herbicide rotational tool to prevent weed shifts and the need for new weed management systems.

Table VI.1. Important Weeds Labeled for Control by Liberty® Herbicide in Cotton Provided are the common and scientific names.

Grass Weeds	
Barnyardgrass	*Echinochloa crus-galli*
Crabgrass, large	*Digitaria sangulinalis*
Johnsongrass	*Sorghum halepense*
Texas Panicum	*Panicum texanum*
Watergrass	*Echinochloa oryzoides*
Broadleaf Weeds	
Pigweed species	*Amaranthus spp.*
Sicklepod	*Cassia obtusifolia*
Cocklebur, common	*Xanthium strumarium*
Prickly Sida	*Sida spinosa*
Black nightshade	*Solanum nigrum*
Morning glory species	*Ipomoea spp.*
Sunflower	*Helanthus annuus*

Some of the more important herbicides used today require high dosage uses (452-888 g ai/acre) for weed control. Liberty Herbicide will be recommended with much lower dose rates (400-560 gm ai/acre). Effective Liberty Herbicide dose rates are variable depending upon the weed species and weed stage of growth. Some herbicides can cause injury to crop. Liberty Herbicide has a 4X tolerance when used on LibertyLink Cotton varieties.

Table VI. 2. Liberty Cotton System Compared to Conventional Herbicide Regime.

Treatment	Conventional Practice		Roundup Ready Practice		With Liberty Herbicide	
	Ingredient	Amount g a.i./acre	Ingredient	Amount g a.i./acre	Ingredient	Amount g a.i./acre
Preplant Burndown	Roundup	339	Roundup	339	Roundup	339
Preplant	Trifluralin or Pendamethalin + Fluometuron	375 + 452	Trifluralin or Pendamethalin	375	Trifluralin or Pendamethalin	375
Early Post-emergence over the top or directed	MSMA	452	Roundup	452	Liberty	189
	Pyrithiobac	20				
Mid Post-emergence over-the –top or directed	MSMA + fluometuron	452 + 339	Roundup	452	Liberty	189
Layby	Prometryn	266	Prometryn	266	Prometryn	266
Total g a.i./acre		2656		1845		1319

Over the past few years cotton prices have lowered and this has resulted in a demand for increased yield to offset the potential income loss. This has resulted in an increase in low quality cotton production and a significant premium for high quality cotton. Fibermax® cotton genetics are highly regarded for their yield potential and for their fiber quality characteristics. Fibermax genetics continually achieve high premiums for quality, which improves the profitability and sustainability of cotton growers. The introduction of the LibertyLink gene into Fibermax cotton allows the use of a more effective and environmentally sound herbicide program than can be used in Fibermax cotton today. The combination of superior genetics with a safe, unique and effective herbicide system will provide greater sustainability for cotton growers in the future.

There are almost as many opinions as to the best weed control program in cotton as there are cotton growers and consultants. In conventional weed control systems in nontransgenic cotton, the herbicides used are dictated by the predominant weed species present.

LibertyLink Cotton and Glufosinate-ammonium may positively impact current agronomic practices in cotton by 1) offering broad spectrum, post-emergence weed control with a wide application window, which allows treatment only when weeds reach economical thresholds; 2) providing the opportunity to continue to move away from pre-emergent and residually active compounds; 3) providing a new herbicidal mode of action that allows for improved weed shift and resistance management; 4) decreasing cultivation needs; 5) allowing the application of less total pounds of active ingredient than used presently, and, 6) providing a more profitable and sustainable cotton system for cotton growers.

VII. Statement of Grounds Unfavorable

Data generated from field and laboratory tests indicate that no unfavorable grounds are associated with LibertyLink Cotton transformation event LLCotton25. Therefore, Aventis requests that LibertyLink Cotton transformation event LLCotton25, and any progeny derived from crosses of event LLCotton25 with traditional cotton varieties, and any progeny derived from crosses of event LLCotton25 with transgenic cotton varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340.

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IX. Appendices

APPENDIX A

1999, 2000 and 2001 (INTERIM) USDA FIELD TRIAL TERMINATION REPORTS

USDA 1999 Termination Report for
Herbicide Tolerant Event Cot25 Cotton (LLCotton25)
Aventis CropScience USA, LP

Trials Conducted

99-007-08n: MS(1)

Trials Not Planted

99-007-08n: CA(1), NC (1), TX(1)

Planting Date

June 6, 1999 (Washington Co., MS)

Plot Destruction Date

October 28, 1999 (Washington Co., MS)

Purpose

Field trials were conducted to test the efficacy of transgenic herbicide-tolerant cotton, for breeding purposes, and tissue sample collections for transgene product content analysis. Transgenic plants contained the *bar* gene expressing the PAT enzyme, which confers resistance to the broad-spectrum herbicide glufosinate-ammonium.

General Field Observations

Experienced personnel qualified in cotton cultivation performed all plot observations. Recorded observations for transgenic and non-transgenic control plots were provided from first square through first open boll growth stages.

Noted insect pest species were Green stinkbug (*Nezara viridula*), Boll weevil (*Anthonomus grandis*), and Cotton boll worm (*Helicoverpa zea*). There were no differences in diversity of pest species found between transgenic and non-transgenic plants.

Beneficial insects listed included lacewings (*Chrysopa sp.*), assassin bugs (Hemiptera: Reduviidae), and ladybugs (*Hippodamia convergens*). As with the pest species, no differences were noted between the two plot types.

Following these observations, both transgenic and non-transgenic plots were sprayed with Pounce®, Decis®, and Curacron®.
No disease pressure was observed within either of the plot types throughout the study period.

Weather at the study site was described as normal in both rainfall and temperature.

Plant emergence patterns were uniform within both plots.

No morphological differences were noted between transgenic and non-transgenic plants. The only in-field phenotypic difference observed between the two genotypes was their respective levels of tolerance to Glufosinate.

Final Disposition

Plant materials remaining at the close of the study were shredded and tilled under.

Post-Trial Monitoring

The field was rotated the following growing season to Round Up Ready Soybeans. No volunteers were found during the following season.

Daryl W. Maddox

USDA 2000 Termination Report for Herbicide Tolerant Cotton Event Cot025

Aventis CropScience USA, LP

Trials Conducted

00-074-14n: AR (Crittenden), NC (Wayne), MS (Washington), TX(Wharton)
00-108-10n: TN(Shelby), MS(Washington (2), Issaquena)
00-119-05n: MO (Stoddard), MS (Washington), TX (Lubbock)
00-258-02n: PR (Juana Diaz)

Trials Not Planted

00-074-14n: CA (Fresno), FL (Escambia), TN (Shelby)
00-108-05n: MS(Washington)

Planting Dates

May 15, 2000 (Wayne Co., NC) through June 1, 2000 (Stoddard Co., MO)
October 20, 2000 (Juana Diaz, Puerto Rico)

Harvest Dates

September 14, 2000 (Wharton Co., TX) through December 5, 2000 (Lubbock Co., TX)

Plot Destruction Dates

October 1, 2000 (Issaquena Co., MS) through December 5, 2000 (Lubbock Co., TX)

Purpose

Field trials were conducted to test the efficacy of transgenic herbicide-tolerant cotton, for breeding purposes, residue analysis, and tissue sample collections for transgene product content analysis. Transgenic plants contained the *bar* gene expressing the PAT enzyme, which confers resistance to the broad-spectrum herbicide glufosinate-ammonium.

General Field Observations

Experienced personnel qualified in cotton cultivation performed all plot observations. Recorded observations for transgenic and non-transgenic control plots were provided from first square through open boll growth stages.

Plant emergence patterns were uniform within both plots.

No morphological differences were noted between transgenic and non-transgenic plants. The only in-field phenotypic difference observed between the two genotypes was their respective levels of tolerance to Glufosinate.

Insect pest species noted included fall armyworm (*Spodoptera frugiperda*), beet armyworm (*Spodoptera exigua*), green stinkbug (*Nezara viridula*), boll weevil (*Anthonomus grandis*), Thrips (taxa ?), cotton aphids (taxa ?), whiteflies (Aleyrodidaae: taxa ?), plant bugs (*Lygus lineolaris*), cutworms (*Agrotis upsilon*), and cotton boll worm (*Helicoverpa zea*). There were no differences in diversity of pest species found between transgenic and non-transgenic plants.

Beneficial insects listed included lacewings (*Chrysopa sp.*), assassin bugs (Hemiptera: Reduviidae), honeybees (*Apis melifera*), parasitic wasp (taxa ?), and ladybugs (*Hippodamia convergens*). As with the pest species, no differences were noted between the two plot types.

Only one case of bacterial blight was recorded at the Lubbock Co., TX site. No plant diseases were recorded within either plot type at any of the remaining sites throughout the study period.

Weather at the majority of the study sites was described as normal in both rainfall and temperature. However, extremely hot and dry conditions were persistent at the Washington Co., MS site while severe winds and hail were recorded during the emergence period at the Lubbock Co., TX site.

Final Disposition

Plant materials remaining at the close of the study were shredded and tilled under.

Post-Trial Monitoring

Volunteer plants were recorded for sites located in Issaquena and Washington Co's., MS and also Lubbock Co., TX. No site listing volunteers recorded more than 1 to 10 plants. Volunteer control was accomplished by herbicide treatment (Roundup®), hand weeding and mechanical cultivation.

Daryl W. Maddox
December 7, 2001

USDA 2001 Interim Termination Report for Herbicide Tolerant Cotton Event Cot025 (LLCotton25)

Aventis CropScience USA, LP

Trials Conducted by State and County

01-075-17n:	AL: Macon
	AR: Crittenden, Drew, Jackson
	GA: Colquitt, Mitchell, Tift
	LA: LA: Tensas Parish
	MS: Noxubee, Tate, Washington
	NC: Wayne
	SC: Barnwell, Horry, Marion
	TN: Shelby
	TX: Lubbock, Wharton, Waller, Burleson, Uvalde
01-102-21n:	TX: Scurry
01-108-05n:	GA: Worth
01-271-05n:	PR: Juana Diaz

Trials Not Planted

01-075-17n:	AR: Desha, Mississippi
	AZ: Maricopa, Pinal
	CA: Fresno
	GA: Jefferson, Lee, Tift
	LA: Madison
	MS: Quitman, Washington
	NC: Halifax, Johnston
	TN: Madison
	TX: Brazos

Planting Dates

May 4, 2001 (Lubbock Co., TX) through October 30, 2001 (Juana Diaz, PR).

Harvest and Plot Destruction Dates

September 6, 2001 (Macon Co., AL) through November 26, 2001 (Washington Co., MS).

Purpose

Field trials were conducted to test the efficacy of transgenic herbicide-tolerant cotton, for breeding purposes, residue analysis, and tissue sample collections for transgene product content analysis. Transgenic plants contained the *bar* gene expressing the PAT enzyme, which confers resistance to the broad-spectrum herbicide glufosinate-ammonium.

General Field Observations

Experienced personnel qualified in cotton cultivation performed all plot observations. Recorded observations for transgenic and non-transgenic control plots were provided from first square through open boll growth stages.

Plant emergence patterns were uniform and vigorous within both plot types. Fifteen-Day Germination rates ranged from 80% (Crittenden Co., AR) to 95% (Jackson Co., AR).

No morphological differences were noted between transgenic and non-transgenic plants. The only in-field phenotypic difference observed between the two genotypes was their respective levels of tolerance to glufosinate-ammonium.

Insect pest species identified included budworm (*Spodoptera sp.*), boll weevil (*Anthonomus grandis*), thrips (taxa ?), cotton aphids (taxa ?), plant bugs (*Lygus lineolaris*), whiteflies (Homoptera: Aleyrodidae), cutoworms (*Agrotis upsilon*), stinkbug (Hemiptera: Pentatomidae), and cotton boll worm (*Helicoverpa zea*). There were no differences recorded in either diversity or density of insect pest species found between transgenic and non-transgenic plants.

Beneficial insect species listed included lacewings (*Chrysopa sp.*), honeybees (*Apis melifera*), big-eyed bug (Hemiptera: Lygaeidae), parasitic wasps (? taxa), and ladybugs (*Hippodamia convergens*). As with the pest species, no differences were noted between species profiles for the two plot types.

