

**Petition for the Determination of Non-regulated Status for Event FG72**  
OECD Unique Identifier MST-FGØ72-3

The undersigned submits this petition under 7 CFR 340.6 to request that the Administrator make a determination that the article should not be Regulated under 7 CFR 340.

(b)(6)

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Contributors (MSTech):

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November 20, 2009

## COMPANY NAMES

On June 3, 2002, Bayer CropScience was formed by the acquisition of Aventis CropScience by Bayer AG. From that date, Bayer CropScience is the agricultural business unit of Bayer that is engaged in the research, development, and marketing of crop protection, seed technology, turf and ornamentals, professional pest and vector control, and home and garden products.

On December 15, 1999, Aventis S.A. was formed by the completion of the merger between Hoechst AG and Rhône-Poulenc S.A. Aventis CropScience was formed as part of a worldwide merger between Rhone-Poulenc S.A. and Hoechst AG. A portion of that merger created Aventis CropScience Holding S.A. that included interests from Hoechst AG and Schering AG. Hoechst AG and Schering AG were the parent companies of AgrEvo USA Company which were all merged into the Aventis companies.

Some of the activities described in this petition were undertaken before the merger and acquisition. Consequently, the names Aventis CropScience, AgrEvo USA Company, AgrEvo, and Hoechst Schering AgrEvo GmbH may appear throughout this petition.

M.S. Technologies, LLC, is an Iowa limited liability company, with offices at 103 Avenue D, West Point, Iowa 52656, U.S.A. The FG72 transformation event is owned by M.S. Technologies, LLC.

In November of 2007, M.S. Technologies, LLC and Bayer CropScience AG entered into an agreement for the joint development of herbicide tolerant soybeans, including the FG72 transformation event.

Some of the activities described in this report were undertaken in the context of the agreement between Bayer CropScience AG and M.S. Technologies, LLC. For example, some of the field activities, described in this petition were conducted by M.S. Technologies, LLC.

## RELEASE OF INFORMATION

The information in this petition is being submitted by Bayer CropScience and M.S. Technologies, LLC for review by the USDA as part of the regulatory process. By submitting this information, Bayer CropScience and M.S. Technologies, LLC do not authorize its release to any third party except to the extent it is requested under the Freedom of Information Act (FOIA), 5 U.S.C., Section 552 and 7 CFR 1, covering all or some of this information. Except in accordance with FOIA, Bayer CropScience and M.S. Technologies, LLC do not authorize the release, publication or other distribution of this information without Bayer CropScience's and M.S. Technologies' prior notice and consent.

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CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

**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 is unfavorable to the petition.

(b)(6)

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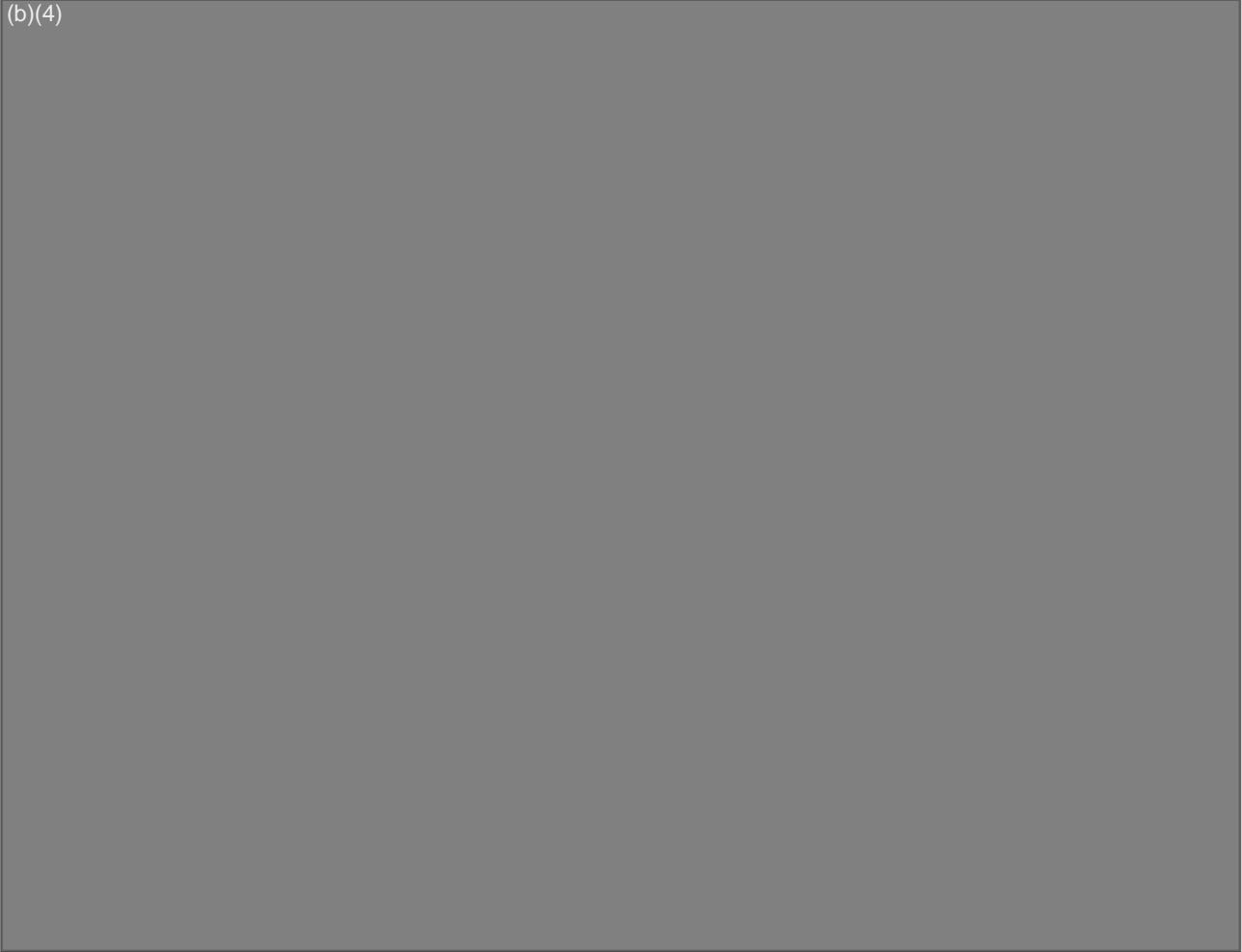
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## SUMMARY

Bayer CropScience (BCS) and M.S. Technologies, LLC (MSTech) are submitting a Petition for the Determination of Non-regulated Status under 7 CFR 340 to USDA Animal and Plant Health Inspection Service (APHIS) for double-herbicide-tolerant soybean event FG72 (Event FG72), any progeny, and crosses of this event with other non-regulated soybean lines.

(b)(4)

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presented herein demonstrate that FG72 soybeans: 1) exhibit no plant pathogenic properties; 2) are no more likely to become a weed than non-modified soybeans; 3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species; 4) do not cause damage to processed agricultural commodities; and 5) are unlikely to harm other organisms that are beneficial to agriculture.

## ACRONYMS AND SCIENTIFIC TERMS

ai	active ingredient	(b)(4)	
A	acre		
ADF	Acid Detergent Fiber	kg	kilogram
ANOVA	Analysis Of Variance	L	liter
APHIS	Animal and Plant Health Inspection Service	LB	Left Border
BCS	Bayer CropScience	lb	pound (1 pound = 0,454 kg)
BLASTP	Basic Local Alignment Search Tool	LC/MS	Liquid Chromatography/Mass Spectroscopy
BLASTx	BLAST search of protein databases using a translated nucleotide query	LD <sub>50</sub>	lethal dose for 50% of animals
BLOSUM	BLOcks SubstitUtion Matrix	LOQ	Limit of Quantitation
bp	base pairs	M	million
bu/ac	bushels/acre	mg	milligram
CAC	Codex Alimentarius Commission	mL	milliliter
DAD	DDBJ Amino acid sequence Database	µg	microgram
DDBJ	DNA Data Bank of Japan	NA	Not Applicable
dw	Dry weight	ng	nanogram
DNA	DeoxyriboNucleic Acid	ND	Not Detectable: Below the limit of detection
<i>E. coli</i>	<i>Escherichia coli</i>	NDF	Neutral Detergent Fiber
ELISA	Enzyme Linked Immunosorbent Assay	nm	nanometer
EMBOSS	European Molecular Biology Open	nt	nucleotide
(b)(4)		OECD	Organization for Economic Co operation and Development
(b)(4)			
		PDB	Protein DataBase
		PIR	Protein Identification Resources
		RAC	Raw Agricultural Commodity
		RB	Right Border
		RCB	Randomized complete block
		RBS	Ribosome Binding Site
		RR	Roundup Ready
		SD	Standard Deviation
		SDS-PAGE	Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis
		SGF	Simulated Gastric Fluid
		SIF	Simulated Intestinal Fluid
		SIM	Selected Ion Monitoring
		Subsp.	Subspecies
		T <sub>1</sub> , T <sub>2</sub> , etc	generations after T <sub>0</sub> (transformation)
		T-DNA	transfer DNA from Agrobacterium
		TDN	Total Digestible Nutrients
		TEP	Total Extractable Protein
		TrEMBL	Translated Sequences from the European Molecular Biology Laboratory Nucleotide Sequence Database
		US	United States of America
		USDA	United States Department of Agriculture
		WHO	World Health Organization
		wt	Wild type
		<i>Z. mays</i>	<i>Zea mays</i> , corn
FDA	United Nations Food and Drug Administration		
FGENESH	Find GENES using Hidden markov model		
FIFRA	Federal Insecticide Fungicide and Rodenticide Act		
FRAC	Fractionated Raw Agricultural Commodity		
fw	Fresh weight		
INCAP	Institution Of Nutrition Of Central America And Panama		
g	gram		
GetORF	EMBOSS database for ORFs		
<i>G. max</i>	<i>Glycine max</i>		
GM	Genetically Modified		
(b)(4)			
HRP	Horseradish Peroxidase		
ID	identification		

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## **I. RATIONALE**

### **I.A. Basis for the Request for Determination of Non-regulated status**

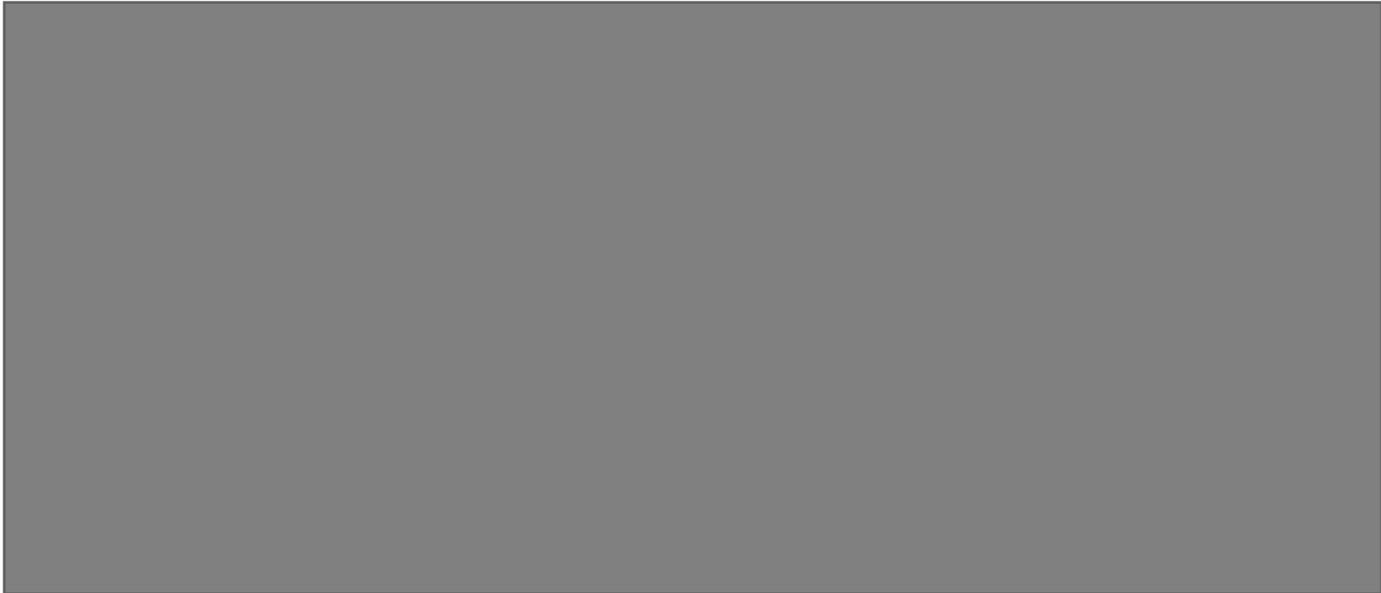
The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Services (APHIS) is responsible for protection of the US agricultural infrastructure against noxious pests and weeds. Under the Plant Protection Act (7 USC § 7701-7772) APHIS considers plants altered or produced by genetic engineering as restricted article under 7 CFR 340 which cannot be released into the environment without appropriate approvals. APHIS provides that petitions may be filed under 7 CFR §340.6 to evaluate data to determine that a particular regulated article does not present a risk as a noxious pest or weed to agricultural infrastructure. Should APHIS determine that the submitted article does not present a plant pest risk, the article may be deregulated and released without further restrictions.

### **I.B. Double-herbicide-tolerant soybean event FG72**

Bayer CropScience (BCS) and M.S.Technologies, LLC (MSTech) have developed double-herbicide-tolerant soybean event FG72 which produces the 2mEPSPS and HPPD W336 proteins which confer tolerance to the herbicides glyphosate and isoxaflutole (IFT), respectively. The combination of the two herbicide tolerances in a single plant provides an effective, broad spectrum weed control option using glyphosate and IFT.

### **I.C. Rationale for the development of event FG72 and benefits**





**I.D. Adoption of event FG72**

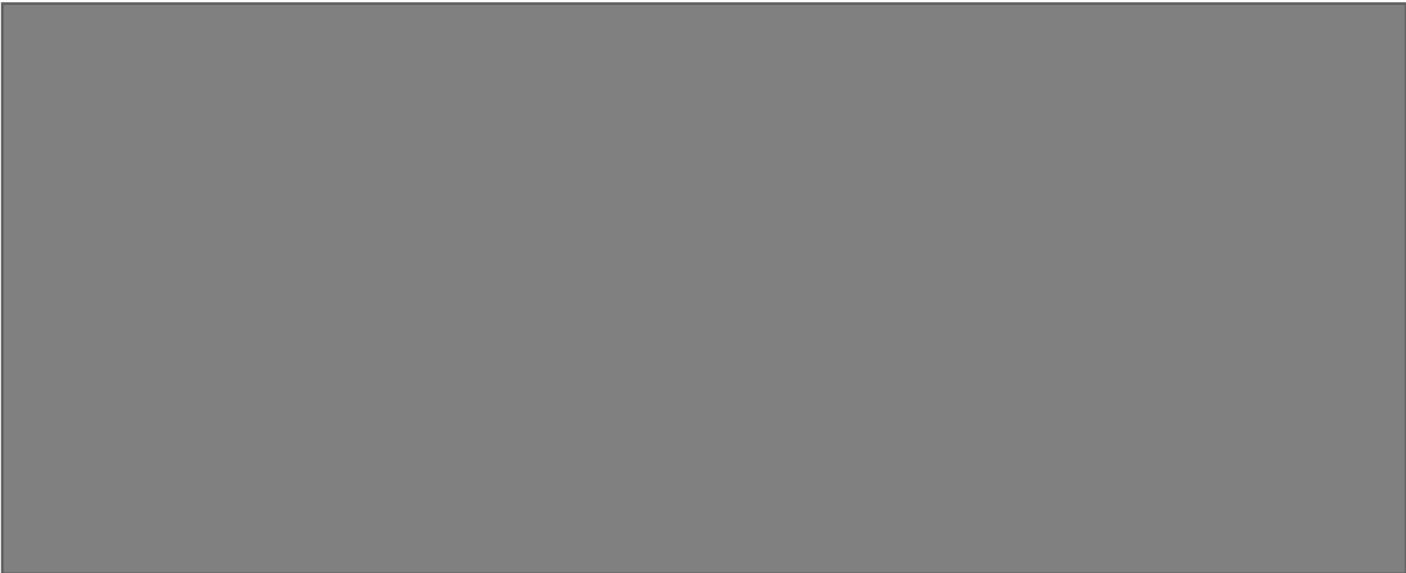
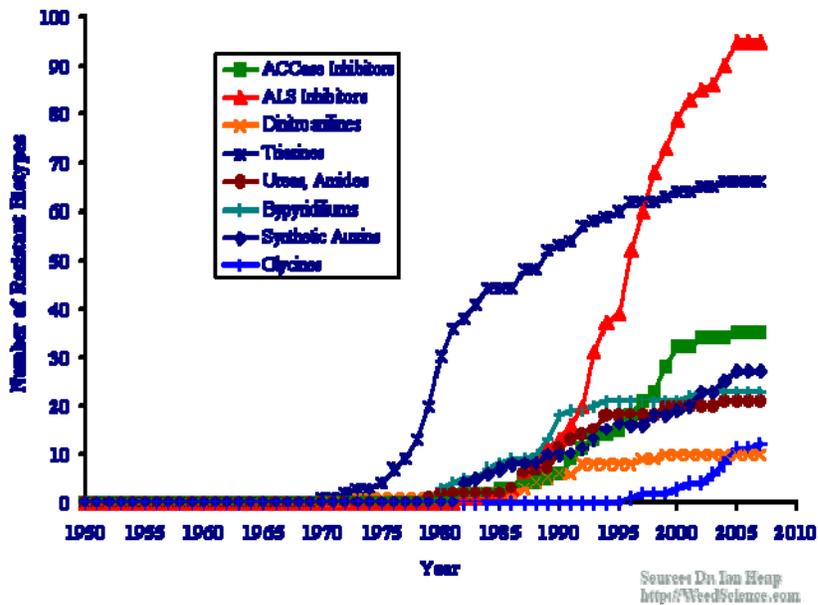


Figure 1. Rise in glyphosate resistance among different biotypes



#### I.E. Submissions to other regulatory agencies

##### Food and Drug Administration

FG72 soybean is within the scope of the 1992 US FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (FDA, 1992). In compliance with this policy, BCS and MSTech will submit a food and feed safety and nutritional assessment summary for FG72 soybean to the US FDA.

##### Foreign Governments

BCS and MSTech intend to submit dossiers to request import of FG72 soybean to the proper regulatory authorities of foreign governments that have regulatory processes in place. These may include submissions to the relevant Regulatory Authorities in Canada, Mexico, EU, Japan, China and others. FG72 soybean has been, or is currently, in field trials in soybean growing regions around the world.

## II. THE BIOLOGY OF SOYBEAN

### II.A. Biology of soybean

The scientific name of soybean is *Glycine max* L. The genus *Glycine* is classified under the tribe *Phaseoleae*, subfamily *Papilionoideae*, and the family *Leguminosae* (*Fabaceae*).

The OECD consensus document (OECD, 2000) and the CFIA biology document (CFIA, 1996) provide information pertaining to the following aspects of soybean biology:

- General description, including taxonomy and morphology and use as a crop plant;
- Agronomic practices;
- Centers of origin of the species;
- Reproductive biology;
- Cultivated *Glycine max* as a volunteer weed;
- Inter-species/genus crosses, introgression into relatives
- Interactions with other organisms;
- Summary of the ecology of *Glycine max*.

### II.B. Characteristics of the recipient soybean cultivar

The publicly available cultivar, Jack, was used as the recipient line for the generation of soybean event FG72. The variety was originally developed at the Illinois Agricultural Experimental Station and commercially released in 1989 (Nickell *et al.*, 1990). Jack is classified as maturity group II and is best adapted to approximately 40 to 42 degrees of Northern latitude. It has white flowers, gray pubescence, brown pods at maturity, and seeds with dull yellow coat and yellow hila. Jack was developed and released because of its resistance to soybean cyst nematode (Races 3 and 4) and higher yield when compared with cultivars of similar maturity. It is susceptible to *Phytophthora sp.* rot (Races 1, 4, and 7).

Jack is extensively used in soybean transformation because of its high embryogenic capacity (Stewart *et al.*, 1996; Santarem *et al.*, 1998; Yan *et al.*, 2000). Somatic embryos can be induced from immature cotyledons, proliferated, and maintained in liquid medium until transformation.

### III. DEVELOPMENT OF DOUBLE-HERBICIDE-TOLERANT SOYBEAN EVENT FG72

#### III.A. Description of the transformation system



#### III.B. Parent line



#### III.C. Breeding Diagram

The breeding diagram of soybean event FG72 is shown in Figure 2.

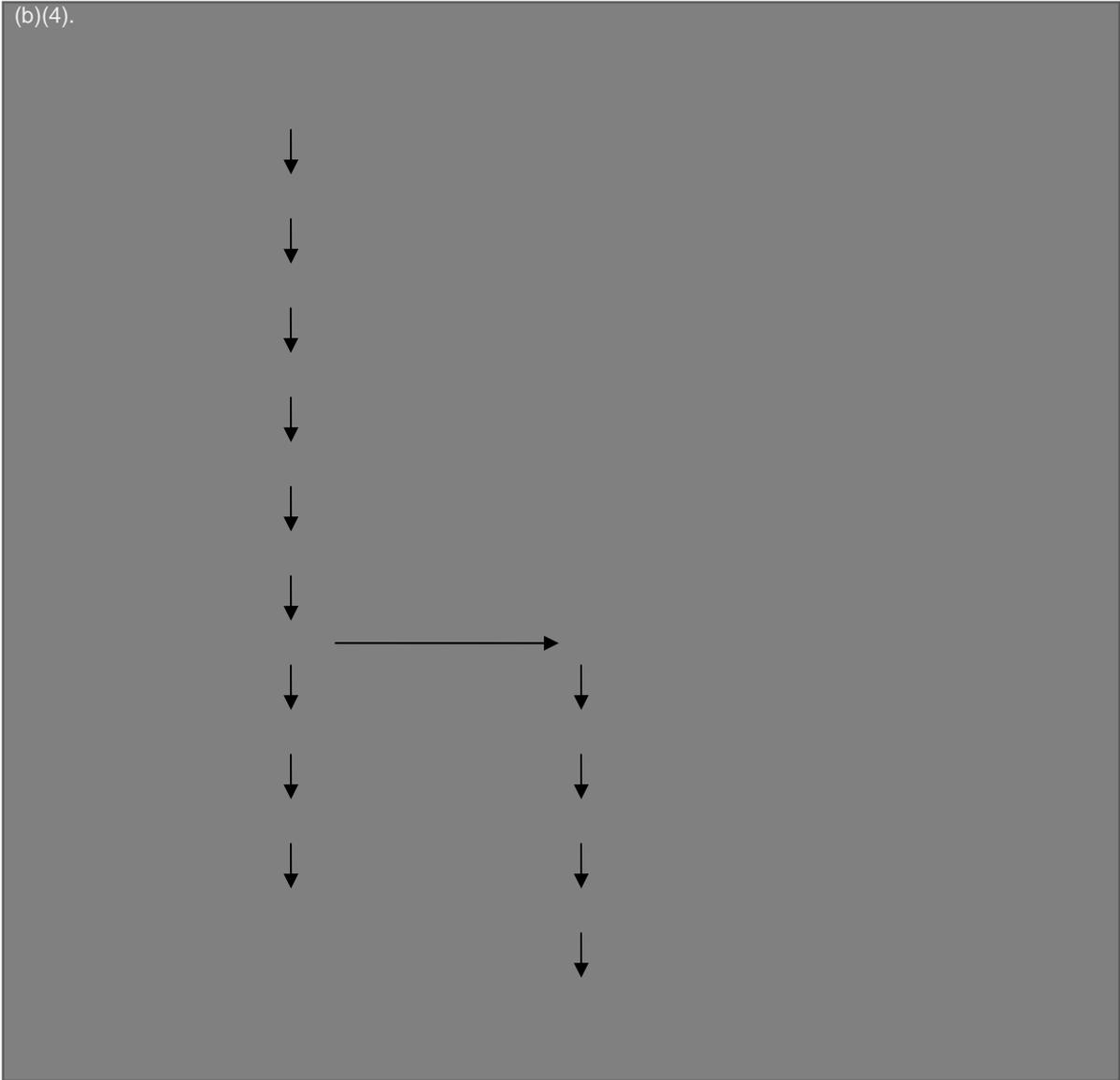
#### III.D. Generations Used for Analysis

The generations used for the studies to analyze soybean event FG72 are described in Table 1.

**Table 1. Generations used for analysis of event FG72**

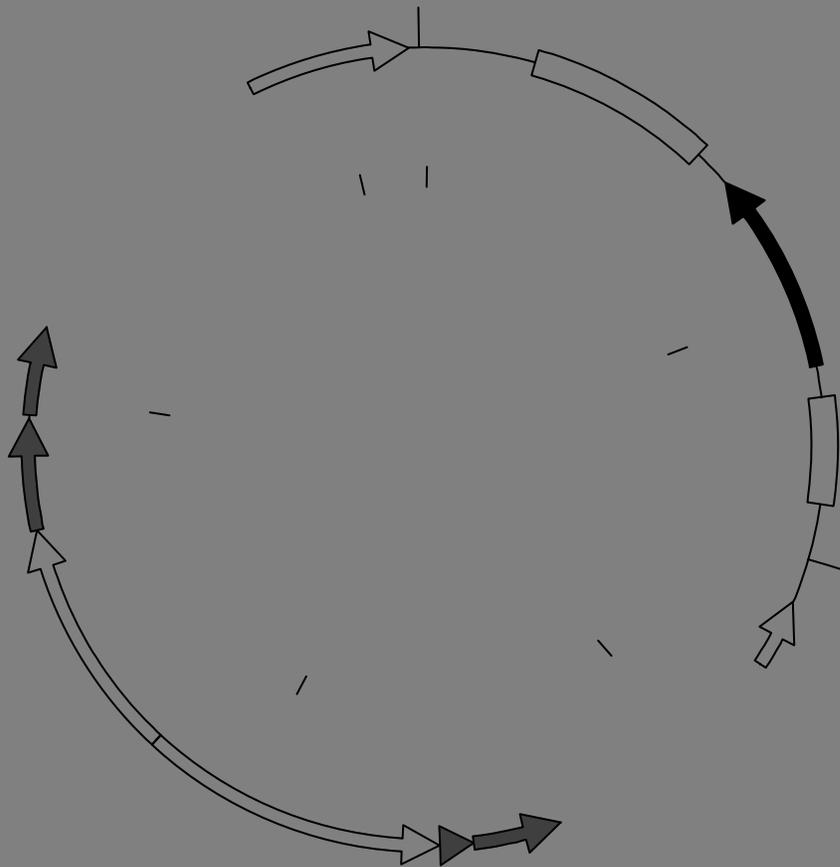
Generation	Study
	

**Figure 2. Breeding Diagram of event FG72**



#### IV. GENETIC MATERIAL USED FOR TRANSFORMATION OF EVENT FG72

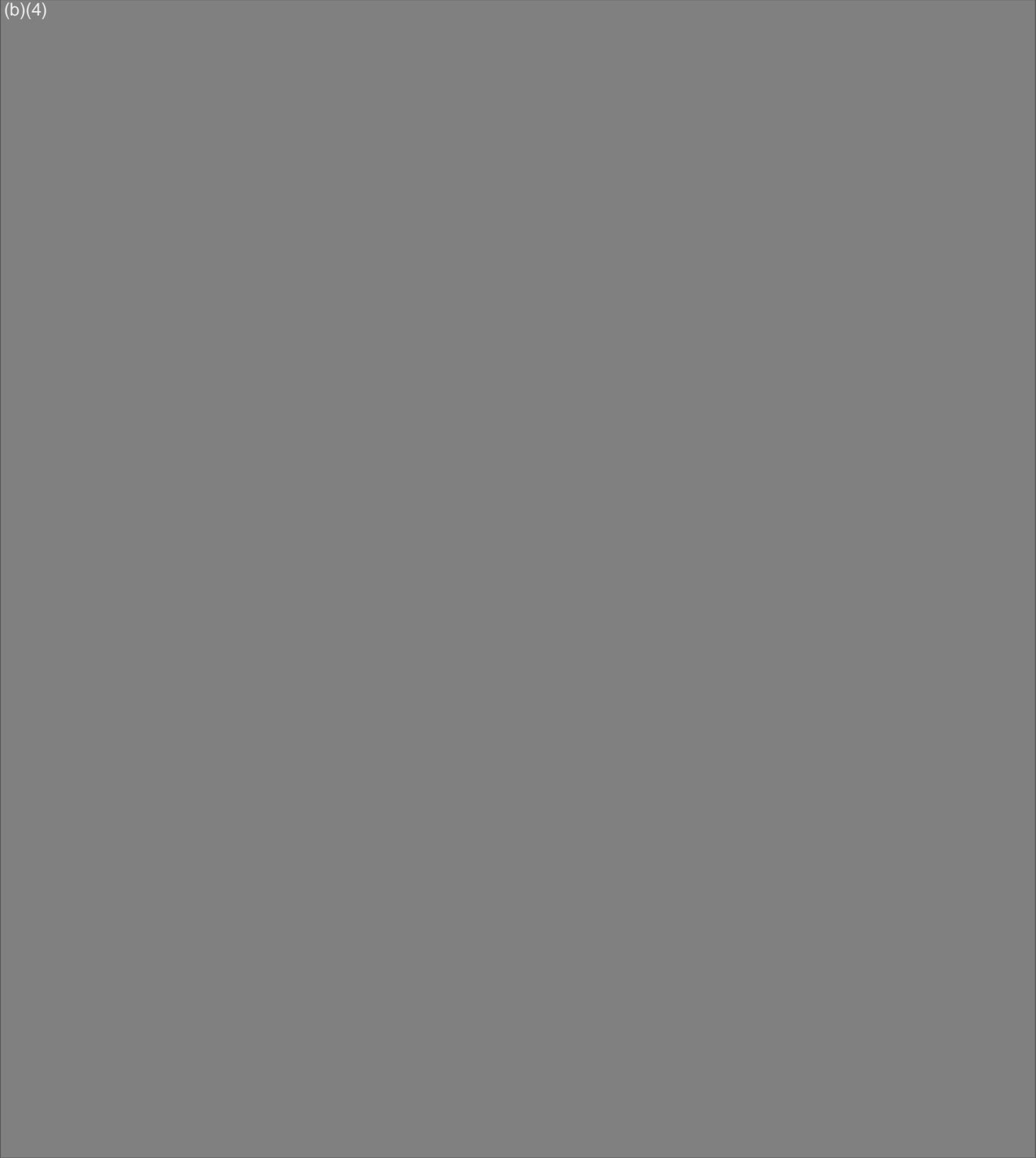
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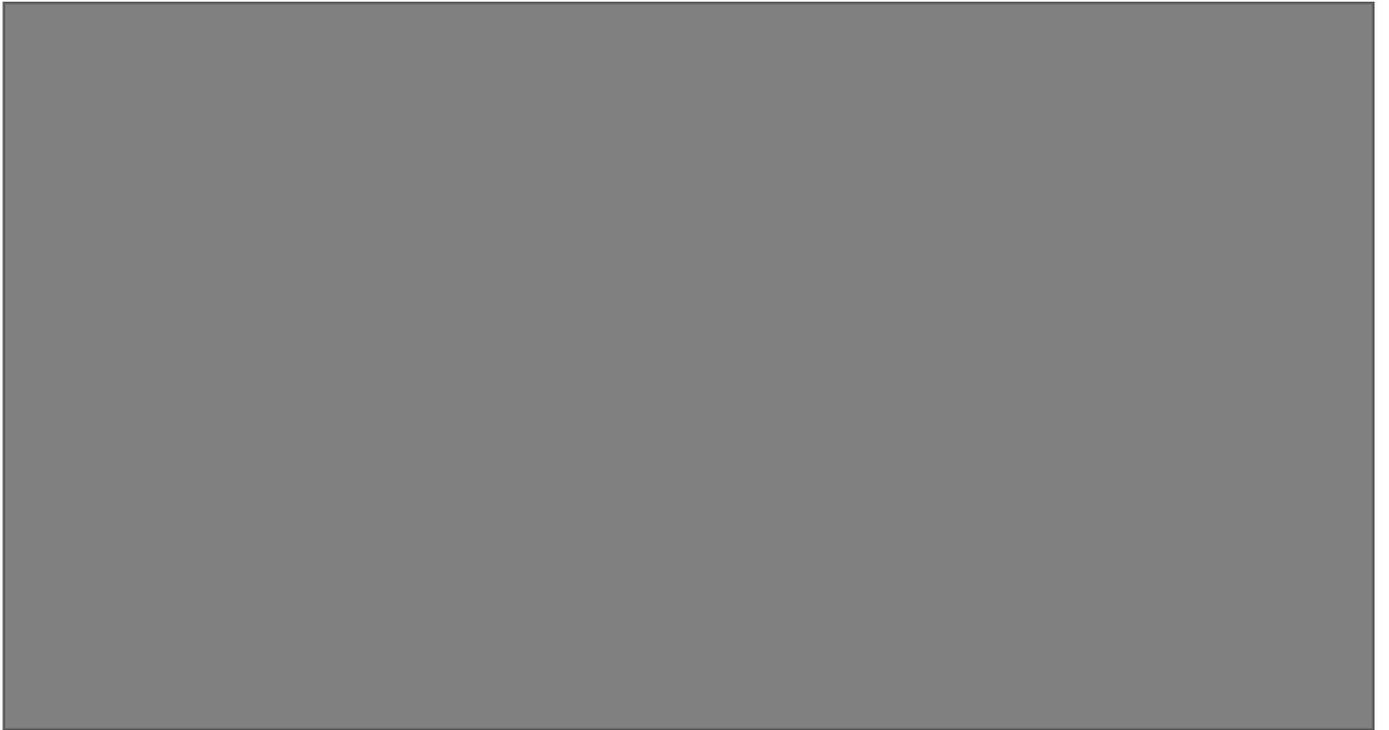


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**IV.B. Donor genes and associated regulatory regions**

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**IV.C. Identity and source of the genetic material**



**Table 2. Genetic elements located on insert**

Nt Positions	Orientation	Origin
		

**Table 2.**

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**V. GENETIC CHARACTERIZATION OF EVENT FG72**

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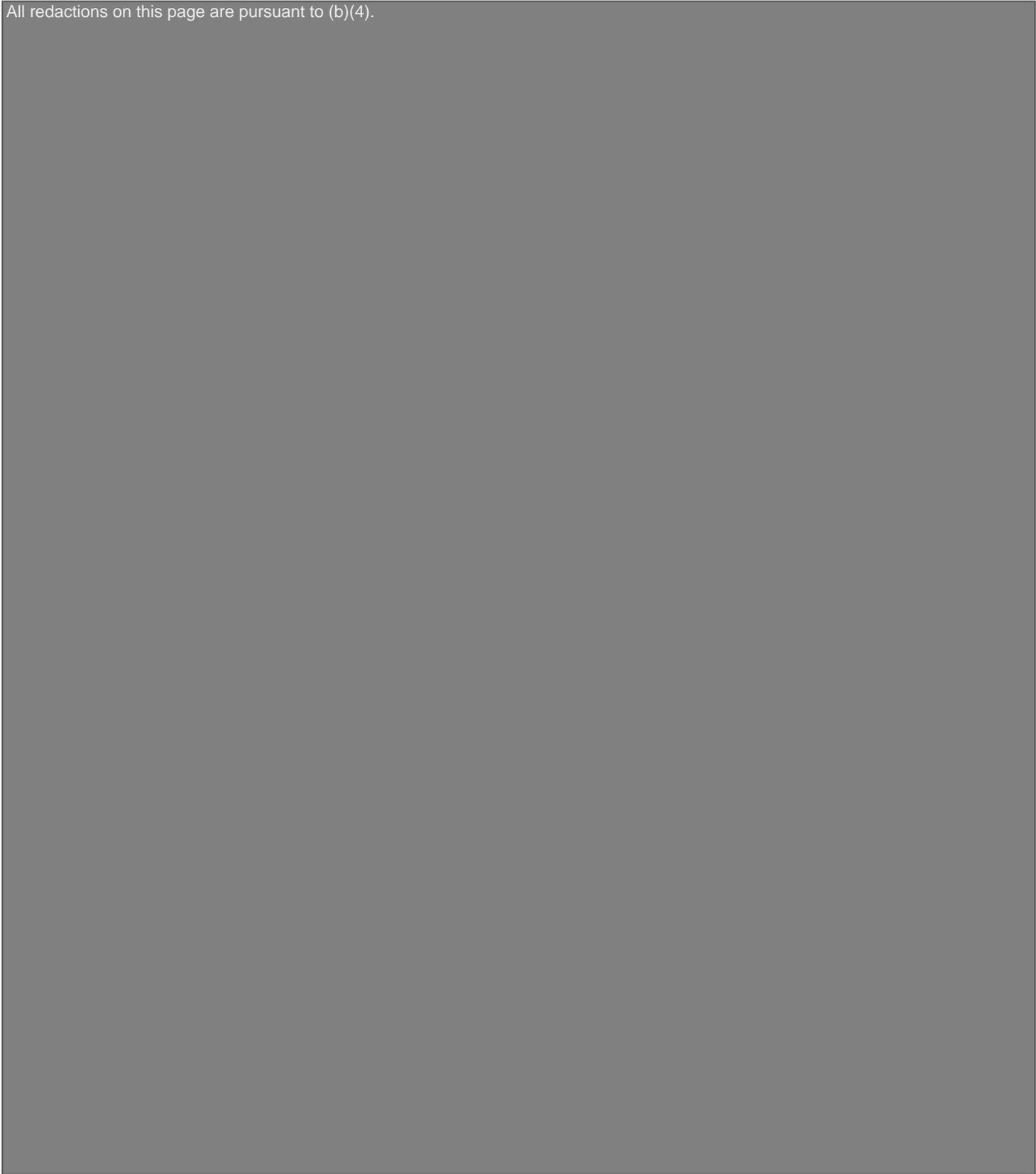
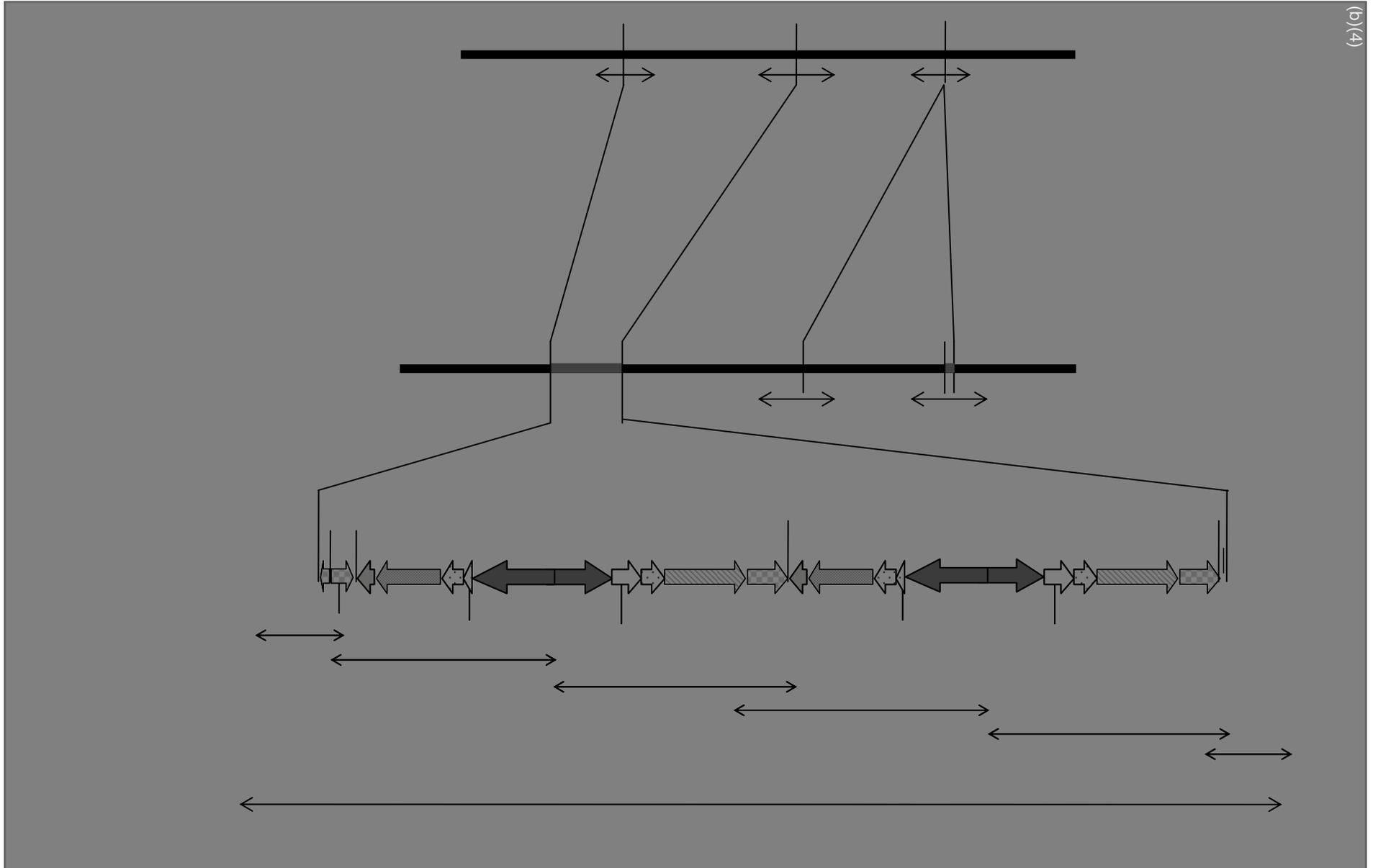


Figure 4. Event FG72 insert diagram



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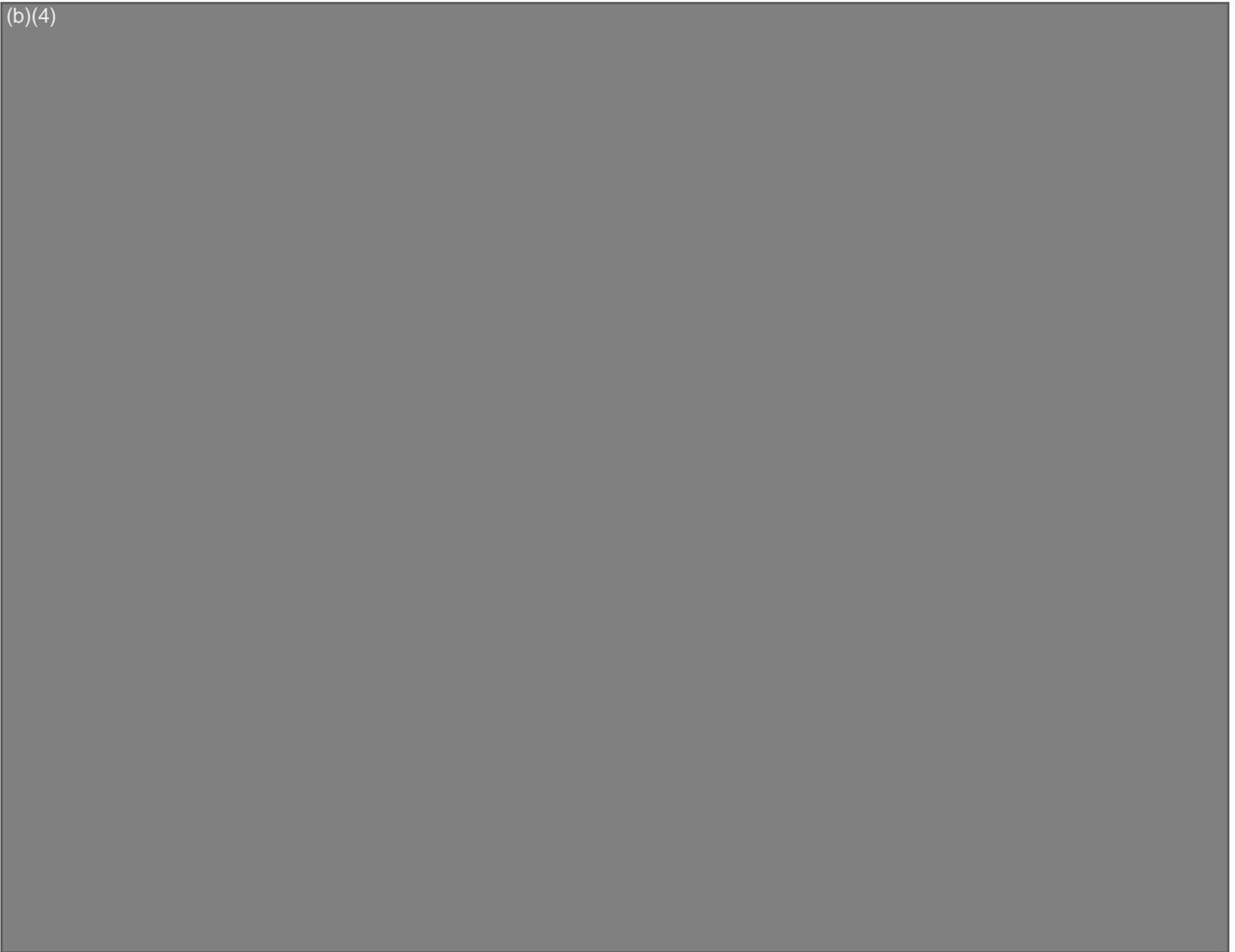
**V.D. The flanking regions of the inserted sequence(s)**

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**V.E. Mendelian inheritance**

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**Table 3**

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**Table 4.**

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**V.F. Stability across and within generations**