"Leaf disease" (? taxa) was listed for Washington Co., MS. In addition, *Rhizoctonia sp.* and *Phymototricum sp.* were recorded at Uvalde Co., TX. No plant diseases were recorded within either plot type at any of the remaining sites throughout the study period.

Weather at the majority of the study sites was described as normal in both rainfall and temperature. However, hot and dry conditions occurred toward the end of the growing season at the Washington Co., MS and Lubbock Co., TX sites.

Final Disposition

Plant materials remaining at the close of the study were mowed and disced under.

Post-Trial Monitoring

Volunteer monitoring is in progress and is incomplete at this writing.

Daryl W. Maddox
December 7, 2001

APPENDIX B

AGRONOMIC PERFORMANCE OF LIBERTY® TOLERANT COTTON BASED UPON TRANSFORMATION EVENT LLCOTTON25 IN THE 2000 USA PRODUCTION SEASON

Title

Agronomic Performance of Liberty® tolerant Cotton based upon transformation event LLCotton25 in the 2000 USA production season

Author

M. Freyssinet

Completed On

25 January 2002

Testing Facility

AVENTIS CropScience

55 Avenue René-Cassin

69009 LYON, France

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).
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Company: Aventis CropScience USA LP

Company Agent: _____ _____

A Name
Function, Department

Date (ddMONyyyy)

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The last page of this report is number 28.

SUMMARY

Liberty® tolerant Cotton
based upon transformation event LLCotton25
in the 2000 USA production season

Objectives

Liberty®-tolerant cotton line, C312 LL25, treated or not treated with Liberty® herbicide, was grown together with its non-transgenic counterpart, Coker 312 in order to:

1) compare plant morphology and agronomic performance and fiber characteristics between C312 LL25/T_5 and its non-transgenic counterpart;

2) evaluate the tolerance to glufosinate applications of transformation event LLCotton25 and potential impact upon plant morphology or agronomic performance.

Summary of Findings

1) no differences were observed when the line C312 LL25 is compared to Coker 312 under conventional herbicide regimes.
2) Crop tolerance is excellent, no plant damage was observed following Liberty herbicide appliations.

SEASON 2000

PROTOCOL

Trial names

FGH004
FGH005
FGH006

Locations

Permit # 00-108-10n
Issaquena County, MS (1 site)
Washington County, MS (2sites)
Shelby County, TN (1 site)

Objective

The purpose of these trials is to compare agronomic performance and fiber characteristics between the Liberty®-tolerant cotton Coker312 LL25/T_5 and its non-transgenic counterpart. The objective of these trials is to evaluate LL25 transgene efficacy and subsequently determine if the various glufosinate applications affect plant morphology or agronomic performance.

Trial design

The statistical design is a complete randomized block design. Nearest Neighbor restrictions and analyses are encouraged. Plots can be 1 or 2 rows with 3-4 replications, depending upon seed availability.

Agronomic treatments

Typical agronomic inputs for conventionally grown cotton for the area, including, but not limited to:

- Conventional herbicide treatments, both pre- and post-planting
- Granular insecticide and/or fungicide application at planting
- Fertilizer applications
- Necessary in-season insecticide applications
- Growth regulator application (this should be done sparingly if at all)
- Additional hand weeding as necessary
- Chemical defoliation without boll-opening desiccants

Test treatments

Test treatments involve over-the-top field applications of glufosinate (Liberty® herbicide).
Treatments include (see field plot layout):

- 0X (no Liberty®)
- 1X (28 oz product per acre) Liberty® at the 3-5 leaf stage followed by an additional 1X treatment at 20% bloom which approximates 75 days pre-harvest
- 4X (112 oz product per acre) Liberty® at the 3-5 leaf stage followed by an additional 4X treatment at 20% bloom which approximates 75 days pre-harvest

APHIS requirements

These trials contain the glufosinate resistance transgene LL25 that is currently regulated by the USDA and EPA. The trials are to be planted, conducted, harvested, and plant material disposed of as mandated by APHIS and ACS requirements. The USDA requires certain information regarding the field release of regulated transgenic plants to be collected and reported.
ALWAYS - refer to and follow directions of the Compliance Notebook.

Data to be collected

- Stand count at 28 days
- Yield: Lbs lint per acre
- Seed index
- Lint Percent
- Plant height
- Total number of nodes
- Height to node ratio
- Sympodia length
- Boll retention
- Number first position bolls

- Nodes above cracked boll
- Percent open bolls
- Days to bloom
- Fertility rating
- Plant morphology rating (leaf, flower, bolls)
- Disease ratings
- Fiber Micronaire
- Fiber Length
- Fiber strength

Note: Appendix D to the LLCotton25 USDA petition provides a listing of parameter definitions.

TRIAL DESCRIPTION

Washington County (ACSI Location - Leland)

This efficacy trial was planted on May 24, 2000 in a soil type that ranges over Boskett very fine sandy loam to Dundee very fine silt loam. This trial contained three reps in complete randomized block design. The trial consisted of C312LL25 plots with Liberty® applications of a 1x rate (28 oz/acre), 1x rate at the 3 to 5 leaf stage +1x rate at 20% bloom, 4x rate of Liberty® at the 3 to 5 leaf stage, and 4x rate of Liberty® at the 3 to 5 leaf stage + 4x rate at the 20% bloom stage during the growing season. Controls plots were non-transgenic C312 and transgenic C312LL25 that did not receive a Liberty® treatment. Observations were made on all plots in the trial at seven, fourteen, twenty-one, and twenty-eight days after planting to determine seedling emergence and vigor. The first Liberty® application was made when the plants reached the 3 to 5 leaf stage on June 20, 2000. The plots that received the Liberty® treatment were rated for herbicide injury. Plots which received a second application of herbicide were sprayed on July 26, 2000 and again the plants were observed and rated after seven days to determine if there was any damage caused by the herbicide.

Data was collected from the plots through a very detailed plant mapping. Ten plants from each plot were mapped at three stages during the growing season. The first plant map was taken when the plants were at the prebloom stage on July 10, 2000. The second mapping occurred when the plants reached the bloom stage on August 2, 2000. The final mapping occurred when the plants reached boll stage on September 14, 2000.

Washington County (Livingston Site)

This efficacy trial was planted on May 24, 2000 in a soil type that is a Boskett very fine sandy loam. This trial contained three reps in complete randomized block design. The trial consisted of C312LL25 plots with Liberty® applications of 1x rate (28 oz/acre), 1x at the 3 to 5 leaf stage +1x rate at 20% bloom, 4x rate of Liberty® at the 3 to 5 leaf stage, and 4x rate at the 3 to 5 leaf stage + 4x rate at the 20% bloom stage during the growing season. Controls plots were non-transgenic C312 and transgenic C312LL25 that did not receive a Liberty® treatment. Observations were made on all plots in the trial at seven, fourteen, twenty-one, and twenty-eight days after planting to determine seedling emergence and vigor. The first Liberty® application was made when the plants reached the 3 to 5 leaf stage on June 14, 2000. The plots that received the Liberty® treatment were rated for herbicide injury. Plots which received a second application of herbicide were sprayed on July 26, 2000 and again the plants were observed and rated after seven days to determine if there was any damage caused by the herbicide.

Data was collected from the plots through a very detailed plant mapping. Ten plants from each plot were mapped at three stages during the growing season. The first plant map was taken when the plants were at the prebloom stage on July 10, 2000. The second mapping occurred when the plants reached the bloom stage on July 31, 2000. The final mapping occurred when the plants reached boll stage on September 14, 2000.

Issaquena County (Fitler Site)

This efficacy trial was planted on May 23, 2000 in a soil type that is a Dundee very fine silt loam and grown under dryland conditions. This trial contained three reps in complete randomized block design. The trial consisted of C312LL25 plots with Liberty® applications of 1x rate (28 oz/acre), 1x at the 3 to 5 leaf stage +1x rate at 20% bloom, 4x rate of Liberty® at the 3 to 5 leaf stage, and 4x at the 3 to 5 leaf stage + 4x rate at the 20% bloom stage during the growing season. Controls plots were non-transgenic C312 and transgenic C312LL25 that did not receive a Liberty® treatment. Observations were made on all plots in the trial at seven,

fourteen, twenty-one, and twenty-eight days after planting to determine seedling emergence and vigor. The first Liberty® application was made when the plants reached the 3 to 5 leaf stage on June 19, 2000. The plots that received the Liberty® treatment were rated for herbicide injury. Plots which received a second application of herbicide were sprayed on July 20, 2000 and again the plants were observed and rated after seven days to determine if there was any damage caused by the herbicide.
Data was collected from the plots through a very detailed plant mapping. Ten plants from each plot were mapped at three stages during the growing season. The first plant map was taken when the plants were at the prebloom stage on July 18, 2000. The second mapping occurred when the plants reached the bloom stage on July 27, 2000. The final mapping occurred when the plants reached boll stage on September 5, 2000.

Shelby County (Tennessee Site)

This efficacy trial was planted on May 22, 2000 in a soil type that is a Dundee very fine silt loam. This trial contained three reps in complete randomized block design. The trial consisted of C312LL25 plots with Liberty® applications of 1x rate (28 oz/acre), 1x at the 3 to 5 leaf stage +1x rate at 20% bloom, 4x rate of Liberty® at the 3 to 5 leaf stage, and 4x at the 3 to 5 leaf stage + 4x rate at the 20% bloom stage during the growing season. Controls plots were non-transgenic C312 and transgenic C312LL25 that did not receive a Liberty® treatment. Observations were made on all plots in the trial at seven, fourteen, twenty-one, and twenty-eight days after planting to determine seedling emergence and vigor. The first Liberty® application was made when the plants reached the 3 to 5 leaf stage on June 26, 2000. The plots that received the Liberty® treatment were rated for herbicide injury. Plots which received a second application of herbicide were sprayed on July 22, 2000 and again the plants were observed and rated after seven days to determine if there was any damage caused by the herbicide.
Data was collected from the plots through a very detailed plant mapping. Ten plants from each plot were mapped at three stages during the growing season. The first plant map was taken when the plants were at the prebloom stage on July 10, 2000. The second mapping occurred when the plants reached the bloom stage on August 7, 2000. The final mapping occurred when the plants reached boll stage on September 20, 2000.

Statistical Tables for ACROSS LOCATIONS Comparison
Coker 312 LL25 (T_5) and Coker 312

t (2-sided a=0.010, 58df) = 2.6633
Means followed by a letter (a,b,...) differ, by a 2-sided LSD, from the means of check entries denoted by the same letter.

Lint percent
LSD = 9.8391 MSE = 52.94341

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	38.35%	19.3	-
C312 LL25 b	0	37.61%	15.9	-
C312 LL25	1x	39.18% b	17.4	-
C312 LL25	1x1x	38.85% b	12.1	-
C312 LL25	4x	38.24%	15.4	-
C312 LL25	4x4x	38.50%	15.2	-

Lint yield (Lbs/Acre)
LSD = 285.4004 MSE = 63159.09177

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	527.22	48.0	-
C312 LL25 b	0	531.42	42.3	-
C312 LL25	1x	523.60	52.0	-
C312 LL25	1x1x	527.13	44.1	-
C312 LL25	4x	469.67	47.3	-
C312 LL25	4x4x	545.03	50.2	-

Yield seed cotton (Lbs / 2x40' rows)
LSD = 3.31 MSE = 8.53011

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	7.53	35.1	-
C312 LL25 b	0	7.77	33.3	-
C312 LL25	1x	7.41	40.4	-
C312 LL25	1x1x	7.78	43.8	-
C312 LL25	4x	6.55	37.9	-
C312 LL25	4x4x	7.51	41.0	-

Seed index (average g weight of 100 seed)
LSD = 1.0908 MSE = 0.92263

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	10.55	8.9	-
C312 LL25 b	0	10.73	8.4	-
C312 LL25	1x	10.36	9.9	-
C312 LL25	1x1x	10.82	10.8	-
C312 LL25	4x	11.18	7.8	-
C312 LL25	4x4x	10.73	7.3	-

Plant height (inches)
LSD = 10.8579 MSE = 91.41549

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	34.45	28.5	-
C312 LL25 b	0	34.55	29.1	-
C312 LL25	1x	31.91	29.0	-
C312 LL25	1x1x	34.91	26.5	-
C312 LL25	4x	35.36	25.6	-
C312 LL25	4x4x	35.73	25.2	-

Height to node ratio
LSD = 0.3325 MSE = 0.08575

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.91	15.8	-
C312 LL25 b	0	1.91	15.8	-
C312 LL25	1x	1.82	22.2	-
C312 LL25	1x1x	1.91	15.8	-
C312 LL25	4x	2.09	14.4	-
C312 LL25	4x4x	2.00	0.0	-

Sympodia length (inches)
LSD = 0.8001 MSE = 0.49633

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	2.73	27.9	-
C312 LL25 b	0	2.80	25.4	-
C312 LL25	1x	2.56	30.9	-
C312 LL25	1x1x	2.82	20.1	-
C312 LL25	4x	2.76	28.1	-
C312 LL25	4x4x	2.51	23.5	-

Days to first bloom

LSD =9.4618 MSE = 69.4185

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	57.64 b	21.6	-
C312 LL25 b	0	52.91 a	8.4	-
C312 LL25	1x	54.45 a	12.1	-
C312 LL25	1x1x	57.18 b	18.0	-
C312 LL25	4x	53.91 a	13.2	-
C312 LL25	4x4x	53.64 a	12.5	-

Boll retention

LSD = 27.9431 MSE = 605.44738

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	59.55	39.8	-
C312 LL25 b	0	63.36	39.3	-
C312 LL25	1x	67.82	25.8	-
C312 LL25	1x1x	62.45	31.0	-
C312 LL25	4x	55.27	48.1	-
C312 LL25	4x4x	57.64	54.0	-

Days to 50% open bolls

LSD = 20.1665 MSE = 315.34550

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	113.09	15.0	-
C312 LL25 b	0	113.36	15.2	-
C312 LL25	1x	112.64	15.6	-
C312 LL25	1x1x	112.73	16.0	-
C312 LL25	4x	112.64	15.7	-
C312 LL25	4x4x	113.73	15.2	-

Stand count 28 days after planting % stand

LSD = 16.5581 MSE = 212.59296

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	64.55	22.4	-
C312 LL25 b	0	56.18	25.7	-
C312 LL25	1x	55.18	13.8	-
C312 LL25	1x1x	64.64	21.8	-
C312 LL25	4x	59.82	27.5	-
C312 LL25	4x4x	65.39	28.3	-

Herbicide injury

All values for herbicide injury are the same - no variation for analysis.

Overall plant morphology

All values for plant morphology are the same - no variation for analysis.

Disease susceptibility

All values for disease susceptibility are the same - no variation for analysis

Plant fertility

All values for fertility are the same - no variation for analysis.

Plant vigor (scale 1-4, 1=best possible rating)

LSD = 0.7457 MSE = 0.43119

Event	Herbicide Treatmen	Means	Cv	Significance
Control C312 a	0	1.73	37.4	-
C312 LL25 b	0	1.64	41.2	-
C312 LL25	1x	1.73	45.5	-
C312 LL25	1x1x	1.36	49.4	-
C312 LL25	4x	1.36	49.4	-
C312 LL25	4x4x	1.36	37.0	-

Micronaire
LSD = 0.6127 MSE = 0.29113

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	4.31	13.6	-
C312 LL25 b	0	4.48	13.4	-
C312 LL25	1x	4.49	9.7	-
C312 LL25	1x1x	4.53	11.5	-
C312 LL25	4x	4.60	12.0	-
C312 LL25	4x4x	4.65 a	10.9	-

Fiber elongation %
LSD = 0.5904 MSE = 0.27026

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	6.47	7.7	-
C312 LL25 b	0	6.74	8.8	-
C312 LL25	1x	6.45	8.6	-
C312 LL25	1x1x	6.41	6.6	-
C312 LL25	4x	6.77	6.5	-
C312 LL25	4x4x	6.68	8.5	-

Fiber strength (g / tex)
LSD = 1.6327 MSE = 2.06608

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	21.85	9.9	-
C312 LL25 b	0	21.79	7.3	-
C312 LL25	1x	22.09	5.5	-
C312 LL25	1x1x	22.50	4.4	-
C312 LL25	4x	21.85	7.0	-
C312 LL25	4x4x	22.05	4.3	-

Fiber length (inches)
LSD = 0.0289 MSE = 0.00065

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.18	2.8	-
C312 LL25 b	0	1.17	1.8	-
C312 LL25	1x	1.16	1.8	-
C312 LL25	1x1x	1.17	2.5	-
C312 LL25	4x	1.17	2.3	-
C312 LL25	4x4x	1.18	1.8	-

Statistical Tables for BY LOCATION Comparison
Coker 312 LL25 (T_5) and Coker 312

Means followed by a letter (a,b, ...) differ, by a 2-sided LSD, from the means of the check entries denoted by the same letter.

Lint percent

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 4.1238 MSE = 1.04600

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	31.5	2.2	-
C312 LL25 b	0	33.7	1.3	-
C312 LL25	1x	32.4	2.6	-
C312 LL25	1x1x	36.1 a	5.9	+
C312 LL25	4x	33	0.9	-
C312 LL25	4x4x	34.2	0.0	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 2.5352 MSE = 0.95981

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	33.4	5.3	-
C312 LL25 b	0	32.87	1.3	-
C312 LL25	1x	35.15	1.8	-
C312 LL25	1x1x	35.87 ab	2.9	+
C312 LL25	4x	34.07	0.7	-
C312 LL25	4x4x	34.73	3.1	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 3.2326 MSE = 1.56056

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	36.87	3.0	-
C312 LL25 b	0	35.90	2.6	-
C312 LL25	1x	35.57	0.9	-
C312 LL25	1x1x	36.50	2.5	-
C312 LL25	4x	37.13	2.4	-
C312 LL25	4x4x	36.30	5.7	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 3.0860 MSE = 1.42222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	49.33	1.2	-
C312 LL25 b	0	46.67	2.5	-
C312 LL25	1x	49.33	2.3	-
C312 LL25	1x1x	46 a	0.0	+
C312 LL25	4x	47	3.7	-
C312 LL25	4x4x	47.33	2.4	-

Lint yield (Lbs / Acre)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 299.1760 MSE = 5505.30729

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	396.67	26.6	-
C312 LL25 b	0	507.64	32.0	-
C312 LL25	1x	462.61	10.9	-

G312 LL25	1x1x	435.35	12.4	-
G312 LL25	4x	370.70	12.6	-
G312 LL25	4x4x	372.64	0.0	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 119.3154 MSE = 2126.01052

Event	Herbicide Treatment	Means	Cv	Significance
Control G312 a	0	514.90	5.0	-
G312 LL25 b	0	514.08	15.9	-
G312 LL25	1x	488.56	5.6	-
G312 LL25	1x1x	699.78 ab	3.6	+
G312 LL25	4x	564.78	7.9	-
G312 LL25	4x4x	630.29	12.7	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 168.0753 MSE = 4218.71251

Event	Herbicide Treatment	Means	Cv	Significance
Control G312 a	0	297.48	20.3	-
G312 LL25 b	0	298.72	3.4	-
G312 LL25	1x	234.58	39.6	-
G312 LL25	1x1x	217.46	31.8	-
G312 LL25	4x	231.20	9.3	-
G312 LL25	4x4x	232.37	26.4	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 517.6096 MSE = 40010.75556

Event	Herbicide Treatment	Means	Cv	Significance
Control G312 a	0	856.33	26.5	-
G312 LL25 b	0	797.33	26.3	-
G312 LL25	1x	888.33	19.4	-
G312 LL25	1x1x	725.33	12.3	-
G312 LL25	4x	679.00	38.5	-
G312 LL25	4x4x	887.33	4.0	-

Yield seed cotton (Lbs / 2x40'row)

a) Leland (MS

t (2-sided a=0.010, 5df) = 4.0321 LSD = 5.3430 MSE = 1.75590

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	6.98	28.8	-
C312 LL25 b	0	8.34	33.2	-
C312 LL25	1x	7.88	8.3	-
C312 LL25	1x1x	6.65	6.6	-
C312 LL25	4x	6.21	13.4	-
C312 LL25	4x4x	6.01	0.2	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.7745 MSE = 0.47024

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	8.53	5.3	-
C312 LL25 b	0	8.65	17.2	-
C312 LL25	1x	7.79	7.7	-
C312 LL25	1x1x	10.79 ab	6.6	+
C312 LL25	4x	9.11	8.7	-
C312 LL25	4x4x	10.01	10.0	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 2.3988 MSE = 0.85931

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	4.45	17.8	-
C312 LL25 b	0	4.60	1.1	-
C312 LL25	1x	3.44	39.1	-
C312 LL25	1x1x	3.30	32.8	-
C312 LL25	4x	3.44	10.4	-
C312 LL25	4x4x	3.52	22.6	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 5.6431 MSE = 4.75556

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	10.00	26.5	-
C312 LL25 b	0	9.67	23.9	-
C312 LL25	1x	10.67	14.3	-
C312 LL25	1x1x	10.00	20.0	-
C312 LL25	4x	7.33	28.4	-
C312 LL25	4x4x	10.00	10.0	-

Seed index (average g weight of 100 seed)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 3.1693 LSD = 1.5616 MSE = 0.15000

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	10.5	6.7	-
C312 LL25 b	0	11	0.0	-
C312 LL25	1x	9.5	7.4	-
C312 LL25	1x1x	12	0.0	-
C312 LL25	4x	11	0.0	-
C312 LL25	4x4x	10.5	6.7	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 2.3464 MSE = 0.82222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	9.67	6.0	-
C312 LL25 b	0	10	10	-
C312 LL25	1x	9.33	6.2	-
C312 LL25	1x1x	9.67	6.0	-
C312 LL25	4x	10.67	14.3	-
C312 LL25	4x4x	10	0	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.1246 MSE = 0.0.18889

Event	Herbicide Treatment	Means	Cv	Significance
Control C312. a	0	10.33	5.6	-
C312 LL25 b	0	10.33	5.6	-
C312 LL25	1x	11	7.4	-
C312 LL25	1x1x	10	0.0	-
C312 LL25	4x	11	0.0	-
C312 LL25	4x4x	11	0.0	-

d) Shelby County (TN)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.0564 MSE = 0.16667

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	11.67	4.9	-
C312 LL25 b	0	11.67	4.9	-
C312 LL25	1x	11.33	5.1	-
C312 LL25	1x1x	12	0.0	-
C312 LL25	4x	12	0.0	-
C312 LL25	4x4x	11.33	5.1	-

Plant height (inches)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 21.9866 MSE = 29.73333

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	38.50	20.2	-
C312 LL25 b	0	40.50	19.2	-
C312 LL25	1x	37.50	5.7	-
C312 LL25	1x1x	38.50	12.9	-
C312 LL25	4x	41.00	0.0	-
C312 LL25	4x4x	38.00	0.0	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 11.8489 MSE = 20.96667

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	39.00	8.9	-
C312 LL25 b	0	39.00	17.8	-
C312 LL25	1x	37.00	10.8	-
C312 LL25	1x1x	40.00	11.5	-
C312 LL25	4x	41.00	4.2	-
C312 LL25	4x4x	41.00	8.8	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 6.0809 MSE = 5.52222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	20.33	11.4	-
C312 LL25 b	0	20.33	5.7	-
C312 LL25	1x	18.67	6.2	-
C312 LL25	1x1x	21.67	14.1	-
C312 LL25	4x	22.00	9.1	-
C312 LL25	4x4x	22.67	14.2	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 12.6507 MSE = 23.90000

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	41.33	9.8	-
C312 LL25 b	0	40.33	6.2	-
C312 LL25	1x	36.33	18.7	-
C312 LL25	1x1x	40.67	11.6	-
C312 LL25	4x	39.33	14.5	-
C312 LL25	4x4x	42.00	10.9	-

Height to node ratio

a) Leland (MS)

All values for height to node ratio are the same - no variation for analysis.