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**V.G. Conclusion**



**VI. CHARACTERIZATION OF THE INTRODUCED PROTEINS**

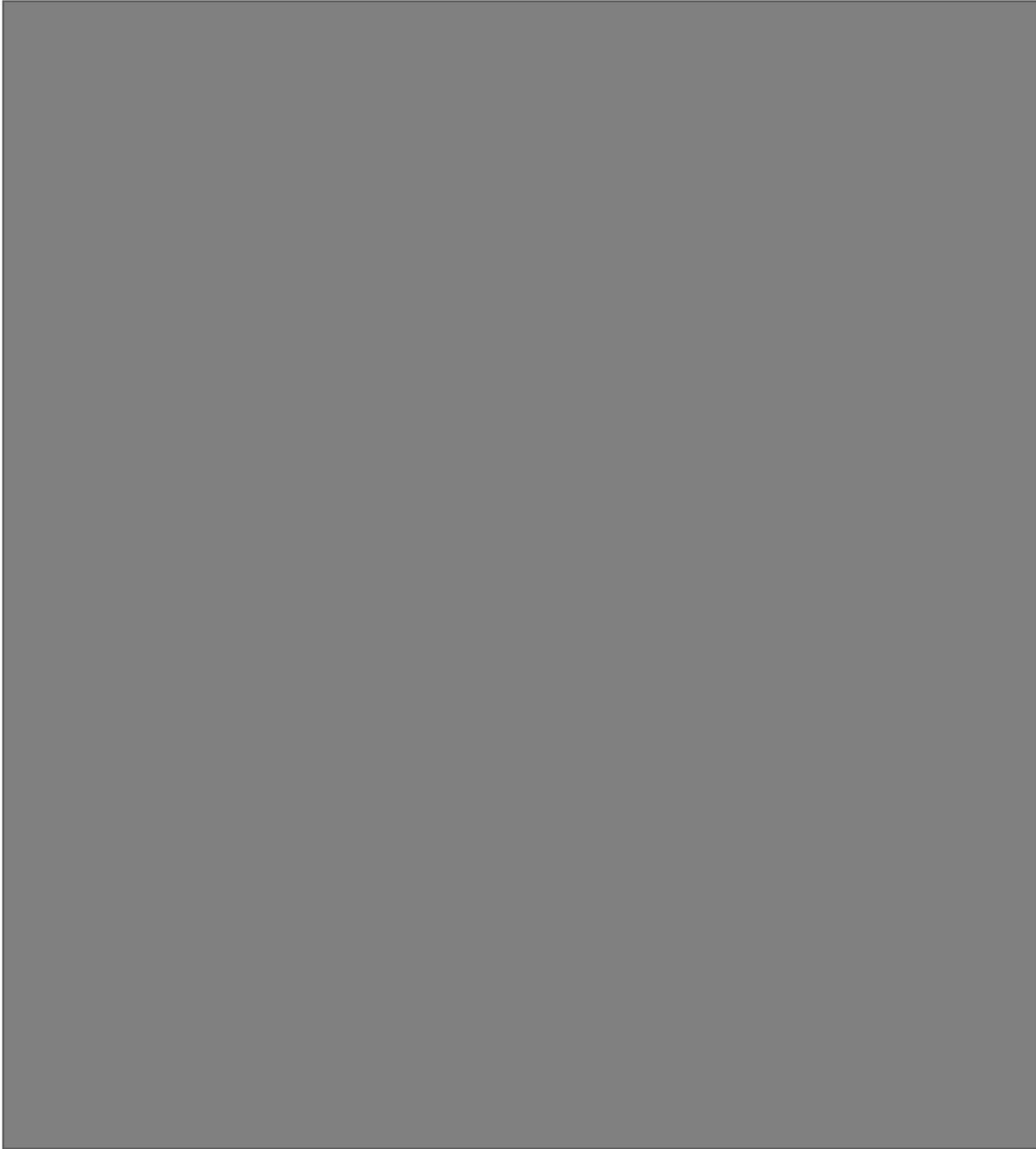
**VI.A.** 

**VI.A.1. History and Background**

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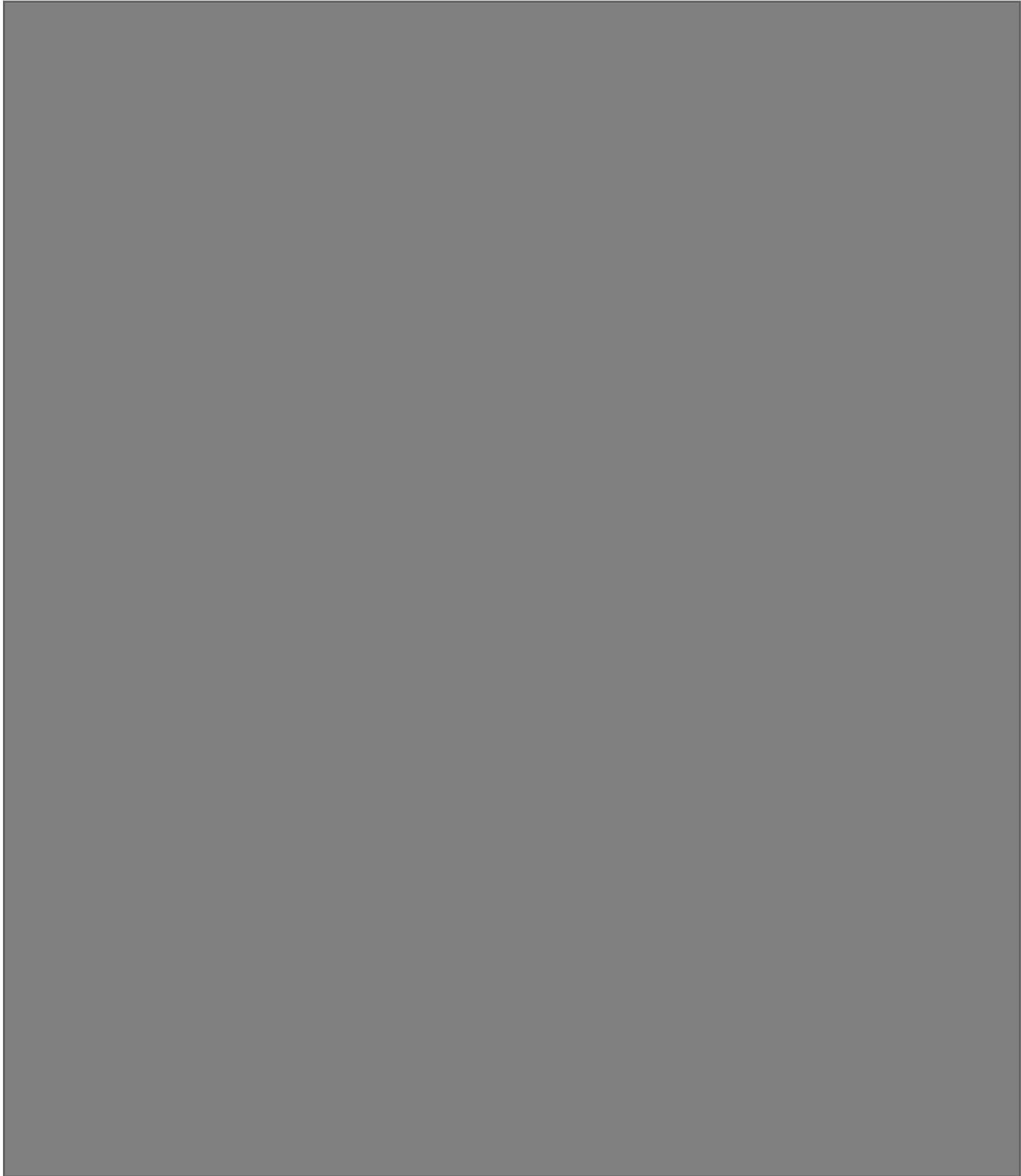
**VI.A.2.** 

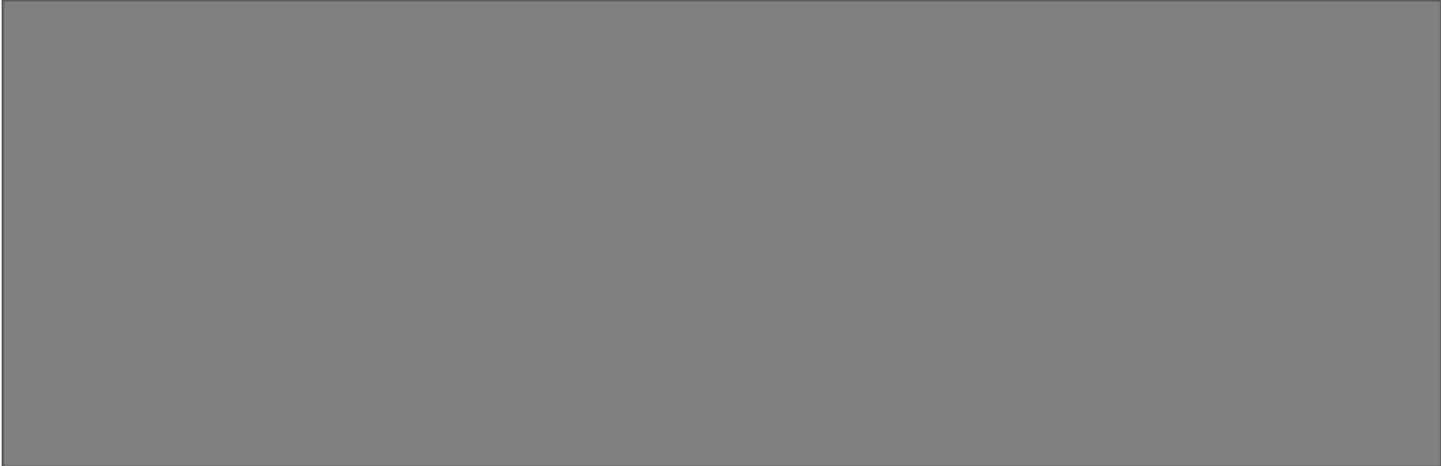
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History of safe use







**VI.B.**

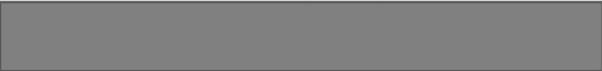


**VI.B.1. History and background**



**VI.B.2. The function of the gene product**

**VI.B.2.1.**



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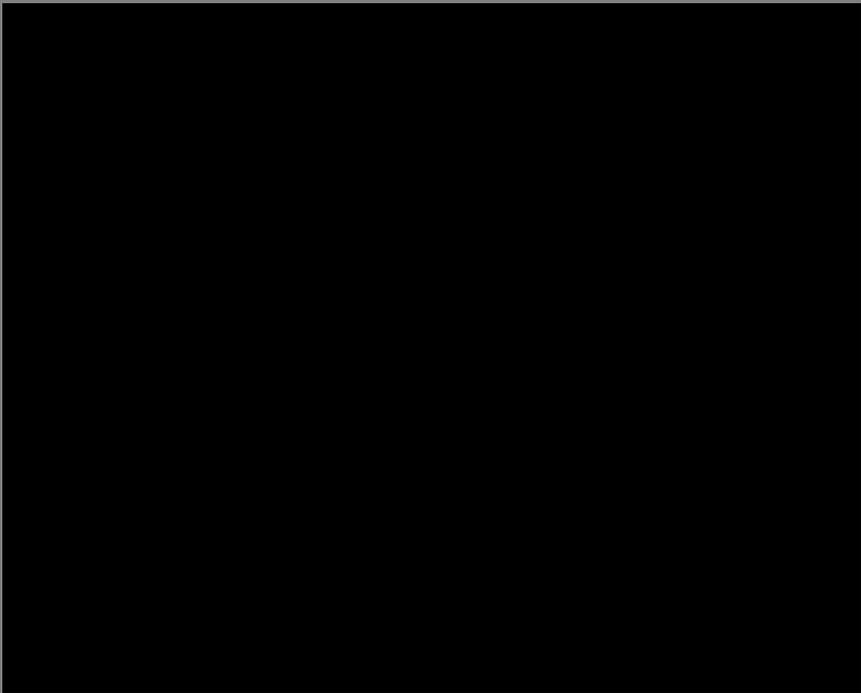
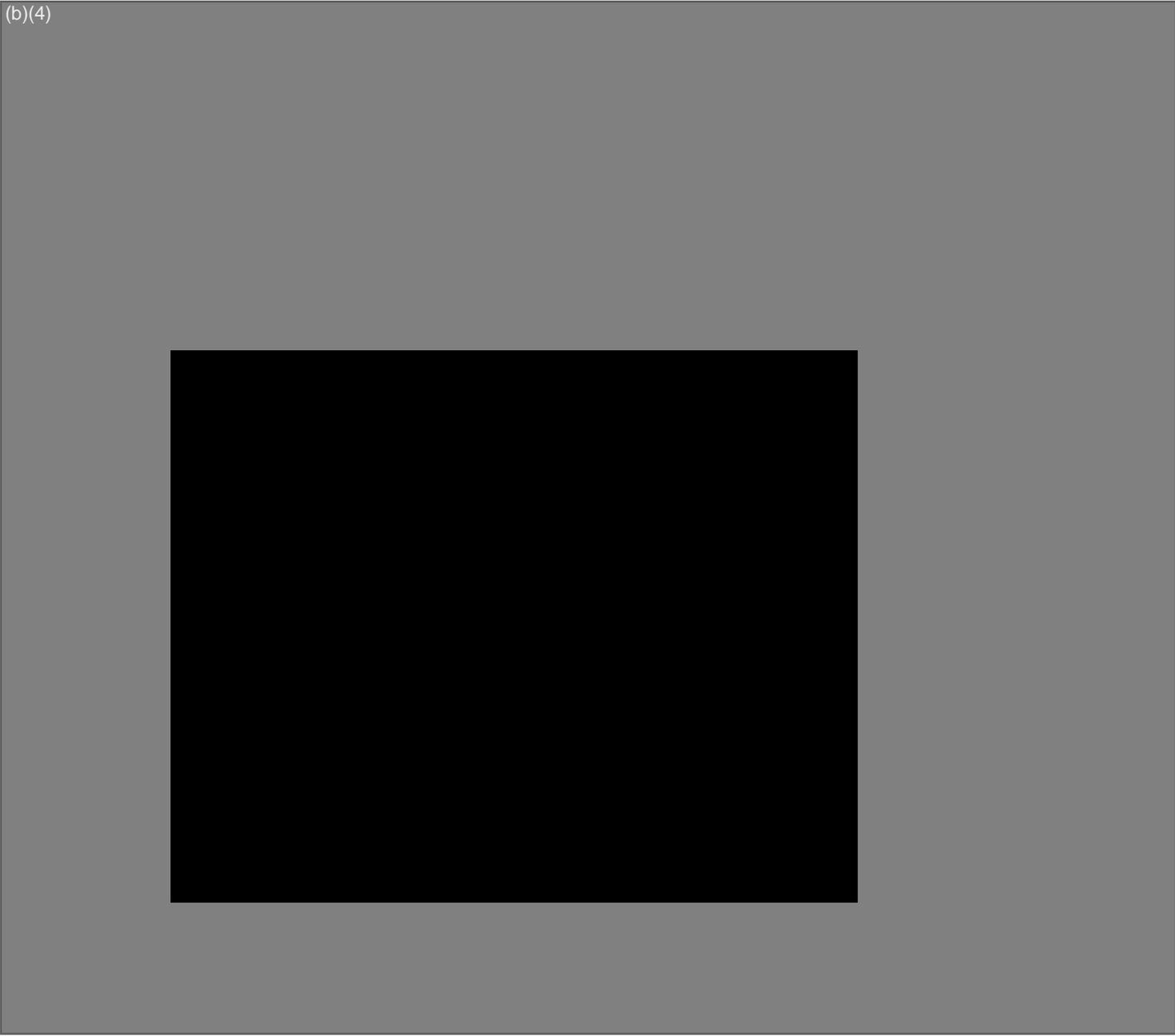
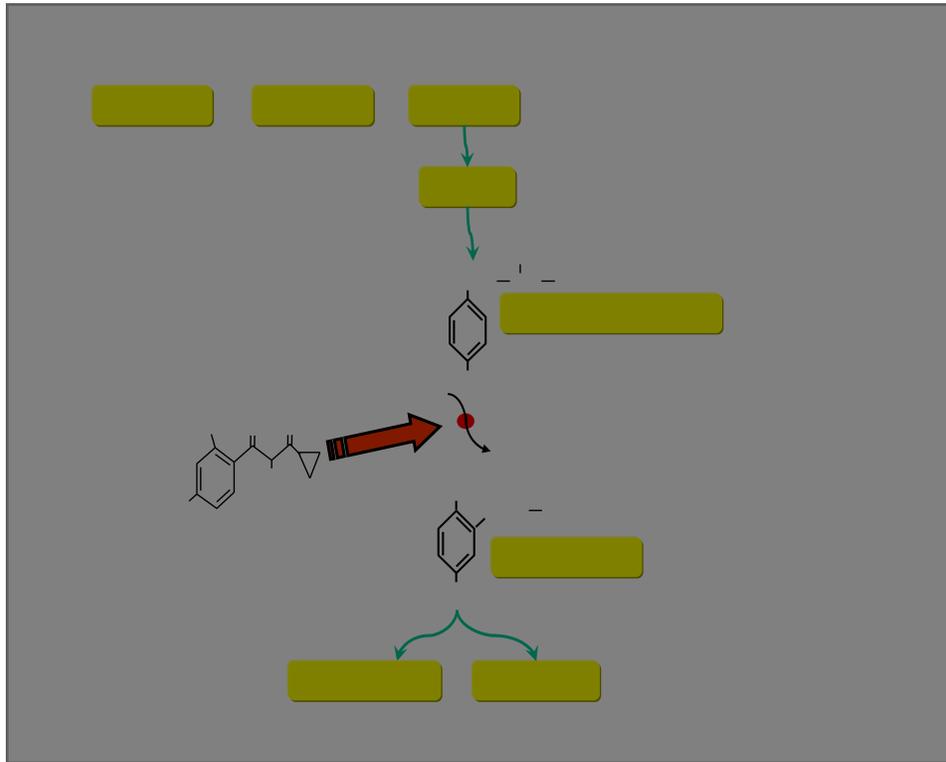
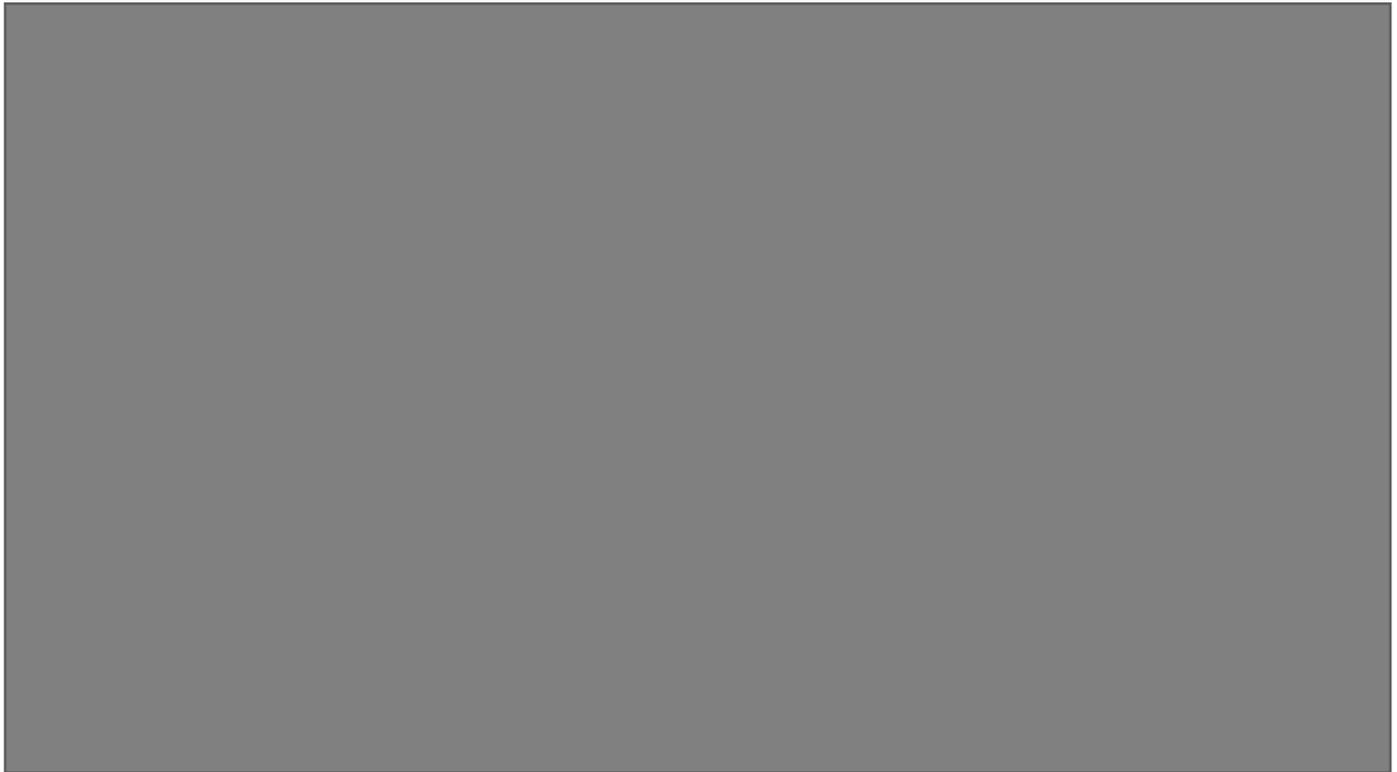


Figure 6.



VI.B.2.2. Source of the gene



### VI.B.2.3. History of safe use of the source organism

#### Risk Group Classification

*P. fluorescens* (*Pf*) strains are generally classified as non-pathogenic bacteria in several national classifications for microorganisms (Table 5).

**Table 5. Risk group classification of *P. fluorescens***

<b>USA</b>	Not classified. <a href="http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm">http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm</a> , accessed on March 02, 2009.
<b>Canada</b>	Non-pathogenic organism. <a href="http://www.phac-aspc.gc.ca/ols-bsl/pathogen/organism-eng.php">http://www.phac-aspc.gc.ca/ols-bsl/pathogen/organism-eng.php</a> , accessed on March 02, 2009.
<b>European Union</b>	Not classified. Directive 2000/54/EC
<b>Belgium</b>	Risk Group 2 plant pathogen. Belgian Monitor 01.04.2004 18362-18442. 2004
<b>Switzerland</b>	1 + opportunistic pathogen. <a href="http://www.bafu.admin.ch/publikationen/index.html?action=show_publ&amp;lang=de&amp;id_t_hema=6&amp;series=VU&amp;nr_publ=4401">http://www.bafu.admin.ch/publikationen/index.html?action=show_publ&amp;lang=de&amp;id_t_hema=6&amp;series=VU&amp;nr_publ=4401</a> ; accessed on March 02, 2009.
<b>France</b>	Not classified. Commission de Genie Génétique
<b>Germany</b>	Risk Group 1 + - opportunistic pathogen. Classification of bacteria and archaea bacteria into risk groups – TRBA 466. 2005

#### Pathogenicity to humans

*P. fluorescens* can be an opportunistic pathogen in immunocompromised patients (McKellar, 1982). Some cases of septicemia have been reported due to *P. fluorescens* contamination of transfused blood and blood products, given its ability to grow at 5°C (Gibb *et al.*, 1995, Puckett *et al.*, 1992). Some *P. fluorescens* strains were also reported to create biofilms on compounded sterile products like catheters and have led to rare infections in immunocompromised populations (Gershman *et al.*, 2008). However, the general virulence of *P. fluorescens* is low, due to its inability to multiply rapidly at body temperature and having to compete with defense mechanisms of the host (Liu, 1964).

#### Pathogenicity to animals

*P. fluorescens* can infect a wide range of animals including horses, chickens, marine turtles, and many fish and invertebrate species. However, since it is unable to grow at elevated temperatures, it is probably only an opportunistic pathogen for warm-blooded animals (OECD, 1997).

#### Pathogenicity to plants

Generally *P. fluorescens* is considered saprophytic but it may be an opportunistic pathogen causing soft rot in plants (OECD, 1997).

### Allergenicity

In general fluorescent pseudomonads have not been described as allergens. However, they do possess an endotoxin (lipopolysaccharide) which may induce an allergic response in some individuals (OECD, 1997).

### History of safe use

*P. fluorescens* is a ubiquitous bacterium frequently present in water, soil and the plant rhizosphere (Bossis *et al.*, 2000). It can be isolated from water, animals, human clinical specimens, the hospital environment, and spoiled foodstuffs such as fish and meat. The survival of *P. fluorescens* is affected by number of biotic and abiotic factors such as soil density, temperature, pH, humidity (OECD, 1997).

*P. fluorescens* is used in agriculture as growth-promoting agent (Fließbach *et al.*, 2009; OECD, 1997). It can enhance plant growth through production of siderophores, which efficiently complex environmental iron, making it unavailable to other components of the soil microflora. In addition, *P. fluorescens* is used as a biopesticide on certain crops and fruits to prevent the growth of frost-forming bacteria on leaves and blossoms (Compant *et al.*, 2005; Raaijmakers *et al.*, 2006; US-EPA, 2008a). It is also used as seed treatment agent for damping off diseases caused by fungi (Haas and Defago, 2005; Thrane *et al.*, 2001; Voisard *et al.*, 1989) and nematodes (Hamid *et al.*, 2003). This pesticide activity of *P. fluorescens* is attributed to three mechanisms: competition for an ecological niche or a substrate, production of inhibitory chemicals, and induction of systemic resistance in host plants to a broad spectrum of pathogens (Compant *et al.*, 2000; Haas and Defago, 2005).

Naturally occurring strains of *P. fluorescens* have been registered commercially for the control of frost injury and fire blight on pear (Wilson and Lindow, 1993). Since 1992, 4 end products containing *P. fluorescens* strains as active ingredients have been approved by US-EPA (US-EPA, 2008b). US-EPA has recognized that this bacterial active ingredient is not expected to have any adverse health effects on humans, based on various studies that found no evidence that these *P. fluorescens* strains are harmful to mammals (US-EPA, 2008a). In addition, US-EPA has established a tolerance exemption for residues of *P. fluorescens* in or on raw agricultural commodity mushrooms (US-EPA, 1994).

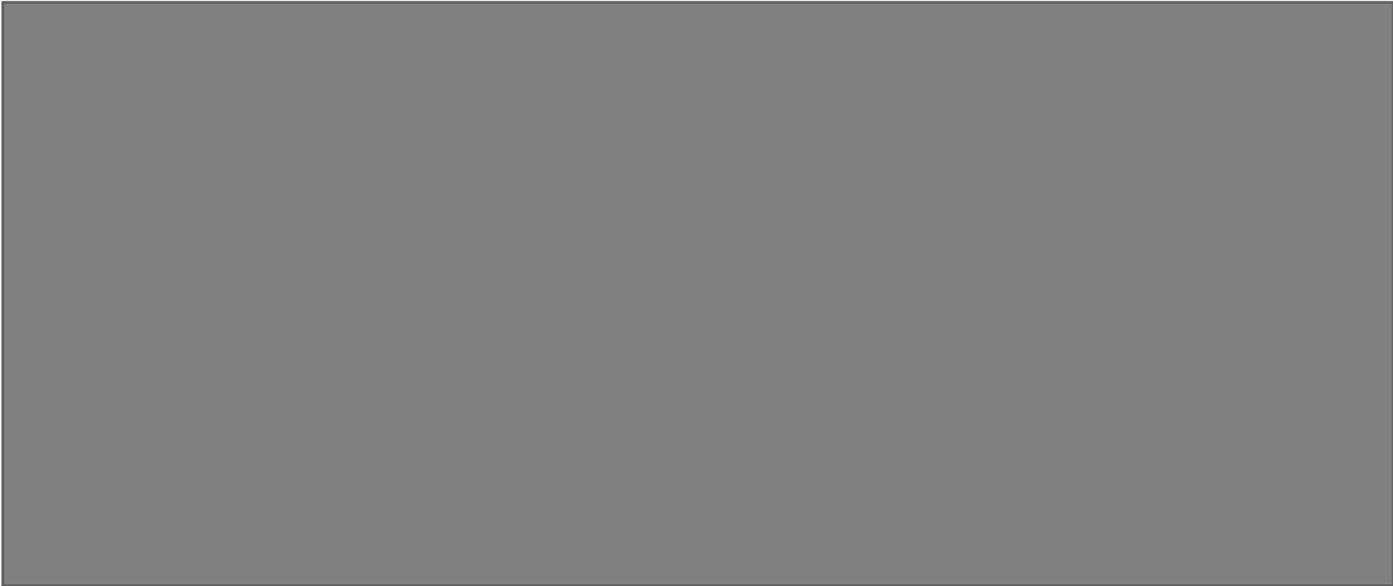
Moreover, strains of *P. fluorescens* have been genetically modified to encapsulate crystal  $\delta$ -endotoxins (Cry proteins) from the bacterium *Bacillus thuringiensis* (*Bt*) (Downing *et al.*, 2000, Peng *et al.*, 2003). The Cry proteins encapsulated by *P. fluorescens* showed high insecticidal activity and retained their activity for two to three times longer than conventional *Bt* formulations (Peng *et al.*, 2003).

In pharmaceutical uses, *P. fluorescens* produces the antibiotic pseudomonic acid (also called mupirocin), which is used to prevent *Staphylococcus aureus* infections (Hothersall *et al.*, 2007; Tacconelli *et al.*, 2003).

Finally, due to the metabolic diversity of *P. fluorescens*, it may be used in bioremediation applications. *P. fluorescens* is able to degrade a wide variety of compounds, including 3-chlorobenzoic acid, naphthalene, phenanthrene, fluorene and fluoranthene, chlorinated aliphatic hydrocarbons, styrene, pure hydrocarbons and crude oil (OECD, 1997).

The source organism of the *hppd* gene, *P. fluorescens*, is ubiquitous in the environment, including soil, water and food. It has many beneficial uses in agriculture, human health and bioremediation. Despite this widespread presence, it is not described as allergenic, toxic or pathogenic to healthy humans and animals and has an overall history of safe use.

**VI.B.2.4. Familiarity of the gene product**



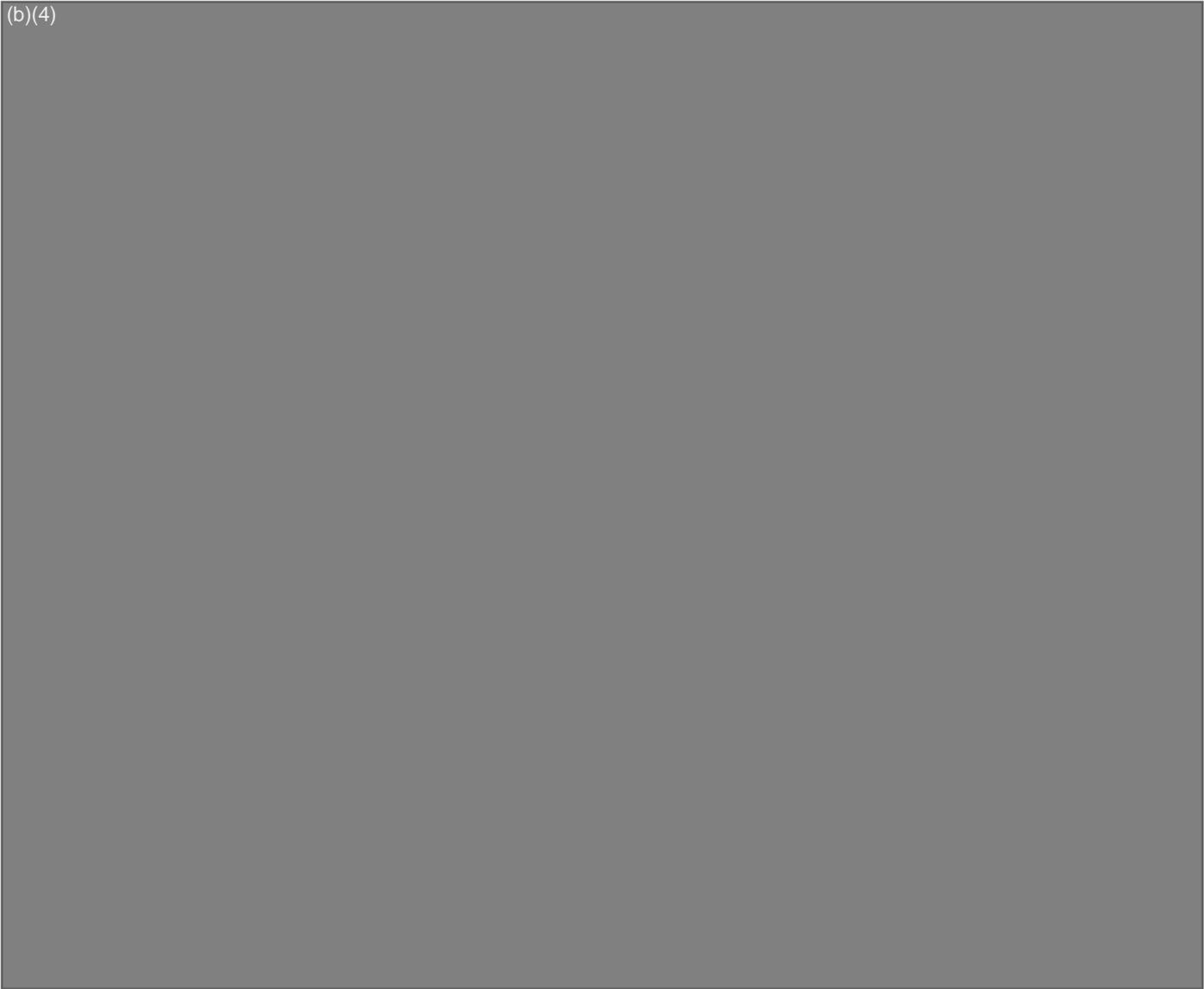
**VI.C.**

**VI.C.1.**





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**Table 7.**

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**Table 8.**

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[Redacted Table Content]

**VI.D.**

[Redacted]

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**VI.D.1.**

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**VI.D.2.**

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## **VI.E. Summary of the Food and Feed Safety Assessment of the 2mEPSPS Protein**

### **VI.E.1. Familiarity to the protein**

EPSPS is the 6<sup>th</sup> enzyme of the shikimate pathway, the metabolic pathway for the biosynthesis of aromatic compounds found in microorganisms and in plants (Herrmann *et al.*, 1995). As such, it has been shown that EPSPS enzymes are ubiquitous in nature and are present in foods derived from plant and microbial sources.

In addition, insensitivity of some EPSPS enzymes to glyphosate also exists in nature at various levels and has been specifically studied for the development of the glyphosate tolerance trait in plants (Van der Klis *et al.*, 2006).

It is apparent that these proteins have a long history of safe use as endogenous components of food and feed. Essentially, there is no evidence suggesting that these proteins may be related to any type of allergenicity or toxicity to humans or other animals. Thus, exposure to the known EPSPS proteins can be deemed as innocuous as exposure to other naturally occurring proteins without inducing adverse effects.

The 2mEPSPS, which contains only two amino acid substitutions of the maize wt EPSPS protein, was modified in such a way that the enzymatic characteristics remain as much as possible unchanged with the exception of the insensitivity to glyphosate (Schultz *et al.*, 1985). Therefore, it is expected to have the same safety profile as the wild-type protein.

### **VI.E.2. Potential allergenicity**

#### **VI.E.2.1. Homology search to known allergens**

The overall amino acid sequence homology search was carried out by using FASTA algorithm, which compares the complete amino acid sequence of the 2mEPSPS protein with all protein sequences present in the public allergen database AllergenOnline ([www.allergenonline.com](http://www.allergenonline.com); release 8.0, 1313 sequences) (Capt, 2008 a and b). The criterion indicating potential allergenicity was 35% identity over at least 80 consecutive amino acids with an allergenic protein.

In addition, an allergenic identity search (80-mer amino acid sequence homology) was performed to compare the query sequence subdivided into 80 amino acid blocks, with all known allergens present in the AllergenOnline database. The criterion indicating potential allergenicity was 35 % identity with an allergenic protein.

Furthermore, the amino acid sequence of the 2mEPSPS protein, subdivided into 8 amino acid blocks, was compared with all known allergens present in the allergen database (epitope search). The algorithm used was FindPatterns and the criterion indicating potential allergenicity was 100 % identity on a window of 8 amino acids with an allergenic protein.

The overall and 80-mer identity searches showed no relevant similarity between the 2mEPSPS sequences and any known allergenic sequences from the allergen database. In addition, the

epitope search showed no identity between all the blocks (8 amino acids) of the 2mEPSPS protein and known allergens.

Although very conservative, this homology search confirms that it is unlikely that the 2mEPSPS protein possesses any allergenic properties.

#### **VI.E.2.2. Potential N-glycosylation sites**

Potential N-glycosylation sites were determined using *in silico* search of the 2mEPSPS protein sequence for the presence of the consensus epitope Asn-Xaa-Ser/Thr (N-X~P-S/T), where Xaa = any amino acid except Pro (P), and Asn-Xaa-Cys (N-X-C) (Capt, 2008 a, Larsen *et al.*, 1998).

Two potential N-glycosylation sites were identified on the amino acid sequence of the 2mEPSPS protein. However, the biological relevance of those potential N-glycosylations in eliciting allergic response is not proven. The 2mEPSPS protein is not expected to be glycosylated, since chloroplastic proteins targeted directly to the chloroplast do not transit through the Endoplasmic Reticulum (ER) where glycosylation occurs in eukaryotes (Mousdale and Coggins, 1985, Pattison and Amtmann, 2008). In bacteria, protein glycosylation is rare (Sherlock *et al.*, 2006).

Furthermore, in the specific case of event FG72, it has been shown that the 2mEPSPS protein is not glycosylated (see Section D.1.). Therefore, potential allergenicity triggered by the presence of N-glycosylation sites is a remote possibility.

#### **VI.E.2.3. *In vitro* digestibility in human simulated gastric fluid**

The 2mEPSPS protein was assayed for digestibility in SGF containing pepsin at pH 1.2 for incubation times from 0.5 to 60 minutes (Rouquié, 2006a).

The test protein was incubated at 37°C in SGF with pepsin at a final concentration of 10 units of pepsin per µg test protein, at pH 1.2, and samples were taken for analysis at time-points of 0, 0.5, 2, 5, 10, 20, 30 and 60 minutes. The resulting protein solution was analyzed for presence of the test proteins and potential stable protein fragments by SDS-PAGE followed by Coomassie blue staining. Appropriate controls included the test protein at pH 1.2 without pepsin and SGF without the test protein.

Coomassie blue staining analysis showed that the 2mEPSPS protein was very rapidly digested in pepsin at pH 1.2, within 30 seconds of incubation. No fragment bands were found to result from digestion of the 2mEPSPS protein.

In conclusion, the 2mEPSPS protein is very rapidly degraded in SGF. This minimizes the likelihood that this protein could survive in the human digestive tract and cause an allergic reaction.

#### **VI.E.2.4. *In vitro* digestibility in human simulated intestinal fluid**

The 2mEPSPS protein was further tested for stability in SIF with pancreatin at pH 7.5 for incubation times from 0.5 to 60 minutes, using a protocol adapted from the SGF assay (Rouquié, 2006b). A solution of the test protein was incubated with SIF, a porcine pancreatin solution at pH 7.5, at approximately 37°C. Then samples were analyzed at time-points of 0, 0.5, 2, 5, 10, 20, 30 and 60 minutes for the presence of the 2mEPSPS protein or potential stable protein fragments by western blot. The immunodetection was performed using a polyclonal antibody directed against the 2mEPSPS protein. Appropriate controls included 2mEPSPS protein in buffer without pancreatin and SIF without 2mEPSPS protein.

Western blot analysis showed that the 2mEPSPS protein band was not visible anymore at time 0 and all subsequent incubation times, indicating that the 2mEPSPS protein was degraded within a few seconds in the presence of pancreatin.

In conclusion, a complete digestion of the 2mEPSPS protein was observed within a few seconds of incubation with SIF, in presence of pancreatin, at pH 7.5.

Rapid degradation of the 2mEPSPS protein in the SGF and SIF indicates a minimal likelihood that the protein could survive and be absorbed through the gastrointestinal system. In case the protein survives in the stomach, 2mEPSPS would be rapidly degraded in the intestine.

#### **VI.E.2.5. *In vitro* stability to heat**

Highly purified (>99%) 2mEPSPS protein produced in *E. coli* (batch LEJ5837) was tested for structural stability at temperatures of 60, 75 or 90°C for periods of 10, 30 or 60 minutes. The protein was examined by SDS-PAGE followed by Coomassie blue staining or by western blot analysis (Rouquié, 2007). The immunodetection was performed using a polyclonal antibody directed against 2mEPSPS protein.

The Coomassie blue-stained SDS-PAGE showed no visible changes of the band intensity at 60°C and 75°C from 10 up to 60 minutes. After 30 minutes of incubation at 90°C, the band was visible with a lower intensity than other heated samples at 60°C and 75°C. After 60 minutes at 90°C, the band was still visible, with a marked decrease in intensity compared to all other samples, including the unheated sample.

The western blot analysis showed an unchanged intensity of the intact 2mEPSPS band after incubation at 60°C or 75°C for 10 up to 60 minutes. At 90°C, the intensity of the intact 2mEPSPS band was unchanged after 10 and 30 minutes, but was decreased after 60 minutes, in accordance with the results obtained by SDS-PAGE analysis after Coomassie blue staining.

In conclusion, the 2mEPSPS protein is partially heat-stable up to 90°C for 60 minutes.

### **VI.E.3. Homology search to known toxins**

The overall amino acid sequence identity search was carried out by using BLASTP algorithm, which compared the complete amino acid sequence of the 2mEPSPS protein with all protein sequences present in the following large reference databases: Uniprot\_Swissprot, Uniprot\_TrEMBL, PDB, DAD and GenPept (Capt, 2008c). The scoring matrix used was BLOSUM62. The overconservative criterion for selecting similar proteins was a threshold E-value of 1.0. Matched sequence proteins were further examined for potential toxicity records in literature in order to assess their biological relevance.

The results showed no sequence identity of the 2mEPSPS protein with known toxins.

In conclusion, it is unlikely that the 2mEPSPS protein would exhibit any toxic properties.

### **VI.E.4.. Acute toxicity study in the mouse**

A group of 5 female OF-1 mice were treated by oral gavage with the 2mEPSPS protein produced in *E. coli* (>99% purity) at a dose level of 2000 mg/kg body weight (Rouquié, 2006c). Another group of 5 female OF-1 mice were treated by oral gavage with bovine serum albumin at the same dose level as the negative control. All animals were observed for clinical signs daily for 15 days, with special attention given during the first 4 hours. Their body weights were measured weekly. At study termination, animals were subjected to a necropsy including a macroscopic examination.

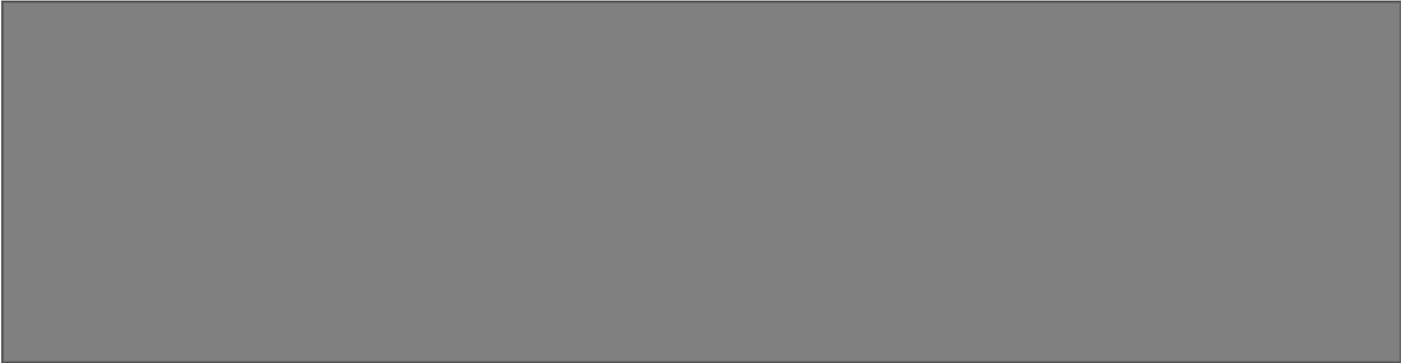
There were no mortalities, no clinical signs or treatment-related effects in female OF1 mice.

In conclusion, a single administration of the 2mEPSPS protein at 2000 mg/kg body weight *via* the oral route did not produce signs of systemic toxicity in the OF1 female mouse. The acute oral LD<sub>50</sub> of 2mEPSPS was found to be greater than 2000 mg/kg body weight in mice.

These results taken together with the results of the homology search with known toxins indicate that it is unlikely that the 2mEPSPS protein would exhibit any toxic properties.

## **VI.F.**

### **VI.F.1. Familiarity to the protein**



**VI.F.2. Potential allergenicity**

**VI.F.2.1. Homology search to known allergens**



**VI.F.2.3. *In vitro* digestibility in human simulated gastric fluid**



**VI.F.2.4. *In vitro* digestibility in human simulated intestinal fluid**



**VI.F.2.5. *In vitro* stability to heat**



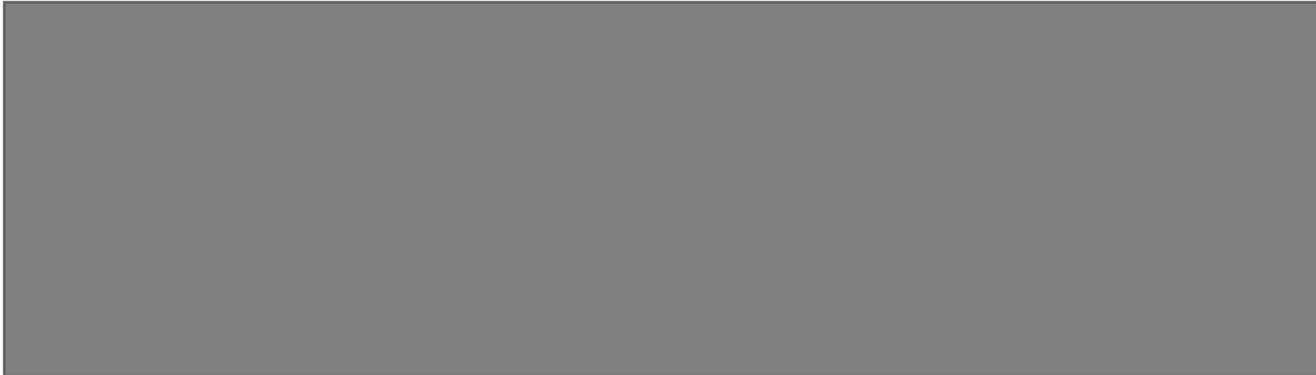
**Figure 7.**





**VI.F.3. Homology search to known toxins**





**VI.F.4.**





## VII. AGRONOMIC AND PHENOTYPIC EVALUATION

### VII.A. History of field activities



**Table 10. Summary of field activities under USDA notifications for event FG72**

USDA Notification #	Planting / Harvest Dates	Number of Locations	Type of Trial	Locations
---------------------	--------------------------	---------------------	---------------	-----------



**Table 11. Summary of field activities under** [REDACTED]

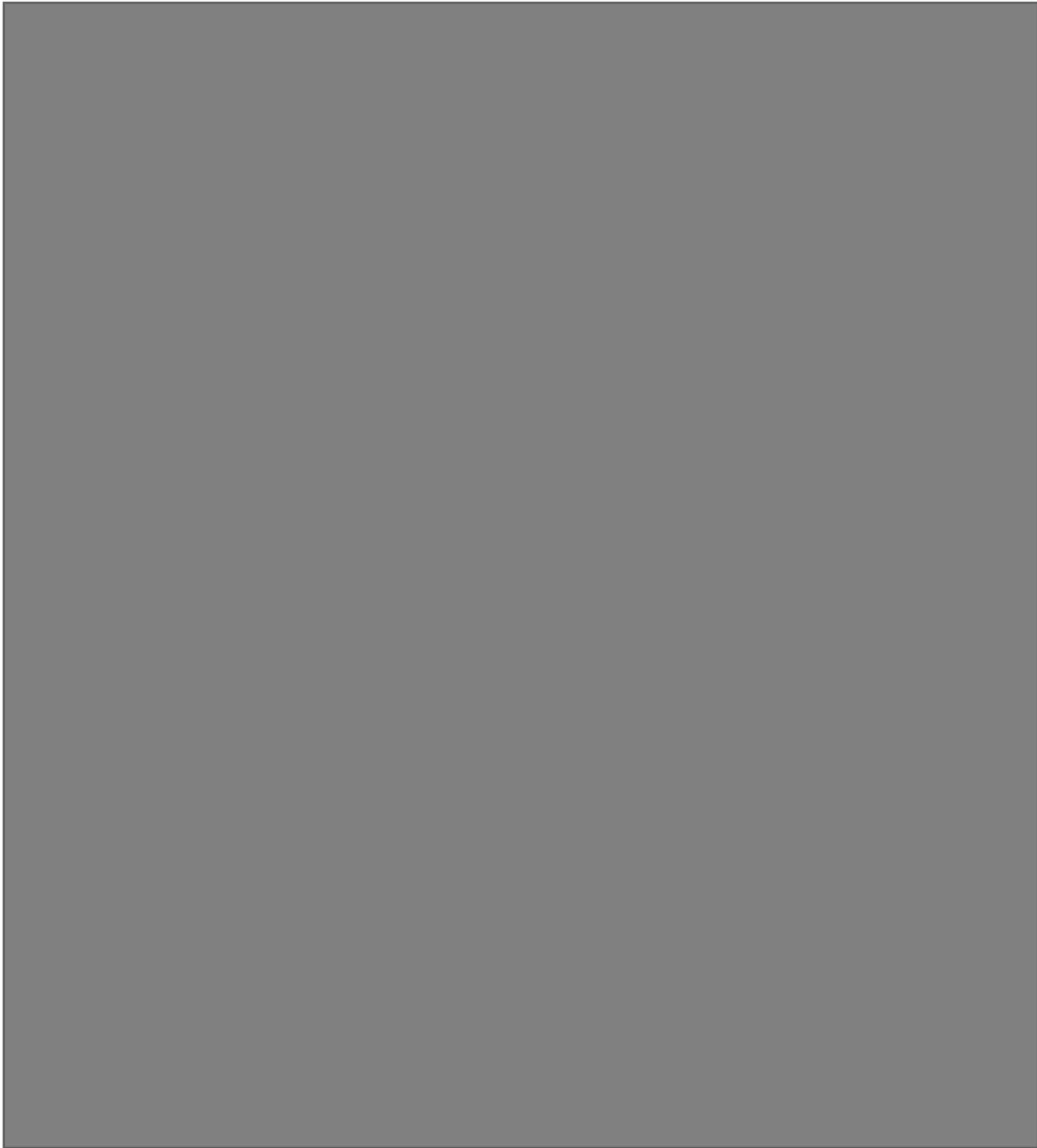


**Table 12. Summary of field activities under** [REDACTED]



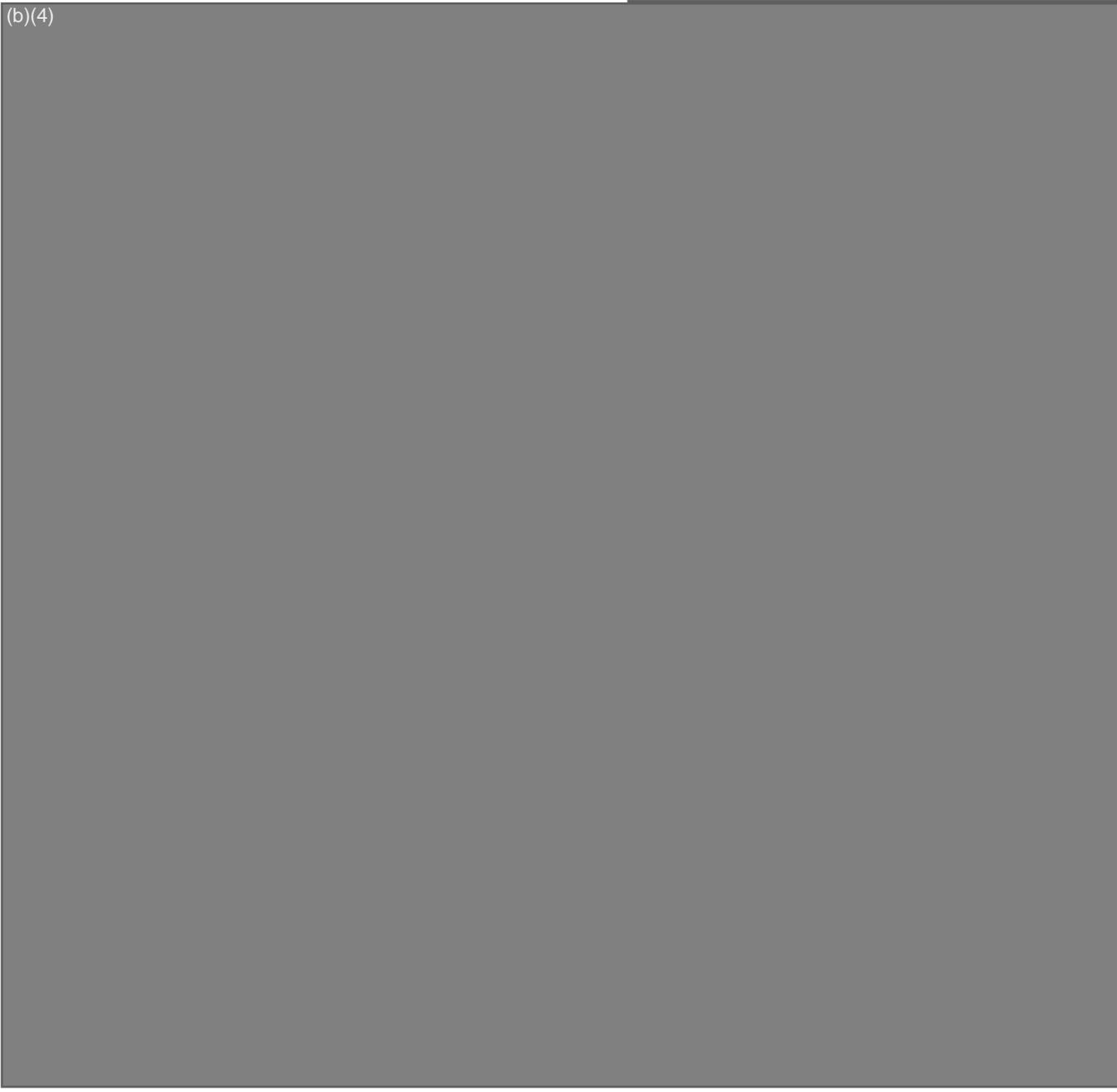


**VII.B. Agronomic and phenotypic evaluation**



**Table 13. Summary of performance characteristics** (b)(4)

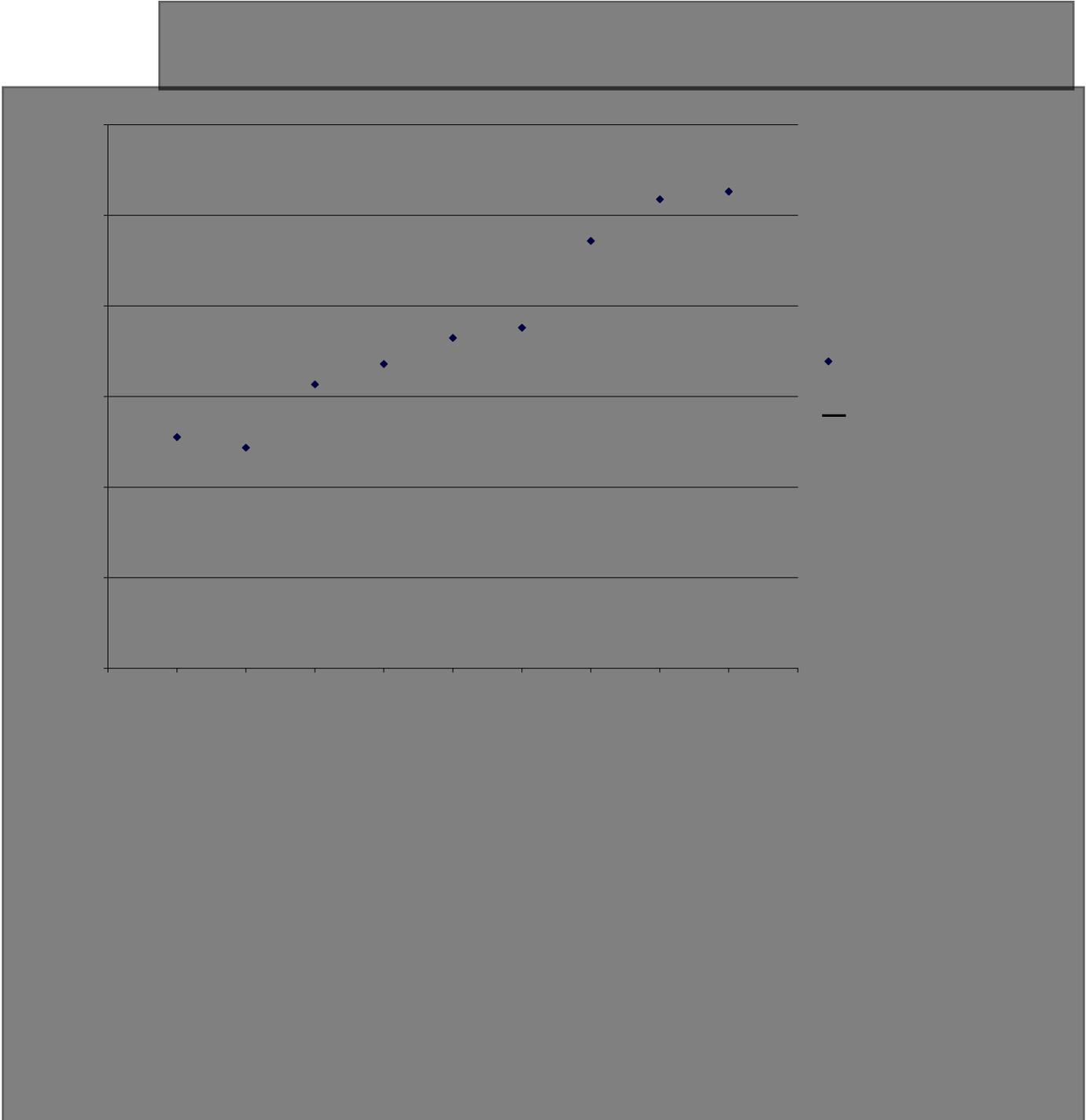
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**Figure 8. Yield of event FG72 – Event FG72 and location means**



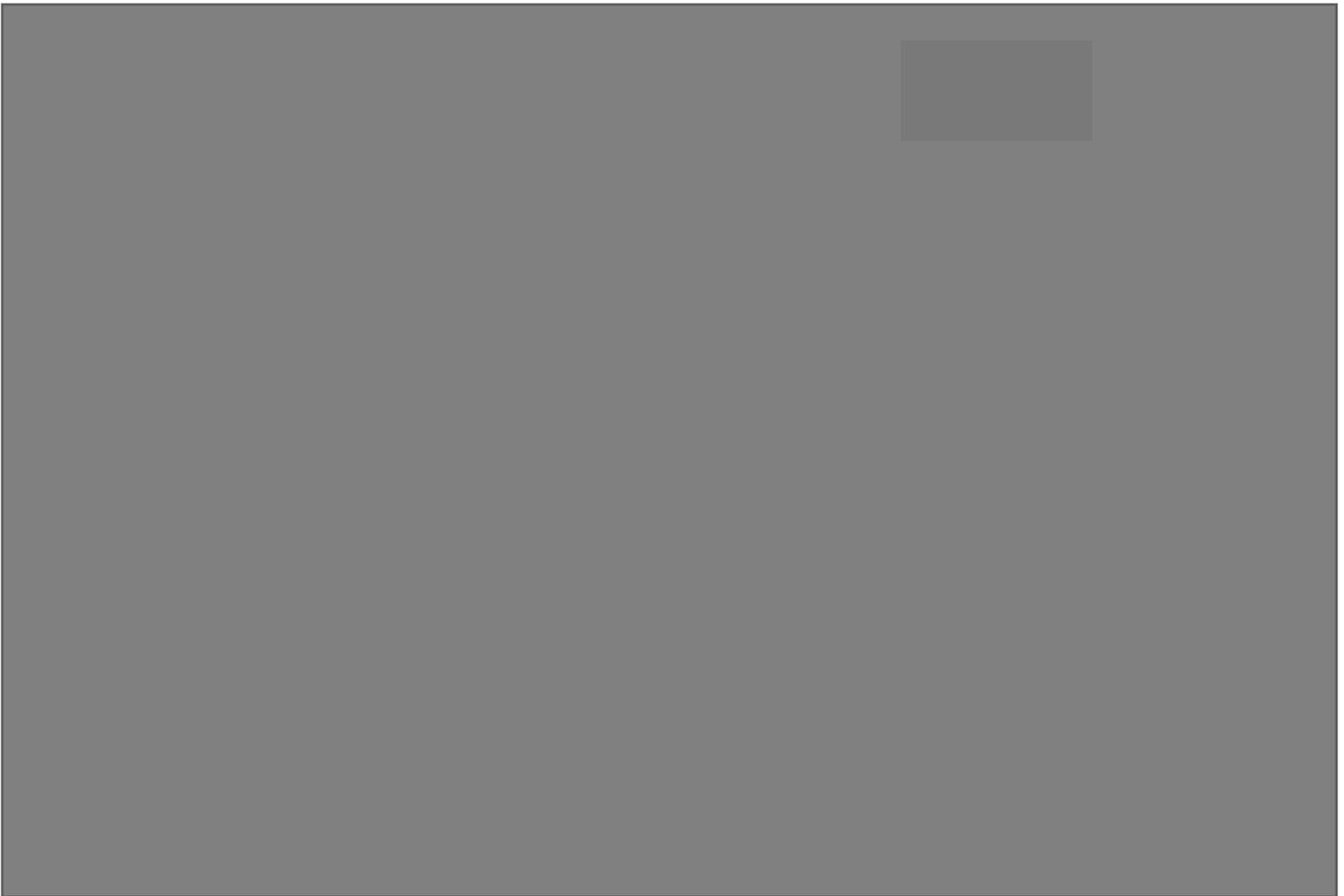


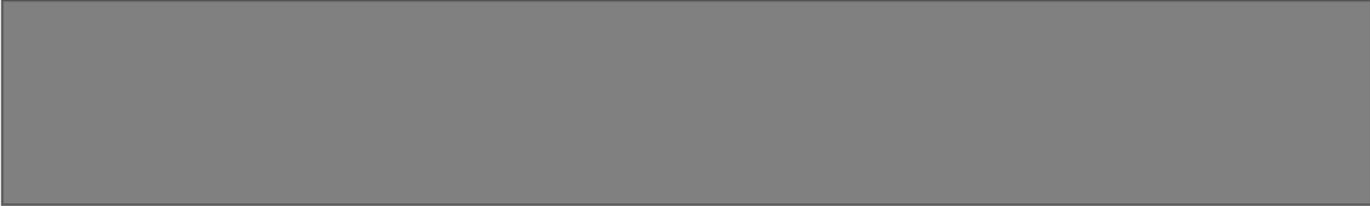
**VII.C.2.**

**VII.C.2.1.**



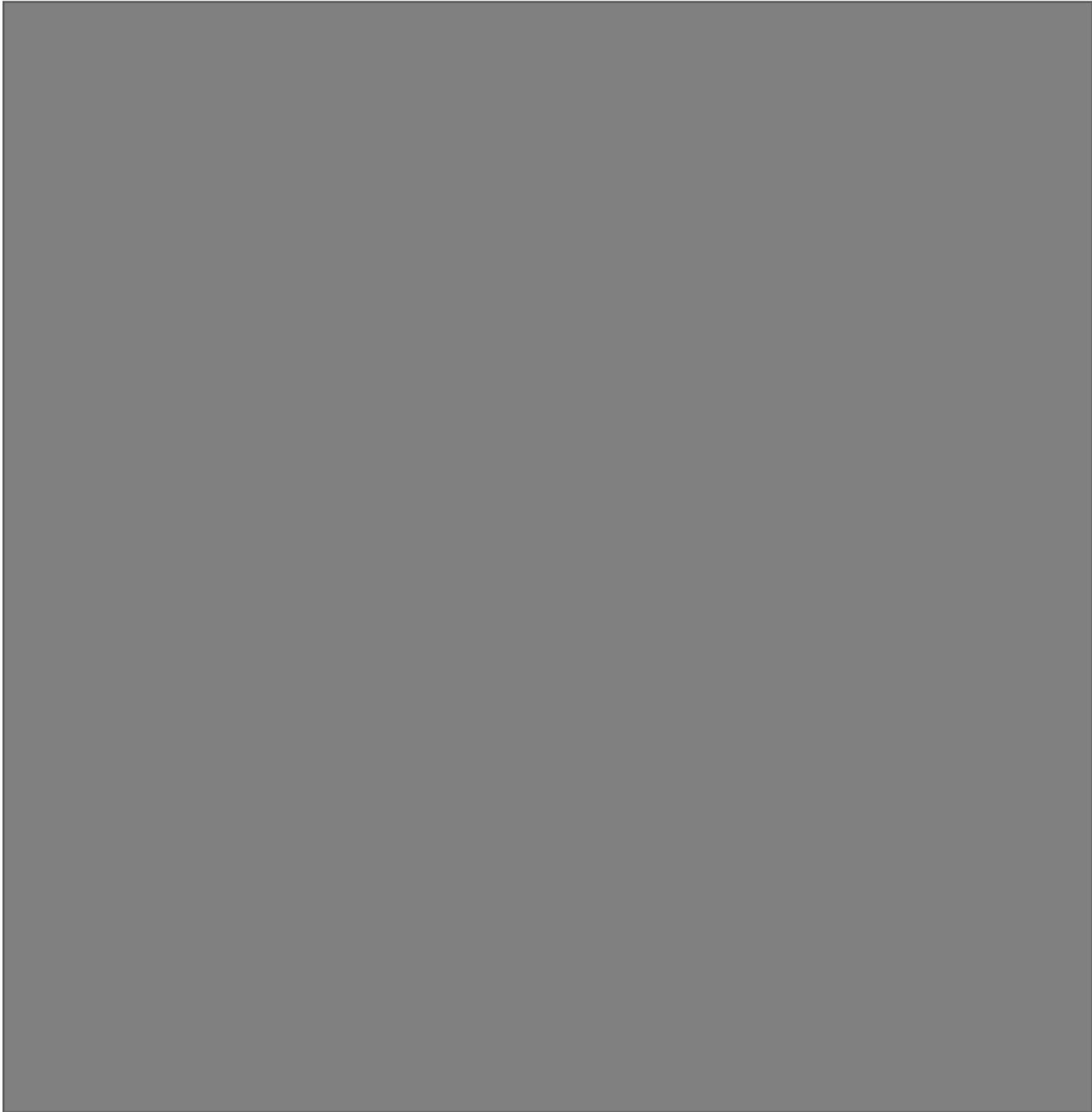
**Table 16. Trial site location for the equivalence field tests**

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**Table 18. Summary of agronomic performance for event FG72 and Jack**

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**VII.C.2.2. Objective variety description**



(b)(4)

**Table 19. General plant descriptors comparison for event FG72 and Jack**

Morphology characters	Jack	FG72
Flower color	White	White
Pubescence color	Gray	Gray
Pod color	Brown	Brown
Seed coat	Dull Yellow	Dull Yellow
Hilum color	Yellow	Yellow
Canopy architecture	Medium	Medium
Leaf shape	Oval	Oval
Growth habit	Indeterminate	Indeterminate

**VII.C.2.3. Seed characteristics**

Observations of the phenotypic characteristics of soybean event FG72 and the parent variety Jack included seed characteristics that are commonly used to describe soybean varieties (Objective Description of Variety, Soybean, *Glycine max* (L.) Merr). Following the convention described by the USDA, National Genetic Resources Program, the following observations for event FG72 and Jack seed were made: seed size hilum color, mottling score, seed coat color, seed coat luster, seed quality and seed shape. The measurement of seed size was made using four independent samples of 100 seed each from each of the ten locations. The four independent samples were also examined for other seed characteristics. In all locations and for all characters, Jack and FG72 are identical (Table 20).

In addition to the morphological characteristics, the USDA Plant Variety Protection Office requests information concerning variety maturity, height, fatty acid profile and total oil and protein content when applying for registration of a new soybean variety. The information compiled in Table 21 follows the template provided by the USDA Plant Variety Protection (PVP) Office. The comparison demonstrates that Jack and event FG72 are similar in all characteristics, with the exception of height. There have been eight generations of selection of lines and seed increase since the transformation of Jack to create event FG72. Modern soybean varieties are shorter than the older variety Jack, and we can see evidence of the breeder's eye at work in the 12 cm difference in plant height.

FG72 soybean is similar to Jack for all parameters considered by Objective Variety Description, with the exception of plant height. The only distinction between event FG72 and Jack is the addition of the *2mepsps* and *hppd* genes to confer double herbicide tolerance to GLY and IFT.

**Table 20. Seed phenotypic characteristics**

Seed Characteristics	FG72 (mean $\pm$ std) <sup>a</sup>	Jack (mean $\pm$ std) <sup>a</sup>
Seed size <sup>b</sup>	13.1 $\pm$ 1.4	12.3 $\pm$ 1.0
Hilum color	Yellow	Yellow
Mottling <sup>c</sup>	1.9% $\pm$ 1.6	1.5% $\pm$ 1.7
Seed coat color	Yellow	Yellow
Seed coat luster	Dull	Dull
Seed quality <sup>d</sup>	7.3 $\pm$ 0.9	7.0 $\pm$ 0.9
Seed shape <sup>e</sup>	Spherical	Spherical
L/W	1.1	1.2
L/T	1.0	1.1
T/W	1.1	1.1

<sup>a</sup> Mean and standard deviation for the replicate measurements from 10 locations (2008).

<sup>b</sup> Seed size was recorded as the weight in grams of 100 seed.

<sup>c</sup> Mottling is the number of mottled seeds in each 100 seed reported as %

<sup>d</sup> Seed quality rating is a numerical score of 1-9 based on visual appearance of the seed (9 = best quality, 1 = worst quality)

<sup>e</sup> The seed shape is measured as the length and width of the seed with the hilum facing up (L and W) and the width with the hilum on the side (T). A seed shape is scored as spherical when the L/W, L/T and T/W ratio is less than or equal to 1.2.

**Table 21. Paired comparison of variety characteristics**

Paired comparison	# Days to maturity	Plant height in cm	% Linoleic acid (18:2)	% Oleic acid (18:1)	% Linolenic acid (18:3)	% Palmitic acid (16:0)	Total oil % dw	Crude protein % dw
Jack	128	106	54%	22%	8%	10%	19%	38%
FG72	128	94	54%	24%	8%	9%	19%	38%

VII.D. (b)(4)

(b)(4)

**Table 22. Plant diseases and syndromes observed**

Phytopathology observed	Causal agent
Downy mildew	<i>Peronospora manshurica</i>
Bacterial blight	<i>Pseudomonas syringae</i> pv. <i>Glycinea</i>
Cercospora leaf blight	<i>Cercospora kikuchii</i>
Brown spot	<i>Septoria glycines</i>
Frogeye leafspot	<i>Cercospora sojina</i>
Powdery mildew	<i>Microsphaera diffusa</i>
Top die back	cause unknown, syndrome described by Iowa State University as plants dying from the top down
Sudden death syndrome	<i>Fusarium virguliforme</i>

The variety registration of Jack, claims resistance to soybean cyst nematode (SCN) (Races 3 and 4) (*Heterodera glycines* Ichinohe) and susceptibility to *Phytophthora* rot (Races 1, 4, and 7) caused by *Phytophthora megasperma* (Drechs.) f. sp. *glycinea* T. Kuan & D.C. Erwin. Neither of these phytopathologies was observed consistently in the trials, so it was not possible to confirm expression of these variety traits. At the Perry location, one plant of event FG72 was presumed to have died of *Phytophthora* root rot.

No insect susceptibility or disease susceptibility or resistance differences were observed between the event FG72 soybean plants and Jack.

There was no evidence of a change in characteristics that would enhance survival of event FG72 soybean plants when compared to Jack.

VII.E. (b)(4)

(b)(4)



**Table 23. Summary of agronomic performance for FG72 and commercial varieties**

The table content is completely redacted with a large grey box.

#### **VII.F. Seed dormancy evaluation**

To provide an evaluation of seed dormancy potential of event FG72 and the variety Jack, measurements of seed germination and dormancy were conducted. The hypothesis was to test seed samples harvested from plants grown to maturity at 10 locations. These seed samples would represent the physiological state of seed that might fall into a field at the end of the season. The seed tests were completed by the Iowa State Seed Lab using the standard test (warm germination) which is used for seed lot evaluations of field emergence under favorable conditions. Seeds are rolled into germination towels, placed into germination trays and incubated at 25°C and 90% relative humidity for five days. Germination is scored on day 6. A minimum of 400 seed were evaluated from each location. Appendix 2.F. describes the materials and methods for the germination studies.

A very small difference in germination (94% vs. 96%) at day 6 was observed (Table 24). In cases where hard seed were observed on day 6, the germination study was extended to 13 days, and in every case, the hard seed germinated. The percent of viable seed from each of the samples (total viable; 95% vs. 96%) was the same.

No dormant seed were identified. Although small difference was observed between the event FG72 and the parent line Jack, the differences were not more than one standard deviation. No impact of the production environment or harvest conditions was observed.

**Table 24. Seed germination test results**

ID	Location	Germination <sup>a</sup> (%)	Hard seed	Dormant seed	Total viable	Abnormal seedlings	Dead seed	Days tested
FG72	Mediapolis	92	2	0	94	3	3	13
FG72	Perry	89	3	0	92	8	0	13
FG72	Sharpsville	82	6	0	88	10	2	13
FG72	Adel	97	1	0	98	2	0	6
FG72	Fithian	90	0	0	90	9	1	13
FG72	Marcus	97	0	0	97	3	0	6
FG72	Glidden	95	0	0	95	5	0	13
FG72	Winterset	99	0	0	99	0	1	6
FG72	Osborn	97	2	0	99	1	0	13
FG72	Iowa Falls	99	0	0	99	1	0	7
	<b>Mean</b>	<b>93.7</b>	<b>1.4</b>	<b>0</b>	<b>95.1</b>	<b>4.2</b>	<b>0.7</b>	
	<b>StDev<sup>b</sup></b>	<b>5.4</b>	<b>2.0</b>	<b>0.0</b>	<b>4.0</b>	<b>3.6</b>	<b>1.1</b>	
Jack	Mediapolis	97	0	0	97	1	2	
Jack	Perry	96	0	0	96	3	1	6
Jack	Sharpsville	93	3	0	96	4	0	13
Jack	Adel	94	0	0	94	5	1	6
Jack	Fithian	94	0	0	94	6	0	6
Jack	Marcus	98	0	0	98	2	0	6
Jack	Glidden	98	0	0	98	2	0	6
Jack	Winterset	99	0	0	99	1	0	6
Jack	Osborn	98	0	0	98	2	0	6
Jack	Iowa Falls	95	0	0	95	4	1	6
	<b>Mean</b>	<b>96.2</b>	<b>0.3</b>	<b>0</b>	<b>96.5</b>	<b>3</b>	<b>0.5</b>	
	<b>StDev<sup>b</sup></b>	<b>2.1</b>	<b>0.9</b>	<b>0.0</b>	<b>1.8</b>	<b>1.7</b>	<b>0.7</b>	

<sup>a</sup> Warm germination – 8 reps of 50 seed<sup>b</sup> StDev; standard deviation

## **VII.G. Composition analysis**

### **VII.G.1. Introduction**

Analysis of the nutritional composition of the double-herbicide-tolerant soybean event FG72 was performed for soybean grain harvested from 10 different locations in the soybean growing areas of North America (Mackie, 2009). The study was conducted during the 2008 growing season using seed of the T<sub>8</sub> generation. Planted at each of the 10 locations were three entries:

- Entry A; the control counterpart variety Jack, which was treated with conventional herbicides registered for use on soybean; (designated as Jack in Tables 25-29)
- Entry B; the test entry event FG72 treated with conventional herbicides (designated as FG72 in Tables 25-29)
- Entry C; the test entry event FG72 treated with the intended herbicides (IFT + GLY) (designated as FG72 treated in Tables 25-29).

Each of the three entries was planted in a RBC design with three replications per location. Three commercial soybean varieties were planted along side the test and control entries at the same locations. These three commercial soybean varieties provided reference values to establish ranges of natural variation for the nutritional components analyzed in this study.

The nutritional composition analysis conducted was based on the OECD guidance document for soybean (OECD, 2001). The nutritional endpoints selected were proximates, fiber compounds, total amino acids, fatty acids, anti-nutrients and isoflavones.

For comparative purposes, the values obtained for the commercial reference lines were used to establish in-study ranges in addition to the ranges reported in the published literature (OECD 2001; ILSI 2007). Together, these two sets of ranges were used to evaluate the nutritional composition results of event FG72 soybean. Nutrient component means that fell within the limits of the commercial or literature reference ranges were considered to be within the normal variation for commercial soybeans.

The test plots were each 15 ft by 20 ft in size and contained 6 rows spaced 30 inches apart. At maturity, grain samples were harvested from the two interior rows of each plot.

### **VII.G.2. Nutritional composition of soybean grain**

Tables 25-29 show the comparisons of the pooled results of the two test and counterpart control entries from all locations, with reference ranges calculated from three commercial soybean varieties. Appendix 2.G. describes the materials and methods for the composition analysis. All mean values typically fell within the respective commercial variety or literature reference ranges and are not considered to be of biological concern or due to the intended modification of event FG72 (Rattemeyer, 2009).

The analysis of proximates and fiber between the test and the counterpart control entries were similar. All mean values were within the calculated commercial variety and literature reference ranges (Table 25).

Analysis of the total amino acid profile for all 18 amino acids between the two test and counterpart control entries were found to be similar (Table 26).

Levels of 24 fatty acids were measured for the two test entries, the counterpart control Jack, and the three commercial varieties. Seventeen of these fatty acids; C08:0, C10:0, C12:0, C14:0, C14:1, C15:0, C15:1, C16:1, C17:1, C18:3 (gamma), C18:4, C20:2, C20:3, C20:4, C20:5, C22:1, C22:5 and C22:6 were below the limit of quantification (LOQ = 0.02 % fw) in all soybean seed samples. These minor fatty acids of soybean were not statistically analyzed and are not reported in Table 26.

The results of the fatty acid analysis of the two test entries and Jack are shown in Table 27. All mean values for the fatty acids listed in Table 27 fell within both the calculated commercial variety and literature reference ranges. The sum of all detected fatty acids was 99.9% and accounts for nearly all fatty acids present in the oil.

The level of the anti-nutrient phytic acid in Jack and event FG72 soybean grain entries fell within the commercial variety and the literature reference ranges (Table 28).

The levels of the two low molecular weight carbohydrates raffinose and stachyose in the two test and control entries were within the range of the commercial lines tested (Table 28).

The analysis of the levels of lectins in the two test and isoline control entries were found to be similar, and fell within the commercial variety and literature reference ranges (Table 28).

The trypsin inhibitor mean values for the two test and control entries fell within the commercial variety and reference literature ranges (Table 28).

Soybeans contain isoflavones which are glucosides and esters of three aglycones (daidzein, genistein and glycitein). The mean values and range reported for isoflavone content in the test and counterpart entries were very similar in numerical value, and all mean values fell within the ranges for the commercial lines and literature references (Table 29).

In summary, no safety related issues were identified in the analysis of the nutrient composition of event FG72 soybean grain. All components measured were comparable to either the commercial soybean varieties grown at the same locations as the test and control entries, or were within the cited literature reference ranges.

**Table 25. Proximate and fiber components**

Component		Jack	FG72	FG72 Treated	Commercial Lines <sup>b</sup>	Literature Reference <sup>c</sup>
Moisture % fw	Mean <sup>a</sup>	9.51	9.65	9.45		
	Range	6.57-10.50	7.90-11.50	6.51-10.90	8.00 – 10.60	5.6-12
Protein % dw	Mean	38.2	38.2	38.1		
	Range	36.2-40.3	36.8-39.8	36.5-39.6	35.8 – 40.1	32 – 45.5
Fat % dw	Mean	19.3	18.9	19.2		
	Range	17.9-21.4	16.6-21.0	17.1-21.6	15.1 – 21.4	8.1 – 24.7
Ash % dw	Mean	5.24	5.07	5.06		
	Range	4.38-6.07	4.17-5.56	4.50-5.68	4.89 – 5.73	3.9 – 7.0
Carb. % dw	Mean	37.3	37.9	37.6		
	Range	34.3-39.3	35.6-39.7	35.3-40.0	34.8 – 41.6	29.6- 50.2
ADF % dw	Mean	17.8	18.1	17.9		
	Range	14.2-22.4	14.1-23.5	15.2-21.4	13.6 – 23.5	7.8 – 18.6
NDF % dw	Mean	19.8	20.3	20.0		
	Range	16.8-24.5	16.9-25.4	17.4-23.0	16.1 – 24.8	5.0 – 21.3

<sup>a</sup> Least square mean<sup>b</sup> Reference ranges of the 3 analyzed commercial soybean lines<sup>c</sup> Literature ranges from OECD (2001) and ILSI (2007)

**Table 26. Amino acids**

Amino acid % dw		Jack	FG72	FG72 Treated	Commercial Lines <sup>b</sup>	Literature Reference <sup>c</sup>
Alanine	Mean <sup>a</sup>	1.68	1.68	1.68		
	Range	1.60-1.75	1.60-1.74	1.60-1.72	1.55 – 1.78	1.51 – 2.10
Arginine	Mean	2.94	2.97	2.95		
	Range	2.71-3.20	2.77-3.14	2.74-3.10	2.69 – 3.13	2.17 – 3.40
Aspartic acid	Mean	4.40	4.38	4.37		
	Range	4.15-4.70	4.08-4.60	4.13-4.55	4.06 – 4.67	3.81 – 5.12
Cystine	Mean	0.58	0.58	0.59		
	Range	0.53-0.63	0.51-0.62	0.49-0.63	0.50 – 0.63	0.37 – 0.81
Glutamic acid	Mean	6.75	6.77	6.74		
	Range	6.30-7.24	6.30-7.21	6.34-7.03	6.32 – 7.23	5.84 – 8.20
Glycine	Mean	1.68	1.68	1.68		
	Range	1.60-1.76	1.60-1.75	1.60-1.74	1.53 – 1.76	1.46 – 2.27
Histidine	Mean	1.05	1.05	1.05		
	Range	1.00-1.10	0.99-1.09	0.98-1.09	0.93 – 1.07	0.84 – 1.22
Isoleucine	Mean	1.81	1.80	1.79		
	Range	1.73-1.92	1.69-1.87	1.67-1.86	1.62 – 1.96	1.54 – 2.32
Leucine	Mean	2.99	2.99	2.98		
	Range	2.84-3.18	2.84-3.13	2.81-3.09	2.71 – 3.13	2.2 – 4.0
Lysine	Mean	2.48	2.48	2.47		
	Range	2.37-2.62	2.34-2.58	2.33-2.56	2.34 – 2.64	1.55 – 2.84
Methionine	Mean	0.54	0.54	0.54		
	Range	0.49-0.60	0.49-0.58	0.46-0.58	0.50 – 0.58	0.43 – 0.76
Methionine	Mean	0.54	0.54	0.54		
	Range	0.49-0.60	0.49-0.58	0.46-0.58	0.50 – 0.58	0.43 – 0.76
Phenylalanine	Mean	1.97	1.98	1.96		
	Range	1.89-2.13	1.87-2.09	1.83-2.05	1.83 – 2.08	1.60 – 2.39
Proline	Mean	1.82	1.83	1.82		
	Range	1.68-1.97	1.72-1.98	1.65-1.94	1.71 – 1.94	1.69 – 2.33
Serine	Mean	1.97	1.98	1.99		
	Range	1.82-2.14	1.75-2.10	1.83-2.11	1.77 – 2.13	1.11 – 2.48
Methionine	Mean	0.54	0.54	0.54		
	Range	0.49-0.60	0.49-0.58	0.46-0.58	0.50 – 0.58	0.43 – 0.76
Phenylalanine	Mean	1.97	1.98	1.96		
	Range	1.89-2.13	1.87-2.09	1.83-2.05	1.83 – 2.08	1.60 – 2.39
Proline	Mean	1.82	1.83	1.82		
	Range	1.68-1.97	1.72-1.98	1.65-1.94	1.71 – 1.94	1.69 – 2.33
Serine	Mean	1.97	1.98	1.99		
	Range	1.82-2.14	1.75-2.10	1.83-2.11	1.77 – 2.13	1.11 – 2.48
Threonine	Mean	1.55	1.54	1.53		
	Range	1.48-1.66	1.45-1.61	1.44-1.62	1.44 – 1.62	1.14 – 1.89
Tryptophan	Mean	0.45	0.44	0.44		
	Range	0.40-0.50	0.38-0.48	0.39-0.50	0.39 – 0.54	0.36 – 0.67
Tyrosine	Mean	1.40	1.40	1.40		
	Range	1.28-1.49	1.33-1.46	1.30-1.46	1.32 – 1.48	0.10 – 1.61
Valine	Mean	1.89	1.88	1.87		
	Range	1.80-2.01	1.78-1.98	1.75-1.95	1.66 – 2.03	1.50 – 2.44

<sup>a</sup> Least square mean; <sup>b</sup> Reference ranges of the 3 analyzed commercial soybean lines, <sup>c</sup> Reference ranges from OECD (2001) and ILSI (2007)

**Table 27. Fatty acids**

Fatty Acid % relative		Jack	FG72	FG72 Treated	Commercial Lines <sup>b</sup>	Literature Reference <sup>c</sup>
<b>Saturated</b>						
C16:0 (palmitic)	Mean <sup>a</sup>	10.1	9.34	9.38	9.78 – 11.40	7 – 16
	Range	9.75-10.9	9.02-9.58	9.03-10.4		
C18:0 (stearic)	Mean	4.28	4.52	4.51	3.49 – 4.81	2 – 5.9
	Range	4.07-4.70	4.23-5.05	3.80-5.08		
C20:0 (arachidic)	Mean	0.31	0.32	0.32	0.25 – 0.35	< 0.10 - 0.48
	Range	0.28-0.36	0.30-0.37	0.27-0.38		
C22:0 (behenic)	Mean	0.32	0.33	0.33	0.25 – 0.35	0.28 – 0.60
	Range	0.30-0.34	0.31-0.35	0.26-0.36		
C24:0 (lignoceric)	Mean	0.113	0.119	0.122	< 0.10 – 0.15	0.15
	Range	< 0.10 -0.16	< 0.10 -0.17	< 0.10 -0.17		
<b>Sum of the saturated</b>		<b>14.9</b>	<b>14.5</b>	<b>14.5</b>	<b>13.8 – 17.2</b>	<b>9.43 – 23.55</b>
<b>Mono-unsaturated</b>						
C18:1 (oleic)	Mean	21.97	24.65	24.12	21.10 – 24.10	14 – 34
	Range	20.10-25.00	23.20-27.20	22.40-26.30		
C20:1 (eicosenoic)	Mean	0.16	0.16	0.17	< 0.10 – 0.18	0.14 – 0.35
	Range	0.14- 0.19	0.15-0.19	0.15-0.19		
<b>Sum of mono-unsaturated</b>		<b>22.13</b>	<b>24.81</b>	<b>24.29</b>	<b>21.10 – 24.28</b>	<b>14.14 – 34.83</b>
<b>Poly-unsaturated</b>						
C18:2 (linoleic)	Mean	54.56	52.65	53.08	51.50 – 55.40	48 – 60
	Range	51.70-55.90	50.60-53.70	51.20-54.70		
C18:3 (α-linolenic)	Mean	8.27	7.94	8.01	7.59– 10.30	2 – 10
	Range	7.37-9.14	7.24-8.65	7.22-8.82		
<b>Sum of poly-unsaturated</b>		<b>62.83</b>	<b>60.59</b>	<b>61.09</b>	<b>59.09 – 65.70</b>	<b>50 - 70</b>
<b>Sum of all the fatty acids</b>		<b>99.93</b>	<b>99.91</b>	<b>99.92</b>		

<sup>a</sup> Least mean square<sup>b</sup> Reference ranges of the 3 analyzed commercial soybean lines<sup>c</sup> Reference ranges from OECD (2001) and ILSI (2007)

**Table 28. Anti-nutrients**

Anti-nutrients (dw)		Jack	FG72	FG72 Treated	Commercial Lines <sup>b</sup>	Literature Reference <sup>c</sup>
Phytic Acid (%)	Mean <sup>a</sup>	1.40	1.37	1.35		
	Range	1.03-1.70	0.89-1.91	0.79-1.87	0.96 – 1.50	0.63 – 2.74
Raffinose (%)	Mean	0.361	0.378	0.379		
	Range	0.286-0.428	0.280-0.526	0.295-0.511	0.290 – 0.504	0.11 – 1.28
Stachyose (%)	Mean	2.49	2.42	2.50		
	Range	2.04-2.91	2.09-2.88	2.06-2.90	2.23 – 2.96	1.21 – 6.30
Lectin (HU/mg)	Mean	1.74	1.40	1.54		
	Range	0.91-4.29	0.66-3.08	0.88-2.63	0.46 – 8.63	0.11 - 129
Trypsin inhibitor	Mean	33.0	30.1	33.9		
	Range	23.3-47.6	19.6-42.4	23.6-43.4	23.5 – 60.1	19.59-118

<sup>a</sup> Least mean square<sup>b</sup> Reference ranges of the 3 analyzed commercial soybean lines<sup>c</sup> Reference ranges from OECD (2001) and ILSI (2007)**Table 29. Isoflavones**

Isoflavones mg/kg dw		Jack	FG72	FG72 Treated	Commercial Lines <sup>b</sup>	Literature Reference <sup>c</sup>
Daidzin	Mean	1035	1034	994		
	Range	480-1850	416-1690	400-1810	568 – 2530	60.0 – 2454
Genistin	Mean	1817	1682	1640		
	Range	839-2760	627-2460	609-2400	1130 – 3290	144 – 2837
Glycitin	Mean	365	414	400		
	Range	298-445	345-511	169-492	142 – 315	15.3 – 1070
Daidzein <sup>e</sup>	Mean	-----	-----	-----		
	Range <sup>d</sup>	< 10 – 17.5	< 10 – 15.1	< 10 – 14.6	< 10 – 14.0	5 – 35
Genistein <sup>e</sup>	Mean	-----	-----	-----		
	Range <sup>d</sup>	< 10 – 17.2	< 10 – 15.7	< 10 – 12.2	< 10 – 20.6	0.3 – 46
Glycitein	Mean	<LOQ	<LOQ	<LOQ	<LOQ	
	Range <sup>d</sup>	< 10	< 10	< 10	< 10	1.1 – 80
<b>Total Isoflavones</b>	<b>Mean<sup>a</sup> Range</b>	<b>2010 1040-3130</b>	<b>1953 930-2860</b>	<b>1891 881-2890</b>	<b>1160 - 3390</b>	<b>679 – 3733</b>

<sup>a</sup> Least mean square<sup>b</sup> Reference ranges of the 3 analyzed commercial soybean lines<sup>c</sup> Reference ranges from ILSI (2007)<sup>d</sup> some or all values reported below the limit of quantification<sup>e</sup> Mean not calculated as some samples were below LOQ

## VII. H. Poultry feeding study

A 42-day broiler chicken feeding study was conducted using diets containing 20% toasted soybean meal from event FG72 soybean, Jack soybean and a non-commercial soybean line (Stafford, 2009). Broiler chicken is very sensitive to minor differences in nutrient quality, since it undergoes an approximate 15-fold increase in body weight during the first 21 days of life.

All chickens were monitored at least daily for health status, overt signs of toxicity, and mortality. Effects of diets on health, survival, live body weight, total weight gain, feed consumption, food conversion, marketable carcass weight and muscle tissue weight and yield (breast, thigh, leg, wing), and abdominal fat pad weight were compared among groups. Gross post-mortem examination findings were reported as appropriate.

After 42 days of daily exposure, no differences were observed between the event FG72 group and the control groups. Minor statistical differences were recorded and were considered not treatment-related. Overall, the growth and health of chickens were similar in all groups.

In conclusion, there was no evidence that the group of broiler chickens fed event FG72 soybean toasted meal were adversely affected in any manner. The toasted meal with event FG72 soybean incorporated at 20 % was as safe and nutritious as the meals made with 20 % of control group soybeans.

## VII.I. Conclusion for agronomic evaluation of event FG72

A thorough review of double-herbicide-tolerant soybean event FG72 was conducted over the 2003 and 2008 crop seasons. During these field studies, more than 20 different agronomic parameters were identified and evaluated to assess the impact of event FG72 on the soybean plant. Development and maturity, environmental susceptibility to biotic and abiotic stressors, and the yield potential and quality of the soybean grain were all evaluated to determine if event FG72 differed from the parent line Jack and other conventional soybean varieties of the same type.

In addition to the agronomic evaluation, event FG72 was analyzed for its main nutritional components and compared to the parent line Jack and commercial soybean varieties. The compositional analysis demonstrated that the intended modification in event FG72 did not change the compositional make-up and the nutritional profile of event FG72 is similar to that of the Jack and within the range of commercial soybeans lines and the established literature ranges.

The overall conclusion is that there are no agronomically meaningful differences between the transformed double-herbicide-tolerant soybean event FG72 and other soybean varieties evaluated. The resulting conclusion is that the introduction of event FG72 soybean poses no new agronomic plant pest risks.

## VIII. ENVIRONMENTAL SAFETY AND IMPACT ON AGRONOMIC PRACTICES

### VIII.A. Environmental assessment of the introduced proteins

The presence of the 2mEPSPS and HPPD W336 proteins introduced in FG72 soybean will not present adverse environmental effects, as both are derived from common, naturally occurring proteins and (b)(4)

(b)(4)

The naturally occurring EPSPS protein is universally expressed in plants and microorganisms, and has been a safe component of food and feed for a long history of consumption. In addition, the substitution of two amino acids was not expected to change the protein's safety or its potential for toxicity and allergenicity. And recent studies conducted by BCS have confirmed the safety profile of the 2mEPSPS protein (Section VI).

Moreover, the safety of the 2mEPSPS protein, which is also present in the genetically modified herbicide tolerant maize Event GA21 and in GlyTol™ cotton, has been evaluated by several regulatory agencies.

### VIII.B. Potential for horizontal or vertical gene transfer

Soybean is a self pollinating crop. Anthers mature in the floral buds and directly pollinate the stigma of the same flower (cleistogamy). Natural cross-pollination with near-by soybean plants is reported to be less than 1% (OECD 2000). The extent of outcrossing can be influenced by the distance between individual plants, floral characteristics of different varieties, environmental conditions and insect activities. In seed production fields, the occurrence of cross pollination is

so low that the standards of certified seed production require isolation distances to prevent mechanical mixture (7CFR 201.76) and are based upon the width of the harvest machinery.

Soybean is a non-native crop of the Americas. The origin of the *Glycine* species is Asia and there are no wild or native soybean relatives in the Americas which could be considered to be potential targets for gene flow.

### **VIII.C. Weediness potential of double-herbicide-tolerant soybean event FG72**

Commercial soybean varieties in the United States are neither problematic volunteer weeds in other cropping systems nor are they found as feral populations on unmanaged lands (OECD 2000). The potential fate of soybean seeds remaining in the field after harvest includes; rot, predation, herbicides from rotational crops, and winter weather. Soybeans generally do not survive over the winter season. When climatic conditions are permissive, volunteer soybeans provide minimal interference in the rotational crop and are not recognized as an economic problem in soybean production. Volunteer soybeans are not competitive and can be managed by existing agronomic practices.

Double-herbicide-tolerant soybean transformation event FG72 is tolerant to two herbicides with different modes of action<sup>i</sup>; class G (glyphosate) and class F (isoxaflutole), and remains sensitive to herbicides registered for pre-plant and pre-emergence use for weed control in soybean and other crops which are common in rotation with soybean. Volunteer soybeans can be treated with a pre-emergence or post-emergence herbicides such as 2,4-D, atrazine, glufosinate, mesotrione, acetochlor, dicamba, and others. These products are also widely used for weed control in the rotational crops of soybean.

As soybean are not difficult to control as volunteers in a subsequent crop, and as FG72 has been shown to be no different from cultivated soybean in any of the traits that might impact weediness, the current practice to control volunteers will be effective.

### **VIII.D. Current agronomic practices for soybean**

The introduction of glyphosate tolerant soybeans in 1996 significantly changed the way growers manage weed control in soybeans. Glyphosate tolerant soybeans were rapidly adopted by growers due to many unique properties of glyphosate enabling the grower to simply, effectively and economically manage their weeds.

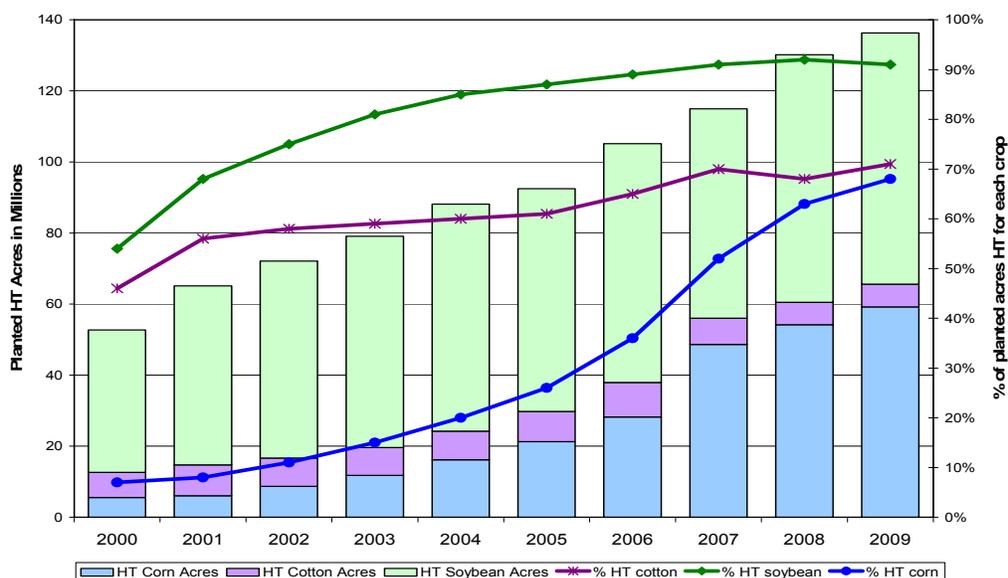
Glyphosate herbicide is exceptionally effective for controlling a broad spectrum of weeds, including many difficult to control weeds, in glyphosate tolerant soybeans with virtually no crop damage. Weed management with glyphosate is also exceptionally simple as the application technique (spray nozzles, spray pattern, carrier volume, and speed of application) has little impact on weed control. Glyphosate herbicide has flexible use rates and patterns and now is more economical since becoming available from generic manufacturers. The use of glyphosate has also resulted in reduction in tillage both prior to planting and in crop cultivation due to its effective weed control (Boerboom and Owen, 2006). This allows growers to forgo tillage which

improves soil conservation, saves labor, and reduces fuel consumption. In addition, the glyphosate tolerant system allows growers to reduce field scouting as effective herbicide applications can be made to large weeds with a wide application window. Furthermore, late season weed control methods for weed escapes such as hand labor, rope wicking, and spot spraying have been virtually eliminated. Additional weed management benefits with glyphosate include no carryover concerns to the following crop, no replanting restrictions, low environmental and human health risks and it is not a restricted-use pesticide (Boerboom and Owen 2006). The glyphosate tolerant trait is widely available and can be found in the highest yielding soybean varieties available on the market.