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.90.47 MSE = 0.12222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.67	34.6	-
C312 LL25 b	0	1.67	34.6	-
C312 LL25	1x	1.33	43.3	-
C312 LL25	1x1x	1.67	34.6	-
C312 LL25	4x	2.00	0.0	-
C312 LL25	4x4x	2.00	0.0	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.6099 MSE = 0.05556

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	2.00	0.0	-
C312 LL25 b	0	2.00	0.0	-
C312 LL25	1x	2.00	0.0	-
C312 LL25	1x1x	2.00	0.0	-
C312 LL25	4x	2.33	24.7	-
C312 LL25	4x4x	2.00	0.0	-

d) Shelby County (TN)

All values for height to node ratio are the same - no variation for analysis.

Sympodia length (inches)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 2.1108 MSE = 0.27403

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	3.73	19.0	-
C312 LL25 b	0	3.43	0.0	-
C312 LL25	1x	3.66	18.4	-
C312 LL25	1x1x	3.73	8.5	-
C312 LL25	4x	3.52	8.7	-
C312 LL25	4x4x	3.13	17.0	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.5993 MSE = 0.38199

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	3.19	11.6	-
C312 LL25 b	0	2.91	38.0	-
C312 LL25	1x	2.30	43.5	-
C312 LL25	1x1x	2.73	3.4	-
C312 LL25	4x	3.33	18.4	-
C312 LL25	4x4x	2.63	10.9	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.8430 MSE = 0.10613

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	2.33	16.2	-
C312 LL25 b	0	2.40	22.5	-
C312 LL25	1x	2.31	12.5	-
C312 LL25	1x1x	2.45	14.5	-
C312 LL25	4x	2.13	27.7	-
C312 LL25	4x4x	1.83	12.0	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 1.4689 MSE = 0.32222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	2.00	0.0	-
C312 LL25 b	0	2.67	21.7	-
C312 LL25	1x	2.33	24.7	-
C312 LL25	1x1x	2.67	21.7	-
C312 LL25	4x	2.33	24.7	-
C312 LL25	4x4x	2.67	21.7	-

Days to first bloom

a) Leland (MS

t (2-sided a=0.010, 5df) = 4.0321 LSD = 7.0802 MSE = 3.08333

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	47.50	1.5	-
C312 LL25 b	0	48.50	1.5	-
C312 LL25	1x	49.00	5.8	-
C312 LL25	1x1x	49.00	0.0	-
C312 LL25	4x	46.50	1.5	-
C312 LL25	4x4x	49.00	5.8	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 8.7711 MSE = 11.48889

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	54.33	10.5	-
C312 LL25 b	0	54.67	1.1	-
C312 LL25	1x	52.00	3.8	-
C312 LL25	1x1x	53.00	1.9	-
C312 LL25	4x	54.00	9.8	-
C312 LL25	4x4x	50.33	4.6	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 4.0274 MSE = 2.42222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	49.33	4.2	-
C312 LL25 b	0	50.00	6.0	-

C312 LL25	1x	50.67	1.1	-
C312 LL25	1x1x	51.00	2.0	-
C312 LL25	4x	49.67	1.2	-
C312 LL25	4x4x	50.00	4.0	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 6.5974 MSE = 6.50000

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	76.00 b	3.5	+
C312 LL25 b	0	57.00 a	9.1	+
C312 LL25	1x	64.33 ab	1.8	+
C312 LL25	1x1x	73.00 b	2.4	+
C312 LL25	4x	63.00 a	6.9	+
C312 LL25	4x4x	63.67 ab	1.8	+

Boll retention

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 24.7684 MSE = 37.73333

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	62.50	30.5	-
C312 LL25 b	0	59.00	38.4	-
C312 LL25	1x	68.00	20.8	-
C312 LL25	1x1x	65.50	11.9	-
C312 LL25	4x	63.50	34.5	-
C312 LL25	4x4x	55.50	19.1	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 52.7190 MSE = 415.05556

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	64.00	32.5	-
C312 LL25 b	0	75.00	28.8	-
C312 LL25	1x	81.00	12.5	-
C312 LL25	1x1x	67.33	7.0	-
C312 LL25	4x	75.67	40.9	-
C312 LL25	4x4x	73.67	16.4	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 40.0634 MSE = 239.70000

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	38.00	38.8	-
C312 LL25 b	0	37.00	5.4	-
C312 LL25	1x	46.00	13.2	-
C312 LL25	1x1x	41.67	67.9	-
C312 LL25	4x	26.33	71.3	-
C312 LL25	4x4x	14.00	7.1	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 40.5195 MSE = 245.18889

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	74.67	40.2	-
C312 LL25 b	0	81.00	31.3	-
C312 LL25	1x	76.33	18.7	-
C312 LL25	1x1x	76.33	7.6	-
C312 LL25	4x	58.33	8.6	-
C312 LL25	4x4x	86.67	13.4	-

Days to 50% open bolls

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 11.9159 MSE = 8.73333

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	107.00	1.3	-
C312 LL25 b	0	107.00	1.3	-
C312 LL25	1x	108.00	3.9	-
C312 LL25	1x1x	107.00	1.3	-
C312 LL25	4x	104.50	0.7	-
C312 LL25	4x4x	107.50	4.6	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 4.0915 MSE = 2.50000

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	98.00	1.0	-
C312 LL25 b	0	96.67	1.2	-
C312 LL25	1x	94.67	0.6	-
C312 LL25	1x1x	94.33	1.2	-
C312 LL25	4x	95.67	2.6	-
C312 LL25	4x4x	95.67	1.6	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 7.3140 MSE = 7.98889

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	106.67	1.4	-
C312 LL25 b	0	108.67	1.9	-
C312 LL25	1x	108.00	1.6	-
C312 LL25	1x1x	108.67	4.3	-
C312 LL25	4x	108.67	1.1	-
C312 LL25	4x4x	111.00	2.4	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 3.3959 MSE = 1.72222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	138.67	1.5	-
C312 LL25 b	0	139.00	0.0	-
C312 LL25	1x	138.33	1.5	-
C312 LL25	1x1x	139.00	1.2	-
C312 LL25	4x	139.00	0.7	-
C312 LL25	4x4x	138.67	0.4	-

Stand count (plants / 18g seed / 40 ft row)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 31.4102 MSE = 60.68333

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	55.50	8.9	-
C312 LL25 b	0	48.00	11.8	-
C312 LL25	1x	52	0.0	-
C312 LL25	1x1x	55.50	8.9	-
C312 LL25	4x	40.50	12.2	-
C312 LL25	4x4x	48	32.4	-

b) Stoneville(MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 51.7964 MSE = 400.65556

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	59.33	33.1	-
C312 LL25 b	0	53.33	30.3	-
C312 LL25	1x	49.33	22.8	-
C312 LL25	1x1x	69	34.3	-
C312 LL25	4x	66.33	40.3	-
C312 LL25	4x4x	84	27.3	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 36.7478 MSE = 201.66667

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	69	25.1	-
C312 LL25 b	0	56.33	37.9	-
C312 LL25	1x	59	0.0	-
C312 LL25	1x1x	61.67	25.5	-
C312 LL25	4x	61.67	7.5	-
C312 LL25	4x4x	59.33	12.6	-

d) Shelby County (TN)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 27.8223 MSE = 115.6

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	71.33	15.8	-
C312 LL25 b	0	64.33	17.5	-
C312 LL25	1x	59.33	12.6	-
C312 LL25	1x1x	69.33	5.8	-
C312 LL25	4x	64.33	17.5	-
C312 LL25	4x4x	64.33	17.5	-

Herbicide injury (scale 1-5, 1=best possible rating)

All values for herbicide injury are the same - no variation for analysis in any location.

Leaf morphology (scale 1-4, 1=best possible rating)

All values for leaf morphology are the same - no variation for analysis in any location.

Flower morphology (scale 1-4, 1=best possible rating)

All values for flower morphology are the same - no variation for analysis in any location.

Overall plant morphology (scale 1-4, 1=best possible rating)

All values for plant morphology are the same - no variation for analysis in any location.

Plant vigor (scale 1-4, 1=best possible rating)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 2.1463 MSE = 0.28333

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.50	47.1	-
C312 LL25 b	0	1.50	47.1	-
C312 LL25	1x	1.50	47.1	-
C312 LL25	1x1x	1.00	0.0	-
C312 LL25	4x	1.00	0.0	-
C312 LL25	4x4x	1.00	0.0	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.4689 MSE = 0.32222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.67	34.6	-
C312 LL25 b	0	1.33	43.3	-
C312 LL25	1x	1.67	34.6	-
C312 LL25	1x1x	1.00	0.0	-
C312 LL25	4x	1.33	43.3	-
C312 LL25	4x4x	1.33	43.3	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.7715 MSE = 0.08889

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.33	43.3	-
C312 LL25 b	0	1.33	43.3	-
C312 LL25	1x	1.00	0.0	-
C312 LL25	1x1x	1.00	0.0	-
C312 LL25	4x	1.00	0.0	-
C312 LL25	4x4x	1.00	0.0	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 1.6815 MSE = 0.42222

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	2.33	24.7	-
C312 LL25 b	0	2.33	24.7	-
C312 LL25	1x	2.67	21.7	-
C312 LL25	1x1x	2.33	24.7	-
C312 LL25	4x	2.00	50.0	-
C312 LL25	4x4x	2.00	0.0	-

Plant fertility (scale 1-4, 1=best possible rating)

All values for fertility are the same - no variation for analysis in any location.

Segregation ratio

All values for segregation ratio are the same - no variation for analysis in any location.

Disease susceptibility (scale 1-4, 1=best possible rating)

All values for disease susceptibility are the same - no variation for analysis for any location.

Chlorosis (scale 1-5, 1=best possible rating)

All values for chlorosis are the same - no variation for analysis in any location.

Micronaire

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 1.6171 MSE = 0.16083

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	3.75	13.2	-
C312 LL25 b	0	4.05	12.2	-
C312 LL25	1x	3.85	5.5	-
C312 LL25	1x1x	4.25	1.7	-
C312 LL25	4x	3.80	3.7	-
C312 LL25	4x4x	4.15	11.9	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.7824 MSE = 0.09142

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	3.83	10.5	-
C312 LL25 b	0	3.87	6.5	-
C312 LL25	1x	4.25	3.5	-
C312 LL25	1x1x	4.07	12.6	-

C312 LL25	4x	4.50	5.9	-
C312 LL25	4x4x	4.33	8.1	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.5722 MSE = 0.04889

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	4.47	2.6	-
C312 LL25 b	0	4.70	4.3	-
C312 LL25	1x	4.67	1.2	-
C312 LL25	1x1x	4.53	4.6	-
C312 LL25	4x	4.53	3.4	-
C312 LL25	4x4x	4.73	8.5	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 0.4457 MSE = 0.02967

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	5.00	2.0	-
C312 LL25 b	0	5.17	4.5	-
C312 LL25	1x	4.97	3.1	-
C312 LL25	1x1x	5.17	4.0	-
C312 LL25	4x	5.30	3.3	-
C312 LL25	4x4x	5.20	1.9	-

Fiber elongation %

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 1.7756 MSE = 0.18958

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	6.82	6.5	-
C312 LL25 b	0	7.38	7.2	-
C312 LL25	1x	7.00	5.1	-
C312 LL25	1x1x	7.00	0.0	-
C312 LL25	4x	6.75	5.2	-
C312 LL25	4x4x	7.13	7.4	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.6541 MSE = 0.06389

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	6.83	4.2	-
C312 LL25 b	0	6.83	4.2	-
C312 LL25	1x	6.25	0.0	-
C312 LL25	1x1x	6.42	6.0	-
C312 LL25	4x	6.25	4.0	-
C312 LL25	4x4x	6.00 ab	0.0	+

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.3240 MSE = 0.26181

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	5.83	4.9	-
C312 LL25 b	0	6.33	12.7	-
C312 LL25	1x	6.00	8.3	-
C312 LL25	1x1x	6.42	6.0	-
C312 LL25	4x	7.08	2.0	-
C312 LL25	4x4x	6.75	6.4	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 1.2275 MSE = 0.22502

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	6.50	3.8	-
C312 LL25 b	0	6.63	6.8	-
C312 LL25	1x	6.75	9.8	-
C312 LL25	1x1x	6.00	0.0	-
C312 LL25	4x	7.00	6.2	-
C312 LL25	4x4x	7.00	7.1	-

Fiber strength (g / tex)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 4.6124 MSE = 1.30850