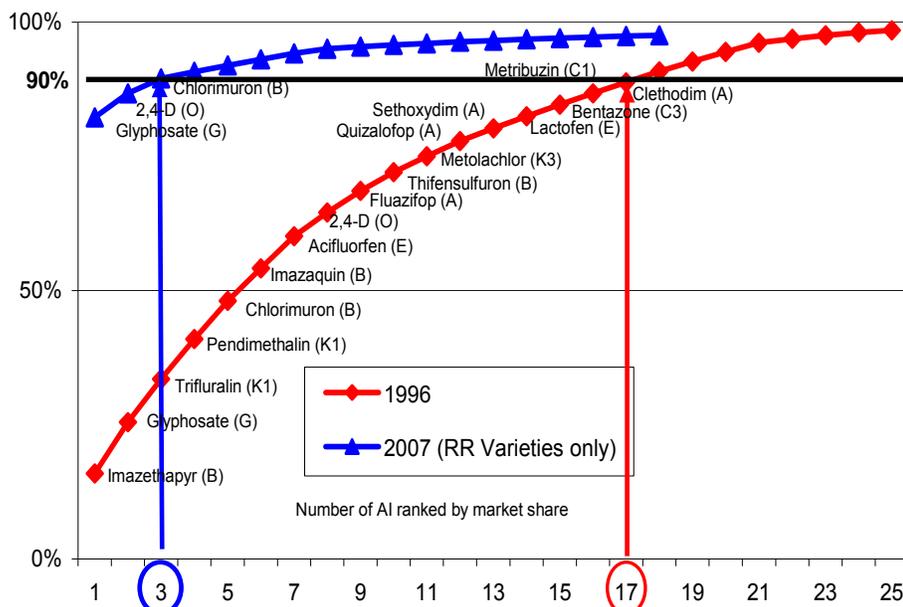
The simple, effective and economical management of weeds with glyphosate in soybean has led to the adoption of a solitary chemical weed control practice at the sacrifice of chemical diversity. By 2007, more than 90% of the US soybean production area was planted to glyphosate herbicide tolerant soybean (USDA 2009). The grower of glyphosate tolerant soybeans makes an average of 1.7 applications of glyphosate to the crop per growing season (USDA 2007). The rapid adaptation of this new technology and the exclusion of other weed control measures set the stage for weed population shifts and the evolution of weeds resistant to glyphosate herbicide. With the application of glyphosate herbicide over most of the soybean production areas, many weeds were exposed to the herbicide and resistant biotypes were enriched

The extensive use of glyphosate was encouraged by the availability of glyphosate tolerance in other crops. Glyphosate tolerant corn is grown on 68% of the 87 million acres of corn in the US. Glyphosate tolerant cotton is grown on 71% of the 9.5 million acres of cotton (Figure 9, USDA 2009). Also available in the market are glyphosate tolerant canola and sugar beet varieties thus, creating unprecedented selection pressure for resistant biotypes. In 1996, seventeen herbicides composed 90% of the market. In 2007, only three herbicides comprised 90% of the market with glyphosate at 80% (Figure 10) for use in soybean production in the USA.

**Figure 9. Growth of herbicide tolerant traits**



Adapted from USDA, NASS Acreage 2001-2009

**Figure 10. Comparison of herbicide use for the years 1996 and 2007**

The declining use of herbicide options lead to the loss of herbicide diversity in soybean weed control in the USA. Indicated in the figure by the red diamond line, seventeen herbicides composed 90% of the market in 1996. In 2007, only three herbicides comprised 90% of the market with glyphosate at 80% and 2,4-D and chlorimuron in minor use. Letters in brackets indicate the mode of action (HRAC 2009).

The over-reliance on a single weed control method can lead to the eventual development of resistant weeds and consequential loss of that particular production system and perhaps even eventually jeopardize the ability to grow a specific crop in a specific field. Because of cost considerations and the additional workload, IWM tactics generally have not been employed until herbicidal efficacy starts to fail and herbicide resistance becomes a problem threatening the economic viability of the farmer. Working preemptively through incorporating integrated weed management measures can lead to the successful prevention of the development of a resistant weed population.

There are currently 189 species of resistant weeds worldwide, 16 of which are resistant to glyphosate (Heap 2009). Growers have been reluctant to alter their weed management practices and return to conventional herbicides in light of the advent of glyphosate resistant weeds. Even with weed resistance, growers will continue to produce glyphosate tolerant crops as glyphosate herbicide remains effective for a number of weeds that are difficult to control with other herbicides. Conventional herbicides generally have a narrower application window, narrower weed spectrum, increased risk of crop injury, various application techniques required, additional time for sprayer cleanout between fields, carryover concerns, replanting restrictions and are considered to be less economical.

However, growers are beginning to alter their farming practices to gain better control of glyphosate resistant weeds. Some growers are utilizing tankmixes with conventional herbicides to help control herbicide-resistant weeds. Conventional tankmix partners have limitations however such as increased cost, increase the risk of injury to the soybeans and limit the application window due to weed size restrictions. Some growers have moved away from reduced or no-till practices as their burndown program no longer provides effective control of glyphosate resistant marestail. Uncontrolled weeds result in soybean yield loss as the weeds compete for soil nutrients, moisture, and sunlight. The impact of glyphosate-resistant weeds firmly impacts a grower's available time and financial resources.

There is an urgency to produce viable alternatives to glyphosate weed control programs in soybeans. There are several HT soybean products available to the US soybean grower (Table 3) however most provide crop tolerance to herbicides for which herbicide resistant weeds are already identified (ALS inhibitors and glyphosate).

Today, the LibertyLink soybean system is the only nonselective alternative to the glyphosate system available for growers. Launched in 2009, there were more than 300,000 acres of LibertyLink soybeans planted in the US. LibertyLink soybeans also must be managed correctly to prevent the development of herbicide-resistant weeds.

#### VIII.E. Potential impact on agricultural practices for soybean

In the near future, soybean growers will have additional options. Table 30 identifies several new soybean events in the process of development and registration. A new herbicide mode of action 4-hydroxy-phenyl-pyruvate-deoxygenase enzyme by specific inhibitors (HPPD inhibitors) was developed during the 1980's. The double-herbicide-tolerant soybean event, FG72 which combines glyphosate tolerance with isoxaflutole tolerance is the first genetic source of crop tolerance to the HPPD inhibiting herbicides.

**Table 30. Sources of genetic-based herbicide tolerance**

<b>Applicant(s)</b>	<b>Event / Trade Name</b>	<b>Trait Description(s)</b>
Bayer CropScience 98-014-01p	A5547-127 / LibertyLink™	Glufosinate tolerant
Pioneer Hi-Bred International 06-271-01p	356042 / Optimum™ GAT™	Glyphosate and ALS inhibitor tolerant
BASF Plant Science 09-015-01p	BPS-CV127-9	ALS tolerant
Bayer CropScience and MS Tech 09-xxx-01p	FG72/ Double-Herbicide- Tolerant soybean	Glyphosate and HPPD tolerant
Monsanto Company	Not announced	Dicamba tolerant

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#### **VIII.F. Weed resistance management**

Weed scientists agree that adopting and implementing best management practices that reduce weed resistance to herbicides is critical (Boerboom and Owen, 2006). We have developed detailed methods for integrated weed management that includes diverse farming practices. Integrated weed management not only improved overall weed control, it provides additional benefits such as improving the overall level and consistency of weed control, adding flexibility in scheduling applications and reducing the risk of yield loss due to weed competition

Ideally integrated weed management should utilize all available tools including herbicides in a well balanced program as the lower the diversity of weed control tools, the higher the risk of selecting resistant biotype becomes. To ensure diversification is maintained in weed control methods, we will also encourage growers to keep detailed records of weed management practices for each field. Our integrated weed management guidelines promote an economically viable, environmentally sustainable and socially acceptable weed control program is fully detailed in Appendix 5. The highlights of our integrated weed management include:

- 1) Correctly identify weeds and look for trouble areas within field to identify resistance indicators.
- 2) Rotate crops.
- 3) Start the growing season with clean fields.
- 4) Rotate herbicide modes of action by using multiple modes of action during the growing season and apply no more than two applications of a single herbicide mode of action to the same field in a two-year period. One method to accomplish this is to rotate herbicide-tolerant trait systems.

- 5) Apply recommended rates of herbicides to actively growing weeds at the correct time with the right application techniques.
- 6) Control any weeds that may have escaped the herbicide application.
- 7) Thoroughly clean field equipment between fields.

#### **VIII.G. Potential impact on farming practices**

Although more than 90% of the soybean acres planted today are glyphosate-tolerant, conventional and organic farming continue to be an important sect of the soybean market. Conventional and organic soybean growers will find no adverse effect on their farming practices with the introduction of FG72 soybeans.

It is not likely that organic farmer or other farmers who choose not to grow FG72 soybeans will be significantly impacted by the expected commercial use of this product. Nontransgenic soybeans varieties will still be available for conventional and organic soybean producers. Soybean is mostly a self-fertilized plant and therefore limits the chance of hybridization to conventional soybean varieties. In addition to the National Organic Program administered by USDA's Agricultural Marketing Service which requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management.

#### **VIII.H. Potential effects on non-target organisms, including beneficial organisms**

No adverse effect on non-target organisms from either the transgenic or non-transgenic plants was observed during any of the trials. Refer to Section VII D for biotic and abiotic stress characteristics.

The FDA issued a finding of "No Concern" for glyphosate tolerant soybeans. As the presence of the 2mEPSPS and the HPPD proteins are the only difference found in FG72 that is not found in conventional soybean, FG72 and its progeny should have no indirect or direct plant pest effects.

#### **VII.I. Threatened and endangered species considerations**

The US Fish & Wildlife Service (FWS) has accountability for endangered species under the Endangered Species Act (ESA), (16 USC 1531). Section 6 of the ESA requires federal agencies who conduct activities which may affect listed species to consult with the FWS to ensure that listed species are protected should there be a potential impact.

It is not anticipated that the use of FG72 soybean will impact any currently listed species of concern. Species of concern that may inhabit areas close to commercial soybean operations would not be impacted by the use of FG72 soybean. Commercial agriculture routinely disturbs the ground in which crops are currently planted. As a result, perennial vegetative species would not grow in these areas. Additionally, because horizontal gene flow to sexually incompatible species is not an issue, there is negligible potential for exposure to the transgenes contained in FG72 soybean through sexual reproduction.

Isoxaflutole is currently registered for weed control use in corn in 18 of the primary corn producing states in the US. Collectively, these states represent approximately 75 % of the planted acres for corn and soybean (a four year average through 2009 - Doane). End use products containing the active ingredient isoxaflutole are listed as "Restricted Use" and are for sale and use only by certified applicators. The approved and proposed end use product labels (e.g., tolerant soybean) also have extensive precautionary and restrictive language statements addressing handling and use of the product including specific endangered species protection requirements. The EPA "Registration Review" process for isoxaflutole is scheduled to be initiated in fiscal year 2011.

Glyphosate is currently supplied to US growers by numerous generic sources of the active ingredient and generic end use products. This active is registered for use on tolerant soybean, corn and cotton as well as on specific non-tolerant crops and non-crop uses. The current Bayer understanding is that future endangered species assessments will be addressed in the "Registration Review" process for this active which was initiated by EPA in July of 2009.

#### **VIII.J. Potential impact on biodiversity**

Soybean is considered a self-pollinated species, propagated commercially by seed. The soybean flower stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high level of self-fertilization and cross pollination is usually less than one percent (Beckie 2007, Palmer *et al.*, 2001).

There is no evidence of genetic transfer and exchange with organisms other than those with which soybean is able to produce fertile crosses through sexual reproduction (Beckie 2007, Stewart *et al.*, 2003). There are no wild *Glycine* species in the United States, nor are their wild or weedy species with which soybeans can produce fertile crosses.

#### **VIII.K. Conclusion**

It has been demonstrated that the presence of the 2mEPSPS and HPPD W336 proteins introduced in FG72 soybean will not present adverse environmental effects. The lack of wild type soybean species or relatives in the Americas in addition to the self pollinating nature of soybean prevents gene transfer into unintended targets. The current practice to control volunteer soybean plants will not be altered by FG72. Current agronomic practices limit weed control diversity tactics. The introduction of FG72 will provide a new mode of action for weed control in soybean to increase improve resistant weed management. It is expected that growers who choose not to grow FG72 will not be impacted by the commercial use of this product. It is also not anticipated that the commercial use of FG72 will have any potential impacts on non-target organisms or on threatened or endangered species.

## **IX. STATEMENT OF GROUNDS UNFAVORABLE**

Bayer CropScience and M.S. Technologies know of no study data and/or observations associated with Event FG72 soybean that will result in adverse environmental consequences for its introduction. The only biologically relevant phenotypic difference between Event FG72 soybean and conventional soybean is the expression of the 2mEPSPS and HPPD W336 proteins which provide tolerance to the application of glyphosate herbicide and isoxaflutole herbicide, respectively. Planting double-herbicide-tolerant soybean varieties, containing transformation event FG72, will provide growers with new options for weed control using IFT herbicide in combination with a glyphosate herbicide. Glyphosate is widely used in herbicide-tolerant soybean and other agricultural production systems. IFT herbicide offers an alternative weed control option for the soybean grower via a new herbicide mode of action for soybeans that is efficacious against many of the herbicide resistant weeds currently found in soybean fields.

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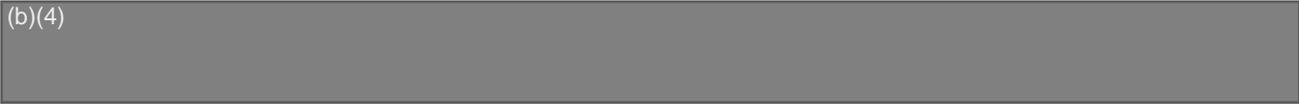
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## **Appendix 1**

### **FIELD TRIAL TERMINATION REPORTS 2001-2008**

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**USDA Field Termination Report**

**Notification No.:**

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**Applicant No.:**

**Permittee:**

**Regulated Article:**

**Site Release Information:**

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Information on each release follows:

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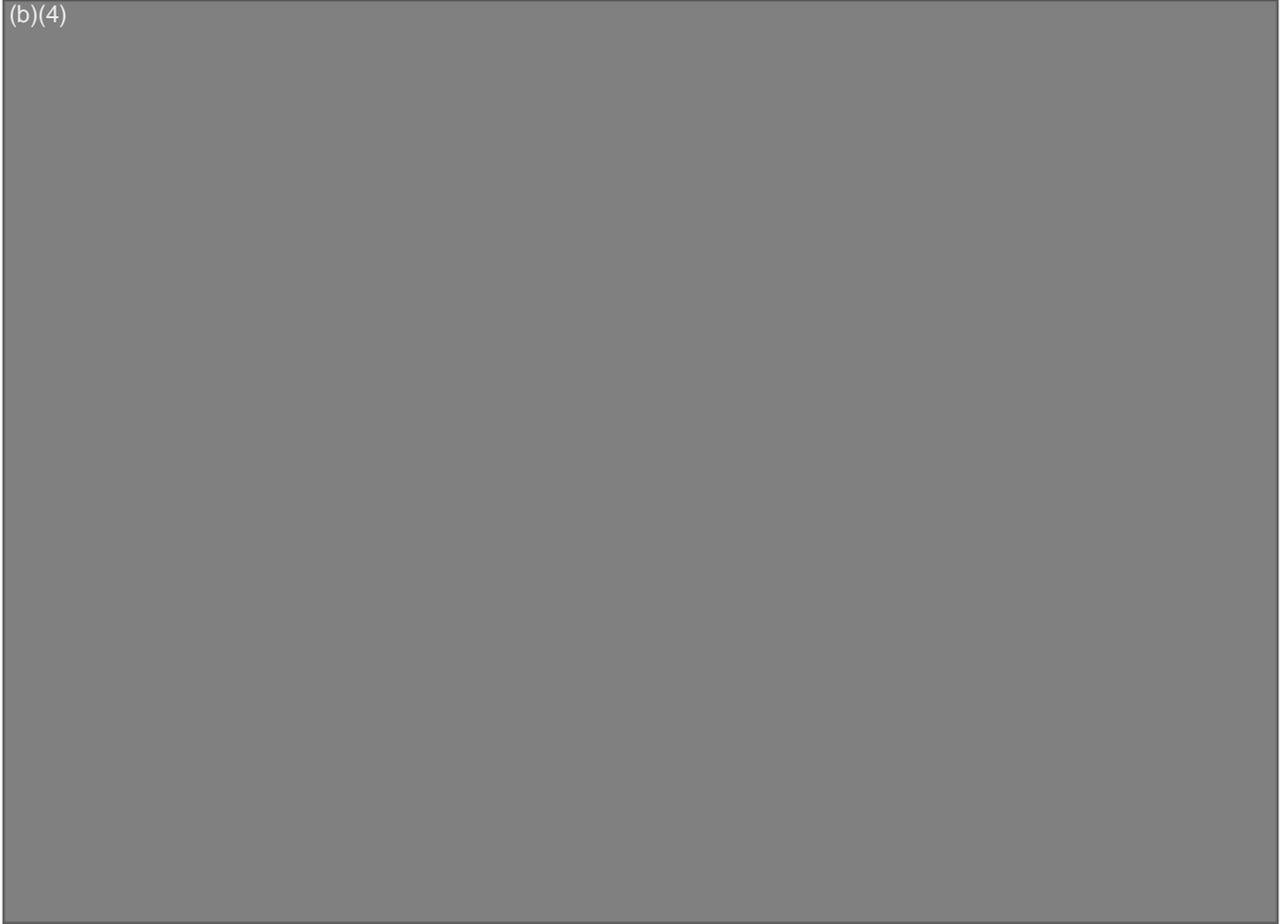
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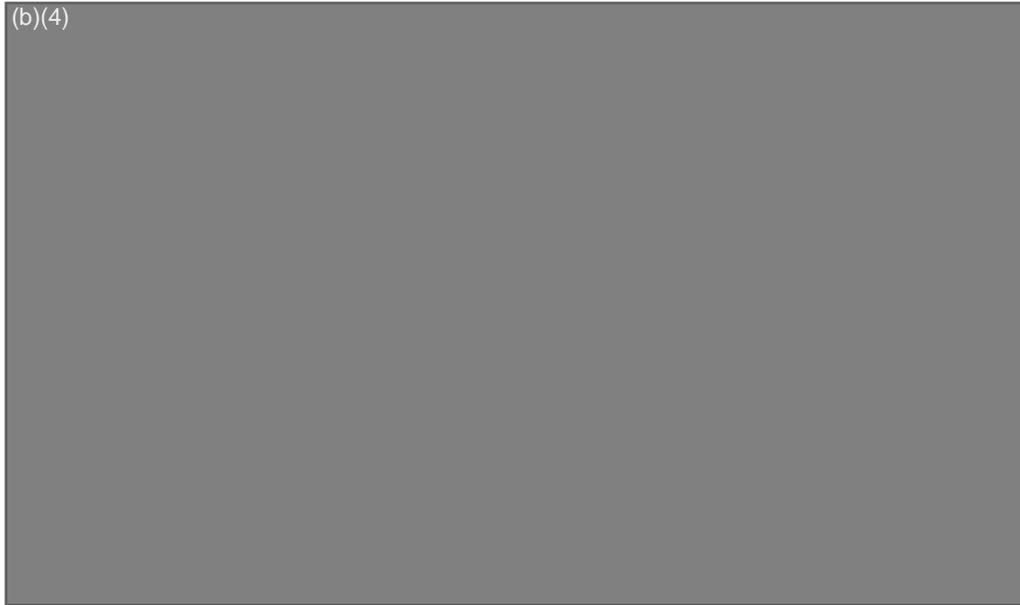
Observations:

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**Results:**

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**Plant Disposition:**

**Volunteer Monitoring:**

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*Field Management:*

*Non-Target Organisms:*

*Weather Synopsis:*

*Containment Measures:*

**USDA Field Termination Report**

**Notification No.:**

**Applicant No.:**

**Permittee:**

**Regulated Article:**

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As follows:

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**Purpose of Release:**

**Observations:**

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**Results:**

**Plant Disposition:**

**Volunteer Monitoring:**

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*Weediness Characteristics:*

*Non-Target Organisms:*

*Weather Synopsis:*

*Containment Measures:*

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**USDA Field Termination Report**

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**Applicant No.:**

**Permittee:**

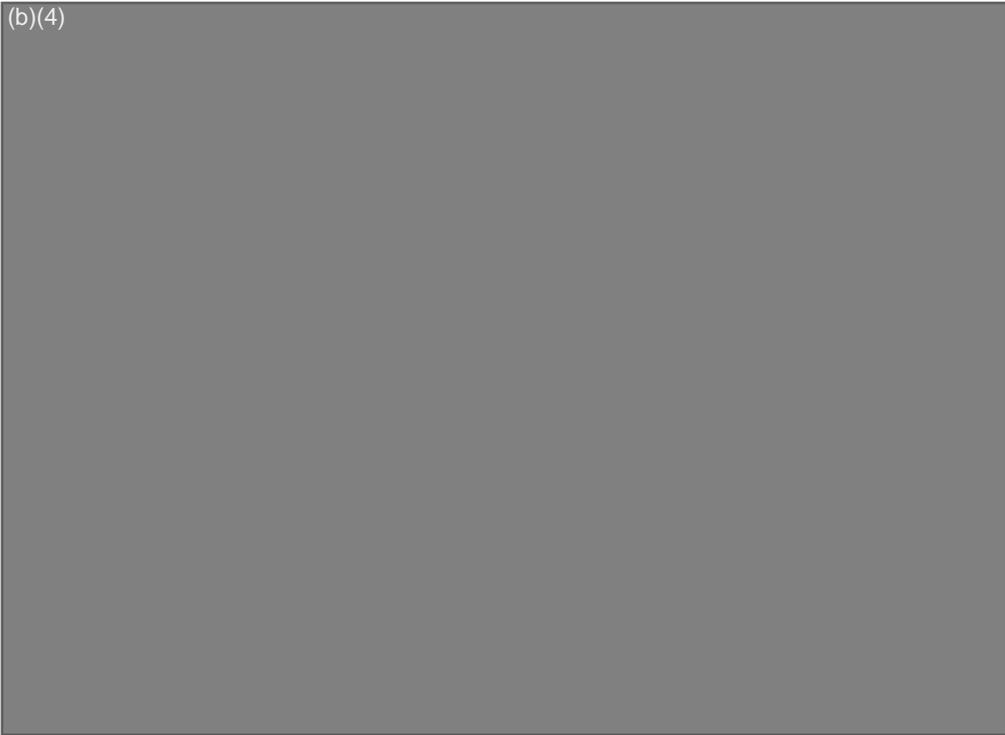
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**Purpose of Release:**

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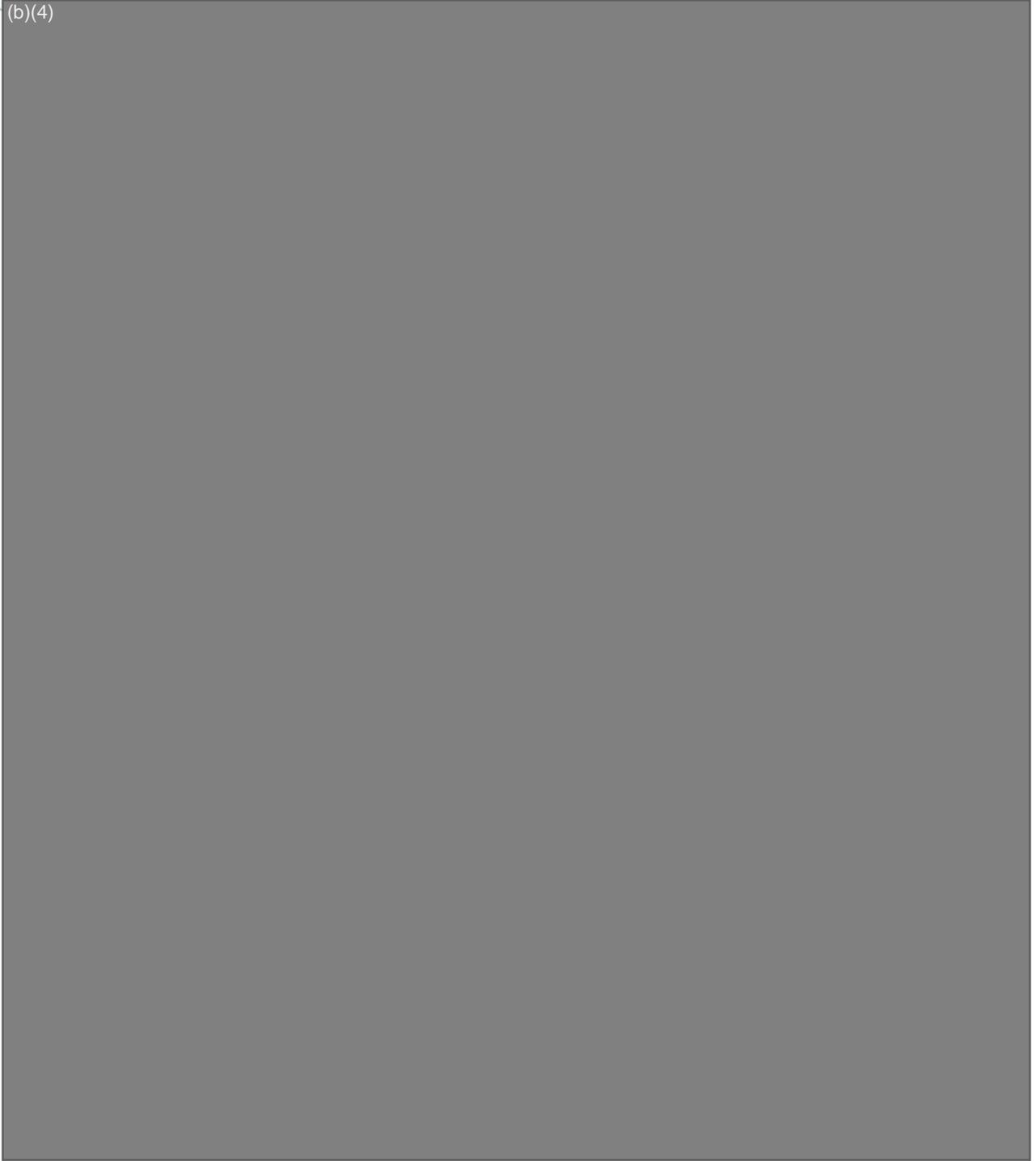
**Observations:**

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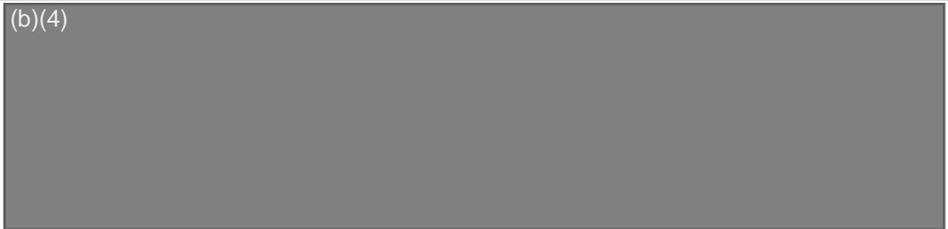


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**Results:**

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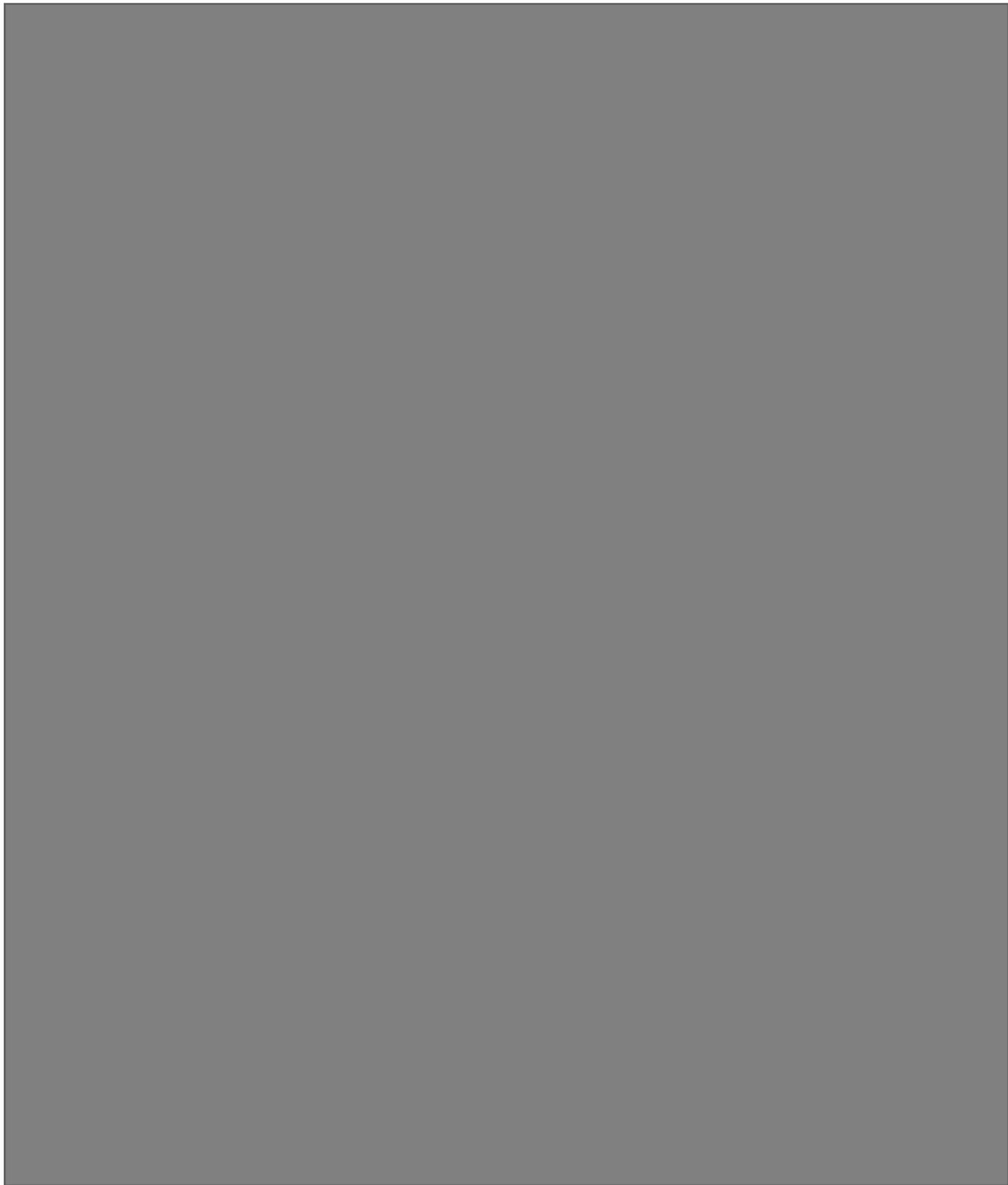


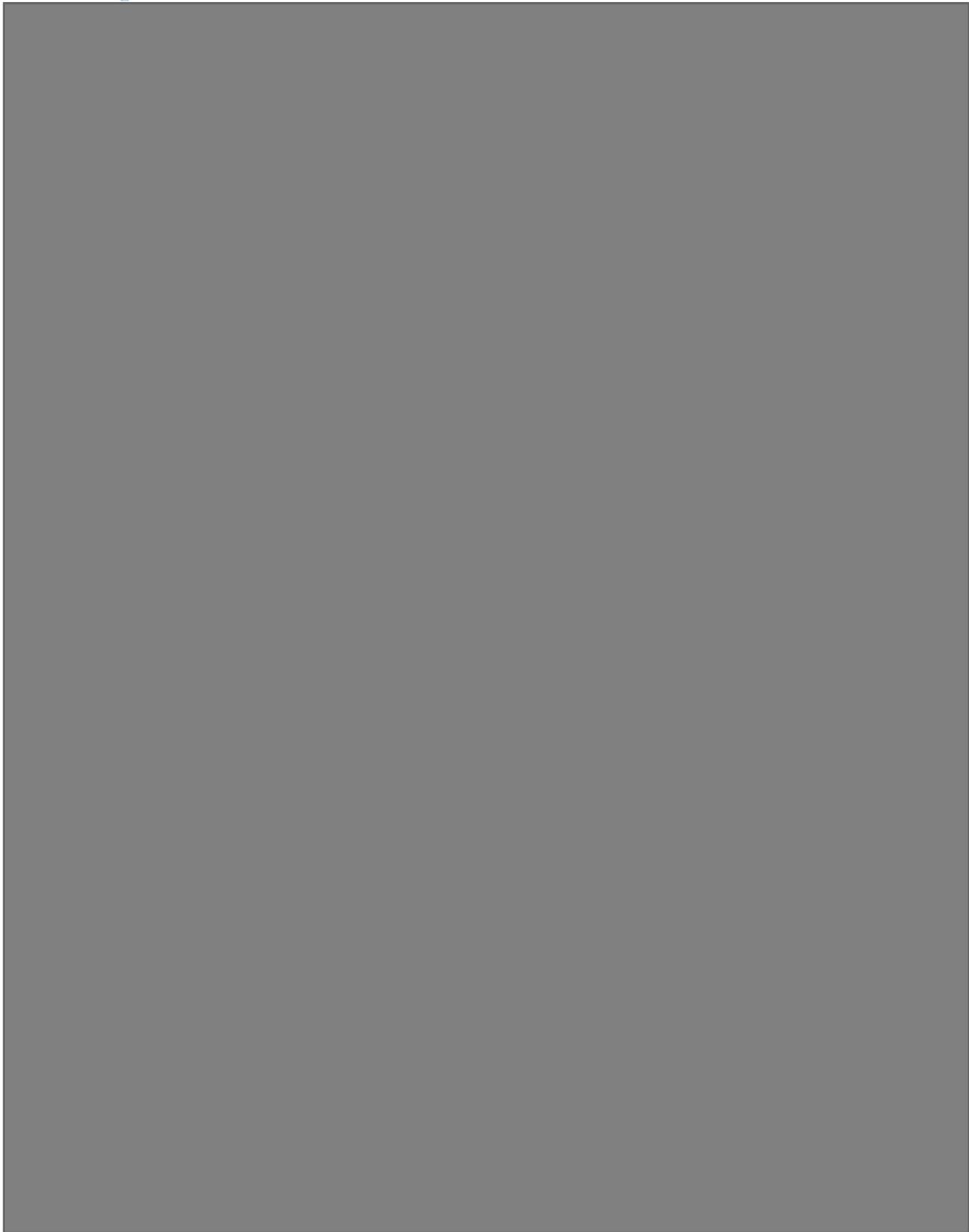
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**Plant Disposition:**

See below:



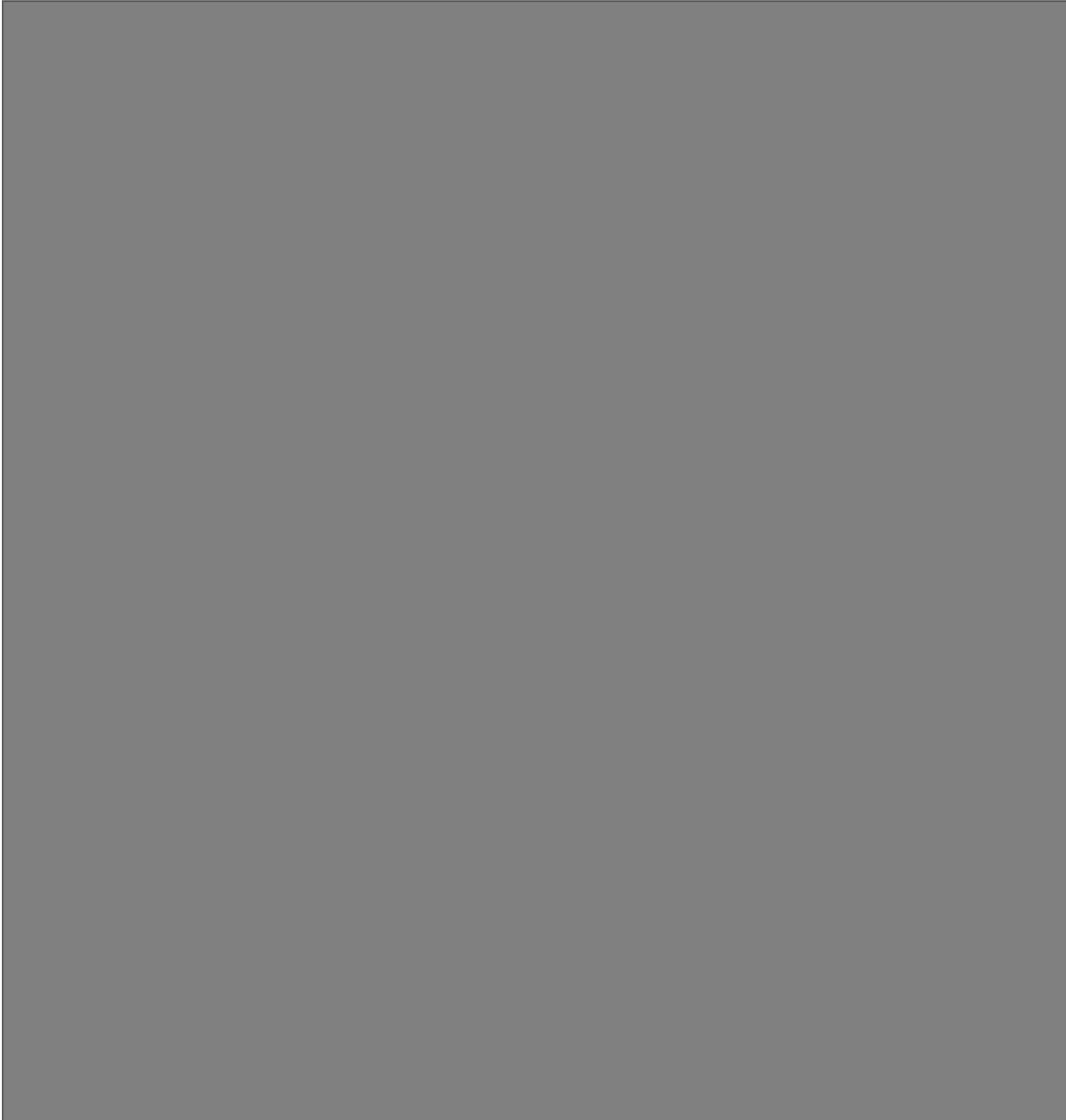


\*Volunteer monitoring of this plot will continue in 2005 to ensure the elimination of all volunteer soybean plants.



*Weediness Characteristics:*

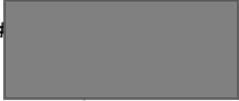
*Non-Target Organisms:*



**USDA Termination Report for Herbicide Tolerant Soybean**

M.S. Technologies, LLC

**Notification#**



**Applicant:**



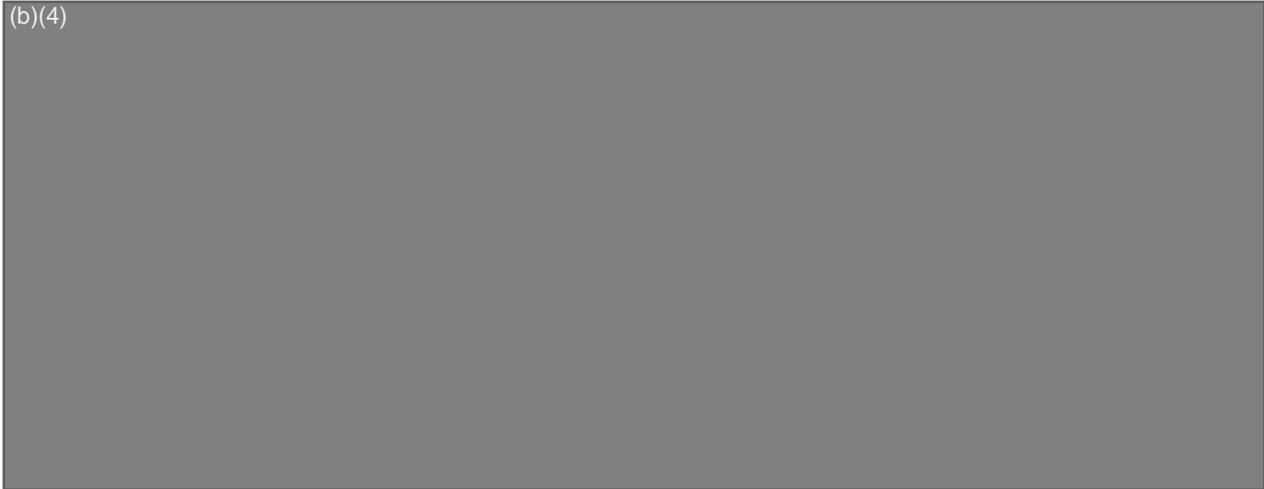
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**Release Site Information:**



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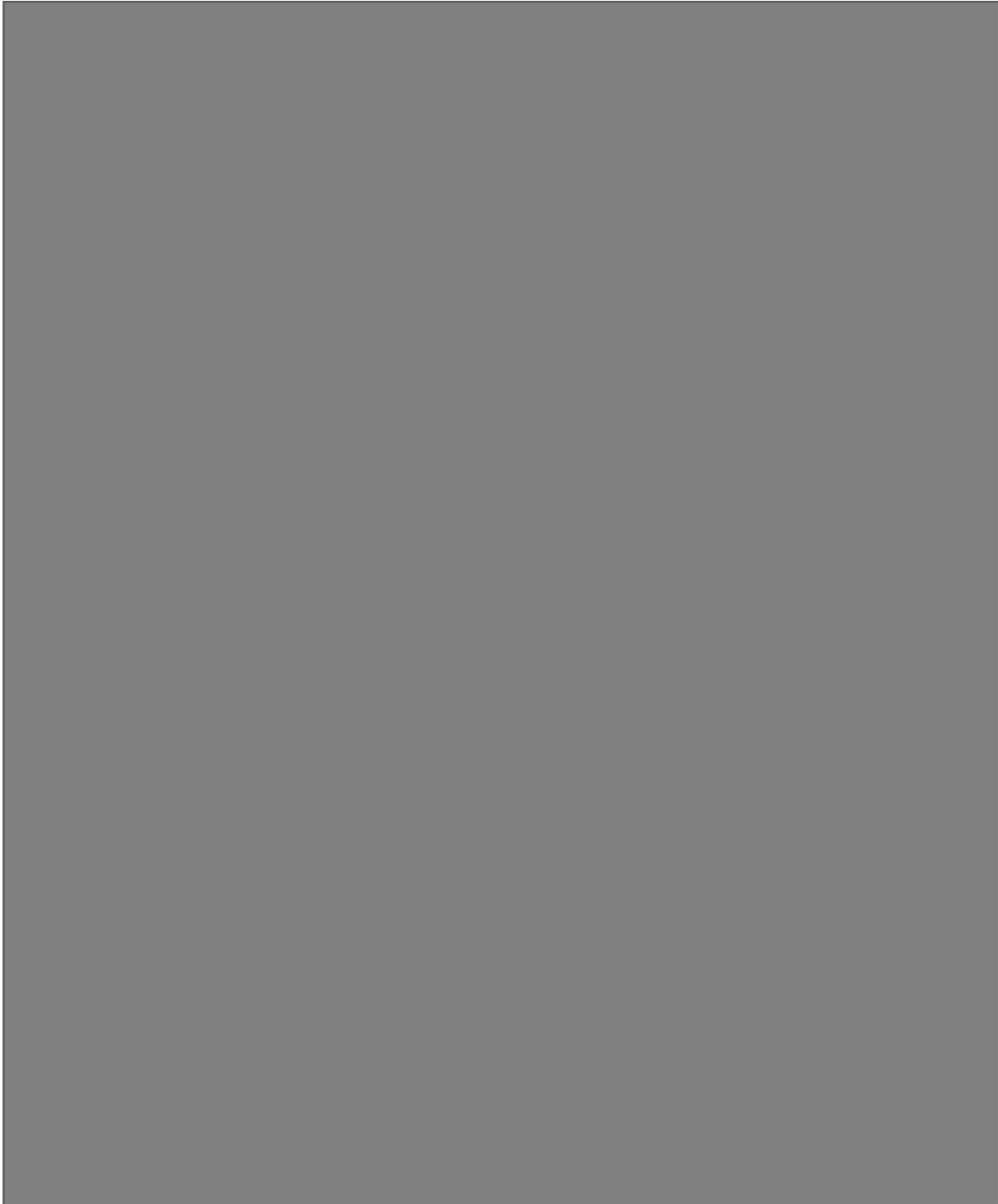


Date 7/14/09



**USDA Termination Report**

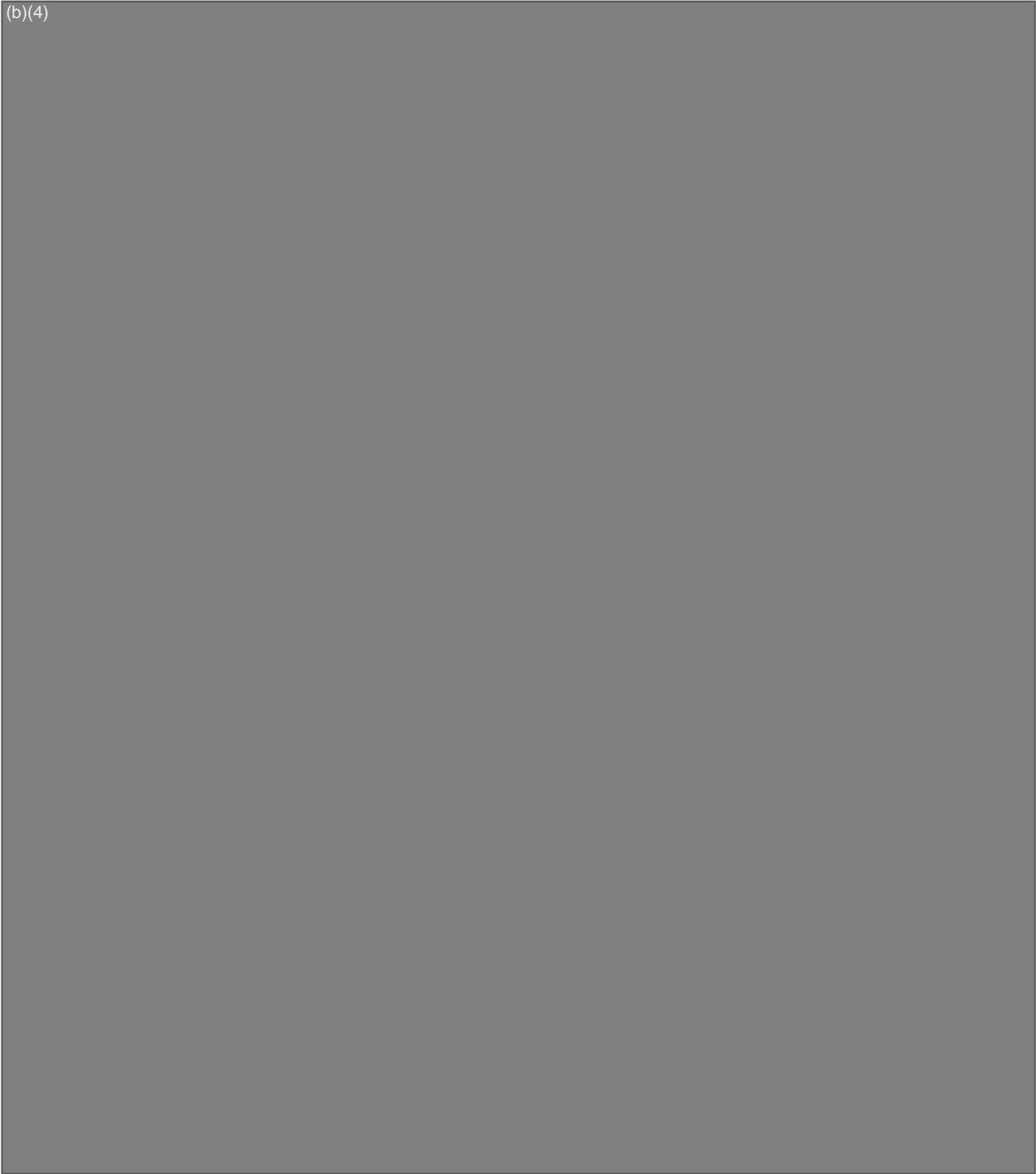
Notification



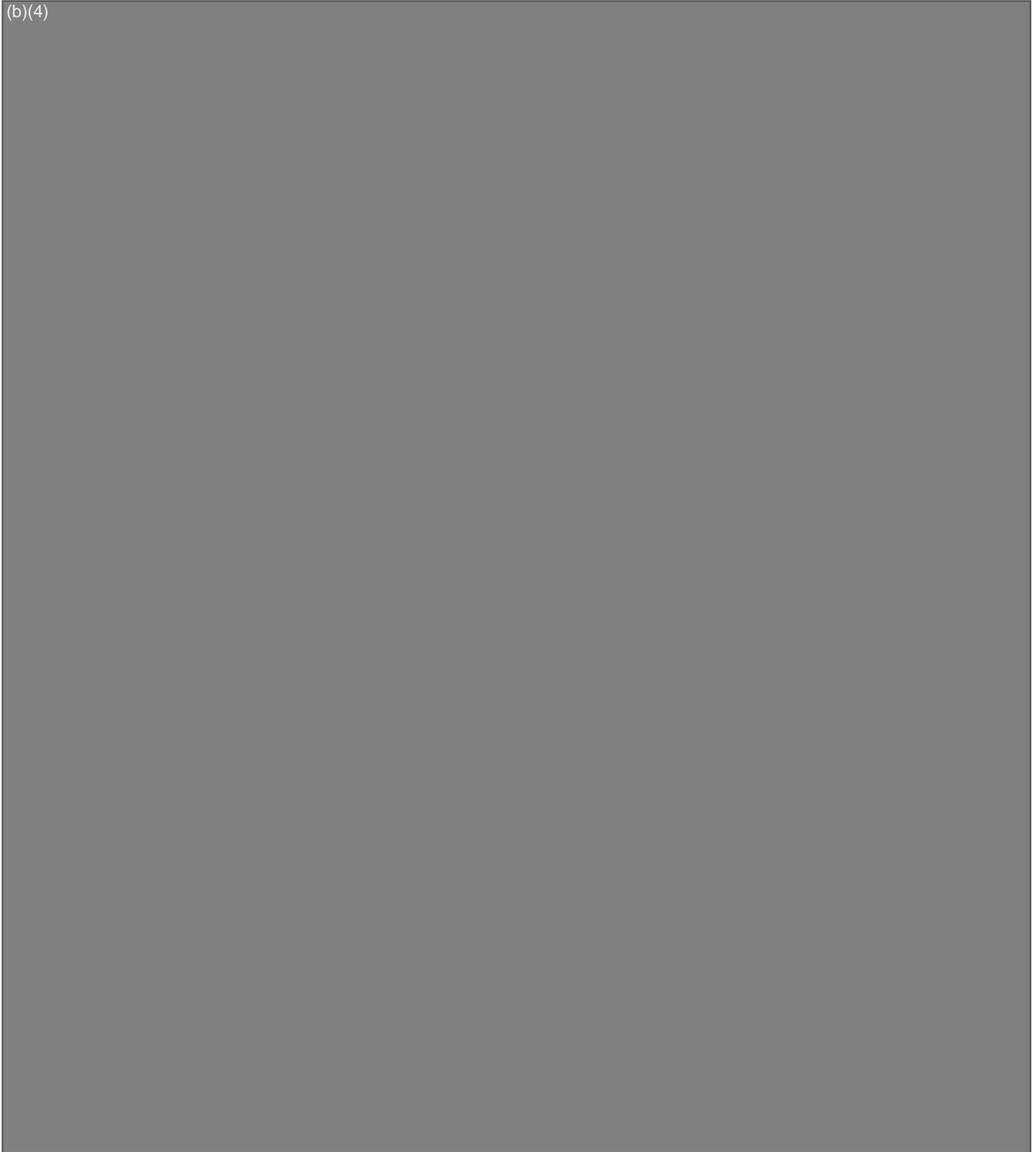
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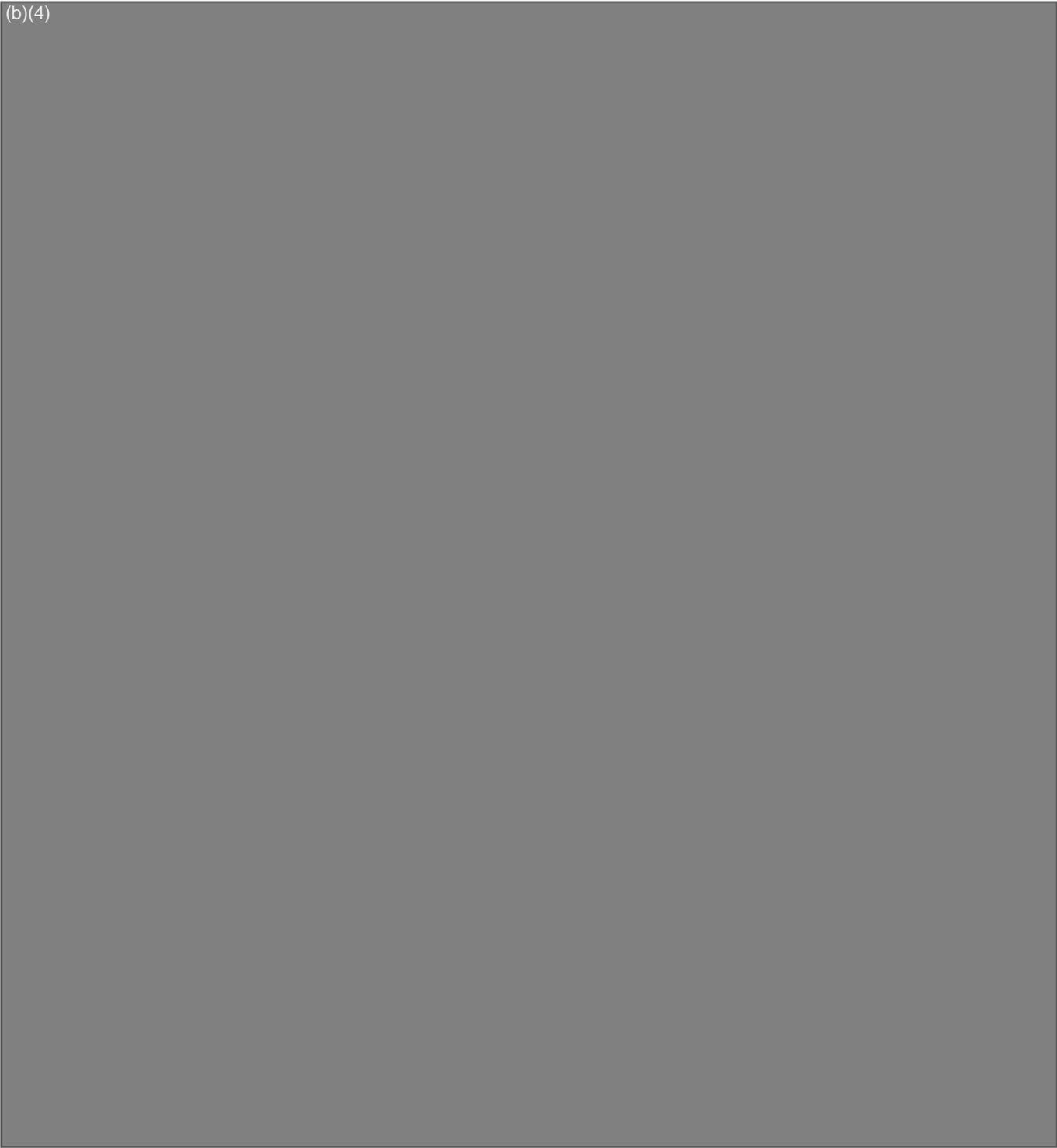
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USDA Termination Report for Herbicide Tolerant Soybean

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**Permit #:**

**Permitter:**

**Permittee:**

**Regulated Article:**

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**Release Site Information:**

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## **Appendix 2**

### **MATERIALS AND METHODS- PRODUCT CHARACTERIZATION**

## 2.A. Materials and methods for molecular characterization - DNA tests

### Materials

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### Identity of the materials

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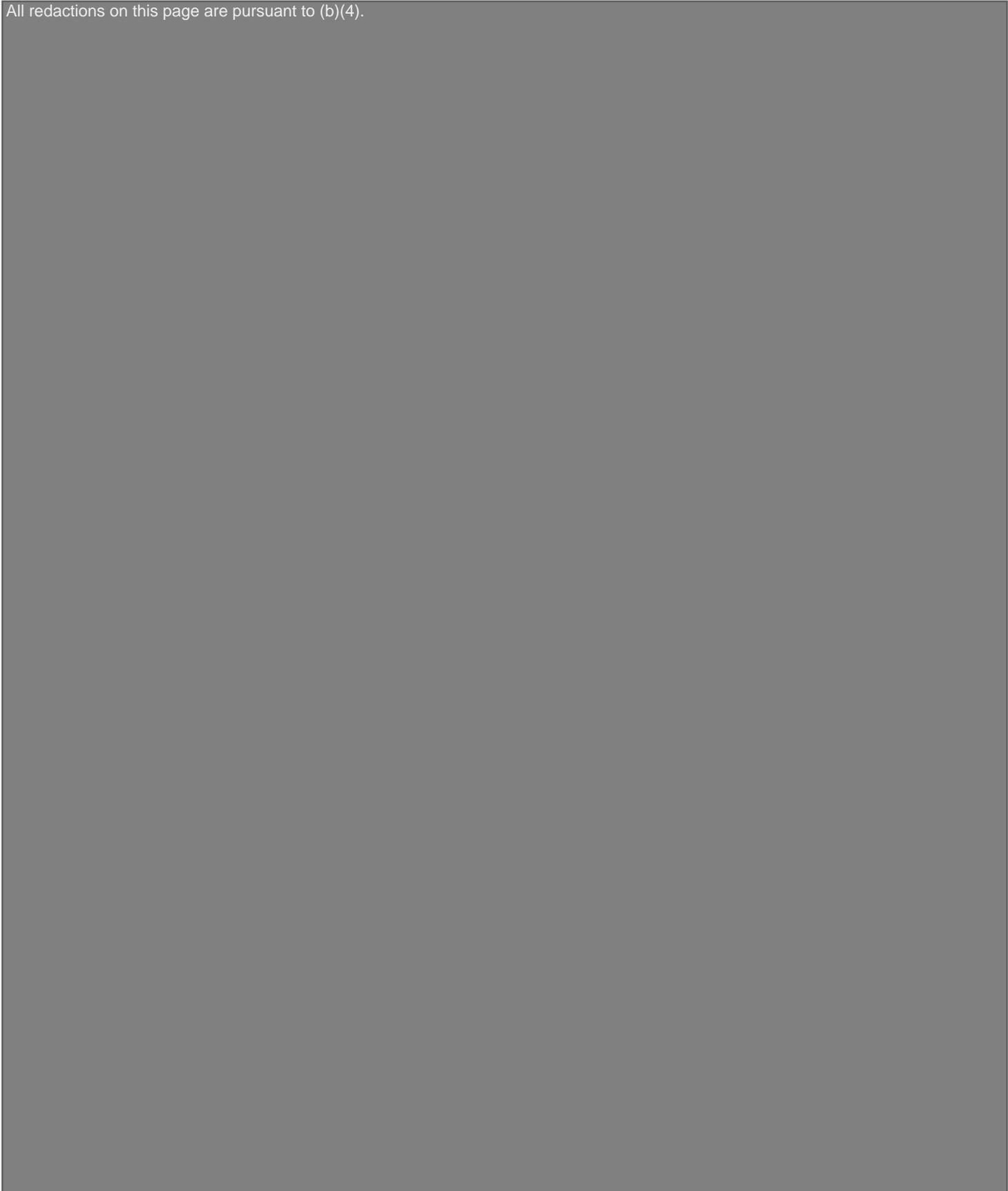
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**2.B. Materials and methods for protein characterization tests**

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**2.C. Materials and methods for protein levels in grain**

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**Sample preparation**

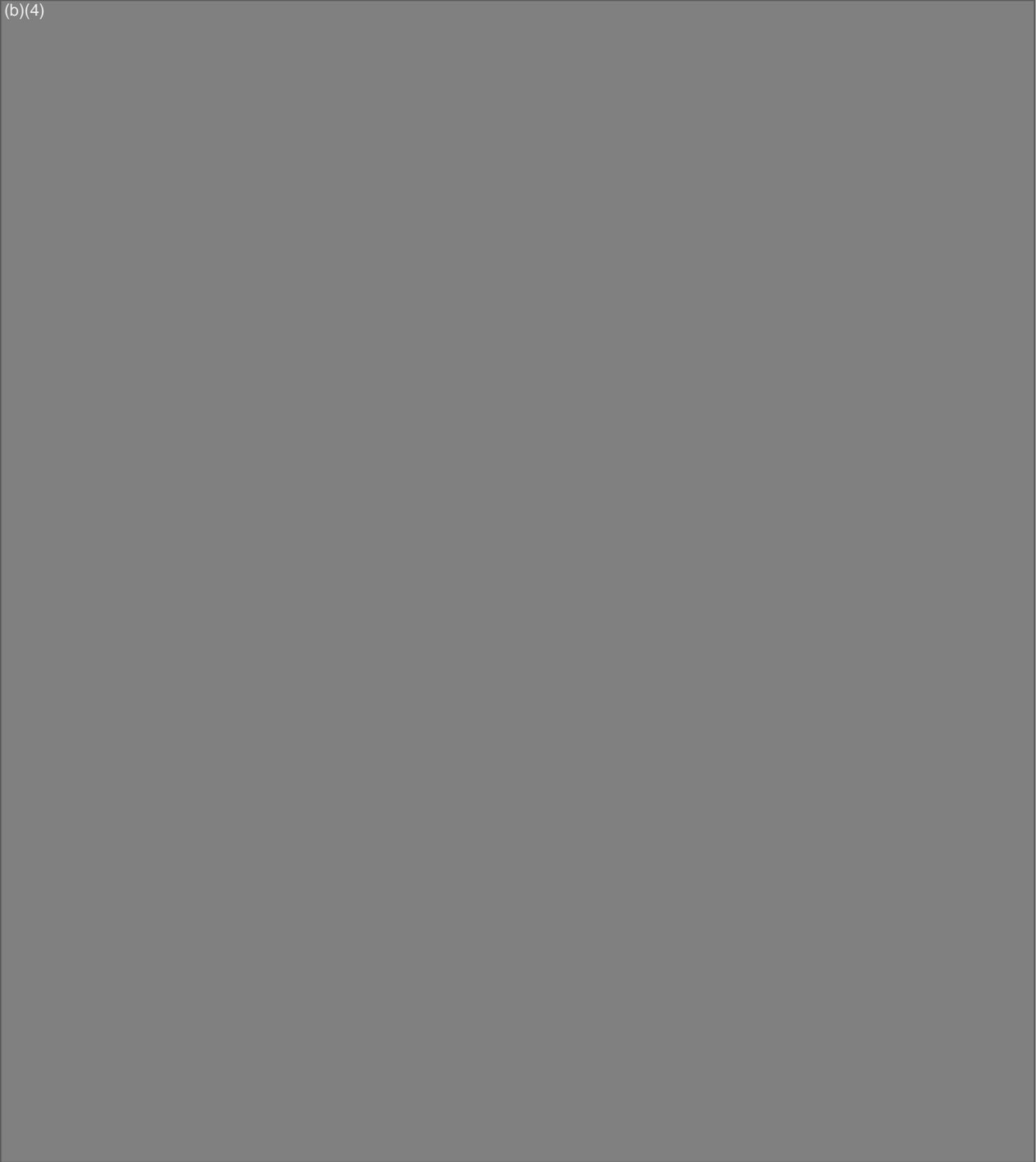
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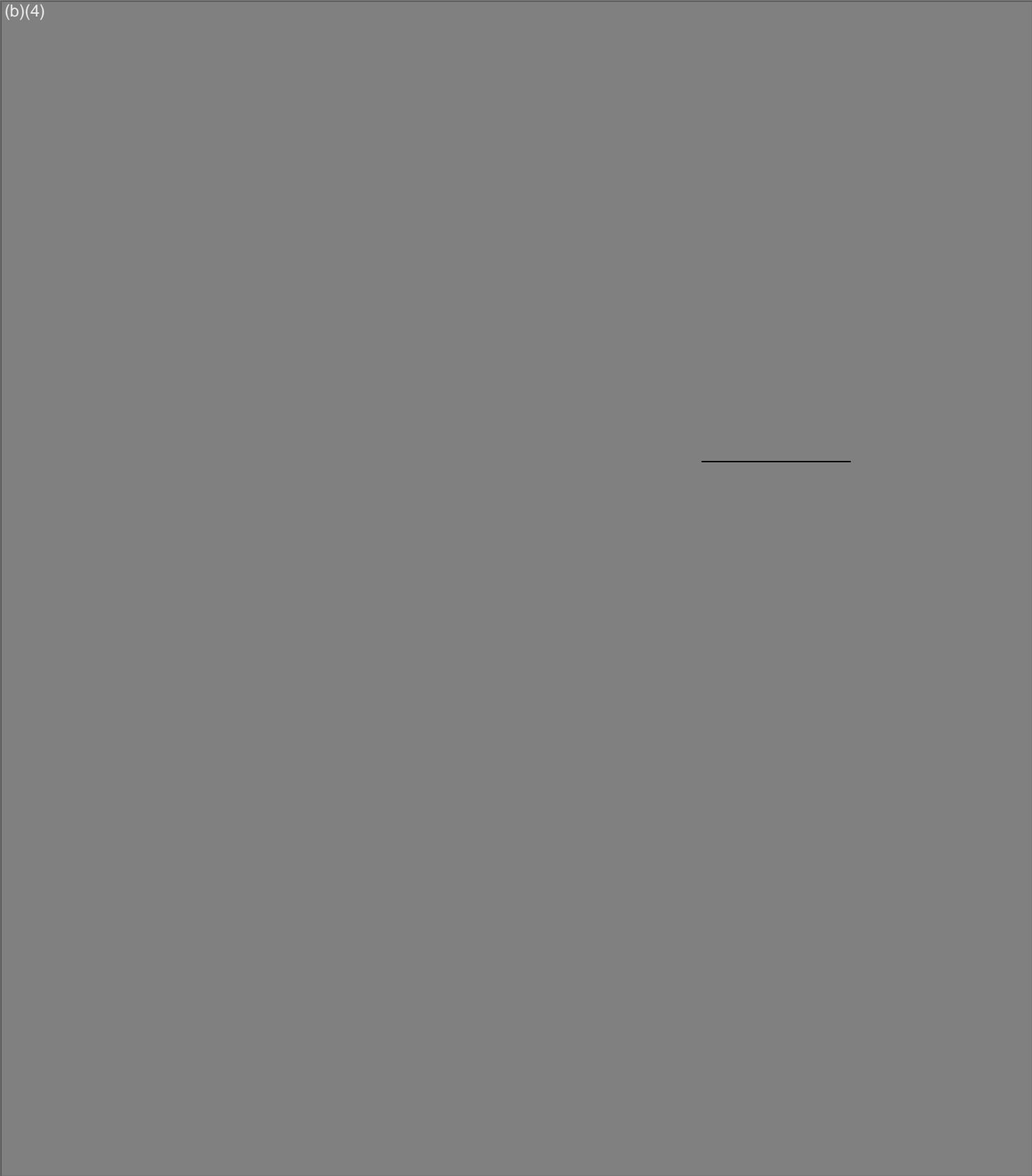
**Bioassay**

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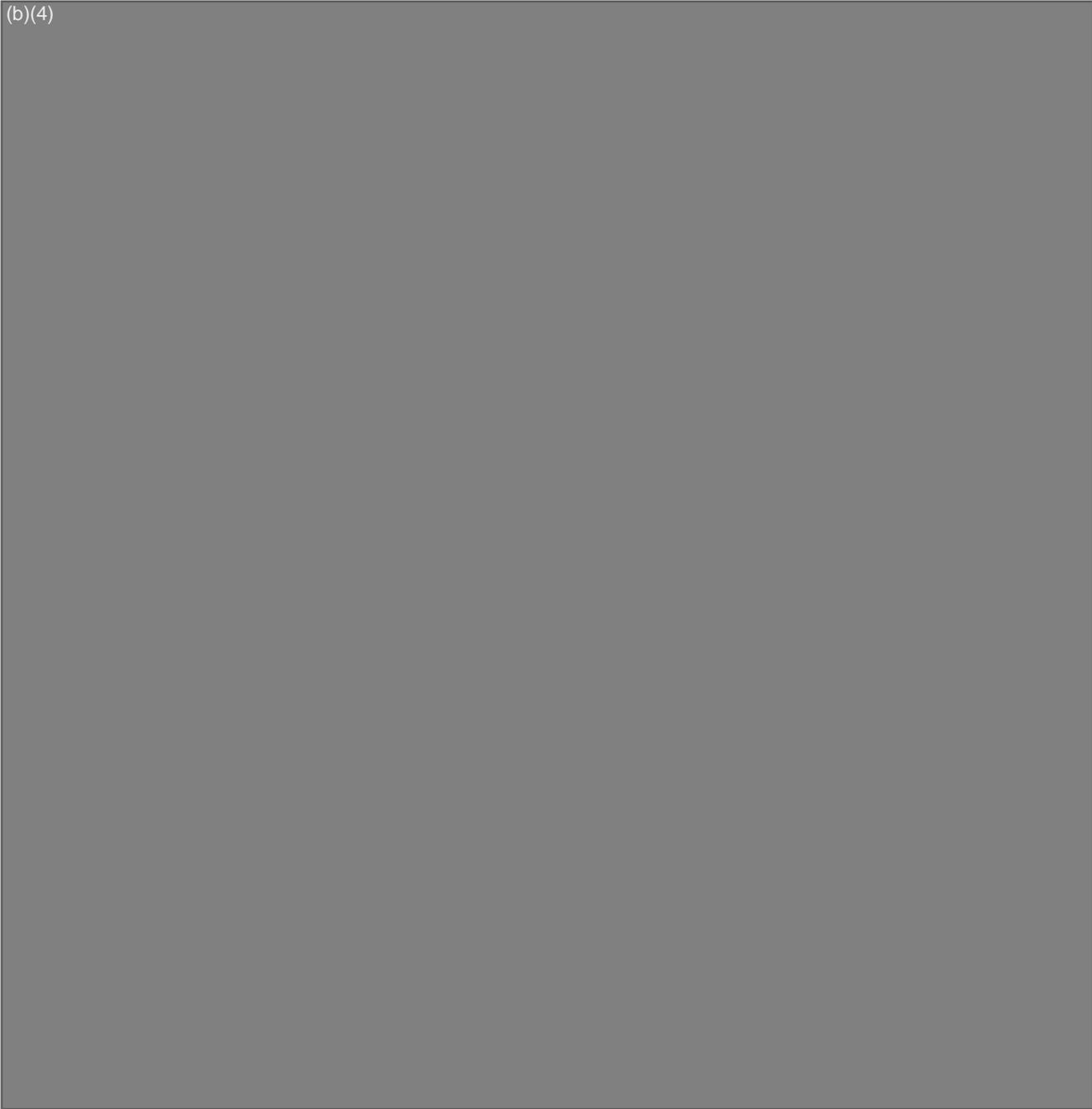
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**2.D. Materials and methods for protein levels in plant parts and during the life cycle**

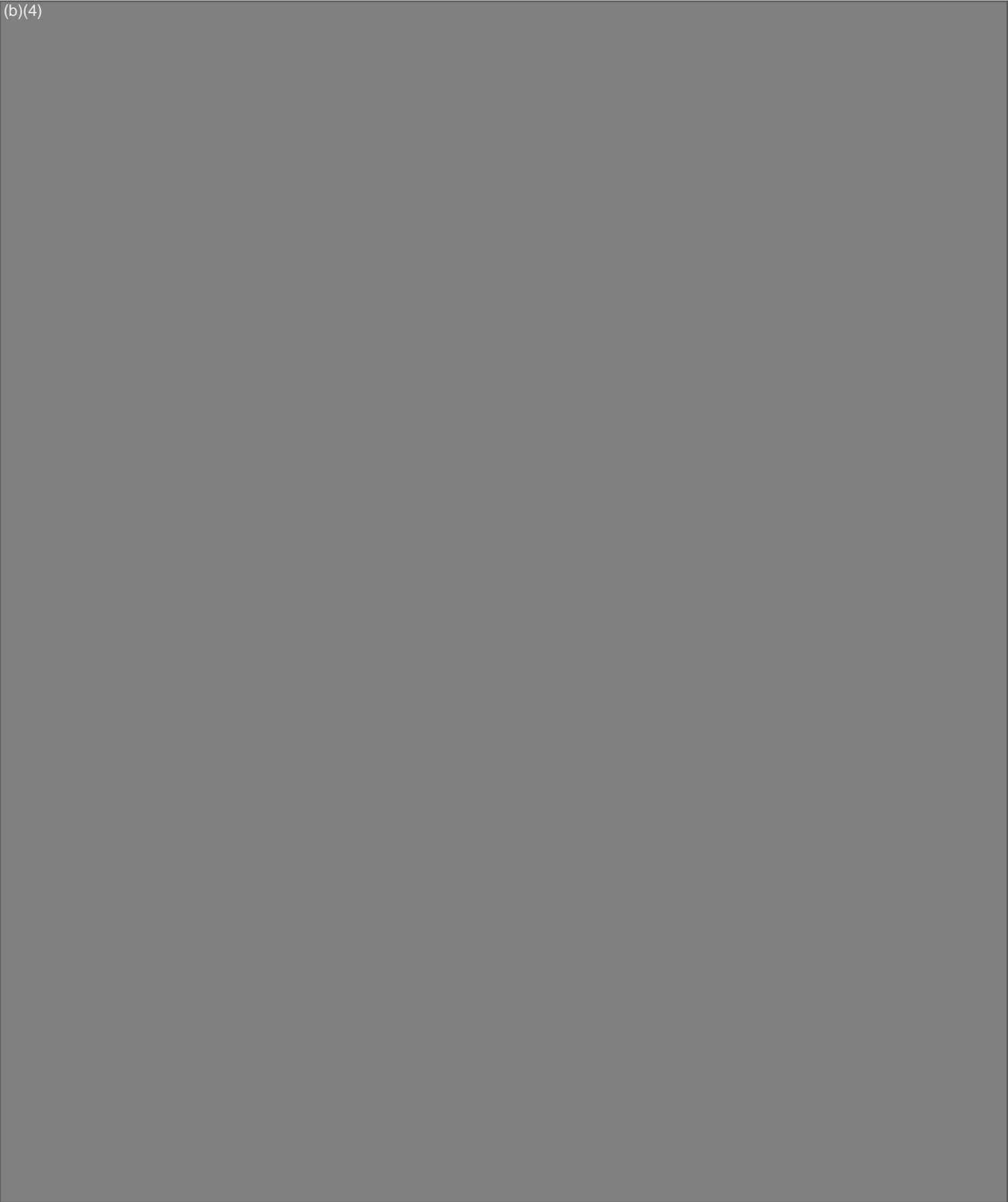
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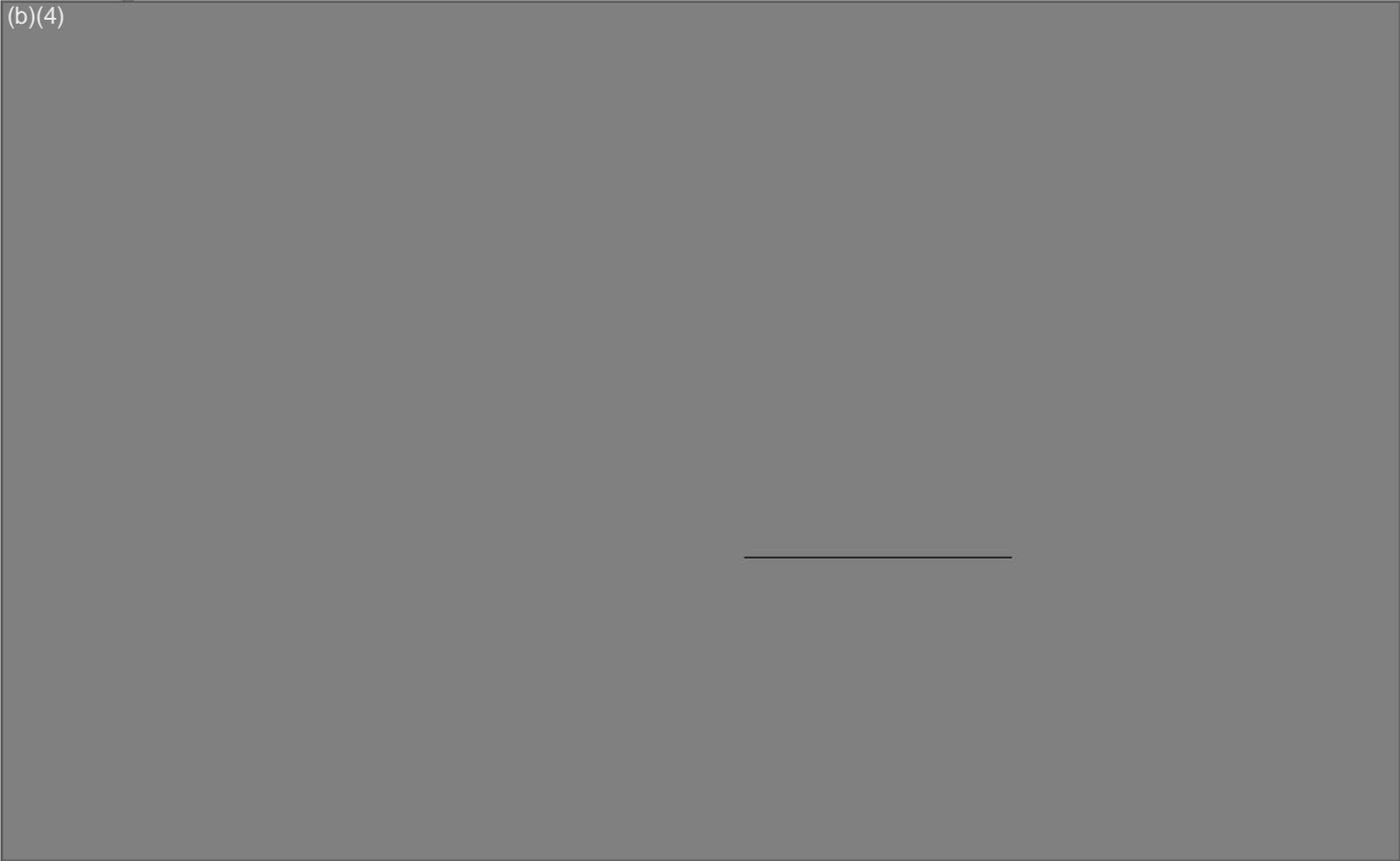
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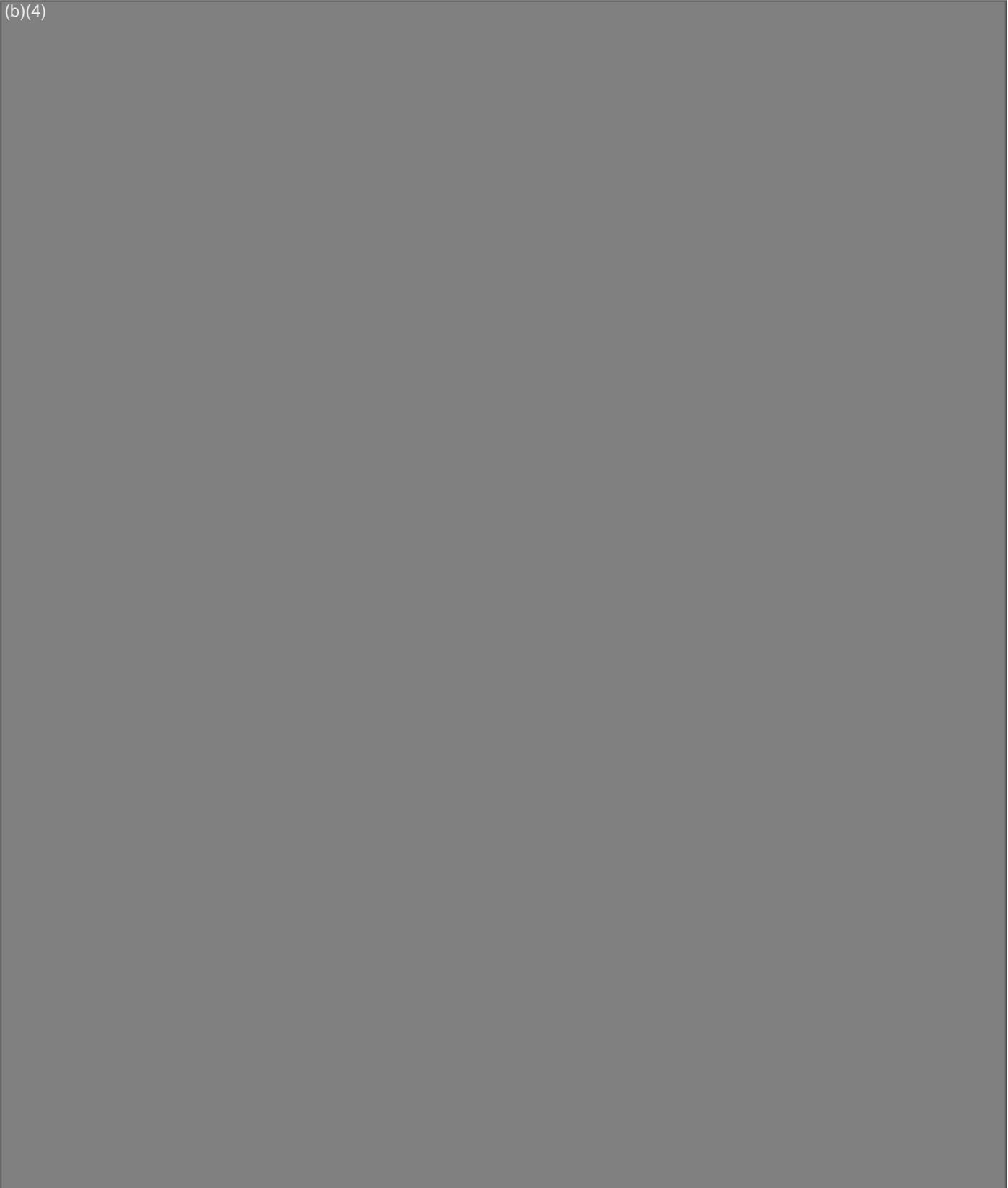


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**2.E. Materials and methods for agronomic studies**

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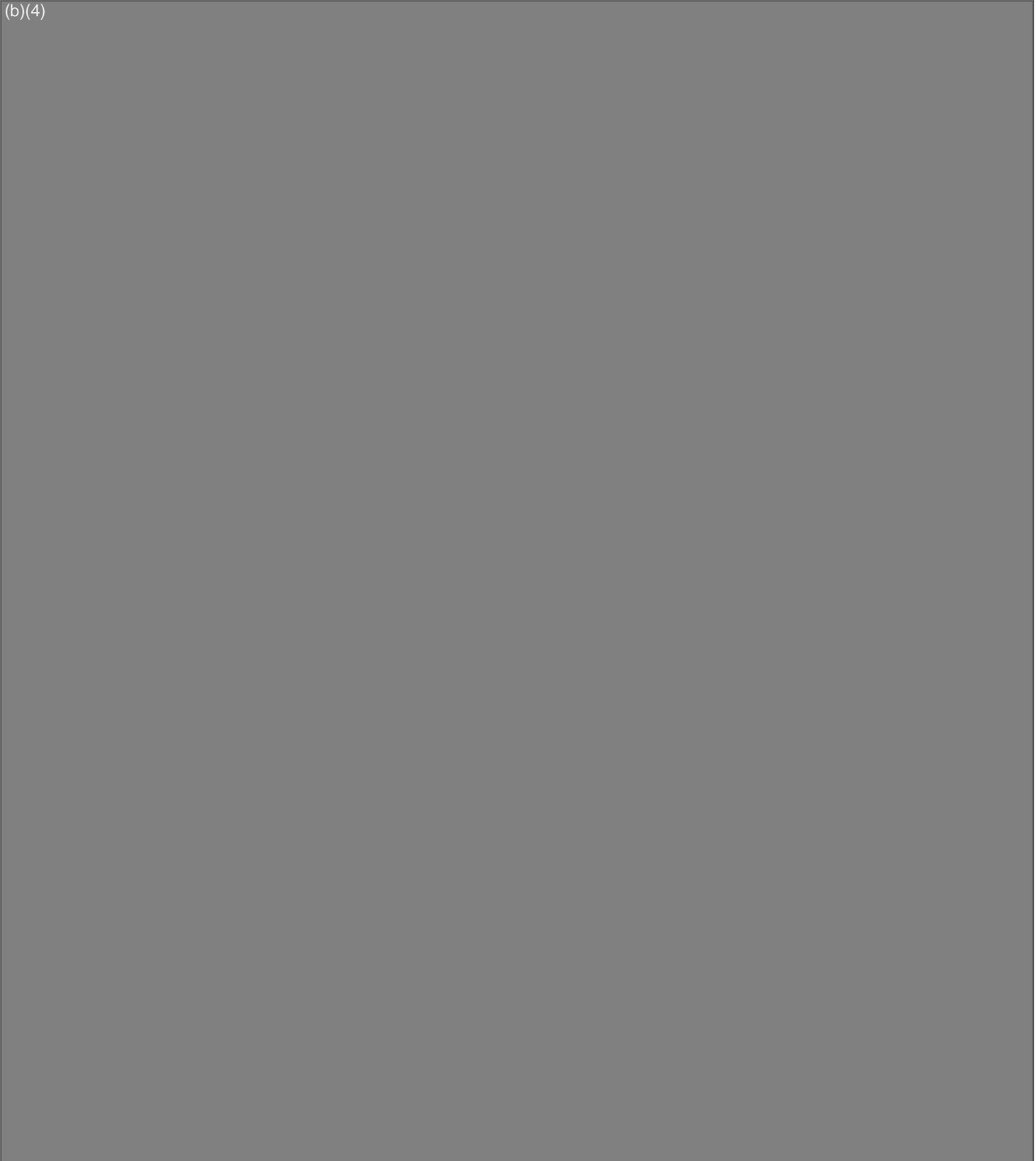
**2.F. Materials and methods for seed germination studies**

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**2.G. Materials and methods for composition analysis**

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**Table 38. Methods used for analysis of soybean grain**

Parameter (Analyte)	Method Mnemonic	Covance Method Reference
Proximates		
Ash	ASHM	AOAC 923.03
Fat	FSOX	AOAC 960.39 and 948.22
Moisture	M100	AOAC 926.08 and 925.09
Protein	PGEN	AOAC 955.04 and 979.09
Carbohydrate (Calculated)	CHO	Difference between 100 and the sum of moisture, crude protein, fat and ash. Agric. Handbook No. 74
Acid detergent fiber	ADF	Agric. Handbook No. 379
Neutral detergent fiber	NDFE	AACC 32.20 and Agric. Handbook No. 379
Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium	ICPS	AOAC 984.27 and 985.01
Vitamin A ( $\beta$ -carotene)	BCLC	AOAC 941.15
Vitamin B <sub>1</sub> (Thiamin)	BIDE	AOAC 942.23, 953.17 and 957.17
Vitamin B <sub>2</sub> (Riboflavin)	B2FV	AOAC 940.33 and 960.46
Folic Acid	FOAN	AOAC 960.46 and 992.05
Vitamin K	VKLC	AOAC 992.27
Tocopherols (single and total)	TTLC/TOIL	HPLC method (see references)
Raffinose and Stachyose	SUGT	Gas-Liquid Chromatography (see references)
Phytic Acid	PHYT	HPLC method (see references)
Trypsin Inhibitor	TRIP	AOCS Ba 12-75
Lectins	LECT	Photometric methods (see references)
Isoflavones	ASOF	AOAC 2001.10
Total Amino Acids	TAA5	AOAC 982.30
Total Fatty Acids	FALC	AOCS Ce 1-62 and Ce 1b-89

**Table 39. Methods used for analysis of soybean hulls**

Analyte	Method Mnemonic	Covance Method Reference
Proximates		
Ash	ASHM	AOAC 923.03
Fat	FSOX	AOAC 960.39 and 948.22
Moisture	M100	AOAC 926.08 and 925.09
Protein	PGEN	AOAC 955.04 and 979.09
Carbohydrate (Calculated)	CHO	Difference between 100 and the sum of moisture, crude protein, fat and ash. Agric. Handbook No. 74
Acid detergent fiber	ADF	Agric. Handbook No. 379
Neutral detergent fiber	NDFE	AACC 32.20 and Agric. Handbook No. 379

**Table 40. Methods used for analysis of soybean meal and toasted meal**

Analyte	Method Mnemonic	Covance Method Reference
Proximates		
Ash	ASHM	AOAC 923.03
Fat	FSOX	AOAC 960.39 and 948.22
Moisture	M100	AOAC 926.08 and 925.09
Protein	PGEN	AOAC 955.04 and 979.09
Carbohydrate (Calculated)	CHO	Difference between 100 and the sum of moisture, crude protein, fat and ash. Agric. Handbook No. 74
Acid detergent fiber	ADF	Agric. Handbook No. 379
Neutral detergent fiber	NDFE	AACC 32.20 and Agric. Handbook No. 379
Raffinose and Stachyose	SUGT	Gas-Liquid Chromatography (see references)
Phytic Acid	PHYT	HPLC method (see references)
Trypsin Inhibitor	TRIP	AOCS Ba 12-75
Lectins	LECT	Photometric methods (see references)
Isoflavones	ASOF	AOAC 2001.10
Total Amino Acids	TAA5	AOAC 982.30

**Table 41. Methods used for analysis of soybean protein isolate**

Analyte	Method Mnemonic	Covance Method Reference
Proximates		
Moisture	M100	AOAC 926.08 and 925.09
Protein	PGEN	AOAC 955.04 and 979.09
Trypsin Inhibitor	TRIP	AOCS Ba 12-75
Lectins	LECT	Photometric methods (see references)
Total Amino Acids	TAA5	AOAC 982.30

**Table 42. Methods used for analysis of soybean crude oil and RBD oil**

Analyte	Method Mnemonic	Covance Method Reference
Vitamin A ( $\beta$ -carotene)	BCLC	AOAC 941.15
Vitamin K	VKLC/VKLP	AOAC 992.27 and 999.15
Tocopherols (single and total)	TTLC/TOIL	HPLC method (see references)
Total Fatty Acids	FALC	AOCS Ce 1-62 and Ce 1b-89

**Table 43. Method used for analysis of soybean lecithin**

Analyte	Method Mnemonic	Covance Method Reference
Phosphatides	LPLC	HPLC method (see reference)

### **Appendix 3**

#### **CHARACTERIZATION OF EVENT FG72 SOYBEAN**

**3.A. Verification of the insert**

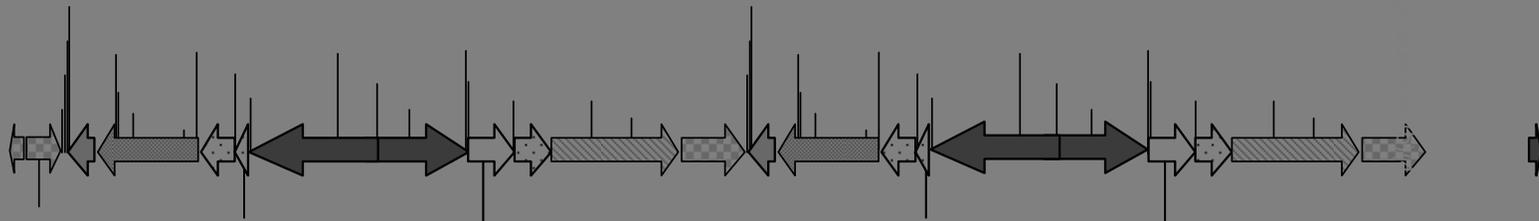
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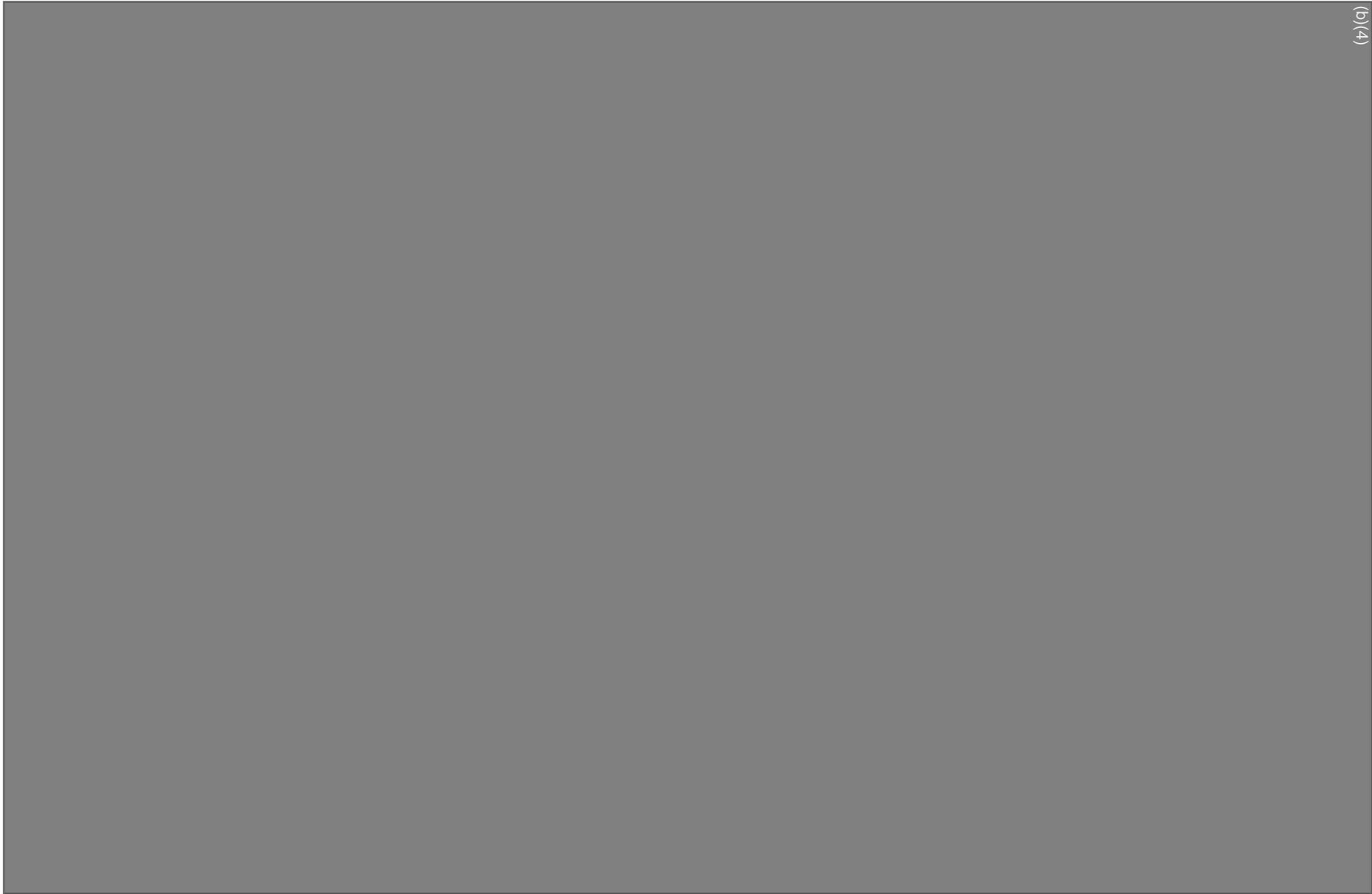
Figure 11