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	26.00	1.4	-
C312 LL25 b	0	24.48	1.0	-
C312 LL25	1x	24.10	6.2	-
C312 LL25	1x1x	22.95	4.0	-
C312 LL25	4x	24.08	9.8	-
C312 LL25	4x4x	23.25	1.5	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 1.9089 MSE = 0.54419

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	20.40	1.7	-
C312 LL25 b	0	21.73	3.7	-
C312 LL25	1x	22.18	1.5	-
C312 LL25	1x1x	22.48 a	5.3	+
C312 LL25	4x	21.65	4.1	-
C312 LL25	4x4x	21.83	0.8	-

c) Issaquena County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 2.1572 MSE = 0.69492

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	21.08	3.1	-
C312 LL25 b	0	20.57	5.2	-
C312 LL25	1x	21.65	1.8	-
C312 LL25	1x1x	22.43	6.7	-
C312 LL25	4x	21.80	2.7	-
C312 LL25	4x4x	22.57	0.5	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 2.1592 MSE = 0.69622

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	21.30	4.8	-
C312 LL25 b	0	21.27	4.4	-
C312 LL25	1x	21.10	2.6	-
C312 LL25	1x1x	22.27	3.0	-
C312 LL25	4x	20.63	3.5	-
C312 LL25	4x4x	20.95	4.0	-

Fiber length (inches)

a) Leland (MS)

t (2-sided a=0.010, 5df) = 4.0321 LSD = 0.1221 MSE = 0.00092

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.18	1.5	-
C312 LL25 b	0	1.18	0.3	-
C312 LL25	1x	1.16	0.3	-
C312 LL25	1x1x	1.18	0.6	-
C312 LL25	4x	1.15	3.7	-
C312 LL25	4x4x	1.17	4.2	-

b) Stoneville (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.0430 MSE = 0.00028

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.22	1.3	-
C312 LL25 b	0	1.19	2.1	-
C312 LL25	1x	1.17 a	0.0	+
C312 LL25	1x1x	1.18	1.0	-
C312 LL25	4x	1.18	1.0	-
C312 LL25	4x4x	1.18	2.0	-

c) Isequanna County (MS)

t (2-sided a=0.010, 10df) = 3.1693 LSD = 0.0501 MSE = 0.00037

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.14	2.0	-
C312 LL25 b	0	1.16	1.9	-
C312.LL25	1x	1.14	1.0	-
C312 LL25	1x1x	1.14	2.7	-
C312 LL25	4x	1.17	0.5	-
C312 LL25	4x4x	1.17	1.3	-

d) Shelby County (TN)

t (2-sided a=0.010, 10 df) = 3.1693 LSD = 0.0569 MSE = 0.00048

Event	Herbicide Treatment	Means	Cv	Significance
Control C312 a	0	1.18	1.0	-
C312 LL25 b	0	1.16	0.7	-
C312 LL25	1x	1.17	2.6	-
C312 LL25	1x1x	1.17	3.3	-
C312 LL25	4x	1.17	3.6	-
C312 LL25	4x4x	1.18	1.1	-

CONCLUSIONS

During the summer of 2000, Liberty®-tolerant Cotton Event 25, in a Coker312 background (Ts), was compared to the non-transgenic counterpart in three different sites in Mississippi and one site in Tennessee (USDA Permit # 00-108-10n). Applications of Liberty® herbicide included:

- 0X (no Liberty®)
- 1X (28 oz product per acre) Liberty® at the 3-5 leaf stage followed by an additional 1X treatment at 20% bloom (approximates 75 days pre-harvest)
- 4X (112 oz product per acre) Liberty® at the 3-5 leaf stage followed by an additional 4X treatment at 20% bloom (approximates 75 days pre-harvest)

The following parameters were evaluated:

- Lint percent
- Lint yield
- Yield seed cotton
- Seed index (average weight of one seed)
- Plant height (inches)
- Height to node ratio
- Sympodia length (inches)
- Days to first bloom
- Boll retention
- Days to 50% open bolls
- Stand count (28 days after planting)
- Herbicide injury
- Disease susceptibility
- Plant fertility
- Plant vigor
- Fiber quality
  - Micronaire
  - Fiber elongation %
  - Fiber strength (g / tex)
  - Fiber length

The statistical analysis of the data across locations and by location show very few significant differences. Even in dryland and high temperature stress conditions (Shelby County TN), LL Cotton 25 was not different from the control.

The overall performance of LL Cotton 25 was equal to or better than that of its non-transgenic counterpart.

APPENDIX C

AGRONOMIC PERFORMANCE OF LIBERTY® TOLERANT COTTON BASED UPON TRANSFORMATION EVENT LLCOTTON25 IN THE 2001 USA PRODUCTION SEASON

Title

Agronomic Performance of Liberty® tolerant Cotton based upon transformation event LLCotton25 in the 2001 USA production season

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Completed On

28 January 2002

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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Company: Aventis CropScience USA LP

Company Agent: _____ _____

A Name
Function, Department

Date (ddMONyyyy)

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SUMMARY

Agronomic Performance of Liberty® tolerant Cotton based upon transformation event LLCotton25 in the 2001 USA production season

Objectives

Liberty®-tolerant cotton LL25, treated or not treated with Liberty® herbicide, was grown together with its non-transgenic counterpart in order to:

1) compare the agronomic performance and fiber characteristics between the LL25 converted sister lines and their respective recurrent parent variety counterpart;
2) evaluate LL25 transgene efficacy and subsequently determine if the various glufosinate applications affect plant morphology or agronomic performance.
3) compare plant morphology and agronomic performance of Coker 312 LL25 - T_6 in 10 different target environments, when treated with conventional herbicide regime (0X) and one application of Liberty (glufosinate) herbicide at the recommended rate (1X), and its untreated non-transgenic counterpart.
4) compare the pollen dissemination and seed germination of Coker 312 LL25 -T_6, and its untreated non-transgenic counterpart.

Summary of Findings

These studies compared transformation event, LLCotton25 in six different genetic backgrounds to their respective recurrent parent variety counterpart. No differences were observed in the measurements taken from plant mapping at the mature boll stage.

As can be expected differences are observed between the genetic backgrounds for some yield components (lint yield, seed per boll, seed index, and sympodia length), some maturity components (node of first fruiting branch and percent open bolls) and some aspects of fiber quality.

Differences between the pairs of similar genetic backgrounds were only observed for stand counts, three LL25-derived lines were lower and fiber quality. One LL25-derived line had improved uniformity and one line had lower strength than its recurrent parent.

A cold treatment is sometimes used to increase the number of germinating seeds. In laboratory germination tests, the cold treatment of seed from two of the five locations resulted in reduced germination of the transgenic C312LL25 compared to that of the non transgenic C312.

A pollen dissemination study conducted in Mississippi in 2001 recorded no incidence of cross pollination from a central 15 x 20 meter plot of C312LL25 into a 12 meter perimeter of Coker 312.

Pages 117-154 have been CBI-deleted.

COKER312-LL25 Performance ACROSS 10 LOCATIONS

PROTOCOL

Locations

Permit # 01-075-17n

Tensas County, LA (1 site)
Washington County, MS (5 sites)
Marion County, SC (1 site)
Shelby County, TN (1 site)
Scurry County, TX (1 site)
Lubbock County, TX (1 site)

Objective

The purpose of these trials is to compare plant morphology and agronomic performance of CokerLL25/T_6 in 10 different target environments when treated with 0X and 1X glufosinate, and its untreated non-transgenic counterpart. The objective of these trials is to evaluate LL25 transgene efficacy and subsequently determine if glufosinate application affects plant morphology or agronomic performance.

Trial design

The preferred statistical design of a split-plot where Liberty® treatment is the main plot and line/transgene is the subplot. A split-strip design may also be used. A complete randomized block design would suffice, but would not provide maximum precision.

1.1.1 Agronomic treatments

Typical agronomic inputs for conventionally grown cotton for the area, including, but not limited to:

- Conventional herbicide treatments, both pre- and post-planting
- Granular insecticide and/or fungicide application at planting
- Fertilizer applications
- Necessary in-season insecticide applications
- Growth regulator application (this should be done sparingly if at all)
- Additional hand weeding as necessary
- Chemical defoliation without boll-opening desiccants

Test treatments

Test treatments involve over-the-top field applications of glufosinate (Liberty® herbicide).
Treatments include (see field plot layout):

- 0X (no Liberty®) Conventional herbicide regime
- 1X (28 oz product per acre) Liberty® at 20% bloom which approximates 75 days pre-harvest

Data requirements

Collect the following data for each plot, each rep, on a per plot basis, where applicable:

- Chlorosis (any yellowing of foliage) at 5-7 days after Liberty® treatment
- Internode length of the top 3 nodes of 10 plants per plot, each rep, 10-14 days after Liberty® treatment
- Strain uniformity: 1=uniform, 2=somewhat variable, 3=highly variable
- Plant type: 1=cluster, 2=intermediate, 3=open
- Leaf pubescence: 1=hairy, 2=semi-smooth, 3=smooth
- Disease reaction (*Verticillium* wilt, bacterial blight, bronze wilt, etc. IF applicable): 1=severe, 2=some symptoms apparent, 3=no symptoms
- Stalk lodging: 1=severely lodged, 2=some lodging, 3=upright
- Number of days to first flower: as an average of the plot
- Number of days to first open boll: as an average of the plot
- Boll type: 1=tight, 2=intermediate, 3=loose
  OR (Storm resistance: 1=very tight, 2=tight, 3=intermediate, 4=loose, 5=falling on ground)
- Number of days to 50% open bolls: as an average of the plot
- Percent open bolls as a visual average when recurrent parent is 40-60% open
- Percent stand count

- Yield in lbs. lint per acre
- Percent lint
- Number of seeds per boll
- Number of seeds per plant
- Seed index (gram weight of 100 seed)
- Fiber properties: micronaire, length, length uniformity, elongation, strength
- Plant mapping: plant map 10 plants per plot, each rep at maturity shortly before defoliation. Plant mapping information and instruction will be provided before that time. Data will include height, number of nodes, and boll position. Information will be collected to reflect overall plant architecture and maturity.

APHIS requirements

These trials contain the glufosinate resistance transgene LL25 that is currently regulated by the USDA and EPA. The trials are to be planted, conducted, harvested, and plant material disposed of as mandated by APHIS and ACS requirements. The USDA requires certain information regarding the field release of regulated transgenic plants to be collected and reported.
ALWAYS - refer to and follow directions of the Compliance Notebook.

TRIAL DESCRIPTION

This efficacy trial contained three reps in complete randomized block design. The trial consisted of C312LL25 plots with a Liberty® application of a 1x rate (28 oz/acre) at 20% bloom. Controls plots were non-transgenic C312 and transgenic C312LL25 that did not receive a Liberty® treatment. Observations were made on all plots in the trial at seven, fourteen, twenty-one, and twenty-eight days after planting to determine seedling emergence and vigor. The plots that received the Liberty® treatment were rated for herbicide injury.
Data was collected from the plots through a very detailed plant mapping. Ten plants from each plot were mapped when the plants reached boll stage.

Statistical Tables for ACROSS LOCATIONS Comparison

Means followed by a letter (a, b, ...) differ, by a 2-sided LSD, from the means of the check entry denoted by the same letter.

Lint percent

t (2-sided a=0.010, 67 df) = 2.6512			LSD = 2.3646		MSE = 0.54602
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	36.27	7.9	-
C312 LL25		0	36.57	10.2	-
C312 LL25		1x	36.97	6.7	-

Lint yield (Lbs / Acre)

t (2-sided a=0.010, 58 df) = 2.6633			LSD = 209.9501		MSE = 65250.75622
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	534.86	48.0	-
C312 LL25		0	558.47	43.9	-
C312 LL25		1x	585.54	43.3	-

Total number of seeds per plant

t (2-sided a=0.010, 58 df) = 2.6633			LSD = 44.4130		MSE = 2919.94131
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	137.75	46.0	-
C312 LL25		0	148.68	30.1	-

C312 LL25		1x	145.50	34.3	-

Seed index (g weight of 100 seeds)

t (2-sided a=0.010, 58 df) = 2.6633 LSD = 0.7030 MSE = 0.73150

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	10.99	7.8	-
C312 LL25		0	10.99	8.6	-
C312 LL25		1x	10.99	7.4	-

Node of first fruiting branch

t (2-sided a=0.010, 85 df) = 2.6349 LSD = 0.5682 MSE = 0.68536

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	4.96	17.2	-
C312 LL25		0	4.79	17.0	-
C312 LL25		1x	4.93	16.1	-

Plant height at maturity (inches)

t (2-sided a=0.010, 85 df) = 2.6349 LSD = 11.5047 MSE = 285.96291

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	51.14	32.4	-
C312 LL25		0	53.28	31.2	-
C312 LL25		1x	56.24	30.3	-

Total number of nodes

t (2-sided a=0.010, 85 df) = 2.6349 LSD = 3.9192 MSE = 33.18618

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	22.39	22.1	-
C312 LL25		0	22.41	27.0	-
C312 LL25		1x	23.40	25.7	-

Height to node ratio

t (2-sided a=0.010, 85 df) = 2.6349 LSD = 0.3764 MSE = 0.30614

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	2.27	20.8	-
C312 LL25		0	2.43	24.9	-
C312 LL25		1x	2.44	22.9	-

Plant fertility

All values for plant fertility are the same - no variation for analysis across the 10 locations

Plant morphology

All values for plant morphology are the same - no variation for analysis across the 10 locations

Herbicide injury

All values for herbicide injury are the same - no variation for analysis across the 10 locations for LL25 cotton

Disease susceptibility

All values for disease susceptibility are the same - no variation for analysis across the 10 locations

Plant vigor (scale 1-4, 1=best possible rating)

t (2-sided a=0.010, 85 df) = 2.6349			LSD = 0.2106		MSE = 0.09582
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	1.00	0.0	-
C312 LL25		0	1.10	27.7	-
C312 LL25		1x	1.13	38.3	-

Days to first bloom

t (2-sided a=0.010, 76 df) = 2.6421			LSD = 2.9562		MSE = 16.90100
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	51.93	8.4	-
C312 LL25		0	54.79	7.0	-
C312 LL25		1x	53.91	7.4	-

Sympodia length (inches)

t (2-sided a=0.010, 58 df) = 2.6633			LSD = 0.5421		MSE = 0.43510
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	2.08	29.1	-
C312 LL25		0	1.95	31.9	-
C312 LL25		1x	2.04	35.3	-

Days to first open boll

t (2-sided a=0.010, 58 df) = 2.6633			LSD =10.6154		MSE = 166.81005
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	110.52	11.9	-
C312 LL25		0	115.07	10.8	-
C312 LL25		1x	114.21	11.0	-

Days to 50% open bolls

t (2-sided a=0.010, 58 df) = 2.6633			LSD = 9.3671		MSE = 129.88727
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	139.38	8.6	-
C312 LL25		0	138.05	8.0	-
C312 LL25		1x	136.73	7.7	-

Percent stand count 28 days after planting

t (2-sided a=0.010, 76 df) = 11.2478 LSD = 11.2478 MSE = 244.66780

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	73.68	21.6	-
C312 LL25		0	69.52	22.8	-
C312 LL25		1x	73.54	19.8	-

Micronaire

t (2-sided a=0.010, 40 df) = 2.7045 LSD = 0.4292 MSE = 0.18889

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	4.38	6.9	-
C312 LL25		0	4.68	10.5	-
C312 LL25		1x	4.43	10.3	-

Fiber length (inches)

t (2-sided a=0.010, 40 df) = 2.7045 LSD = 0.0325 MSE = 0.00108

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	1.19	3.7	-
C312 LL25		0	1.17	2.6	-
C312 LL25		1x	1.18	2.1	-

Fiber length uniformity (%)

t (2-sided a=0.010, 40 df) = 2.7045 LSD = 1.1743 MSE = 1.41396

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	85.34	1.8	-
C312 LL25		0	85.17	1.4	-
C312 LL25		1x	85.06	1.0	-

Fiber elongation (%)

t (2-sided a=0.010, 40 df) = 2.7045 LSD = 1.3351 MSE = 1.82768

Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	7.28	20.2	-
C312 LL25		0	7.70	18.5	-
C312 LL25		1x	7.63	15.3	-

Fiber strength (g / tex)

t (2-sided a=0.010, 40 df) = 2.7045			LSD = 1.6719		MSE = 2.86630
Event		Herbicide Treatment	Means	Cv	Significance
Control C312	a	0	27.85	5.9	-
C312 LL25		0	28.64	7.1	-
C312 LL25		1x	28.68	4.9	-

CONCLUSIONS

During the summer of 2001, Liberty®-tolerant Cotton Event 25, in a Coker312 background (T_6) was compared to the non-transgenic counterpart Coker 312 in ten sites in Louisiana (1 site), Mississippi (5 sites), Southern-Carolina (1 site), Tennessee (1 site) and Texas (2 sites) under USDA Permit # 01-075-17n.
Applications of Liberty® herbicide included:

- 0X (no Liberty®)
- 1X (28 oz product per acre) Liberty® at 20% bloom which approximates 75 days pre-harvest

The following parameters were evaluated:

- Lint percent
- Lint yield
- Number of seeds per plant
- Seed index (average g weight of 100 seeds)
- Stand count 28 days after planting (plant / 11 g seed / 40 ft row)
- Node of first fruiting branch
- Plant height at maturity (inches)
- Total number of nodes
- Height to node ratio
- Plant fertility
- Plant morphology
- Herbicide injury
- Disease susceptibility
- Plant vigor
- Days to first bloom
- Sympodia length (inches)
- Days to first open boll
- Days to 50% open bolls
- Fiber quality
  - Micronaire
  - Fiber length
  - Fiber length uniformity %
  - Fiber elongation %
  - Fiber strength (g / tex)

The statistical analysis of the data show that LL Cotton 25, either treated with Liberty® herbicide or not treated, is not different from the non-transgenic counterpart.
The overall performance of LL Cotton 25 was equal to or better than that of its non-transgenic counterpart.

Seed Germination STUDY

PROTOCOL

Locations
Permit # 01-075-17n
Chula County, GA
Jackson County, AR
Crittenden County, AR
Tate County, MS
Tensas Parish, LA

Objective
To evaluate Liberty® tolerant cotton Event LL25 for potential effects on seed dormancy.

Entries
Coker312 LL25
Non-transgenic Coker 312

Agronomic Treatments
Typical agronomic inputs for conventionally grown cotton for the area, including, but not limited to:

- Conventional herbicide treatments, both pre- and post-planting
- Granular insecticide and/or fungicide application at planting
- Fertilizer applications
- Necessary in-season insecticide applications
- Growth regulator application (this should be done sparingly if at all)
- Additional hand weeding as necessary
- Chemical defoliation without boll-opening desiccants

Test Treatments
No special herbicide treatments are required. Liberty® herbicide is not to be applied for these trials.

Experimental design
One hundred and fifty seeds (fifty seeds from each location) were taken from each entry and divided into two seed lots of 75 seeds each, (LL25 transgenic seed lots A & B and non-transgenic lots A and B). Transgenic and non-transgenic seed lot A was treated overnight at 0°C. Lot B was kept at room temperature overnight. Twenty five seeds from each seed lot were placed into a wet paper towel germination roll and replicated three times. The rolls were placed in a plastic container with drilled air holes in the lid and put into the greenhouse. Percent germination was recorded for each roll and hypocotyls were measured after seven days and again after ten days to show the growth rate of the seedlings.

Entries

- Non-transgenic Coker 312 Lots A & B
- Tansgenic Coker 312 LL25 Lots A & B
- Lot A: cold treated at 0°C for 24 hours
- Lot B: no cold treatment
- 25 seeds per paper towel roll, 3 replicates

DATA

Mean % Germination
T(2 sided a=0.010, 6 df)=3.7074

Location	Event	No treatment		Cold Treatment		Significance
		7 days	10 days	7 days	10 days	
GA	C312a	88	89.33	93.33	93.33	-
GA	C312LL25b	85.33	90.67	92	92	-

Tillar AR	C312a	93.33	93.33	88	93.33	-
Tillar AR	C312LL25b	70.67	80	50.67a	66.67a	+
Proctor AR	C312a	96	97.33	92	96	-
Proctor AR	C312LL25b	94.67	96	76	84	-
MS	C312a	90.67	93.33	93.33	93.33	-
MS	C312LL25b	93.33	96	69.33	73.33	-
LA	C312a	97.33	97.33	97.33	98.67	-
5LA	C312LL25b	90.67	93.33	57.33ab	66.67	+

Mean Hypocotyl length (cm)
T(2-sided a=0.010, 6 df) = 3.7074

Location	Event	No treatment Hypocotyl in cm. 7 days	10 days	Cold Treatment Hypocotyl in cm. 7 days	10 days	Significance
GA	C312a	6.33	7.00	7.00	8.00	-
GA	C312LL25b	8.67	9.33	6.00	6.33	-
Tillar AR	C312a	6.00	6.67	11.00a	11.33a	+
Tillar AR	C312LL25b	8.67	9.33	2.67b	3.00ab	+
Proctor AR	C312a	10.00	10.67	6.67	7.33	-
Proctor AR	C312LL25b	8.67	9.00	3.33a	3.33ab	+
MS	C312a	7.33	7.67	8.33	9.00	-
MS	C312LL25b	9.67	10.00	2.00ab	2.00ab	+
LA	C312a	10.00	11.00	6.33	6.67	-
5LA	C312LL25b	9.00	9.67	1.00ab	1.00ab	+

CONCLUSIONS

One hundred and fifty seeds each of Coker 312 LL25 and the non-transgenic counterpart, collected on five different locations immediately upon harvest. Seed samples were divided into two seed lots of 75 seeds each. The four seed lots were either kept at room temperature, or treated overnight at 0°C (which is common practice for breaking dormancy of cotton seed). The seed lots were then submitted to a standard germination test and evaluated for germination and hypocotyl growth.

Breaking dormancy is not an absolute requirement for cotton seeds, and a cold treatment is sometimes used to increase the number of germinating seeds. The data show that the cold treatment of seed from two of the five locations resulted in reduced germination of the transgenic C312LL25 compared to that of the non transgenic C312.

Pollen DISSEMINATION STUDY

PROTOCOL

Trial name

01LL25SDINC

Location

Permit # 01-075-17n

Washington County, MS Soil type: very fine Boskett sandy loam

Objective

The purpose of this trial is to evaluate possible dissemination of Liberty® resistance via cross-pollination to a cultivated cotton variety of the same genetic background.

Trial design

A seed block consisting of twenty fifty-foot rows of transgenic C312 LL25 T_6 is planted with twelve rows of Coker 312 nontransgenic border rows of cotton planted on all four sides of the perimeter of the seed block. All rows are oriented in the same direction (East-West) to facilitate the movement of pollinators. The spacing between each row is approximately 1 meter (38 inches). So the size of the transgenic plot is approximately 15 x 20m (50 x 60 ft).

Agronomic Treatments:

Typical agronomic inputs for conventionally grown cotton for the area, including, but not limited to:

- Conventional herbicide treatments, both pre and post planting
- Fertilizer applications
- Necessary insecticide applications
- Growth regulator application if necessary

Trial Herbicide Treatment

Two applications of 28 oz Liberty® per acre, one application at the 7 leaf stage and another at 20% bloom on transgenic seed block, the pollen donor rows. Conventional herbicide regime was used for the non-transgenic, pollen receptor rows.

Data Requirement

Each plant within the transgenic seed block was tested via dPCR for the target transformation event, LLCotton25 and for the absence of non- target genes. Four plants from each perimeter border row on all four sides of the seed block will be tagged and harvested on a plant by plant basis. Plant samples represented distance from 1 to 12 meters in one meter increments from the pollen donor plots. Seed from the individual harvested plants will be evaluated by a rolled towel bioassay to identify the Liberty trait as described in Savoy and Berkley (2001) using Liberty® herbicide as the selective agent: 75 seeds from each perimeter plant, positive control, known Coker312LL25, and negative controls will be germinated in the presence of 0.3% Liberty® herbicide. The number of resistant and susceptible plants will be recorded using the length of the hypocotyl at the end of a 10-day period of germination to identify Liberty® resistant plants and susceptible plants.