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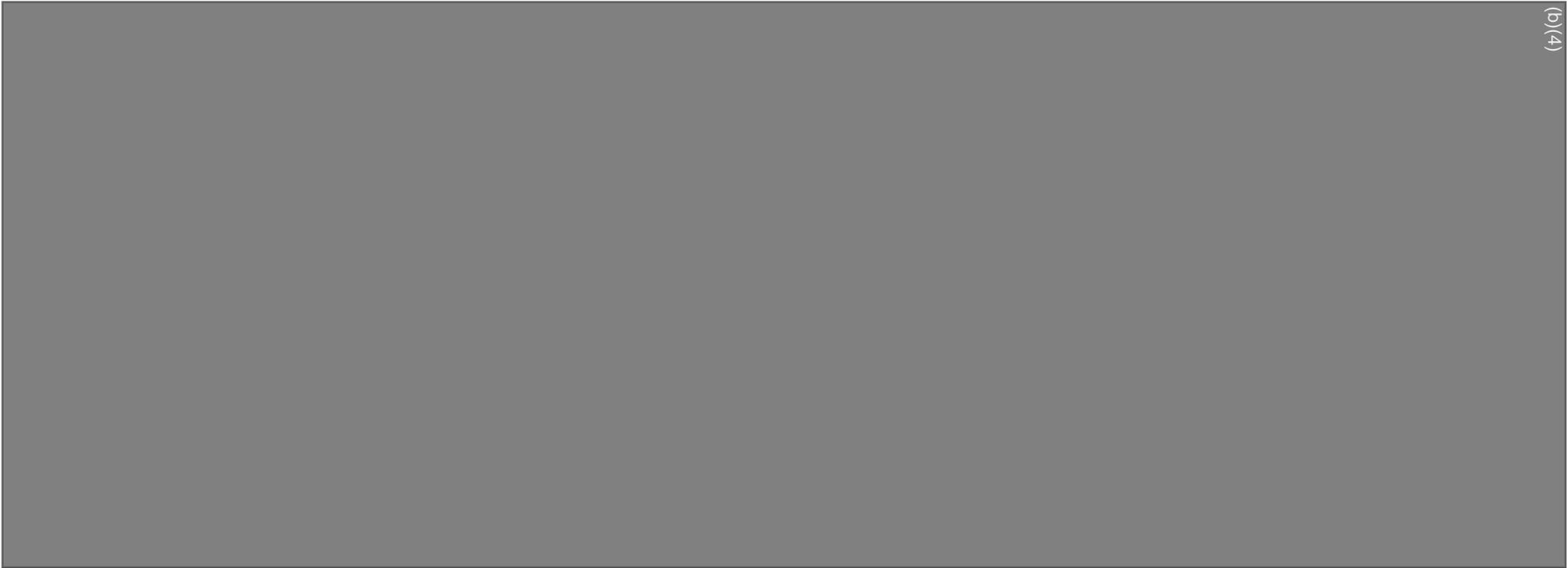


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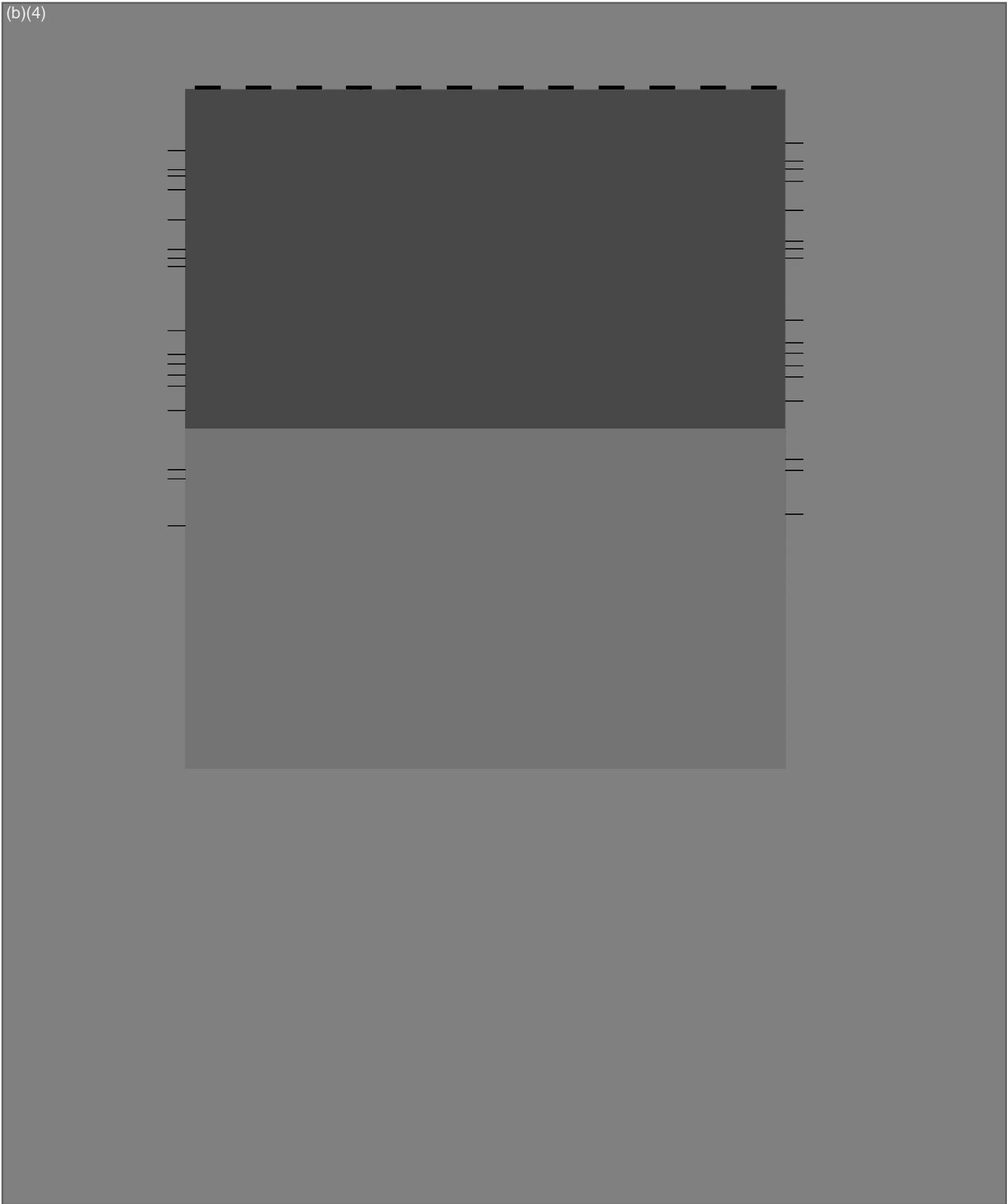


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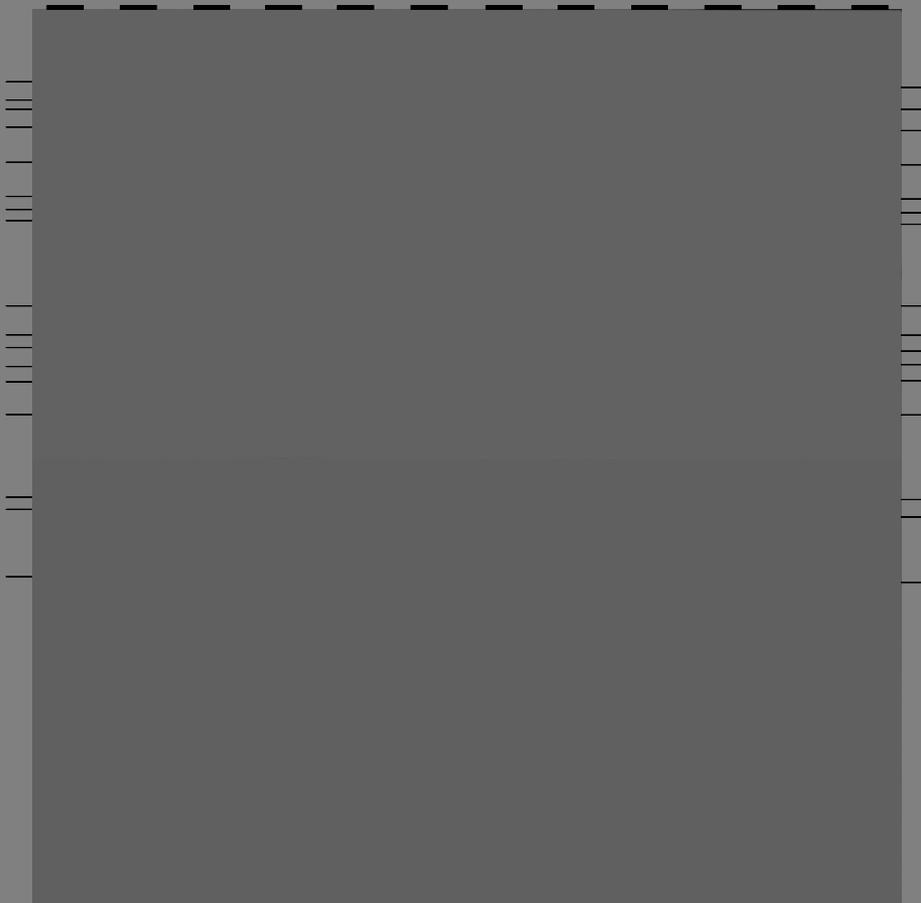
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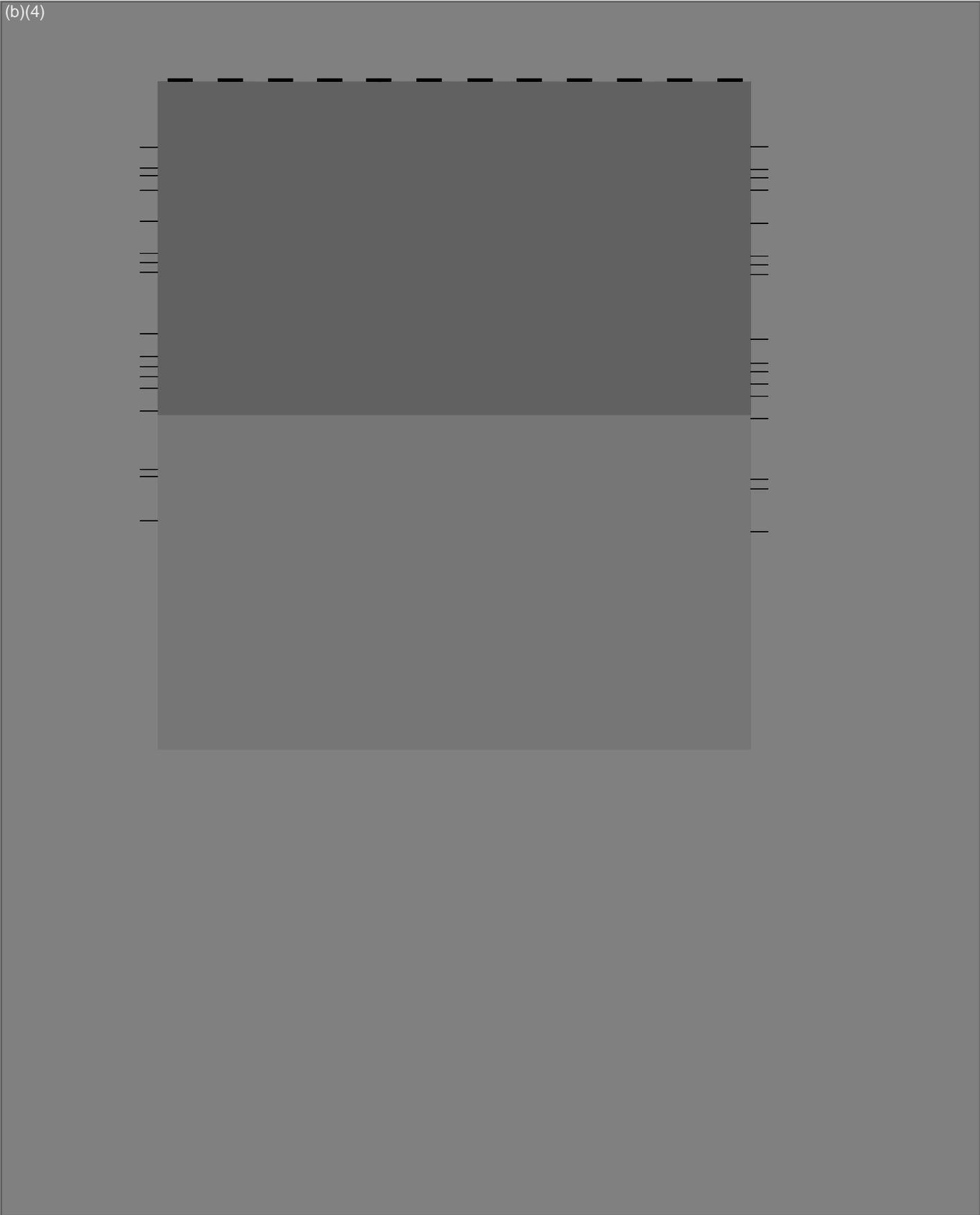
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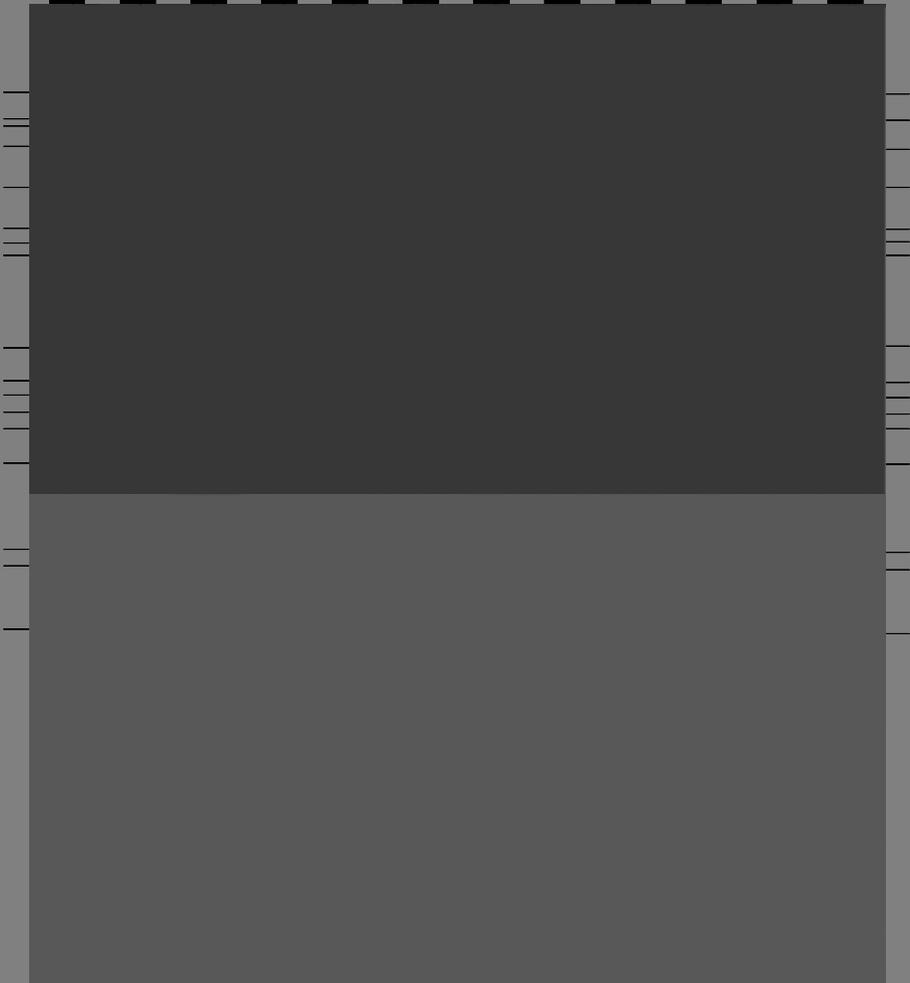
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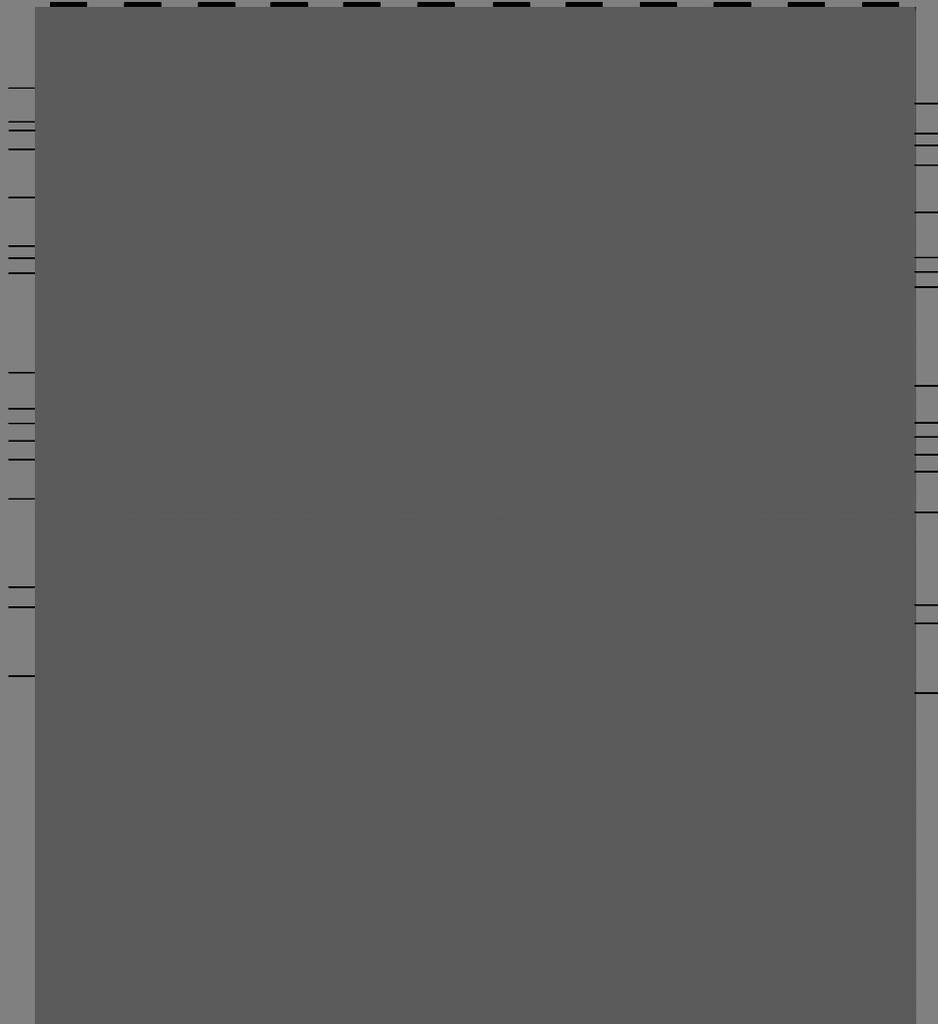
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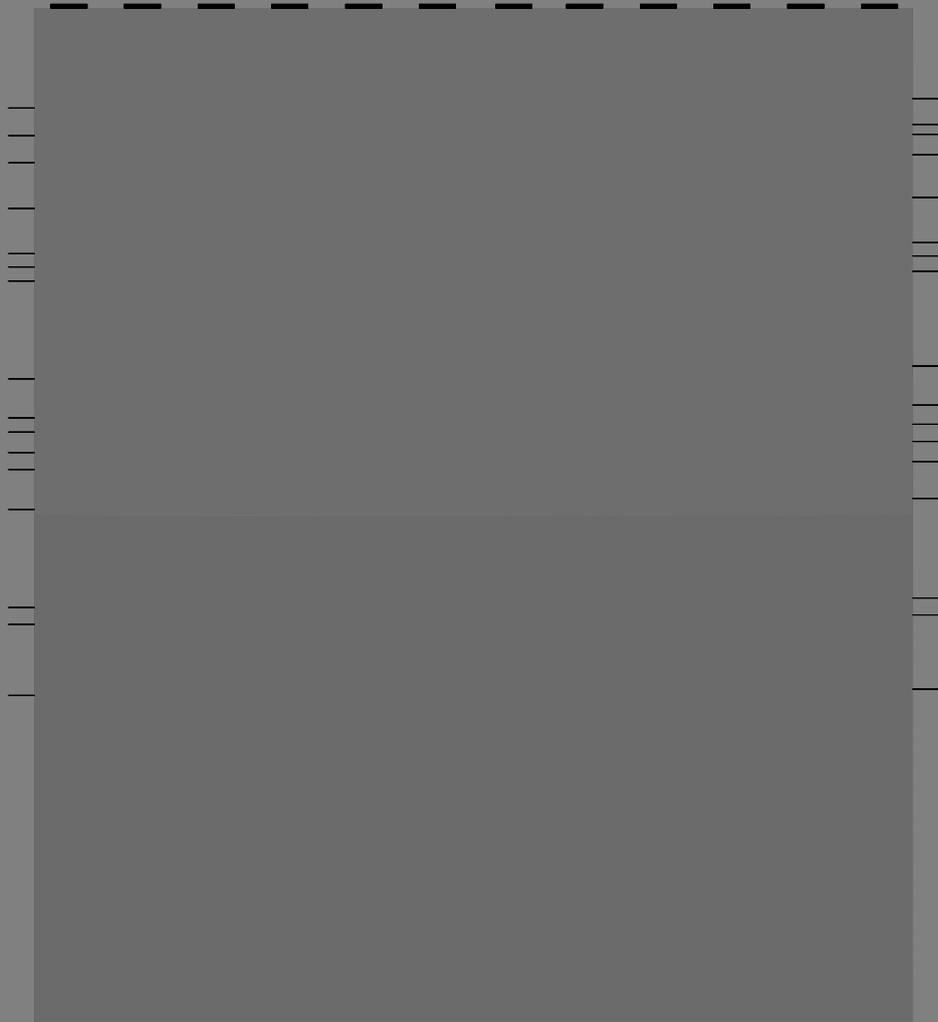
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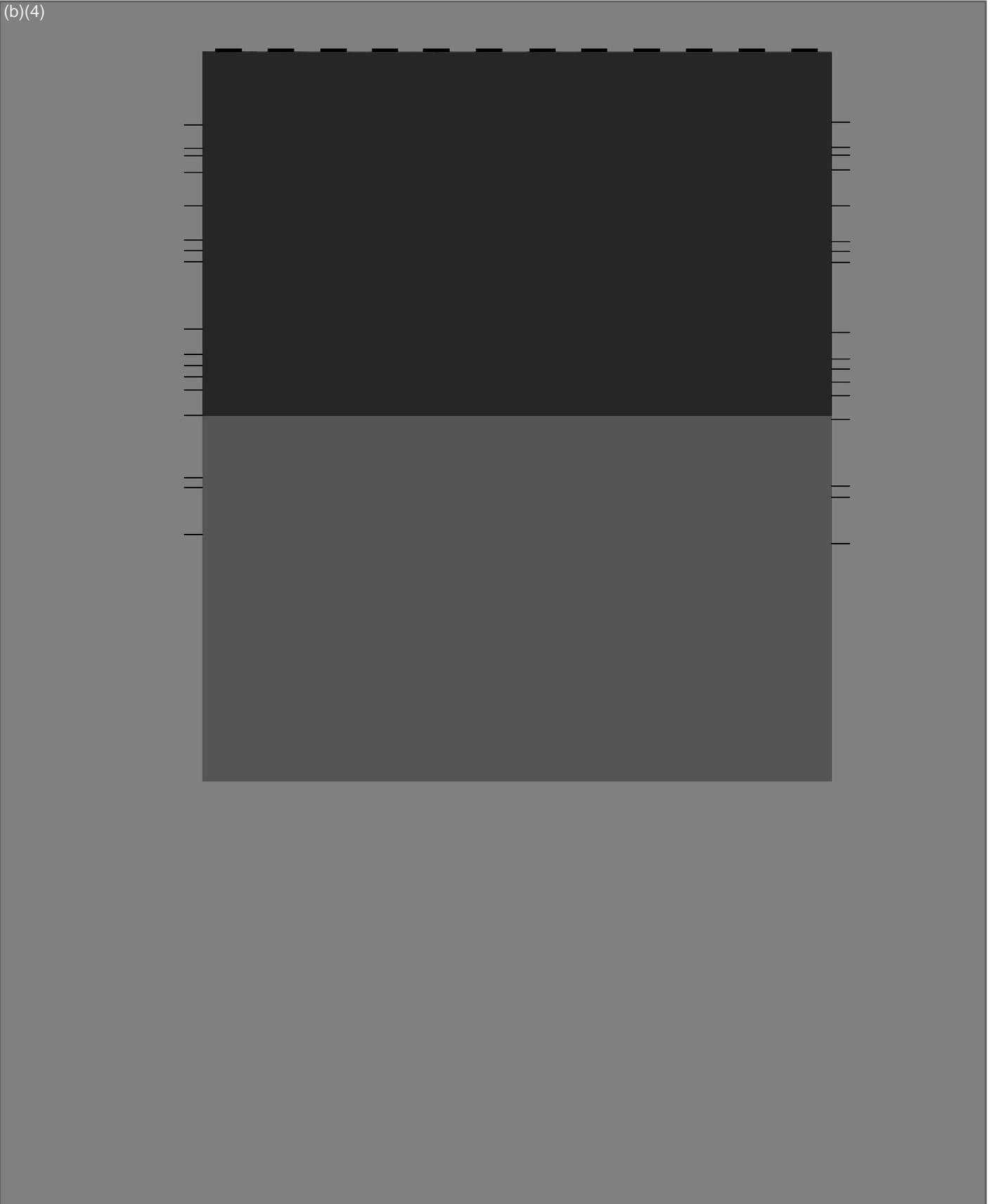
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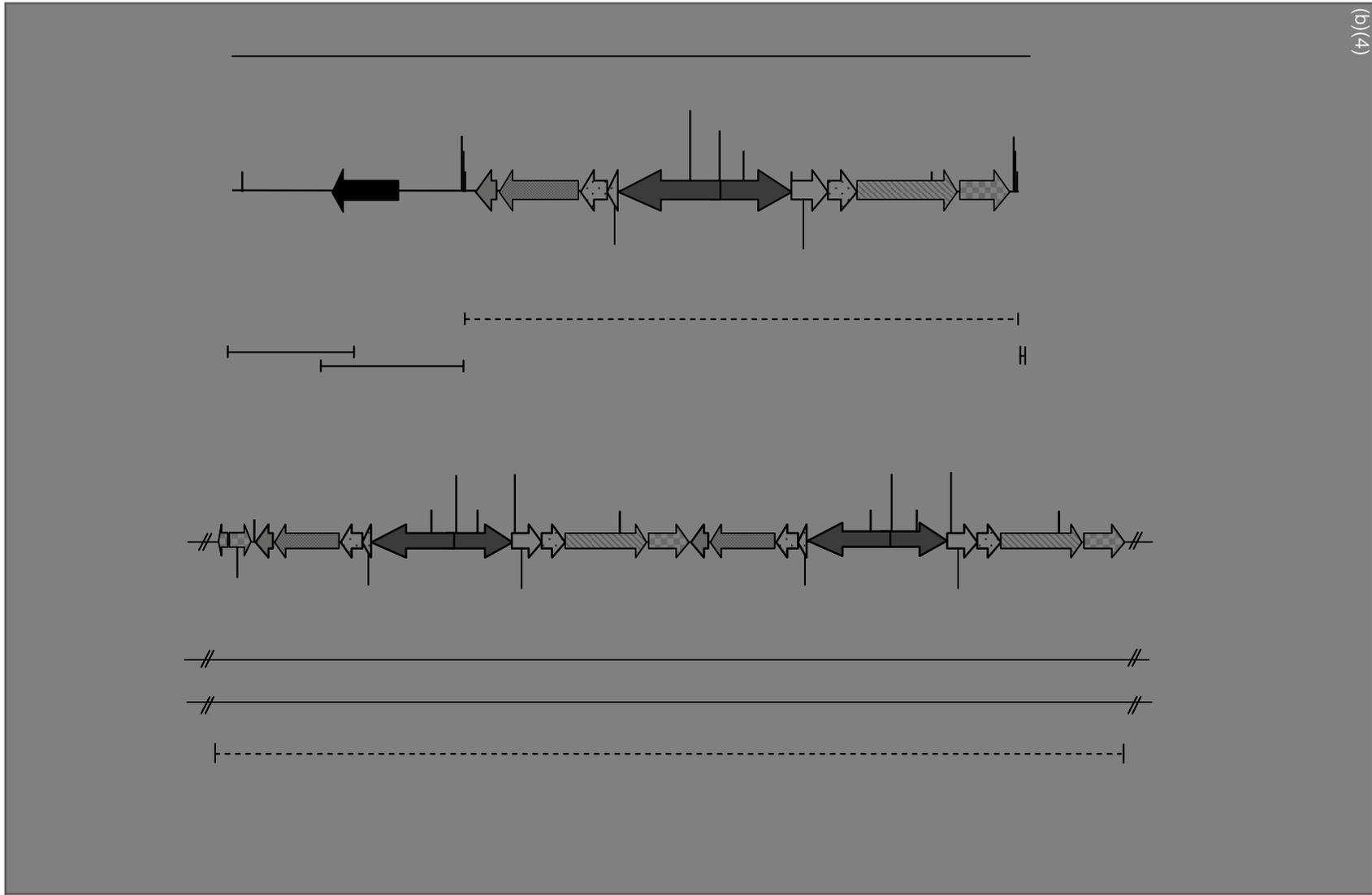


**Figure 20. Southern blot analysis of event FG72 – insert-DNA probe**



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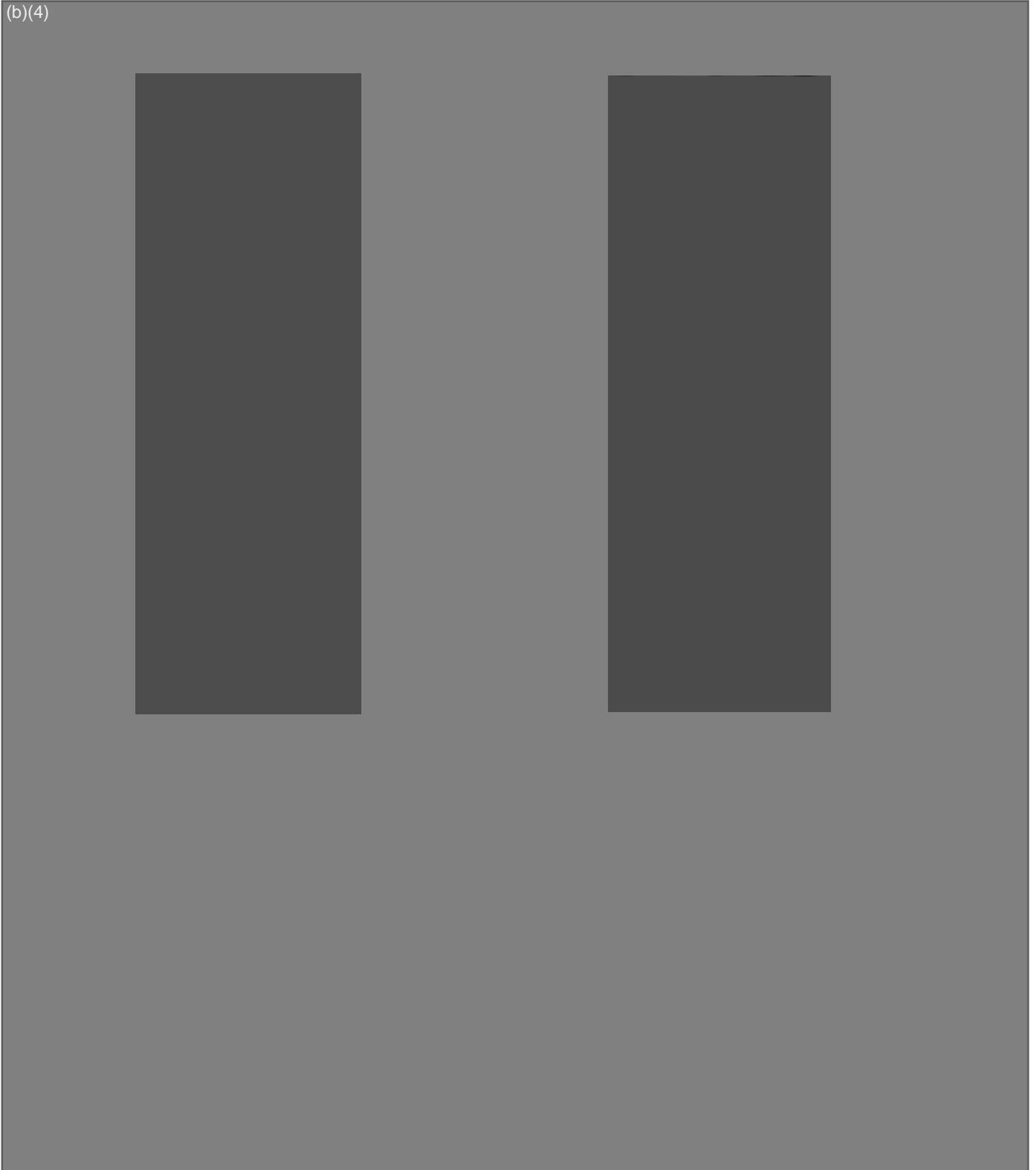


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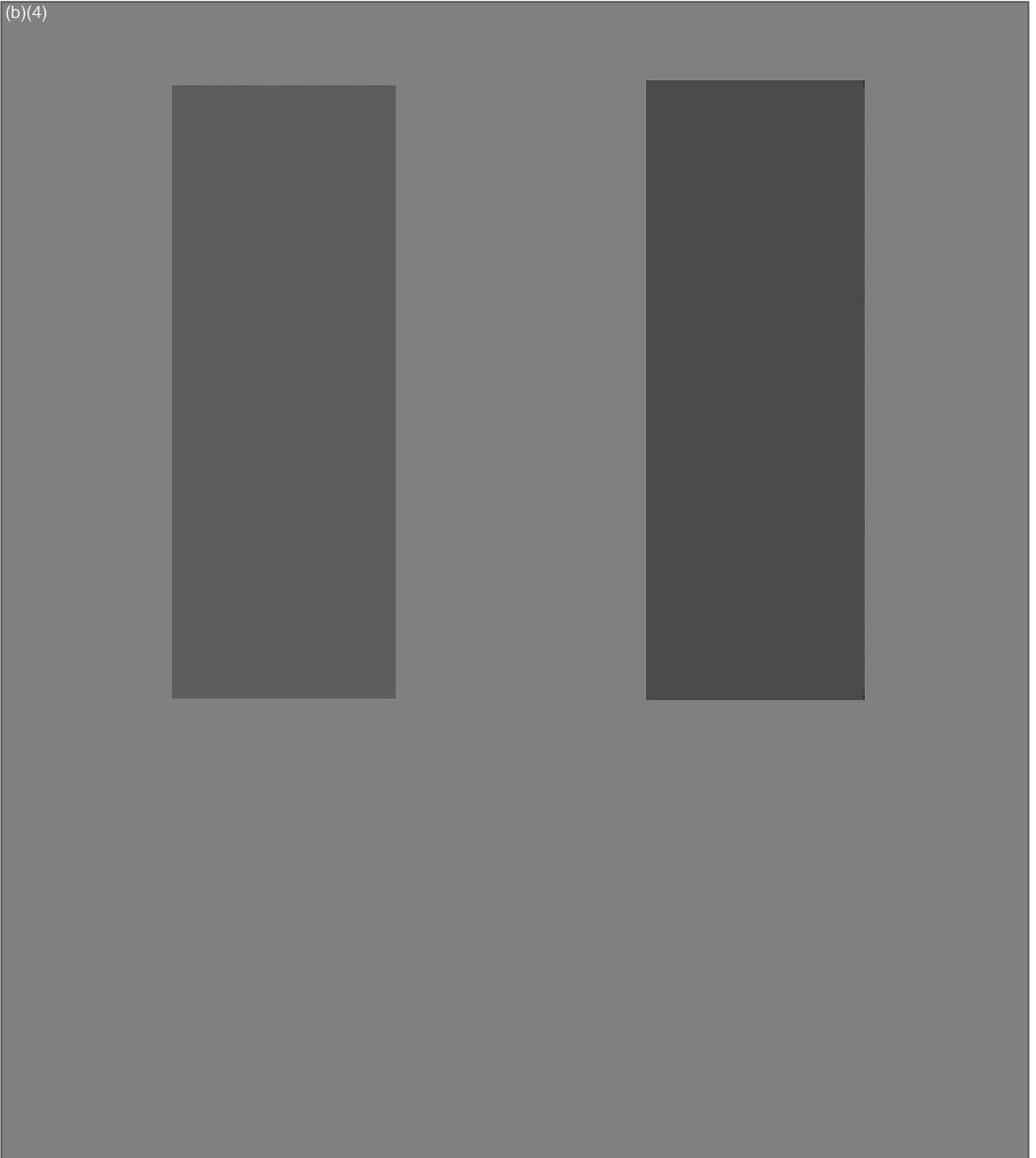
**Figure 22. Southern blot analysis of event FG72 – Absence of vector backbone –**

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**Figure 23. Southern blot analysis of event FG72 – Absence of vector backbone –**

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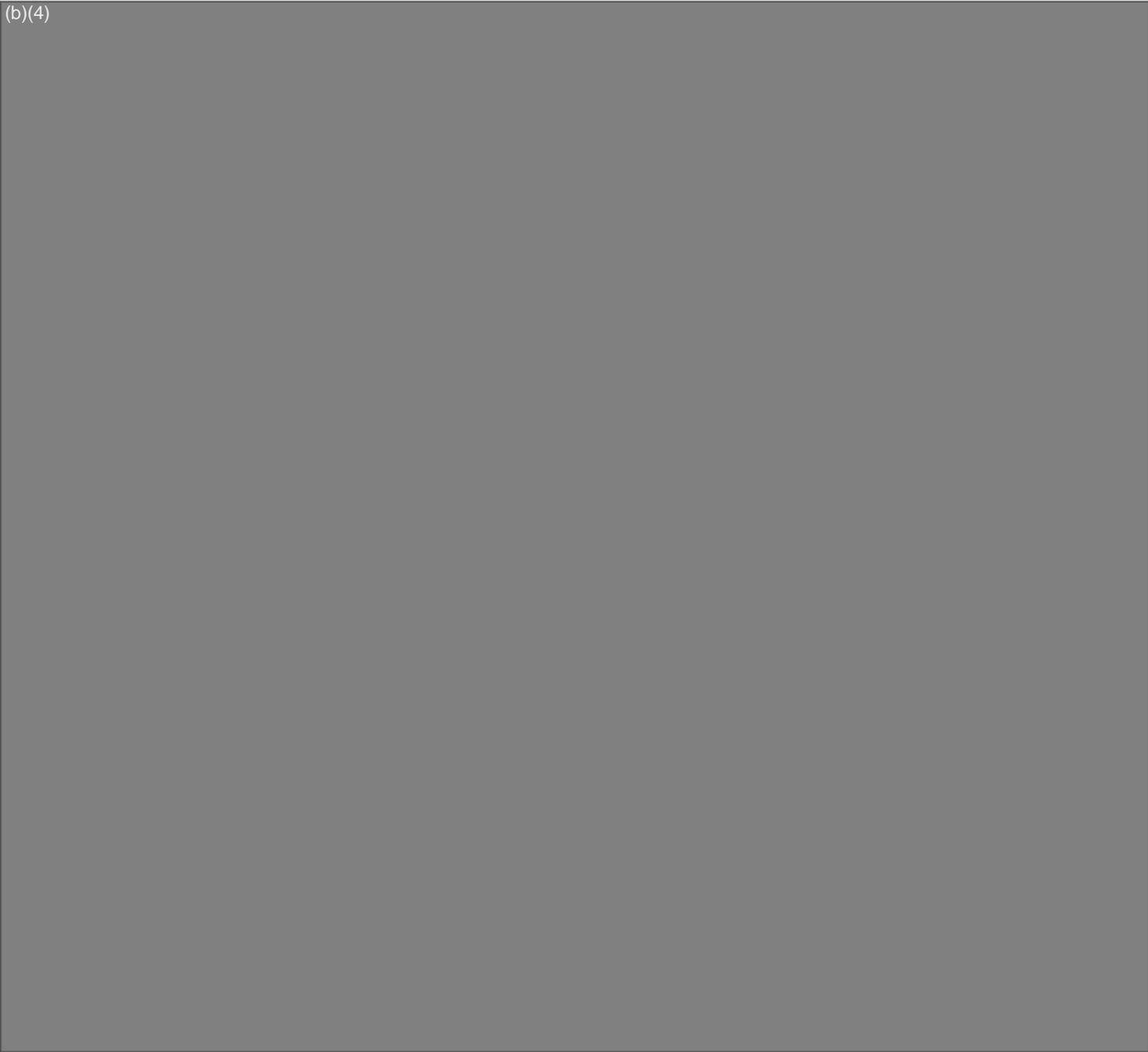


**3.C. Stability across and within generations**

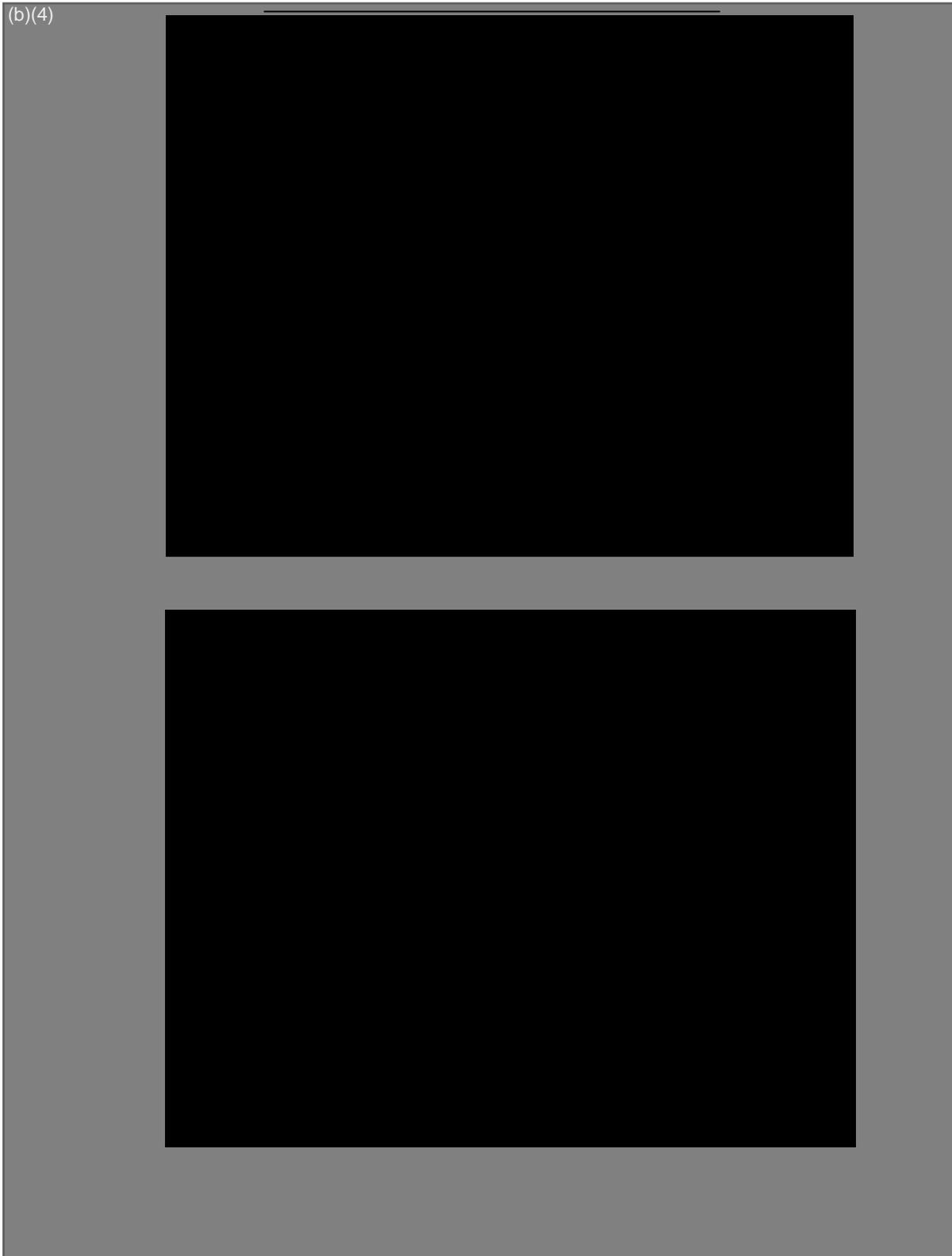
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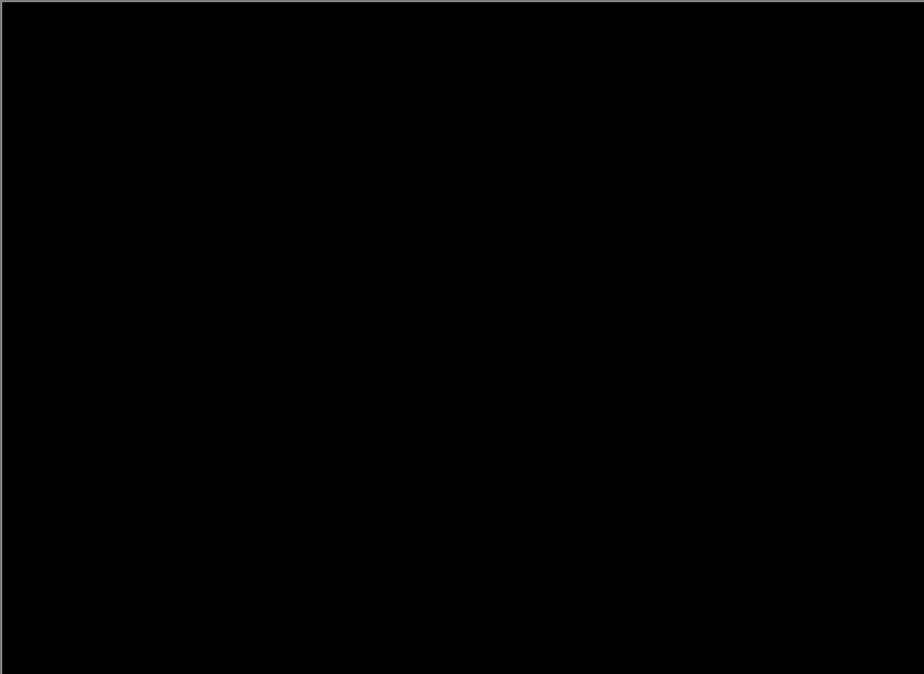
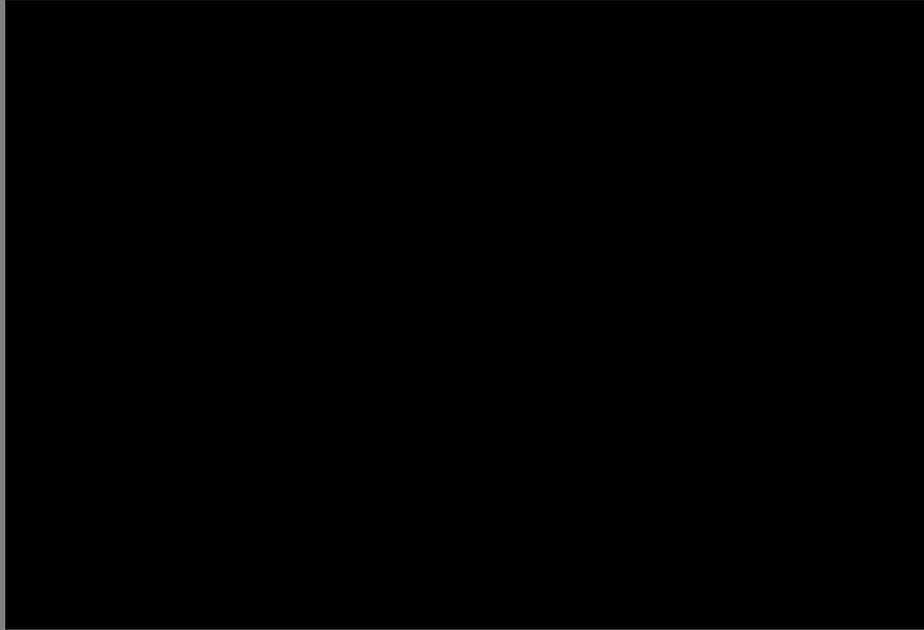
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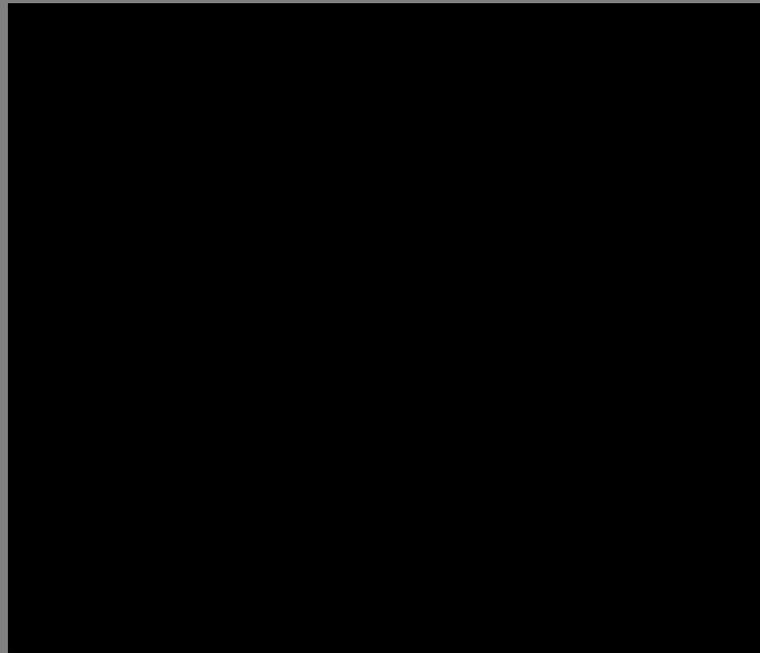
**Figure 24. Environment Adel**