Individual plants were harvested from non-transgenic border rows surrounding a transgenic (Liberty resistant) seed block. Starting from each corner of the seed block, one plant was harvested from each row in a perpendicular line moving away from the center of the transgenic seed block. The plants were named according to which corner they were nearest (NW, NE, SW, or SE) and numbered according to which row they were harvested from (1 being nearest the seed block and 12 being farthest). 38 inches between each row or approximately 1 meter.

The seed cotton from the individual plants was ginned and the seed was acid delinted. 50 seeds from each plant sampled from the field was placed on germinating paper that had been soaked in a 0.3 % Liberty solution for one hour. They were then covered with another sheet of paper that had also been soaked in the Liberty solution and rolled up. This was repeated so that there were three samples from each plant. Non-transgenic C312 was used as a negative control and LL25 C312 was used as a positive control by the same procedures.

Non-transgenic C312 was also used as a positive germination control by placing the seeds on germination paper soaked only in water.

The rolled seeds were placed in a plastic container with ventilation holes bored in the lid and put into a greenhouse. After seven days, the seedlings were evaluated for tolerance to Liberty. Germination rate and lengths of hypocotyls were recorded. Liberty tolerant seeds produce normal seedlings with all essential structures, although growth is usually inhibited by 25-30% compared to a standard germination study. Susceptible seedlings have a shortened hypocotyl-radicle length with characteristic black lesions on hypocotyls. Seed bioassay for resistance to Liberty herbicide

No evidence of pollen movement into the non-transgenic plants. The hypocotyl elongation observed is indicative of susceptible seedlings.

Means followed by a letter (a, b, ...) differ, by a 2-sided LSD, from the means of the check entry denoted by the same letter.

t (2-sided a = 0.010, 39 df) = 2.7079 LSD = 15.9014 MSE = 68.96581

		Number of seed which germinated		Mean hypocotyl length (cm)	CV	Significance
Distance From Transgenic Plot (m)	1	58		0.45 bc	9.3	+
	2	60		0.43 bc	4.8	+
	3	62		0.46 bc	7.5	+
	4	56		0.33 bc	80.3	+
	5	64		0.34 bc	66.8	+
	6	22		0.32 bc	66.9	+
	7	36		0.45 bc	24.8	+
	8	36		0.36 bc	69.0	+
	9	76		0.53 bc	31.1	+
	10	38		0.35 bc	76.2	+
	11	36		0.44 bc	12.0	+
	12	80		0.40 bc	18.8	+
Controls	C312 + Liberty		a	0.36 bc	5.3	+
	C312 + water		b	2.00 a	14.9	+
	C312LL25 + water		c	8.87 a	12.3	+

CONCLUSIONS

A dissemination study was set up by collecting seed from the border rows around a plot of LL Cotton 25. The transgenic plot was approximately 15 x 20m, surrounded by 12 rows of non-transgenic Coker 312, and the spacing between each row was 1 meter. In addition to a Liberty® application on the transgenic plot, transgenic and non-transgenic rows received the typical agronomic inputs for conventionally grown cotton for the area (conventional herbicide treatments, both pre and post planting - post planting only for the plot which did not receive a Liberty® herbicide treatment, fertilizer applications and necessary insecticide applications, growth regulators).

The seeds collected were germinated in the presence of 0.3% Liberty® herbicide, together with the appropriate positive and negative controls.

The data obtained show that all seeds collected on the border rows are as susceptible to Liberty® herbicide as the non-transgenic controls. It can be concluded that, under conventional agronomic inputs, no - or very low dissemination via pollen of the LibertyLink® trait has occurred in the 12-meter perimeter. This is in agreement with literature information: cotton behaves like a self-pollinated crop, and the amount of cross-pollination varies with the insect pollinator population. In typical cotton growing areas, use of insecticides to control insect pests will essentially eliminate cross-pollination by insects.

References

No	Doc No	Report No	Author(s). year. title. source. edition. pages.
No	Doc No	Report No	Savoy B.R., Berkley D.A. 2001. Beltwide Cotton Conferences. 390-391.

APPENDIX D

AGRONOMIC PARAMETERS DEFINED

Agronomic Parameters Defined

Listing of the parameters and description of the methods for measurement.

Boll retention	Number of bolls per plant divided by total fruiting positions, expressed as a percentage, an indication of fruiting efficiency. Fruit retention is an important component of yield.
Days to 50% open bolls	The number of days from planting to 50% open bolls indicates harvest ready, physiological crop maturity.
Days to bloom	The number of days from planting to first bloom is an indication of earliness as it relates to time for the plant to go into reproductive mode.
Days to first open boll	Days to first open boll are an important component of earliness that indicates the length of the boll period.
Disease ratings	A scale rating of susceptibility to disease pressure.
Fertility rating	A scale rating of pollen production and viability.
Fiber elongation %	Fiber elongation is a measure of the deformation of fiber at rupture expressed as percent change in length based on the original fiber length.
Fiber Length	Average length of the longer one-half of the fibers. Fiber length is largely determined by variety, but the cotton plant's exposure to extreme temperatures, water stress, or nutrient deficiencies may shorten the length.
Fiber length uniformity %	Length uniformity is the ratio between the mean length and the upper half mean length of the fibers expressed as a percentage. This is a measure of the natural variation in the length of cotton fibers.
Fiber Micronaire	A measure of fiber fineness and maturity as indicated by specific surface area. An airflow instrument is used to measure the

	air permeability of a constant mass of cotton fibers compressed to a fixed volume. Micronaire measurements (no units – the scale is quadratic) can be influenced during the growing period by environmental conditions such as moisture, temperature, sunlight, plant nutrients, and extremes in plant or boll population.
Fiber strength	The force in grams required to break a bundle of fibers one tex unit in size (1 tex = weight in grams of 1,000 meters of fiber). Fiber strength is largely determined by variety, but plant nutrient deficiency and weather may affect it.
Height to node ratio	Plant height divided by total number of nodes, an indication of plant morphology between plots and a measure of stress tolerance within plots. The accumulation of height and nodes as the season progresses can indicate the presence of yield-limiting factors.
Lint Percent	Lint weight divided by seed cotton weight, expressed as a percentage. This is an important yield component in cotton, where agronomically relevant yield refers to lint yield, not seed yield.
Node of first fruiting branch	The node of first fruiting branch indication of the physiological age of the plant when it begins reproductive mode. Variety, plant population and temperature influence this parameter.
Nodes above cracked boll	Number of reproductive nodes above a cracked (partially open) boll, an indication of determinancy. Cotton is a perennial crop by nature, and the number of reproductive nodes still present as the crop matures indicates tendency to crop termination.
Number first position bolls	Total number of bolls set on first positions of fruiting branches, an indication of yield potential. The highest percentage of yield by weight comes from first position bolls.
Number of seeds per boll	Average number of seeds per boll is an important component of yield for cotton (yield components = seed per acre, weight of fiber/seed). The number of ovules that

	are fertilized and develop into mature seed is an indication of pollination efficiency, most usually affected by heat.
Number of seeds per plant	An expression of yield component combining numbers of seed per boll and average boll retention.
Percent open bolls	Differences in percent open bolls at a given time are an indication of differences in crop maturity.
Plant height	Average plant height from cotyledonary node to terminal, expressed in inches; an indication of plant vigor. Height at a particular node number and the height increase for several nodes reflects the plant's reaction to stress. Stress associated with moisture, seedling disease, and nematodes, cool temperatures, salinity and soil compaction decrease plant height and decrease plant vigor.
Plant morphology rating (leaf, flower, bolls)	A scale rating of leaf, flower and boll type.
Seed index	Average weight in grams of 100 seed, an indication of seed size and maturity.
Seedling vigor	Visual rating of 1-4, 1 is best, rated at stand count.
Stand count 28 days after planting (plant / 11 g seed / 40 ft row)	An indication of germination efficiency, seedling vigor, and/or reaction to disease pressure. These measurements can be influenced by dormancy (commercial seed vs. seed coming from winter nursery).
Sympodia length	Length of fruiting branch from main stem node to first fruiting position is an indication of plant morphology. Variety as well as growth regulating factors can influence differences in sympodia length. Sympodia length is important to the efficient partitioning of carbohydrates to vegetative and reproductive growth.
Total number of nodes	Number of reproductive nodes present on the main stem of the plant. This is an indication of physiological age. Severe stress will only slightly reduce node development against heat unit accumulation, so node number is a reasonable estimate of physiological age.

Yield: Lbs. lint per acre	Productivity expressed as pounds of lint produced per acre.

Plant Mapping Methods

Sampling
- Representative of general field conditions
- Ten plants per plot, choose consecutive plants

Three plant stages for mapping

Pre-bloom
- cut plant before the cotyledonary node
- measure height in inches from the cotyledon to the terminal
- count the nodes above the cotyledon to the terminal

Bloom
- Select plants with white bloom on the first position of a fruiting branch
- cut plant before the cotyledonary node
- measure height in inches from the cotyledon to the terminal
- count the nodes above the cotyledon to the terminal
- at the first position of each node, note the presence of a square (immature bloom), white bloom, young boll or large boll

Mature boll
- Select plants with mature boll on the first position of a fruiting branch
- cut plant before the cotyledonary node
- measure height in inches from the cotyledon to the terminal
- count the nodes above the cotyledon to the terminal
- at the first position of each node, note the presence of a square (immature bloom), white bloom, young boll or large boll

APPENDIX E

LIBERTY AND ROUNDUP ULTRA EFFECTS ON FRUITING IN LIBERTYLINK AND ROUNDUP COTTON

Liberty and Roundup Ultra Effects on Fruiting in Liberty Link and Roundup Ready Cotton

Cotton yield is directly related to the number of bolls set on the plant as well as the quantity of seeds within these bolls. Good pollination of flowers is critical in order to insure adequate seed set and boll retention. When flowers are not adequately pollinated, the resulting boll may not be retained. A number of environmental factors as well as herbicide applications may affect pollination and thus boll retention. Studies are being conducted at North Carolina State University to investigate the effects of herbicide applications on pollination and fruit retention in transgenic Roundup Ready (RR) and Liberty Link (LL) cotton.

Pollen Viability

The quality and viability of pollen are crucial factors to achieve fertilization of ovaries resulting in seed formation.

According to the Roundup Ultra supplemental label for use on RR cotton, applications of 1 qt/A of Roundup Ultra may be applied before the 5-leaf growth stage (over the top) and again later in the season as a post-directed spray. Research has shown that these treatments can substantially reduce the viability of pollen as well as seed set (Table 1). Much of the pollen is arrested at various stages in development by Roundup and is malformed and not viable (Figure 1).

The application window for Liberty applications to Liberty Link cotton is still being developed, but it appears that pollen viability is not affected by Liberty at any of the tested application timings and methods (Table 2). Research in this area is still ongoing.

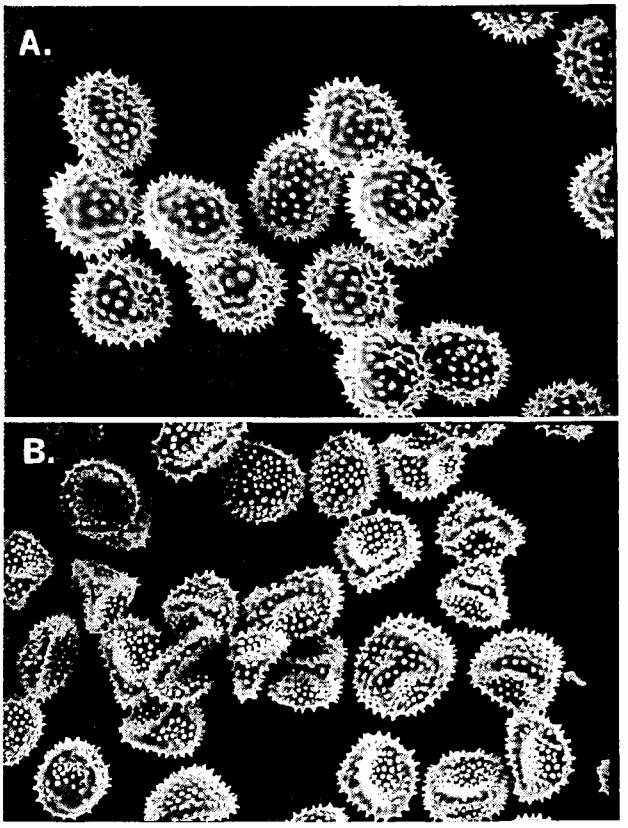

Figure 1. Roundup applications to RR cotton arrest pollen development before maturation resulting in malformed, collapsed pollen grains. A.) Pollen from non-treated DP 5415RR plant. B.) Pollen from DP 5415RR plant treated at the 4 leaf (over the top) and 8 leaf (post-directed) stages with 1 qt/A Roundup.