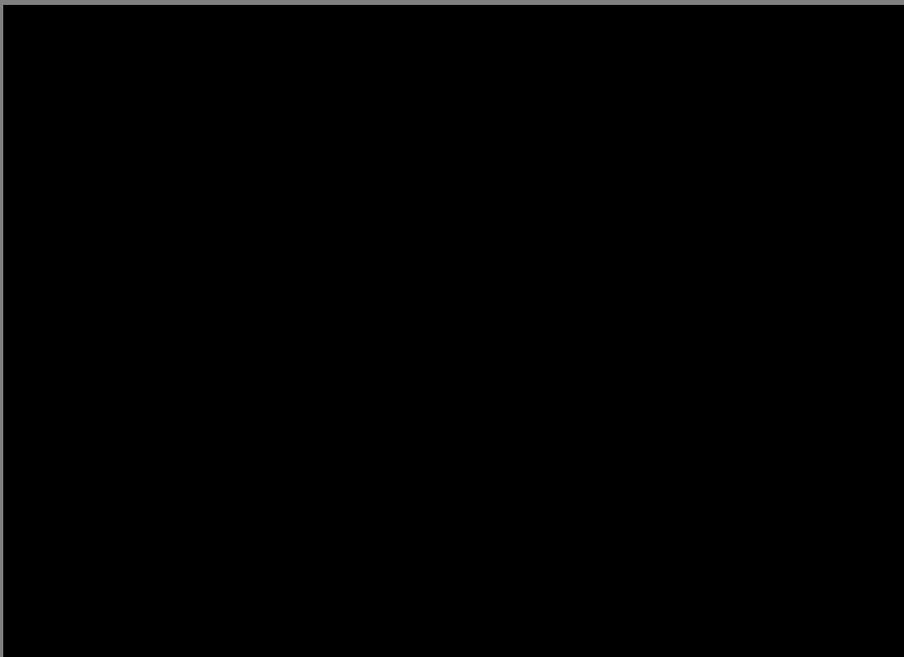
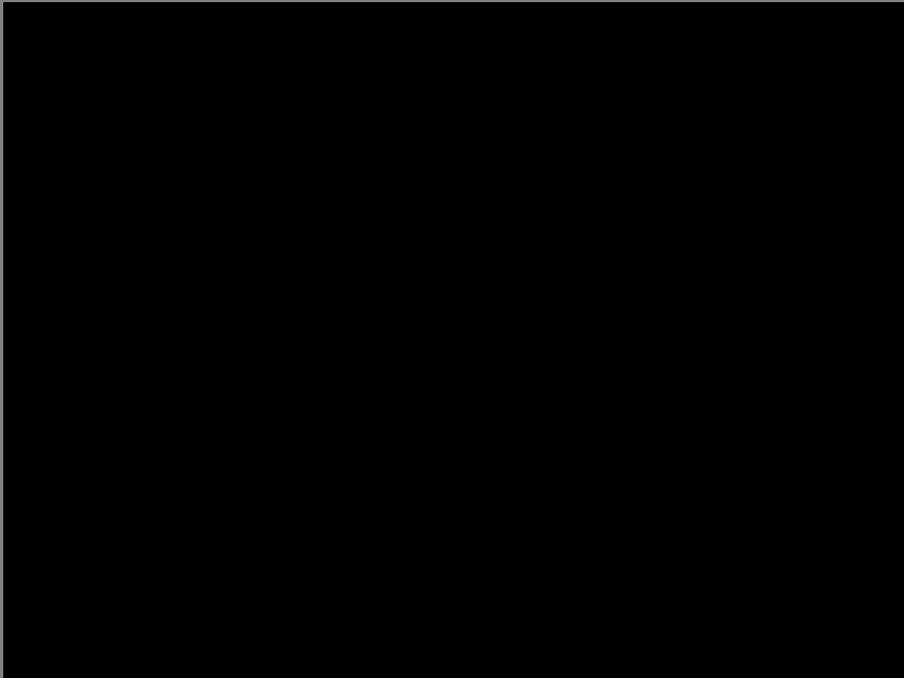
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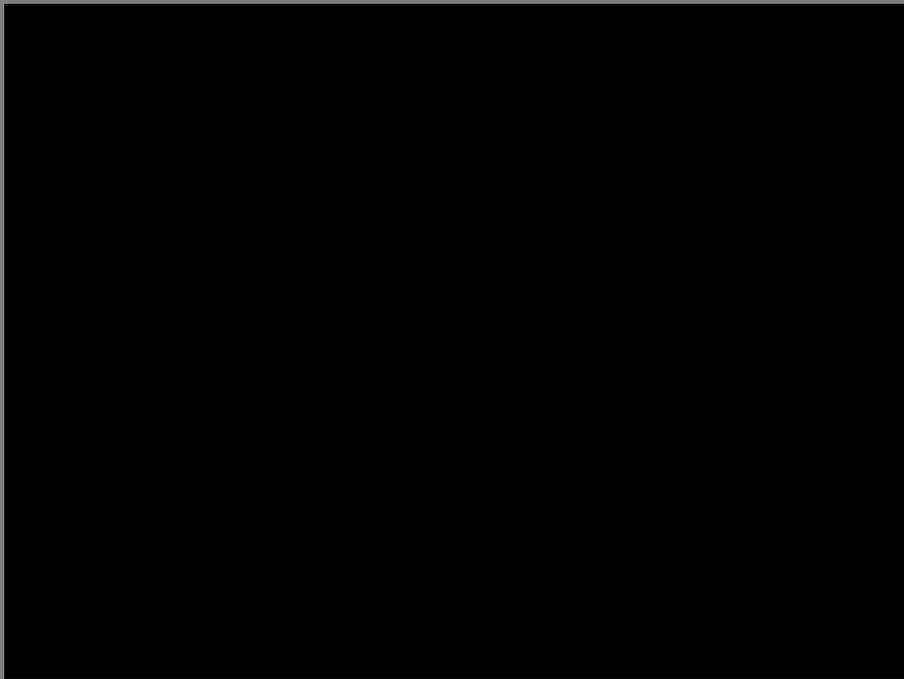
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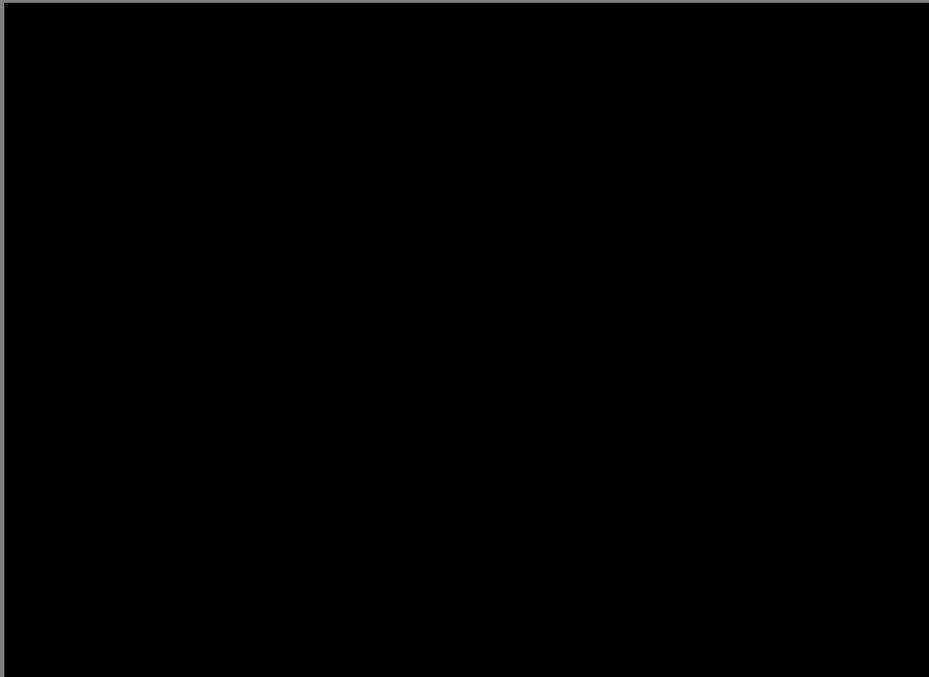
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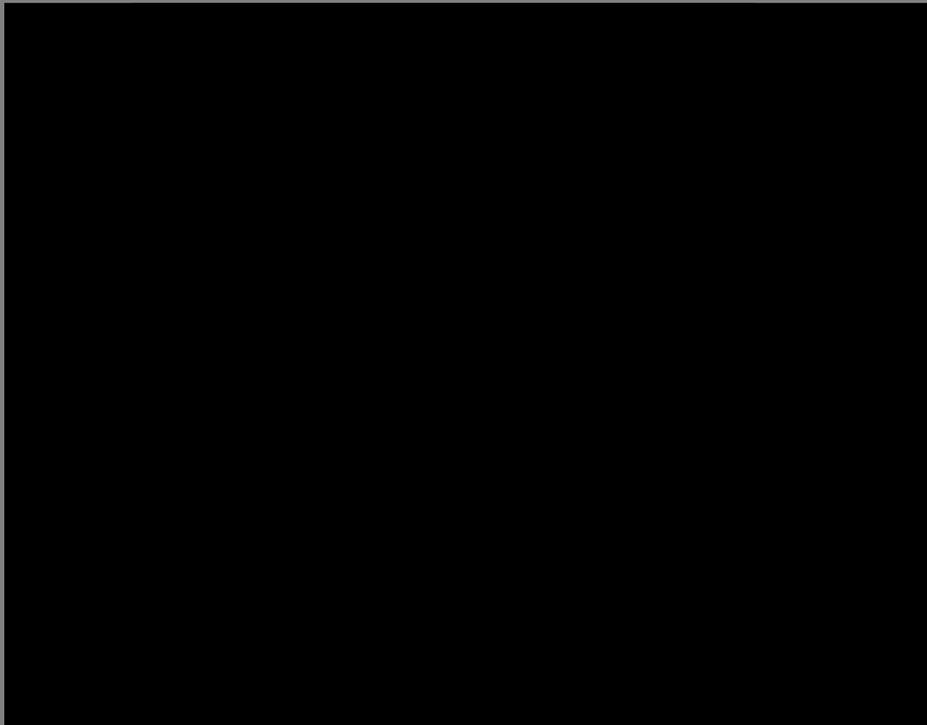
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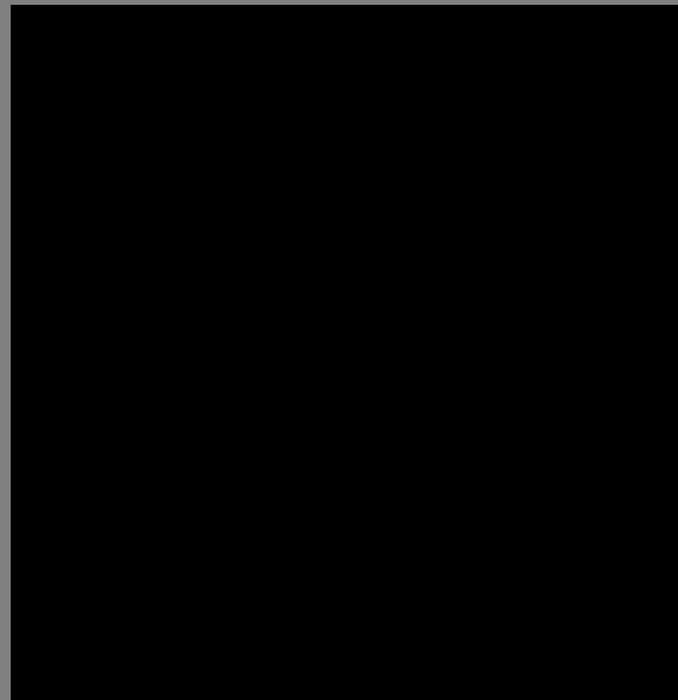
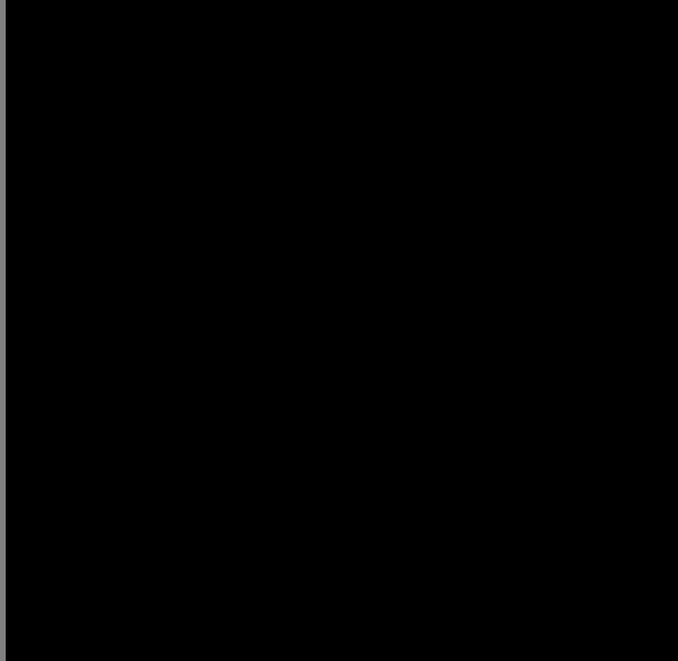
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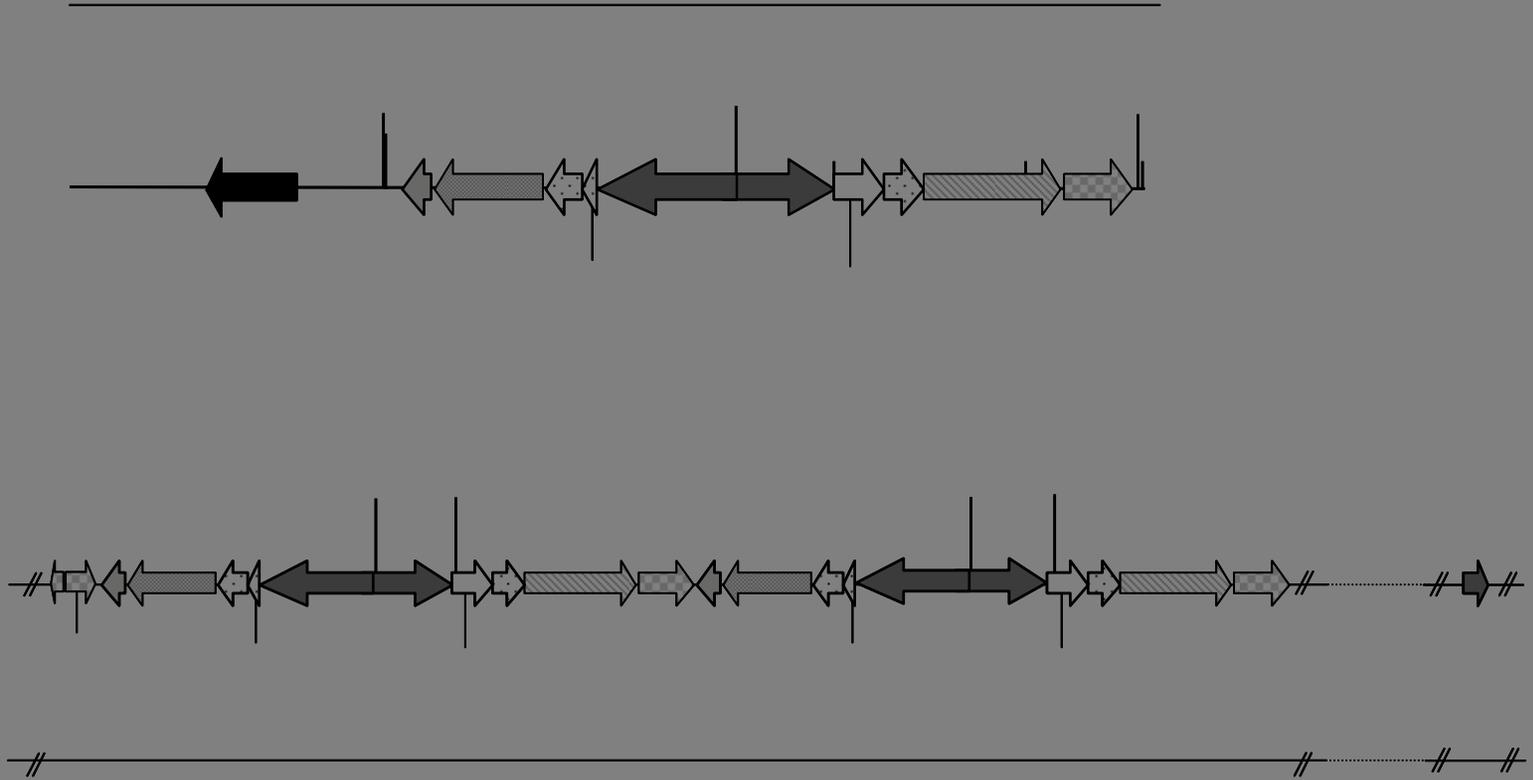


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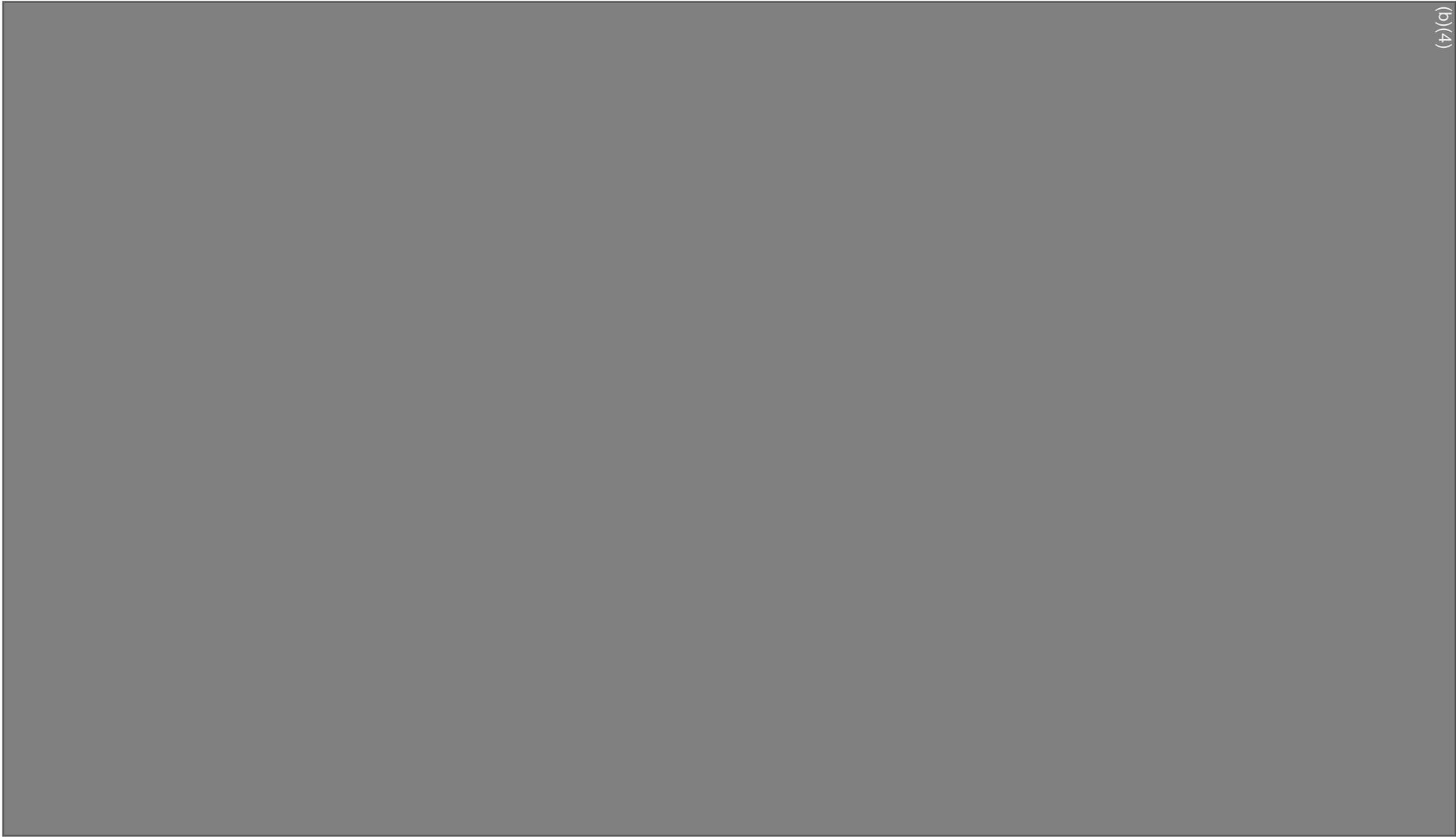




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### 3.D. Demonstration of protein equivalence

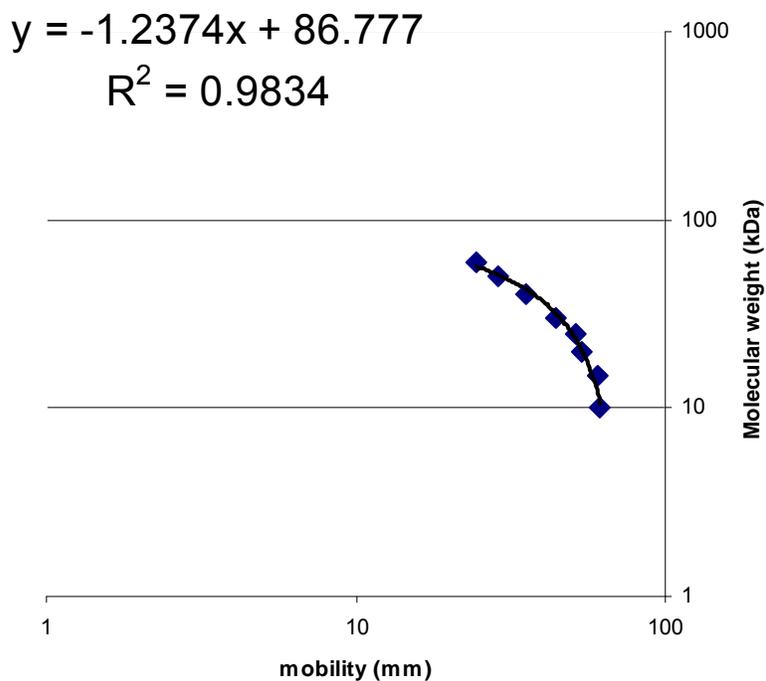
#### 3.D.1 2mEPSPS protein

The SDS-PAGE and western blots demonstrated that the molecular weight, mobility, and immuno-reactivity of the plant-produced and microbially-produced 2mEPSPS proteins are the same. The western blot also indicated that the non-transgenic Jack soybean control sample did not have immunoreactive proteins. The band appearing below the 2mEPSPS band in the plant-produced protein did not appear on the western blot, indicating that the band is not related to the 2mEPSPS protein.

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**Figure 34. Standard curve of electrophoretic mobility versus molecular weight**

The electrophoretic mobility of the protein standards for the SDS-PAGE gel shown in Figure 3.23 were plotted against their respective molecular weights bracketing the 2mEPSPS protein. The equation defining the curve is  $y = -1.2374x + 86.777$ . The  $R^2$  value for this curve is 0.9834. The equation defining this curve was used to calculate an approximate molecular weight of 50.3 kDa for 2mEPSPS isolated from soybean, event FG72. The molecular weight of the *E. coli* produced 2mEPSPS protein calculated from the equation is 49.7 kDa.

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**Table 51. Electrospray LC/MS peptide mapping of the 2mEPSPS protein**

2mEPSPS Residue	Sequence	Theoretical [M+H]	98% cov. <i>E. coli</i> 2mEPSPS protein*	71% cov. FG72 Soybean Leaf*	Non-transgenic Jack Soybean Leaf*
1 to 13	MAGAEIIVLQPIK		700.9 [M+2H]	ND	ND
2 to 13	AGAEIIVLQPI	1268.7	635.4 [M+2H]	635.3 [M+2H]	ND
14 to 20	K EISGTVK	733.8	734.4 [M+H]	734.4 [M+H]	ND
1 to 20	MAGAEIIVLQPIKEISGTV	2113.2	705.4 [M+3H]	ND	ND
21 to 25	K LPGSK	501.6	502.4 [M+H]	502.3 [M+H]	ND
26 to 30	SLSNR	576.6	577.4 [M+H]	577.5 [M+H]	ND
31 to 61	ILLLAALSEGTTVVVDNLLNSEDV HYMLGALR	3342.9	1115.3 [M+3H]	ND	ND
62 to 71	TLGLSVEAD	1033	517.6 [M+2H]	517.5 [M+2H]	ND
72 to 74	K AAK	289	289.5 [M+H]	289.7 [M+H]	289.9[M+H]
75 to 75	R	175	ND**	ND	ND
76 to 84	AVVVGCGG	790	424.5 [M+2H]	424.5 [M+2H]	ND
85 to 91	K FPVEDAK	805.9	ND**	ND**	ND
92 to 106	EEVQLFLGNAGIAMR	1648.9	ND**	ND**	ND
85 to 106	FPVEDAKEEVQLFLGNAGIAMR	2434.2	812.4 [M+3H]	812.5 [M+3H]	ND
107 to 128	SLTAAVTAAGGNATYVLDGVPR	2105	702.9 [M+3H]	702.8 [M+3H]	702.7 [M+3H]
129 to 130	MR	306	307.2 [M+H]	307.1 [M+H]	ND
131 to 142	ERPIGDLVVGLK	1296	649.2 [M+2H]	649.4 [M+2H]	ND
143 to 160	QLGADVDFCLGTDCCPPVR	1907	1011.7 [M+2H]	1011.7 [M+2H]	ND
161 to 171	VNGIGLPGGK	969	485.6 [M+2H]	485.5 [M+2H]	ND
172 to 173	VK	246	246.8 [M+H]	247.2 [M+H]	ND
174 to 204	LSGSISSQYLSALLMAAPLALGD VEIEIIDK	3219.8	ND**	ND	ND
205 to 216	LISIPYVEMTL	1435.8	ND**	ND	ND
217 to 220	R LMER	548.7	ND**	ND	ND
221 to 224	FGVK	450.6	ND**	ND**	ND
225 to 233	AEHSDSWDR	1103	ND**	ND**	ND
174 to 233	LSGSISSQYLSALLMAAPLALGD VEIEIIDKLISIPYVEMTLRLMERF GVKAEHSDSWDR	6681.8	1114.5 [M+6H]	ND	ND
234 to 237	FYIK	570.7	286.2 [M+2H]	286.3 [M+2H]	ND
238 to 241	GGQK	389	390.3 [M+H]	390.1 [M+H]	ND
242 to 243	YK	310	310.8 [M+H]	310.7 [M+H]	ND
244 to 246	SPK	331	332.3 [M+H]	332.2 [M+H]	ND
247 to 286	NAYVEGDASSASYFLAGAATG GTVTVEGCGTTSLQGDVK	3870	1309.8 [M+3H]	1309.8 [M+3H]	1310 [M+3H]
287 to 297	FAEVLEMMGAK	1226	614.6 [M+2H]	614.3 [M+2H]	ND
298 to 312	VTWTETSVTVTGPPR	1631.8	817 [M+2H]	816.9 [M+2H]	ND
313 to 317	EPFGR	605.6	ND	ND	ND
318 to 318	K	147	ND	ND	ND
319 to 321	HLK	397	398.3 [M+H]	398.2 [M+H]	ND
322 to 329	AIDVNMNK	905	453.6 [M+2H]	453.4 [M+2H]	ND
330 to 351	MPDVAMTLAVVVFADGPTAIR	2260.7	ND**	ND	ND
352 to 357	DVASWR	733.8	733.9 [M+H]	734.3 [M+H]	ND
330 to 357	MPDVAMTLAVVVFADGPTAIR DVASWR	2975.5	992.5 [M+3H]	ND	ND
358 to 359	VK	246	246.8 [M+H]	247.2 [M+H]	246.2 [M+H]
360 to 363	ETER	534	535.3 [M+H]	534.8 [M+H]	ND
364 to 368	MVAIR	589.8	590.4 [M+H]	590.3 [M+H]	ND
369 to 373	TELT	591.7	592.4 [M+H]	592.3 [M+H]	ND
374 to 392	LGASVEEEDPDYCIITPPEK	2019	1039.2 [M+2H]	1039.2 [M+2H]	ND
393 to 405	LNVTAITDYDDHR	1533.6	767.9 [M+2H]	768.1 [M+2H]	767.4 [M+2H]
406 to 423	MAMAFSLAACAEVPTIR	1882	ND**	ND**	876.3 [M+3H]
424 to 429	DPGCTR	648.7	ND**	ND**	ND
406 to 429	MAMAFSLAACAEVPTIRDPGC TR (Cys_CAM mod)	2624.2	875.9 [M+3H]	875.9 [M+3H]	876.3 [M+3H]
430 to 430	K	147	ND**	ND**	ND
431 to 444	TFPDYFDVLSFVK	1679.9	841 [M+2H]	840.9 [M+2H]	ND
445 to 445	N	133	ND**	ND**	ND

\* = Average mass reported. ND= Not detected. ND\*\* = Missed cleavage, peptide not detected by full scan analysis.

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**Table 52. Amino acid coverage of the 2mEPSPS protein**

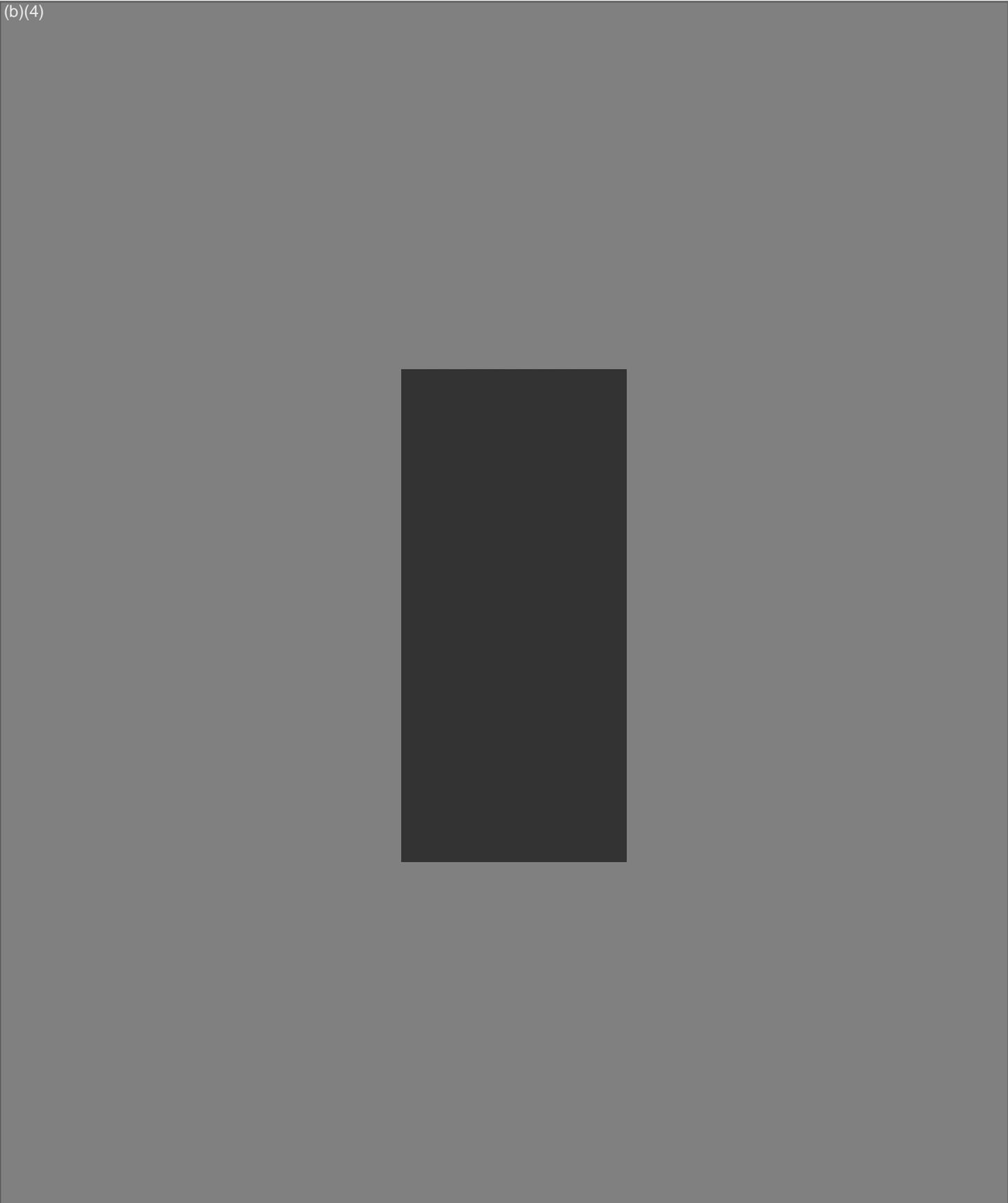
Calculation of % Amino Acid coverage	Number of Amino Acids Not Detected		Residue Number
	<i>E. coli</i> 2mEPSPS	Plant-produced 2mEPSPS	
		1	1-13
		31	31-61
	1	1	75-75
		60	174-233
	5	5	313-317
	1	1	318-318
		28	330-357
	1	1	430-430
	1	1	445-445
Total	9	129	NA <sup>a</sup>
Total number amino Acids	445	445	
% Amino Acid Not Detected or Analyzed	2	29	
% Amino Acid Sequence Coverage	98	71	
% Amino Acid Coverage to 2mEPSPS from <i>E. coli</i> .	100	72	

<sup>a</sup> NA = Not Applicable

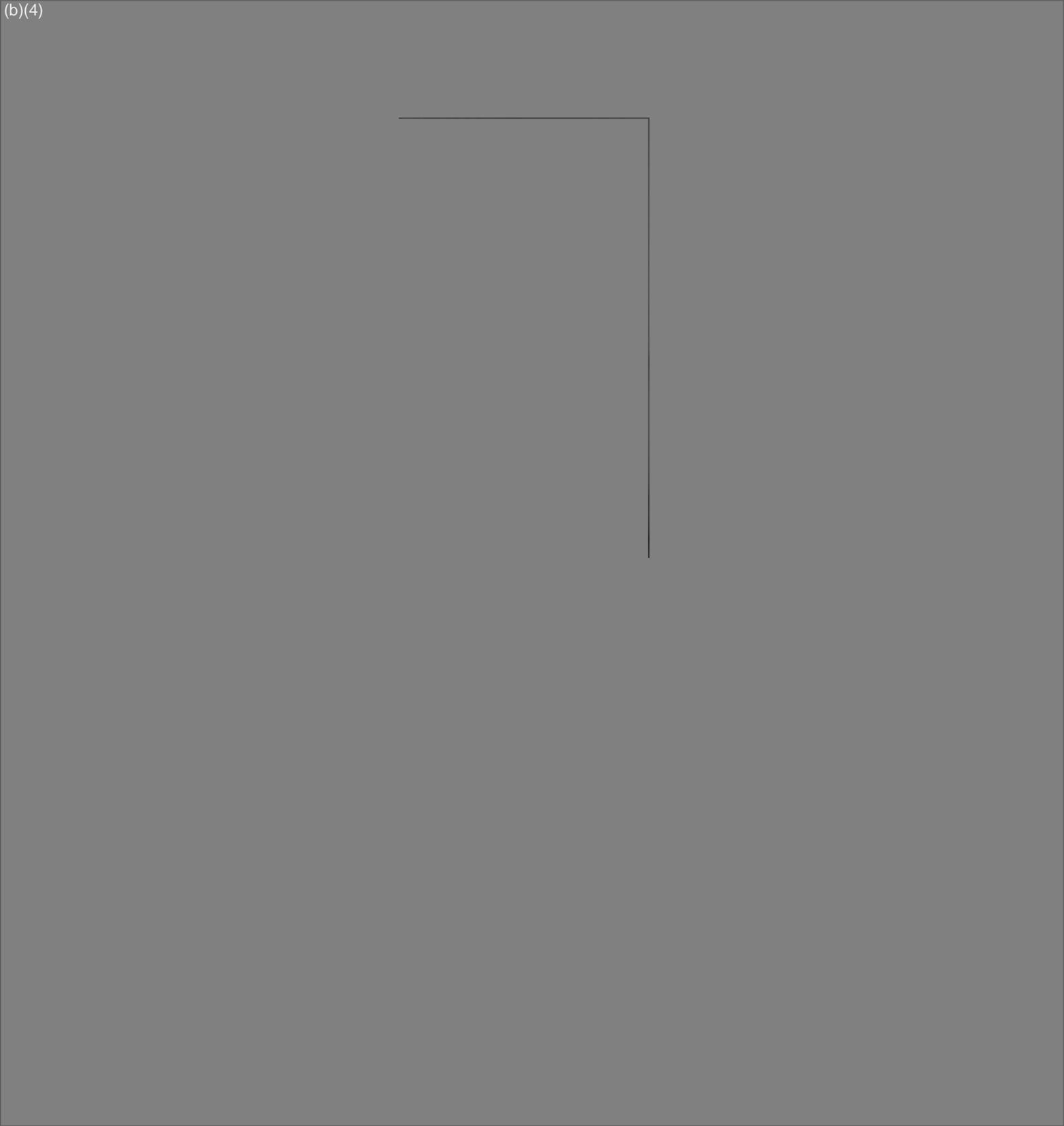
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**3.D.2. HPPD W366 protein**

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**Appendix 4**

**RAW AGRONOMIC DATA FOR 2008**

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## Appendix 5

### HERBICIDE RESISTANCE AND STEWARDSHIP

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## 5.A. Herbicide resistant weeds

Herbicides are the most economical, effective and reliable method of weed control in most crop production systems. Herbicides act by targeting and inhibiting specific plant biochemical processes or pathways. The process of specific activity is termed "mode of action" (MOA). Herbicides are classified into groups based on their MOA (HRAC 2009).

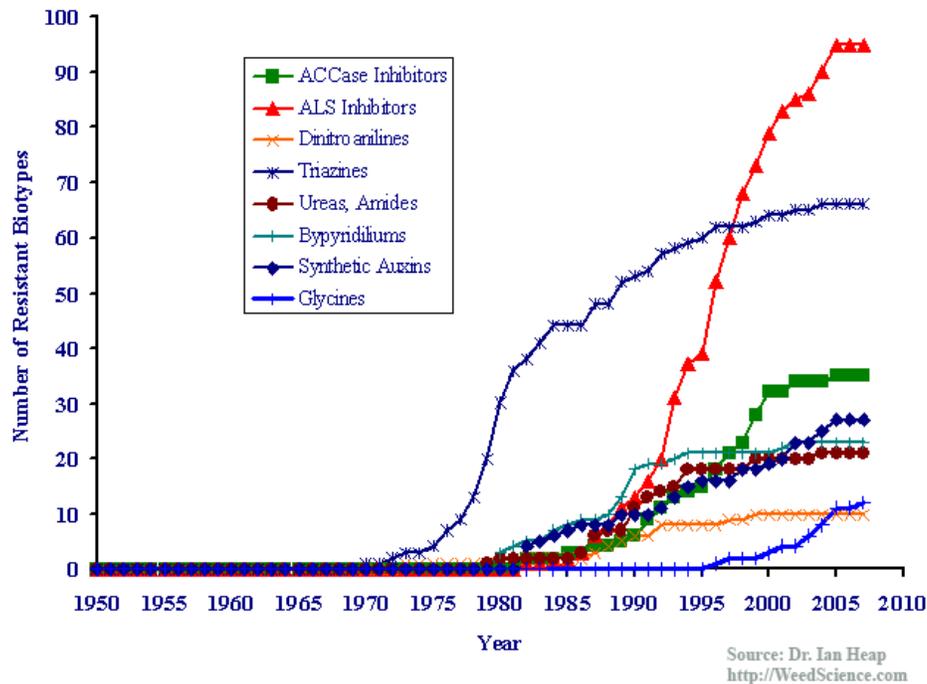
During the past several decades, diversity in weed control methods has been declining. Consolidation of agriculture has occurred at all levels including combining smaller farms to form larger farms. The resulting economic pressures have led to the selection of the most profitable crops and have driven the adoption of monocultures. Tillage, a key cultural practice contributing to a diversified weed management program, has also been severely reduced through the adoption of conservation tillage systems such as no-till and minimum tillage to combat the widespread problem of soil erosion (Anderson, 1996).

Weed control in the absence of complementary cultural control practices has resulted in the use of herbicides as the only weed control tactic. With this decline in use of alternative weed control methods, extensive use of herbicides with a single MOA has not only resulted in weed shifts but also high selection pressure for herbicide resistant weeds. Plants have the ability to adapt to ensure survival, which includes adapting to survive an herbicide application. The development of herbicide resistance is a function of time and exposure and also the genetic capability of the weed population present in a field.

Herbicide resistance is the naturally-occurring inheritable ability of some weed biotypes within a given population to survive an herbicide treatment that should, under normal use conditions, effectively control that weed population (HRAC, 2009).

The first herbicide resistant weed was identified in 1964 (HRAC, 2009). An increase in the number of documented herbicide resistant weeds began a steep incline after the ALS inhibiting herbicides were introduced in the 1980's. ALS herbicides inhibit the plant enzyme acetolactate synthase (ALS) and provide effective control of many grass and broadleaf weed species (Anderson, 1996; Whaley *et al.*, 2007). ALS herbicides were available for a broad number of crops for both post-emergence and residual weed control. Farming practices shifted, as use of ALS inhibitors reduced the amount of tillage needed for weed control. The lack of diversified weed control methods lead to the selection of populations of ALS herbicide-resistant weed species or biotypes.

There are 39 weed species resistant to the ALS class of chemistry in the US today and more than 90 resistant weed species reported world wide (Heap, 2009). Virtually all waterhemp (*Amaranthus rudis*) is considered by university weed scientists to be resistant to ALS inhibiting herbicides, resulting in the conclusion that ALS inhibiting herbicides are considered "obsolete" technology for weed control in soybean (Nordby *et al.*, 2007). In addition to weeds resistant to ALS inhibiting herbicides, resistance has also developed too many other herbicide modes of action as evident in Figure 38.

**Figure 38. Timeline of the development of herbicide resistant weeds**

## 5.B. Managing herbicide resistant weeds

Ideally integrated weed management should utilize all available tools including herbicides in a well balanced program as the lower the diversity of weed control tools, the higher the risk of selecting a resistant biotype becomes. To ensure diversification is maintained in weed control methods, we will also encourage growers to keep detailed records of weed management practices for each field. The following are our integrated weed management guidelines to promote an economically viable, environmentally sustainable, and socially acceptable weed control program:

### Know your weeds, know your fields

Today's herbicides control a broad spectrum of weed species, minimizing the importance of weed identification to a grower. However, identification of weed species will help identify an herbicide program that works best for every acre. Equally important is for the grower to understand the weed pressure and history within each field. Problematic areas like difficult-to-control weeds or dense weed populations should be closely monitored. There are several indications for a grower to consider with weed escapes to identify resistant weeds.

#### *Resistance Indicators*

- The field has been sprayed repeatedly with the same herbicide (mode of action), particularly if there was no mode of action diversity in the weed management system.
- A patch of weeds occurs in the same area year after year and is spreading.
- Many weed species are managed, but one particular weed species is no longer controlled. For example, following a glyphosate application, actively growing marestail can still be seen, in the absence of other weeds.

- Surviving weeds of the problem species may be in a patch where some are dead and some exhibit variable symptoms, but all are approximately the same age.

### Crop rotation

Crop rotation is one of the most important factors in an IWM program. Crop rotation adds weed management diversity through the inherent use of herbicides with different modes of action. In addition, crops vary in their ability to compete for sunlight, water and nutrients with weeds. Different planting times and seedbed preparation techniques can lead to a variety of cultural methods which employ diversity in a weed management program. Reliance on a monoculture crop leads to weed population shifts to fewer weed species but to overall higher densities, which increases the selection pressure for herbicide resistant weeds.

### Start with clean fields

Yields can be significantly reduced by early season weed competition. Proper tillage or the use of a burndown herbicide program should be used to control all emerged weeds prior to planting. Not only does the control of weeds prior to planting aid in the ease of planting, it also eliminates weed competition for soil moisture, light and nutrients.

Regardless of the tillage system (conventional, minimal, or no-till), a pre or early post-emergent soil-applied residual herbicide should be a part of every spray program. A soil-applied herbicide provides residual weed control allowing the crop to get a head start. Residual herbicides minimize the weed pressure and allow a wider post-emergent herbicide application window. Generally, soil-applied herbicides can be included in the burndown herbicide program for residual weed control on no-till acres. A residual herbicide also introduces another mode of action into weed resistance management programs (Nordby *et al.*, 2007).

### Rotate herbicide modes of action

There are three key factors in using herbicides to promote good resistant weed management:

*1. Use multiple modes of action during the growing season*

The use of multiple modes of action during the growing season increases the diversity within the weed control program by reducing the selection pressure of a single mode of action. A planned two pass herbicide (pre followed by post-emergence) program implements multiple modes of action in weed management systems for delaying weed resistance.

*2. Apply no more than two applications of a single herbicide mode of action to the same field in a two-year period*

Repeated, successive use of herbicides with the same mode of action increases the likelihood that resistant plants will reproduce and become dominant in the population. The best way to manage resistant weeds is to prevent them from spreading or populating. Herbicide-resistant weeds become problematic due to overuse of a single herbicide mode of action. To preserve an herbicide's efficacy, maintain its use and reap its benefits, growers should not use more than two applications of a single herbicide mode of action on the same field in a two-year period (Boerboom *et al.* 2006). In addition, rotating crops generally allows additional modes of actions to be used in a weed management program.

### 3. *Rotate herbicide-tolerant trait systems*

To ensure the viability of all traits for the future, rotate the herbicide tolerant trait used in each field each year to increase the chemical diversity used in each field.

#### Correct herbicide application

Product efficacy can be influenced by a multitude of factors. Ensuring correct use rates, weed stage and crop growth, and application technique will maximize weed control (Boerboom *et al.*, 2006).

##### *Apply to Actively Growing Weeds*

Herbicides provide peak performance when applied to actively growing weeds. Weeds that are actively growing absorb more herbicide. Conditions that provide peak growing environment for weeds are adequate soil moisture, sunlight and optimal soil nutrients.

##### *Timing*

The use of pre-emergent residual herbicides will provide key control of early season weeds that result in the greatest crop yield reduction and open a wider application window for post-emergence applications. Post emergence herbicides should be applied after crop emergence when weeds are 3 inches to 4 inches tall for optimal performance. Applying post emergence herbicides to smaller weeds increases crop yield again by eliminating early season weed competition.

##### *Application Technique*

Herbicides differ in the optimal application technique. Read and follow all label instructions to ensure proper application technique is achieved. Factors affecting weed control include: spray coverage, carrier volume, application speed, adjuvants, and tankmix partners.

##### *Product Rate*

The rate listed on the product label has been researched and tested by manufacturers and university researchers to provide the optimal control of the weeds at the height listed on the label. The application of an herbicide at a rate less than listed on the label can result in insufficient control and will have a significant impact on the immediate weed control and therefore the weed seed bank by allowing partially controlled weeds to reproduce and set seed.

#### Control weed escapes

Problematic weeds that escape the herbicide applications should be controlled to reduce weed seed production. A grower should consider spot herbicide applications, row wicking, cultivation or hand removal of weeds to improve weed management for the subsequent growing seasons.

#### Clean equipment

To prevent the spread of herbicide-resistant weeds and potentially introduce new invasive weeds on to the farm, avoid moving equipment that has not been thoroughly cleaned.

### 5.C. Evolution of herbicide resistant weeds

There are currently 9 glyphosate-resistant weeds in the United States. These weeds include palmer amaranth (*Amaranthus palmeri*), marestail (*Conyza Canadensis*), waterhemp

(*Amaranthus rudis*), giant ragweed (*Ambrosia trifida*), common ragweed (*Ambrosia artemisiifolia*), Johnsongrass (*Sorghum halepense*), Italian ryegrass (*Lolium multiflorum*), hairy fleabane (*Conyza bonariensis*), and rigid ryegrass (*Lolium rigidum*). There are an additional 7 glyphosate resistant weeds that can be found in other parts of the world (Heap 2009). Giant ragweed, common ragweed, and waterhemp are 3 of the top 10 most frequently sprayed for weeds in soybeans (Bayer CropScience, 2009).