Floral Morphology

To insure proper pollination, the pollen containing anthers must come in contact with the stigma within the cotton flower.

Roundup applications to RR cotton can cause shortened anthers which increase the distance between the anthers and stigma (Figure 2). An increase in this distance can result in a reduction of pollen deposition and seed set in treated plants (Table 1).

Liberty applications of 34 oz/A do not seem to affect floral morphology in treated Liberty Link cotton (Table 2).

Table 1. Effect of glyphosate treatments and timings on fruiting characteristics of Roundup Ready Cotton.

Cultivar	Seeds/ boll		Abnormal pollen grains		Pollen Viability	
			% of total			
DP 50	36.6	a†	1.8	c	93.6	a
DP 90	33.1	ab	2.6	c	94.0	a
DP 5415RR	37.4	a	1.7	c	95.3	a
DP 5415RR Treated	21.6	d	54.7	a	57.4	b
SG 125RR	29.5	bc	3.5	c	91.6	a
SG 125RR Treated	25.6	cd	45.8	b	60.4	b

† Means within a column followed by the same letter in parentheses are not significantly different at the 0.05 probability level.

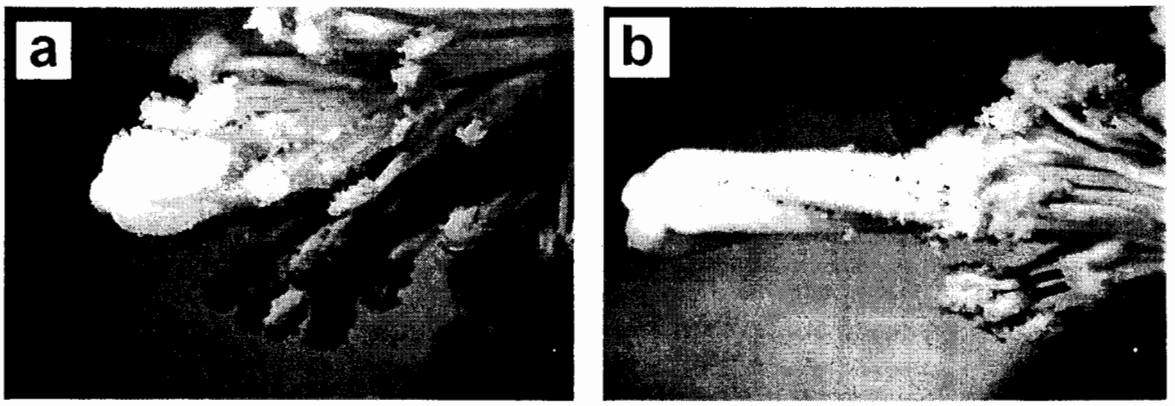

Figure 2. Effect of glyphosate on floral morphology in RR cotton. A. Non-treated B. Glyphosate-treated. Arrows indicate pollen coverage.

Table 2. Effect of glufosinate treatments and timings on fruiting characteristics of Liberty-Link Cotton*.

	Vegetative bolls	Vegetative squares	total bolls	1st position bolls	bolls (nodes 1-6)	Squares	Seeds/boll	Anther-stigma distance	Staminal column	Stigma height	Pollen viability
	Number							mm			%
Untreated	5.8	3.5	13.8	6.8	11.5	15.3	31.2	-0.7	17.1	24.7	96.7
4 POT	4.5	4.3	11.5	5.5	9.5	15.0	25.8	0.3	17.8	25.4	94.7
8 POT	3.8	3.3	13.5	7.5	11.8	13.5	29.0	0.8	17.1	25.5	96.3
4 POT + 8 POT	5.8	6.0	13.5	6.0	11.5	13.0	26.7	0.0	17.3	25.4	95.0
4 POT + 8 PD	1.5	2.3	12.5	6.5	10.8	15.0	24.7	0.1	16.5	24.0	93.1
	NS	NS	NS	NS	NS	NS	NS	1.1	NS	NS	NS

*Plants were mapped during the 4th week of flowering. Seeds per boll were counted on 1st position bolls from fruiting branches 1-6, but did not vary by fruiting branch. Floral morphology and pollen viability measurements were taken 2 times weekly for 4 weeks, but did not vary by week, thus data were combined over the 4 weeks. POT, Postemergence; PD, Postemergence-directed; 4, treatment at 4-leaf stage; 8, treatment at 8-leaf stage.

Glufosinate applications to LL cotton did not cause differences in fruiting characteristics compared to nontreated plants in any of the parameters investigated, with the exception of anther stigma distances. Anther-stigma distances in non-treated plants were 0.7 to 1.5 mm less than treated plants, but this distance would not be likely to cause differences in pollen deposition.

These data suggest that glufosinate treatments to LL cotton may be less injurious to reproductive development than glyphosate treatments to RR cotton because fruiting characteristics were not effected by glufosinate treatments. The study is currently being repeated in the phytotron.

Wendy Pline, John Wilcut, Keith Edmisten, and Randy Wells; North Carolina State University

APPENDIX F

REPRODUCTIVE COTTON DATA GLUFOSINATE-TOLERANT COTTON EVENT LL25

Title

Reproductive Biology Data
Glufosinate-tolerant Cotton Event LL25

Author

M. Freyssinet

Completed On

31 October 2001

Testing Facility

Aventis CropScience

La Dargoire Research Center
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CONFIDENTIALITY STATEMENT

This report is confidential. No part of the report or any information contained herein may be disclosed to any third party without the prior written authorisation of Hoechst Schering AgrEvo GmbH, Hoechst Schering AgrEvo SA, AgrEvo UK Limited, AgrEvo USA Company or their legal successors in the Aventis CropScience group.

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SUMMARY

Reproductive Biology Data (Event LL25)

Glufosinate-tolerant Cotton

The objective of this study was to compare the reproductive characteristics of transgenic, glufosinate-tolerant cotton event LL25 (T5) and its non-transgenic, non-tolerant counterpart (variety Coker 312-17) parental line.

Transgenic LL25 cottons and the non-transgenic counterpart variety Coker 312 were grown in the greenhouse in 2001. The plants were grown under conditions typical of normal greenhouse production practices for cotton, allowing pollen shed. The morphology of seeds and flowers were compared and the viability and germination frequency of the pollen grains were measured.

It was concluded that there are no differences in the morphology of seed, flowers and pollen betweeen the transgenic cotton event LL25 and the non-transgenic counterpart Coker 312. There is therefore unlikely to be any difference in reproductive behavior of the transgenic and non-transgenic cotton examined in this study.

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CONFIDENTIAL BUSINESS INFORMATION JUSTIFICATION

The information claimed as confidential within this application may fall into two categories, namely (1) the genotype/phenotype description and (2) commercial development information. The genotype/phenotype description category includes names and information about the recipient plant, the phenotype of the regulated article, vectors, mode of transformation, gene coding regions, associated regulatory sequences and expressed traits. Commercial development information includes the names and locations of cooperators, collaborators, investigators, and contacts.

This confidential business information justification is submitted by Bayer CropScience LP ("Bayer"). Bayer is the successor in interest of Aventis CropScience USA LP. Bayer is part of the worldwide Bayer CropScience group of companies which also includes Bayer BioScience N.V. (the former Aventis CropScience N.V., which was the former Plant Genetics Systems N.V.) and Bayer CropScience GmbH (the former Aventis CropScience GmbH, which was the former Hoechst Schering AgrEvo GmbH). All of these entities are referred to as Bayer CropScience in the statements given below.

GENOTYPE/PHENOTYPE DESCRIPTION

Central to the commercial value of Bayer CropScience' biotechnology products is the genetic information that confers the desired traits on the plant product, as well as the technical means by which the desired products have been achieved. Bayer CropScience has spent many person years in developing its expertise in the field of plant biotechnology, concurrent with the expenditure of millions of dollars on biotechnology research. In the rapidly growing and highly competitive industry of biotechnology products, Bayer CropScience has a leading edge.

Bayer CropScience has been working on the development of genetically enhanced plants, particularly those with herbicide tolerance, since the early 1980's and can document the large sums of money spent in research and testing costs. The uniqueness of Bayer CropScience' products lies in the transformation and regeneration methods and/or the combination of genetic components in the vectors transferred into the genomes of the recipient plants. The transformation and regeneration methods may be Bayer CropScience proprietary methods or available through licensing of other's proprietary methods. The genetic components in these vectors include the coding sequence for the expression of the trait(s), and regulatory sequences such as promoters, enhancers, introns, termination and polyadenylation sequences. In certain cases the recipient plant strain used is tantamount for regeneration and other desired features. Although the information on the transformation methods, recipient plant strains, or on each of these vector components may be in the public domain, the particular combination of the components put together by Bayer CropScience is unique and represents a great expenditure of time and money.

Competitors (which include Monsanto/DeKalb, Syngenta, DuPont/Pioneer, Dow Mycogen) of Bayer CropScience cannot presently duplicate Bayer CropScience's commercially valuable products without going through the painstaking process of trial and error development and testing of many different combinations of genetic information and plant strains. Access to genotype and/or phenotype description information, including the donor organisms and the recipient plant, for Bayer CropScience's products would allow competitors to create similar products that would result in a market share loss for Bayer CropScience of millions of dollars.

By performing simple copy work, these competitors would avoid the significant expenditure of dollars, research time and effort used by Bayer CropScience to develop its commercial products. Furthermore, the release of genotype and phenotype information would provide competitors with commercially valuable knowledge about particular products that Bayer CropScience is planning to commercialize and the likely timeframe for commercialization. Such information would be extremely useful to these companies in developing their own marketing and development strategies.

COMMERCIAL DEVELOPMENT INFORMATION

The disclosure of information about the names of cooperators, collaborators, investigators, research farm on-site personnel or contacts and the location and characteristics of the field experiments will provide Bayer CropScience's competitors with invaluable information about Bayer CropScience's marketing strategy, and could cause severe harm to Bayer CropScience's competitive standing in the industry.

In particular, release of the choice of cooperators and collaborators provides the competition with knowledge about the individuals and organizations that Bayer CropScience has found, through experience and investigation, to be most expert. Information on the location and characteristics of the field experiments will directly, or with little effort, provide the identity of the cooperators and collaborators. There is no doubt that competitors would seek to use the services of the entities found most expert by Bayer CropScience, and limit or block access of these sources. This could be accomplished by prices for services being increased, or by competitors acquiring exclusive licenses with these individuals and organizations, or by entering into contracts that would essentially tie up the time and facilities of such entities.

Maintaining the good will of the cooperators and collaborators is also a very important consideration for Bayer CropScience's success. The release of information that would directly or indirectly identify these entities could cost Bayer CropScience considerable good will and could cause the breach of an agreement with the entity concerned. This could lead to the loss of the entity as an expert source. If Bayer CropScience is forced to use alternative cooperators and collaborators, it would take time to identify high technical performance, and it would represent a loss of the valuable expertise and understanding built-up with former entities. This, in turn, could result in a delay in bringing products to market, which would cost Bayer CropScience sums into the millions of dollars.

Additionally, the disclosure of information about cooperators and collaborators would provide strong insights into Bayer CropScience's marketing strategy by revealing where Bayer CropScience is planning to introduce the products, and the schedule for such introduction.

Finally, all information deemed confidential is not known to others unless made available by appropriate secrecy agreements. Bayer CropScience takes the necessary precautions to prevent the intentional or unintentional disclosure to others of this information, supplemented with general site security system of gate guards, 24-hr security personnel, employee identification, limited access areas, escorts for visitors and restrictions for visitors, employee secrecy agreements, locked cabinets, files and data rooms, inside mail marked confidential and sealed as well as other security measures.