Marestail, also known as horseweed, is the most widely spread glyphosate-resistant weed in the U.S. Marestail can produce up to 200,000 seeds per plant. In a management study conducted in Michigan, soybean yields could be reduced up to 83% by marestail in untreated check treatments (Bruce and Kells, 1990). Some populations of marestail have become resistant to other available herbicides including atrazines, simazines, diurons, and ALS inhibiting herbicides (Loux *et al.*, 2006; Heap, 2009).

Another glyphosate-resistant weed of concern is giant ragweed. Glyphosate-resistant giant ragweed isn't as widespread today as glyphosate-resistant waterhemp; however, it can be just as difficult to control with alternative herbicides. Giant ragweed can grow up to 17 feet tall and produces allergenic pollen. One giant ragweed plant per 110 square foot can reduce soybean yield 50%. There are also populations of giant ragweed that are resistant to ALS inhibiting herbicides (Johnson *et al.*, 2007).

Waterhemp is likely the weed of most concern in terms of control to soybean growers and university researchers. Waterhemp can produce more than 1 million seeds per plant. Waterhemp can reduce soybean yields by 37 to 44% in 7.5" and 30" rows, respectively (Nordby *et al.*, 2007). Moreover, nearly all populations of waterhemp are also resistant to ALS inhibiting herbicides and some populations are resistant to triazines and PPO inhibiting herbicides (Boerboom and Owen, 2006; Heap, 2009).

Today there are few choices for conventional herbicides that are rated as "good" by University Extension programs for waterhemp control in glyphosate tolerant soybeans. Of those that are rated as good, their use is complicated as described in the following discussion.

#### Soil-applied residual herbicides

Growers applied pre-emergence or pre-plant incorporated herbicides on less than 5% of soybean acres in 2006 (USDA, 2007). The use of residual herbicides declined due to the efficacy and ease of glyphosate use. The seedling growth inhibitors or microtubule inhibitors such as pendimethalin (Prowl<sup>®</sup>), trifluralin (Treflan<sup>®</sup>), and ethalfluralin (Sonalan<sup>®</sup>) which inhibit cell division, provide residual control of waterhemp, however, these herbicides need to be incorporated into the soil for maximum efficacy. Products that contain chloroacetamide herbicides and control waterhemp in soybean include s- metolachlor (Dual II Magnum<sup>®</sup>), s- metolachlor + fomesafen, dimethenamid-P (Outlook<sup>®</sup>) and alachlor (Intro<sup>®</sup>, Micro-tech<sup>®</sup>). Another pre-emergence herbicide for the control of waterhemp is metribuzin (Sencor<sup>®</sup>), which is in the triazinone family but can result in crop damage in certain environmental conditions.

### Post-applied herbicides

The only conventional herbicide mode of action that provides “good” control of waterhemp post-emergence as rated by University Weed Scientists are the PPO inhibitors. These herbicides inhibit the protoporphyrinogen oxidase (PPO) enzyme which is involved in the heme-pigment synthesis pathway. Products that contain PPO inhibitors, such as lactofen (Cobra<sup>®</sup>, Phoenix<sup>™</sup>), fomesafen (Flexstar<sup>®</sup>, Reflex<sup>®</sup>) and s-metolachlor + fomesafen (Prefix<sup>™</sup>) have potential to injure the soybean crop. Applying PPO inhibitors under high temperature and humidity increases the potential crop injury. Also, there are populations of waterhemp that are resistant to PPO inhibiting herbicides (Boerboom and Owen 2006; Heap 2009). In addition, these conventional herbicides have stringent limitations on the size of waterhemp and other weeds that they can control. Environmental situations prevent timely application of conventional herbicides, weed control will be sacrificed.

## **5.D. Characteristics of glyphosate and isoxaflutole herbicides**

### **5.D.1. Glyphosate herbicide**

Glyphosate is a non-selective, broad spectrum systemic herbicide introduced to the marketplace in the 1970's. Glyphosate can be formulated in multiple ways: glyphosate isopropylamine salt (Roundup<sup>®</sup>), glyphosate trimethylsulfonium salt (Touchdown<sup>®</sup>), or glyphosate diammonium salt (Touchdown<sup>®</sup> 4 or Touchdown Pro<sup>®</sup>). Glyphosate is the only member of the glycine herbicide family. Glyphosate inhibits the biosynthesis of the aromatic amino acids in the shikimic acid pathway by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Anderson, 1996; Vencill, 2002).

Glyphosate is labeled for the control of 113 annual broadleaf and grass weeds and additional 62 perennial weeds (Roundup Weathermax<sup>®</sup> label 2006). Glyphosate is likely the most broad spectrum herbicide available today for weed control in row crops. The effectiveness of glyphosate is established; more than 90% of the soybean acres in 2006 were treated with glyphosate at an average use rate of 0.802 lb/A with an average of 1.7 applications per season (USDA, 2007). The lack of effective alternatives is illustrated by the fact that the second most commonly used herbicide in 2006 was 2,4-D 2-EHE which was sprayed on only 7% of the US soybean acres (USDA, 2007). In addition to soybean, glyphosate was applied to 85% of the planted cotton acres in 2007 (USDA, 2008) although resistance to glyphosate has developed in several weed species, the chemical is extremely effective on the vast array of weeds in commercial crops.

(b)(4)

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CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

(b)(4)

### 5.E. Stewardship of double-herbicide-tolerant soybean event FG72

Bayer CropScience (BCS) places a high importance on the sustainability of its technology and has adopted a life-cycle approach to product stewardship. This means that appropriate stewardship principles are applied at every stage of biotechnology development from research through to product discontinuation and as a founding member of Excellence Through Stewardship<sup>®</sup>, BCS is helping advance stewardship best practices throughout the industry. BCS commitment to stewardship extends to our corporate relationships and is evidenced by the stewardship and quality assurance standards that are required in those relationships and is also indicated in the following clause that is now included in third party agreements related to BCS biotechnology traits:

“BAYER is committed to the proper stewardship of its products and expects those with whom it contracts to handle material containing BAYER technology in an appropriate manner. This includes without limitation adherence to the stewardship and quality assurance provisions of this Agreement. BAYER supports and has affirmed its commitment to the Excellence Through Stewardship<sup>®</sup> industry stewardship initiative. Further information relating to this initiative can be found at [www.excellencethroughstewardship.org](http://www.excellencethroughstewardship.org).”

In the BCS organization, our crop market area teams are committed to BCS stewardship principles and are aware of procedures to communicate appropriate information within the BCS crop team matrix to rapidly respond to issues that may develop from use of our technologies. Field development and market support teams are provided the tools necessary to serve the grower as a local and direct contact for any questions related to BCS technologies with regards to product performance or impacts on human and environmental health and safety.

BCS participates in several industry and professional initiatives in support of stewardship:

- Herbicide Resistance Action Committee (HRAC)  
HRAC is an industry initiative which fosters co-operation between plant protection manufacturers, government, researchers, advisors and farmers. The objective of the working group is to facilitate the effective management of herbicide resistance. Weed scientists employed by Bayer CropScience participate as members of the Herbicide Resistance Action Committee and BCS supports the work of this group.
- CropLife America - US  
BCS is active in CropLife, serving on committees and working groups that develop industry-wide approaches to regulatory and technology management issues.
- American Seed Trade Association (ASTA)  
M.S. Technologies, LLC (MSTech) and BCS are active in serving on committees and working groups that set industry standards for seed quality and purity, and product stewardship.
- BIO  
BCS is active in the Biotechnology Industry Organization, serving on committees and working groups that develop industry-wide approaches to regulatory and technology management issues.
- Excellence Through Stewardship  
BCS is active in Excellence Through Stewardship, serving on the board of directors, committees and working groups that develop industry best practices for stewardship.
- Weed Science professional societies  
BCS is active participant in a number of organizations. We maintain active memberships in the Weed Science Society of America, North Central Weed Science Society, Northeastern Weed Science Society, Southern Weed Science Society, and Western Weed Science Society, all of which are professional, non-profit societies, established to promote research, education, and extension outreach activities related to weeds; provide science-based information to the public and policy makers; and foster awareness of weeds and their impacts on managed and natural ecosystems (WSSA 2009).

### 5.E.1. Customer outreach

BCS and MSTech have a commitment to stewardship of all of our products, including herbicide-tolerant trait (HTT) technology. We strive to provide best management practices of HTT technology which includes integrated weed management to our customers (see section 5.B.). Education of integrated weed management is the only practical method for its success. Education starts internally with our own field development, technical service, chemical sales representatives, and seed salesmen. Externally, we collaborate with key influencers to help growers understand the long term economic viability of integrated weed management. Those key influencers include university extension agents, agronomists, consultants, and local retail seed and chemical salesmen. In addition, we directly provide the integrated weed management message to growers through grower meetings, trade shows, and web and mail communications.

A Technology Use Agreement or similar agreement will be developed that will be provided to each grower at the time of seed purchase. By signing the agreement, the grower will agree to best management strategies that are indicated in the agreement. The agreement will contain company contact information including a website for the best management practices and product information. In addition, a toll free hotline for growers to obtain live technical product support will be provided. BCS and MSTech are committed to stewardship principles and procedures, and to communicating appropriate information in order to rapidly respond to any issues that may develop.

Growers may also contact the seed company for product support. The seed company name and contact information will be provided on the label of each bag of seed sold. Each grower purchase of FG72 Soybeans will be recorded by seed company partners. This information will be provided to MSTech which will enable MSTech to maintain a database of all growers utilizing event FG72 products. This database could be used to disseminate updated stewardship information.

## 5.E.2. Additional customer support

### Product information

There are a number of ways that a grower can obtain product information. The product label is the formal legal method of communicating directions for use of an herbicide. BCS's history of including recommendations on product labels for integrated weed management. Here is an example of a BCS product label on this topic.

BALANCE<sup>®</sup> FLEXX Herbicide is also recommended as the first herbicide applied in an integrated weed control program that includes sequential post-emergence herbicide applications.

CORVUS<sup>™</sup> Herbicide may be applied as the first herbicide in an Integrated weed control program that includes sequential post-emergence herbicide applications with products such as LAUDIS<sup>™</sup> Herbicide, or IGNITE<sup>®</sup> 280 SL Herbicide or glyphosate in transgenic field corn.

BCS is committed to supporting research by university institutions to generate local grower recommendations. University Extension Weed Control Handbooks (*2008 Guide for Iowa Corn and Soybean Production, Illinois Agricultural Pest Management Handbook, 2009 Weed Control Guide for Ohio and Indiana*) contain use directions and product information on many BCS herbicides.

### Screening for Herbicide Resistance

Currently, confirmation of weed resistance is commonly conducted by collecting seed of suspected resistant plants. Those seeds are replanted in a greenhouse environment and sprayed with various rates of the herbicide to which resistance is suspected. The survival of the weeds confirms resistance.

BCS invests a significant amount of resources to inform and train our own employees, customers and stakeholders so that they can develop sustainable programs to manage both their resistant and susceptible weed populations. Modern testing conducted in the laboratory such as those employed by Bayer CropScience will in the future allow faster and more reliable herbicide resistance diagnosis. Such methods include testing for metabolic resistance by following the degradation of an active substance in a plant and testing for target-site resistance through PCR analysis coupled with pyrosequencing.

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### **5.E.3. Monitoring of effectiveness of the stewardship plan**

Each grower purchase of event FG72 soybeans will be recorded by the individual seed company making the sale. This information will be provided to MSTech which will enable MSTech to maintain a database of all growers utilizing event FG72 products. BCS regularly utilizes market research surveys to determine market share and adaptation of technology.

Seed company partners will have direct contact with growers and will be able to provide feedback to MSTech regarding the stewardship effectiveness. BCS field representatives will also interact with growers and will be a source of information.

BCS will continue to support ongoing efforts to understand weed resistance to herbicides, to apply up-to-date information to product labels, and to provide information to growers.

## Appendix 6

### REFERENCES – APPENDICES 1-5

CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

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**Petition for the Determination of Non-Regulated Status for MON 87701**

The undersigned submits this petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

March 19, 2009

OECD Unique Identifier: MON-87701-2  
Monsanto Petition Number: 09-SY-194U

**Submitted by**

(b)(6)

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**From:** [Michael T Watson](#)  
**To:** [Rebecca L Stankiewicz-Gabel](#)  
**Subject:** Fw: Letter of Completeness  
**Date:** 12/08/2009 03:05 PM  
**Attachments:** [letter\\_of\\_completeness\\_0908201p.docx](#)

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----- Forwarded by Michael T Watson/MD/APHIS/USDA on 12/08/2009 03:05 PM -----

**Michael T  
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11/25/2009 12:32 PM

Subject Letter of Completeness

Good Afternoon,

Please find the attached letter of completeness for your MON87701 Cry1A(c) soybean. If you have any comments or questions, please contact either Rebecca Stankiewicz Gabel or me at your convenience. Thanks!

Mike



[letter\\_of\\_completeness\\_0908201p.docx](#)

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United States	Animal and	Biotechnology	4700 River Road, Unit 147
Department of	Plant Health	Regulatory	Riverdale, Maryland
Agriculture	Inspection Service	Services	20737-1236

November 19, 2009

Subject: Review for Completeness and Acceptability of Monsanto Petition Number 09-082-01p for a Determination of Non-regulated Status for MON87701 Cry1A(c) soybean

(b)(6)

This letter is in reference to the Petition for Determination of Non-regulated Status for Mon 87701 soybean submitted to the USDA, Animal and Plant Health Inspection Service/Biotechnology Regulatory Services (APHIS/BRS) on March 23, 2009. APHIS/BRS has assigned this petition the number 09-082-01p. After reviewing this petition, APHIS/BRS has determined that there is a need for additional information and clarification before we can declare this petition technically complete. The clock will be stopped on the petition until such time as this information is provided. Please respond to each of the points listed below in the form of a revised petition that includes the information requested as written below. A PDF copy of the complete petition will be posted on our website as part of the petition process. The specific issues can be found under the relevant headings along with page number as they appear in the petition.

#### General Issue:

- Standard deviation (or standard error) values are presented for some analyses in the petition, but not others. Those statistics are useful for reviewers to make inferences about data presented in tables or to cross check petitioner's interpretation of data. Please provide SD (or SE) values for all mean values in tables. Also, provide sample sizes for all measures of central tendency presented in the petition.
- Please submit all final field test reports for those notifications that are cited in the petition. While we recognize that some of are not yet due, we cannot complete our review until all are received.

#### Specific Issues:

(pg. 66) The petition states that, "A comparison between the PCR product generated from conventional soybean and the sequence generated from the 5' and 3' flanking sequences of Mon 87701 indicate there was a 32 bp deletion (bases 1441-1472) and a 14 bp insertion (bases 1987-2000) just 5' to the Mon 87701 insertion site." Please provide the data to support this statement.

(pg 76-77, Figure VI-1) please highlight differences in sequence as described in the caption  
(pg 112) There are errors in the lane numbering in the caption.

### Staff Contact

If you have any questions about the statements and information requested in this letter, please contact Rebecca Stankiewicz Gabel at 301-734-5603 or by e-mail: [rebecca.l.stankiewicz-gabel@aphis.usda.gov](mailto:rebecca.l.stankiewicz-gabel@aphis.usda.gov). Please refer to petition application number 09-082-01p in your correspondence. APHIS looks forward to your reply.

Best Regards,

Michael Watson  
Director, Environmental Risk Analysis Programs  
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## RELEASE OF INFORMATION

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party. In the event the USDA receives a Freedom of Information Act request, pursuant to 5 U.S.C. § 552, and 7 CFR Part 1, covering all or some of this information, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g. responsiveness, confidentiality, and/or competitive concerns. Monsanto expects that no information that has been identified as CBI (confidential business information), will be provided to any third party. Monsanto understands that a CBI-deleted copy of this information may be made available to the public in a reading room and by individual request, as part of a public comment period. Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

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## CERTIFICATION

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

(b)(6)

A large rectangular area of the document is redacted with a solid grey fill. A thin red border surrounds this area. In the top-left corner of the redacted area, the text "(b)(6)" is written in a small, black font.

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## EXECUTIVE SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility under the Plant Protection Act (7 USC § 7701-7772) to prevent the introduction and dissemination of plant pests into the United States. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of non-regulated status in whole for the new biotechnology-derived insect-protected soybean product, MON 87701, any progeny derived from crosses between MON 87701 and conventional soybean, and any progeny derived from crosses of MON 87701 with other biotechnology-derived soybean that has been granted non-regulated status under 7 CFR Part 340.

### **Product Description**

Monsanto Company has developed biotechnology-derived insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal (Cry) protein ( $\delta$ -endotoxin) derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. The Cry1Ac protein provides protection from feeding damage caused by targeted lepidopteran pests. The *cry1Ac* gene was transferred into the genome of soybean cells using *Agrobacterium tumefaciens*-mediated transformation. The MON 87701 product concept is to reduce or replace current insecticide applications to control lepidopteran pests in tropical and subtropical soybean production regions where these insects cause significant plant damage and yield loss. MON 87701 will offer growers in these regions an effective pest management tool and help to maintain soybean yield potential.

Soybean production in the U.S. can be impacted by insect pests that require insecticide treatments to control infestations that reach economic thresholds. The impact and severity of insect pest infestations vary greatly across soybean production regions primarily due to the different climate and weather conditions, insect species distributions, insect species environmental tolerances, and agricultural practices. In the U.S., the most economically important soybean lepidopteran pests are the defoliating and pod-feeding insects. The most damaging lepidopteran defoliators are velvetbean caterpillar, *Anticarsia gemmatalis*; soybean looper, *Pseudoplusia includens*; and green cloverworm, *Plathypena scabra*.

Analysis of Cry1Ac protein levels in over-season leaf indicate that relatively high levels of the Cry1Ac protein are expressed throughout the entire growing season in MON 87701, providing exceptional control of targeted lepidopteran pests, such as velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*). In general, insect pressure is greatest on soybean grown in the southern U.S., especially the southeastern states bordering the Gulf of Mexico and Atlantic Ocean, in which the tropical and sub-tropical weather favors pest infestation. According to USDA-NASS statistics, about 16% of the approximately 75 million U.S. soybean acres, those

grown mainly in the southeastern and delta states, received insecticide applications in 2006 (USDA-NASS, 2007b). Given the limited number of acres in the U.S. that consistently have sufficient lepidopteran insect pressure to require the use of insecticides or other insect control practices, Monsanto will file an application with the EPA to support future breeding and seed multiplication activities in the U.S. This application will request a seed increase registration of the plant-incorporated protectant *Bacillus thuringiensis* Cry1Ac protein and the genetic material (vector PV-GMIR9) necessary for its production in soybean. Under this type of seed increase registration, commercial sale of MON 87701 within the U.S. would be prohibited by law.

In the future, if Monsanto decides to commercially introduce MON 87701 in the U.S., Monsanto would be required to apply to the EPA for a commercial use registration of the plant-incorporated protectant *Bacillus thuringiensis* Cry1Ac protein and the genetic material (vector PV-GMIR9) necessary for its production in soybean. As a condition of a commercial use registration, EPA would require that Monsanto develop, administer, and oversee an EPA-approved insect resistance monitoring (IRM) program. EPA does not require IRM programs for the small acreages used for Section 3 seed increase registrations.

#### **Data and Information Presented to Assess Plant Pest Potential of MON 87701**

The data and information presented in this Petition demonstrate the familiarity of MON 87701 as compared to conventional soybean and, moreover, show that MON 87701 is not likely to pose an increased plant pest potential, including weediness or adverse environmental impact, compared to conventional soybean. The overall safety of MON 87701 was confirmed based on multiple, well established lines of evidence:

1. A detailed molecular characterization of the inserted DNA, where the results confirm the insertion of a single functional *cry1Ac* expression cassette at a single locus within the soybean genome.
2. An extensive set of biochemical evaluations that demonstrate the identity of the full-length Cry1Ac produced in MON 87701.
3. An assessment of toxicity and allergenicity potential of the Cry1Ac protein based on extensive information collected and evaluations performed on Cry1Ac. The results demonstrate that the Cry1Ac protein is not likely to be a toxin or allergen.
4. The compositional and nutritional assessment confirmed that MON 87701 harvested seed and forage are compositionally and nutritionally equivalent to and as safe as those of conventional soybean.
5. An extensive evaluation of the MON 87701 phenotypic and agronomic characteristics and environmental interactions that demonstrate MON 87701 is not likely to have increased plant pest potential compared to conventional soybean.
6. An assessment on the potential impact to non-target-organisms (NTOs) and endangered species concludes that MON 87701 is unlikely to have adverse effects on these organisms under normal agricultural practices.

### **Weediness Potential of Soybean**

The commercial soybean species in the U.S. (*Glycine max* L.) does not exhibit weedy characteristics and is not effective in invading established ecosystems. Soybean is not listed as a weed in major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Soybean does not possess any of the attributes commonly associated with weeds (Baker, 1965), such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Due to the lack of dormancy, soybean seed can germinate quickly under adequate temperature and moisture and potentially can grow as a volunteer plant. However, a volunteer plant likely would be killed by frost during autumn or winter of the year it was produced. If it did become established, a volunteer plant would not compete well with the succeeding crop, and could be controlled readily by either mechanical or chemical means (OECD, 2000). In addition, since wild populations of *Glycine* species are not known to exist in the U.S., the potential does not exist for MON 87701 to outcross to wild or weedy relatives and alter their weediness potential.

### **Molecular Characterization of Inserted DNA**

MON 87701 was produced by *Agrobacterium*-mediated transformation of soybean with PV-GMIR9, which is a binary vector containing two T-DNAs. The first T-DNA, designated as T-DNA I, contains the *cryIAc* gene expression cassette. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* gene cassette. During transformation, both T-DNAs were inserted into the soybean genome at unlinked loci. The *cp4 epsps* gene was used as the selectable marker (glyphosate tolerance) that was needed for the initial selection of transformed cells and plants. After the transformed cells, and subsequently the plants, were identified, the selectable marker gene was no longer needed. Therefore, traditional breeding and segregation was deployed to isolate plants that only contain the *cryIAc* expression cassette (T-DNA I), thereby producing marker-free MON 87701 plants. Molecular characterization of MON 87701 by Southern blot analyses demonstrated that the DNA inserted into the soybean genome is present at a single locus and contains one functional copy of the *cryIAc* expression cassette. No T-DNA II (*cp4 epsps* gene expression cassette) genetic elements or backbone sequences from the transformation plasmid were detected in MON 87701. In addition, no partial genetic elements, linked or unlinked to the inserted expression cassette were detected. The stability of the integrated DNA (*cryIAc* gene) was demonstrated by confirming the Southern blot fingerprint of MON 87701 and was maintained for five generations tested across the breeding history. The stability was further confirmed by the inheritance of the insect-protected trait in MON 87701 that followed the expected Mendelian segregation pattern.

The inserted T-DNA I in MON 87701 contains left and right border sequences from *Agrobacterium tumefaciens*, which is considered a plant pest. These sequences are well characterized and are only non-coding regions. These regions will not cause MON 87701 to promote plant disease.

### **Characterization of the Cry1Ac Protein**

The expression level of full-length Cry1Ac protein was determined by enzyme-linked immunosorbent assay (ELISA) in MON 87701 tissues produced from five field trials located in U.S. soybean production regions during the 2007 growing season. The results demonstrated that the Cry1Ac protein was expressed and detected in all above-ground tissues tested, including leaf, forage, pollen, and harvested seed. The Cry1Ac level in root was determined to be less than the ELISA assay limit of detection (LOD). The mean Cry1Ac protein levels in MON 87701 across the five sites were 4.7 µg/g dwt in harvested seed and 34 µg/g dwt in forage. In leaf tissue samples harvested throughout the growing season, mean Cry1Ac protein levels in MON 87701 across all sites ranged from 220 to 340 µg/g dwt. The mean Cry1Ac protein level in pollen (anther) from replicate samples collected at a single site was 2.3 µg/g fwt.

A history of safe use and data from multiple evaluations support the safety of the Cry1Ac protein and, by extension, MON 87701. The Cry1Ac protein belongs to a family of Cry proteins from *Bacillus thuringiensis* (Bt). Application sprays of sporulated Bt have a long history of safe use for pest control in agriculture, including organic farming (Cannon, 1993; EPA, 1988; WHO, 1999). Microbial pesticides containing *B. thuringiensis* Cry1A proteins have been used for more than 45 years and have undergone extensive toxicity testing showing no adverse effects to human or animal health (Baum et al., 1999; Betz et al., 2000; EPA, 2000; EPA, 2001; McClintock et al., 1995; Mendelsohn et al., 2003). During the last decade a variety of biotechnology-derived crops containing Cry1 proteins from *B. thuringiensis* have been commercialized; thereby rendering these plants resistant to several insect pests. Commercially available Bollgard® cotton contains Cry1Ac that has 100% amino acid identity to the MON 87701-produced Cry1Ac, with the exception of four additional amino acids at the N-terminus related to the chloroplast transit peptide. A related protein, Cry1Ab, which has ~90% amino acid identity to the Cry1Ac in MON 87701 and Bollgard cotton, is expressed in YieldGard® corn that is used extensively for feed and food. The compositional equivalence of Cry1-containing commercial products to conventional varieties has been demonstrated (Berberich et al., 1996). Detailed human and animal safety assessments and over a decade of safe human and animal consumption of these crops further support the conclusion that these crops are safe for consumption (Betz et al., 2000; Mendelsohn et al., 2003).

Safety assessments were conducted using the full-length Cry1Ac protein that includes the four additional amino acids on the N-terminus. The expression level of the Cry1Ac protein in MON 87701 seed was too low and insufficient for use in the safety evaluations. Therefore, it was necessary to produce the Cry1Ac protein in a high-expressing recombinant host organism, *Escherichia coli* (*E. coli*). The protein produced by *E. coli* was designed to match the exact amino acid sequence of its counterpart expressed in MON 87701. Subsequently, the physicochemical and functional equivalence of the MON 87701-produced and *E. coli*-produced Cry1Ac proteins were examined to ensure that the proteins from the two host sources were equivalent. The proteins were characterized and equivalence was evaluated based on a panel of analytical tests and

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assays. The results of these evaluations provide a detailed characterization of the Cry1Ac protein isolated from MON 87701 and confirmed its equivalence to the *E. coli*-produced Cry1Ac protein.

### **Allergenicity and Toxicity Potential of the Cry1Ac Protein**

The Cry1Ac protein produced by MON 87701 does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that have adverse effects on mammals. This has been shown by extensive assessments with bioinformatics tools, such as a FASTA sequence alignment search and an eight-amino acid sliding window search. With its extremely low and negligible toxicity to mammals, other vertebrates and invertebrates, DEKALB Genetics Corporation previously petitioned the U.S. EPA for an exemption from the requirement of a tolerance for Cry1Ac protein in or on all raw agricultural commodities and the genetic material necessary for its production. In 1997, U.S. EPA established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1Ac protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 180.1155). Additionally, digestive fate experiments conducted with the Cry1Ac protein produced in MON 87701 have demonstrated that the full-length protein is rapidly digested in simulated gastric fluid (SGF), a characteristic shared among many proteins with a history of safe consumption. A small, transiently stable Cry1Ac protein fragment from the SGF digestion was very quickly (within 30 sec) degraded during short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length Cry1Ac protein in SGF and SIF, together with complete degradation of the small, transiently stable fragment in SIF, indicates that it is highly unlikely that the Cry1Ac protein and its fragment will reach absorptive cells of the intestinal mucosa. Mouse acute oral toxicity evaluations have demonstrated that the Cry1Ac protein is not acutely toxic and does not cause any adverse effect, even at the highest dose levels tested, which were 1290 mg/kg body weight for females and 1460 mg/kg body weight for males. The dietary safety assessment based on the acute toxicity data for the Cry1Ac protein and soybean product dietary pattern shows that the margin of exposure (MOE) for the overall U.S. population is  $\geq 2.93 \times 10^6$ . Similarly, for non-nursing infants aged from 6-24 months old, the subpopulation with the highest soybean intake on a bodyweight basis, the MOE is  $\geq 7.71 \times 10^4$ . In the United States, soybean are crushed to produce high protein soybean meal that is used as feed. For the soybean meal produced in U.S., approximately 98% is consumed by the livestock industry (ASA, 2008). From a worst case assessment in feed, the percentage of the Cry1Ac protein consumed as part of the daily protein intake for a dairy cow is 0.0498%, and for both the broiler and pig it is less than 0.0012%. Taken together, these data indicate that food and feed derived from MON 87701 containing the Cry1Ac protein are as safe for consumption as food and feed derived from conventional soybean.

### **Composition and Nutrition of Forage and Grain**

A compositional assessment was conducted on the harvested seed and forage collected from five field sites in U.S. soybean production regions during 2007 to demonstrate that MON 87701 is compositionally equivalent to conventional soybean. Compositional analyses on harvested seed and forage included the significant nutrients, anti-nutrients, and key secondary metabolites, consistent with OECD guidelines (OECD, 2001).

The analytes included protein, fat, carbohydrates, fiber, ash, moisture, amino acids, fatty acids, a vitamin, and anti-nutrients. In each assessment, MON 87701 was compared to an appropriate conventional control, which had a genetic background similar to MON 87701 but did not possess the introduced trait. In addition, the same compositional analytes were assessed in 20 conventional soybean varieties to establish a 99% tolerance interval for each of the analytes. The results show that MON 87701 is nutritionally and compositionally equivalent to, and as safe and nutritious as, conventional soybean. The resulting compositional data on MON 87701 and the conventional soybean control were statistically compared in a combined-site analysis as a first order assessment of biologically relevant changes, followed by individual-site analyses. The combined-site analysis for harvested seed and forage samples showed no significant difference ( $p > 0.05$ ) between MON 87701 and the conventional control for 40 of the 55 comparisons. For the analytes where differences were noted ( $p < 0.05$ ), the magnitude of differences between MON 87701 and the conventional soybean control were generally low (most  $< 5\%$ ), were not observed consistently across all sites (individual-site analyses), and mean values for MON 87701 were within the calculated 99% tolerance interval for the population of commercial conventional soybean varieties grown concurrently at the same time and field sites. Therefore, it is concluded that the statistical differences represent the natural variability for these soybean analytes such that they were not regarded as biologically meaningful. Harvested seed and forage analytical component values also were comparable to published scientific literature and the ILSI Crop Composition Database, further supporting the conclusion that harvested seed and forage from MON 87701 are compositionally equivalent to those of conventional soybean.

### **Phenotypic and Agronomic Characteristics and Environmental Interactions**

The phenotypic, agronomic, and environmental interaction assessment indicates that MON 87701 is comparable to conventional soybean and is unlikely to have an increased plant pest risk. An important element in assessing plant pest potential and environmental impact of MON 87701 is to compare MON 87701 to conventional soybean. The assessment is based initially on the concept of familiarity, which USDA recognizes plays an important role in these assessments. Familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interactions among these factors, and provides a basis for comparative risk assessment between a biotechnology-derived plant and its conventional counterpart. The MON 87701 characteristics assessed include: seed dormancy and germination, pollen morphology, symbiont interactions conducted in the laboratory, and plant phenotypic observations and environmental interaction evaluations conducted in the field.

Seed dormancy and germination characterization indicated that MON 87701 seed had germination characteristics similar to that of the conventional soybean control. In particular, the absence of hard seed, a well-accepted characteristic of weediness affecting seed germination rate and viability, supports a conclusion of no increased weediness potential of MON 87701 compared to conventional soybean for germination and dormancy characteristics. For pollen characteristics and symbiont interactions, there were no significant differences ( $p < 0.05$ ) observed for any of the parameters measured,

including pollen viability, nodule dry weight, and shoot total nitrogen. Collectively, these results support the conclusion that MON 87701 is not likely to exhibit increased weed potential compared to conventional soybean.

The field evaluation of phenotypic, agronomic, and ecological characteristics of MON 87701 also supports the conclusion that MON 87701 is not likely to pose an increased plant pest potential compared to conventional soybean. These evaluations were conducted at 16 replicated field sites across U.S. soybean production regions. The assessments analyzed 14 phenotypic characteristics, plant-insect and plant-disease interactions and plant response to abiotic stressors. The observed phenotypic characteristics were comparable between MON 87701 and the conventional soybean control. No significant differences ( $p < 0.05$ ) were observed for any of the phenotypic characteristics measured, including early stand count, seedling vigor, days to 50% flowering, flower color, lodging, pod shattering, final stand count, seed moisture, seed test weight, and yield. In an assessment of the abiotic stress response, disease damage, and arthropod damage, no significant differences were detected between MON 87701 and the conventional soybean control for 367 of 373 comparisons (including all 109 abiotic stressor comparisons, all 131 disease damage comparisons, and 127 of 133 arthropod damage comparisons, respectively) among all observations at the 16 sites. Of the six significant differences in the arthropod damage category, four of the significant differences were associated with MON 87701 having less damage caused by lepidopteran pests than the control and, thus, were expected since the insect-protected trait controls certain lepidopteran pests. For the two other significant differences, MON 87701 had less damage than the control from bean leaf beetle during a single observation at two separate sites. Bean leaf beetle damage was not consistent across the 16 sites or observation intervals. Thus, the detected differences in arthropod damage ratings are unlikely to be biologically meaningful in terms of increased plant pest potential or indicate an adverse environmental impact of MON 87701 compared to the conventional soybean control. Overall, except for the intended change in resistance to selected lepidopteran insects, the phenotypic, agronomic and ecological characteristics of MON 87701 are consistent with those of conventional soybean.

Similarly, in an assessment of pest and beneficial arthropod abundance, no significant differences were detected between MON 87701 and the conventional soybean control for 70 out of 80 comparisons (including 26 out of 34 arthropod pest comparisons and 44 out of 46 beneficial arthropod comparisons) among the collection intervals conducted at four sites. Seven of the 10 significant differences between MON 87701 and the conventional soybean control in arthropod abundance were for lepidopteran pests, including corn earworms, green cloverworms, soybean loopers, and webworms. These differences were not unexpected since the insect-protected trait expressed in MON 87701 controls certain lepidopteran pests. The remaining three significant differences were for stink bug, *Orius*, and ladybird beetle abundance. None of the significant differences in arthropod abundance were consistent across collection intervals or sites. Thus, the differences are unlikely to be biologically meaningful in terms of increased plant pest potential or indicate an adverse environmental impact of MON 87701 compared to the conventional soybean control. Taken together, these comparative assessments lead to the conclusion that MON 87701 is not likely to increase plant pest potential, including weediness, or to have an increased environmental impact compared to conventional soybean.

### **Non-Target Organisms and Threatened or Endangered Species**

The environmental assessment of MON 87701 and the expressed, Cry1Ac protein indicates that MON 87701 poses no adverse effect on non-target-organisms (NTOs), including threatened or endangered species under normal agricultural practices. The assessment took into consideration several components, including the familiarity of the mode of action of Cry proteins, the activity spectrum of the Cry1Ac protein, the expression level of the Cry1Ac protein in MON 87701, the environmental fate of the Cry1Ac protein, and the feeding tests of Cry1Ac protein or MON 87701 soybean materials to representative NTOs. The tested NTOs include one mammalian species (mice), one avian species (bobwhite quail), soil decomposers (earthworm and two species of Collembola), and four beneficial insect species (honeybee, minute pirate bugs, ladybird beetle, and parasitic wasp). The estimated margins of exposure (MOEs) for the NTO insects exposed to the Cry1Ac protein range from 15 to 322. Additionally, according to information found on the U.S. Fish and Wildlife Service's website on threatened and endangered species (<http://www.fws.gov/endangered/wildlife.html#Species>), no threatened or endangered lepidoptera are known to feed on soybean nor are soybean fields suitable habitat for these organisms. Given that soybean fields are not a critical habitat for threatened and endangered lepidoptera, and given the lack of exposure to threatened and endangered lepidoptera in general through soybean tissues, notably pollen, it is reasonable to conclude there is no adverse impact to threatened and endangered species. Taken together, these data support the conclusion that MON 87701 is unlikely to have an adverse effect on NTOs or endangered species under normal agricultural practices in U.S. soybean production.

The potential for MON 87701 to outcross with sexually compatible species, including threatened or endangered plant species, is unlikely in the U.S., since no known wild *Glycine* species related to cultivated soybean are known to be present in North America. In those world areas where sexually compatible species do exist, the potential to outcross is concluded to be low because soybean is a highly self-pollinated species, with cross-pollination to other soybean varieties occurring at very low frequencies (0.04 to 3.62%) in adjacent plants (Caviness, 1966). Furthermore, in the rare event when cross-pollination may occur, MON 87701 and its progeny are not expected to have a significant environmental impact because, as described above for the Cry1Ac protein, evaluations have shown that the insect-protected trait in MON 87701 is not likely to enhance weediness or other plant pest potential. Therefore, the environmental consequence of pollen transfer from MON 87701 to other *Glycine* species is considered negligible.

### **Soybean Agronomic Practices and Land Use**

Soybean is one of the largest U.S. crops in terms of acreage planted and grain quantity harvested. In 2007, soybean was planted on 64.1 million acres in the U.S., where the harvested soybean seed had an average yield of 41.5 bushels per acre and total productivity was 2.59 billion bushels, resulting in a net value greater than \$26.88 billion (ASA, 2008; Soya and Oilseed Bluebook, 2008). Approximately 3% of the production acres are devoted to soybean breeding and seed multiplication, where the seed is harvested utilizing similar agronomic practices as soybean grown to produce grain.

Soybean fields are typically highly managed agricultural areas that can be expected to be dedicated to crop production for many years. Cultivation of MON 87701 would not be expected to differ from typical soybean cultivation. If commercially cultivated in the U.S., MON 87701 likely would be used in common rotations on land previously used for agricultural purposes. No significant impact would be expected following the introduction of MON 87701 at any scale on current cultivation and management practices for soybean, with the exception of potentially fewer insecticide treatments for the control of targeted lepidopteran pests. MON 87701 has been shown to be no different from conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics and has the same levels of resistance to insects and diseases as current commercial soybean, except for the introduced trait of enhanced protection from feeding damage caused by certain lepidopteran pests. The introduction of MON 87701 would provide growers with a simple and highly effective means for controlling lepidopteran pests. The approach is environmentally benign, helps to preserve beneficial insects, and requires fewer chemical insecticide applications. Based on these considerations, there is no apparent potential for significant impact on land use.

### **Conclusion**

Based on the data and information presented in this Petition, it is concluded that MON 87701 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87701 and any progeny derived from crosses between MON 87701 and conventional soybean or deregulated biotechnology-derived soybean be granted non-regulated status under 7 CFR Part 340.

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## ABBREVIATION AND DEFINITIONS\*

~	Approximately
2T-DNA	Plasmid vector containing two separate T-DNA regions each surrounded by left and right borders of the Ti plasmid
7S $\alpha'$	3' region of the <i>Sphas1</i> gene of <i>Glycine max</i> encoding the 7S $\alpha'$ seed storage protein, $\beta$ -conglycinin, including 35 nucleotides of the carboxyl terminal $\beta$ -conglycinin coding region with the termination codon and the polyadenylation sequence
35S	The promoter and leader from the cauliflower mosaic virus (CaMV) 35S RNA
<i>aadA</i>	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7
AA	Amino acid
ADF	Acid detergent fiber
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
AOCS	American Oil Chemists Society
AOSA	Association of Official Seed Analysts
AOSCA	Association of Official Seed Certifying Agencies
APS	Analytical protein standard
ASA	America Soybean Association
Bt	<i>Bacillus thuringiensis</i>
CaMV	Cauliflower mosaic virus
CAPS	N-Cyclohexyl-3-aminopropanesulfonic acid
CAST	Council for Agricultural Science and Technology, USDA
CBI	Confidential business information
CEQ	The Council on Environmental Quality
CEW	Corn earworm [ <i>Helicoverpa zea</i> (Boddie)]
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
COA	Certificate of analysis
CPB	Cartagena Protocol on Biosafety
Cry	Crystal proteins from <i>Bacillus thuringiensis</i>
<i>cryIAc</i>	Coding sequence for CryIAc protein

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\* Note: Standard abbreviations, e.g., units of measure, are used according to the format described in 'Instructions to Authors' in the *Journal of Biological Chemistry*.

Cry1Ac	A Cry1 class crystal protein from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> .
CSFII	Continuing Survey of Food Intakes by Individuals
CTAB	Hexadecyltrimethylammonium bromide
CTP	Chloroplast transit peptide
DAP	Days after planting
dCTP	Deoxycytidine triphosphate
DEEM-FCID	Dietary exposure evaluation model-food commodity intake database
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DT <sub>50</sub>	Time to 50% dissipation of a protein in soil
DTT	Dithiothreitol
DW	Dry weight
DWCF	Dry weight conversion factor
dwt	Dry weight of tissue
<i>E. coli</i>	<i>Escherichia coli</i>
EC <sub>50</sub>	Effective protein concentration to inhibit the growth of the target insect by 50%
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	United States Environmental Protection Agency
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
EU	European Union
EUP	Experimental Use Permit
FA	Fatty acid
FASTA	Algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
<i>FMV</i>	Figwort mosaic virus 35S promoter
FONSI	Finding of No Significant Impact
FW	Fresh weight
fwt	Fresh weight of tissue
GLP	Good Laboratory Practice
GE	Genetically engineered

GMO	Genetically modified organism
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
ILSI-CCD	International Life Sciences Institute crop composition database
IRM	Insect resistance management
ISO	International Organization for Standardization
LC <sub>50</sub>	LC stands for lethal concentration. LC <sub>50</sub> is the concentration of a substance that causes the death of 50% (one half) of a group of test organisms
Left Border	DNA region from <i>Agrobacterium tumefaciens</i> containing the left border sequence used for transfer of the T-DNA
LOD	Limit of detection
LOQ	Limit of quantitation
MAFF	Ministry of Agriculture, Forestry and Fisheries, Japan
MALDI-TOF MS	Matix-assisted laser desorption/ionization time-of-flight mass spectrometry
MEEC	Maximum expected environmental concentration
MH <sup>+</sup>	Protonated mass ion
MHLW	Ministry of Health, Labor and Welfare, Japan
MMT	Million metric tones
MOE	Margin of exposure
MW	Molecular weight
MWM	Molecular weight marker
N/A	Not applicable
NDF	Neutral detergent fiber
NEPA	National Environmental Policy Act
NFDM	Non-fat dried milk
NMWC	Nominal molecular weight cut-off
NOAEL	No observed adverse effect level
NOEC	No observable effect concentration
NOEL	No observable effect level
NOP	National organic program
NTO	Non-target organism
OECD	Organization for Economic Co-operation and Development
OR	Origin of replication
<i>ori-PBR322</i>	Origin of replication from pBR322 for maintenance of plasmid in <i>E. coli</i>
<i>ori-V</i>	Origin of replication for <i>Agrobacterium</i> derived from the broad host range plasmid RK2

OSL	Overseason leaf
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PIP	Plant-incorporated protectant
PMSF	Phenylmethanesulfonyl fluoride
PPA	Plant Protection Act
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpyrrolidone
PV-GMIR9	Plasmid vector used to develop MON 87701
RbcS4	Promoter, leader, and 5' non-translated region of the <i>Arabidopsis thaliana</i> RbcS4 gene encoding ribulose 1,5-bisphosphate carboxylase small subunit 1A
Right Border	DNA region from <i>Agrobacterium tumefaciens</i> containing the right border sequence used for transfer of the T-DNA
RK2	Broad host range plasmid of Inc-P1 originally isolated in <i>Klebsiella pneumonia</i>
<i>rop</i>	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in <i>E. coli</i>
SAP	Scientific Advisory Panel organized by U.S. EPA
SAS	Statistical Analysis System
SCN	Soybean cyst nematode
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	Standard error
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SOP	Standard operating procedure
TDF	Total dietary fiber
T-DNA I	Transfer DNA containing the <i>cryIAc</i> expression cassette in plasmid vector PV-GMIR9
T-DNA II	Transfer DNA containing the <i>cp4 epsps</i> gene cassette in plasmid vector PV-GMIR9
T-DNA	Transfer DNA
TES	Threatened or endangered species
TFA	Trifluoroacetic acid

TMB	3,3',5,5'-tetramethylbenzidene
Tris	Tris (hydroxymethyl) aminomethane
TSSP	Tissue-specific site pool
Tween-20	Polyoxyethylenesorbitan monolaurate
USDA-APHIS	United States Department of Agriculture – Animal and Plant Health Inspection Service
USDA-ARS	United State Department of Agriculture – Agricultural Research Service
USDA-ERS	United States Department of Agriculture – Economic Research Service
USDA-GRIN	United State Department of Agriculture – Germplasm Resources Information Network
USDA-NASS	United States Department of Agriculture – National Agricultural Statistics Service
USDA-NSHS	United States Department of Agriculture – National Seed Health System
USFWS	United States Fish and Wildlife Service
v/v	Volume per volume
w/v	Weight per volume

## **I. RATIONALE FOR THE DEVELOPMENT OF MON 87701**

### **I.A. Basis for the Request for a Determination of Non-Regulated Status**

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the United States. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of non-regulated status in whole for the new biotechnology-derived insect-protected soybean product, MON 87701, any progeny derived from crosses between MON 87701 and conventional soybean, and any progeny derived from crosses of MON 87701 with other biotechnology-derived soybean that has been granted non-regulated status under 7 CFR Part 340.

### **I.B. Rationale for the Development of Insect-Protected Soybean MON 87701**

Soybean is one of the largest U.S. crops in terms of the acreage planted and quantity harvested. In 2007, soybean was planted on 64.1 million acres in the U.S., where the harvested soybean seed had an average yield of 41.5 bushels per acre and total productivity was 2.59 billion bushels, resulting in a net value greater than \$26.88 billion (ASA, 2008; Soya and Oilseed Bluebook, 2008). There was approximately a 10 million acre drop in the number of acres of soybean planted in 2007 compared to acreage planted in 2006, which hit an all-time high of about 75.5 million acres, largely due to high corn prices, but soybean planted acreage in the U.S. for 2008 rebounded to an estimated 74.8 million acres (USDA-NASS, 2008a).

Over the past 60 years, soybean yield per unit area has almost tripled (Soya Bluebook, 2008). This increase is credited to the introduction of improved soybean germplasm, development of new varieties, the availability of better field equipment, and the use of herbicide and other pesticides that have greatly reduced crop losses caused by weeds and pests (Soya and Oilseed Bluebook, 2008).

On a regional basis, soybean production in certain areas in the U.S., and in other soybean production regions such as South America, can be affected substantially and can suffer considerable economic damage due to the infestation of various soybean pests (Higley and Boethel, 1994; Moscardi, 1993). Generally, insect pressure is greatest on soybean grown in the southeastern states in the U.S., particularly in states bordering the Gulf of Mexico and the Atlantic Ocean in which the tropical and sub-tropical weather favors pest infestation. Soybean insect pest problems in the mid-west and north central states are less severe than in other soybean-producing areas. According to USDA-NASS statistics,

about 16% of the approximately 75 million U.S. soybean acres received insecticide applications in 2006 (USDA-NASS, 2007b). The prevalence and severity of soybean insect pests are very diverse across U.S. soybean growing regions. Reasons for this variability include differences in climatic and weather conditions, pest species distribution, species environmental tolerances, and production practices. Within the U.S., the impact of insect pests on soybean production varies annually and regionally, with the most economically important soybean pests in the southeastern states (which constitute roughly 13% of total U.S. soybean acres) being the defoliating and pod-feeding insects. The most damaging defoliating insects in the South are velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*). It was estimated that 40-50% of the soybean acreage in the southeastern states such as Georgia and Louisiana were treated with insecticides to control lepidopteran pests, with velvetbean caterpillar and soybean looper being the main target pests (Gianessi et al., 2002). Soybean insect pest problems in more northern regions of the U.S. (e.g., the midwest and north central states), where the majority of soybean are grown, are less severe than in other soybean-producing areas and are generally attributable to non-lepidopteran pests. However, due to the large acreage of soybean grown in the midwest, even minor pest problems can have a serious economic impact (Higley and Boethel, 1994).

Chemical insecticides are commonly used for controlling lepidopteran infestations in soybean, but are not always effective. The cryptic habits of the soybean axil borer *Epinotia aporema* larvae protect them from insecticidal sprays, making high rates and careful timing of systemic insecticide applications necessary for effective control (Aragon et al., 1997). The soybean looper *Pseudoplusia includens* has developed resistance to every synthetic class of insecticide used against it (Thomas and Boethel, 1994), and resistance to pyrethroids is widespread across the southern U.S. (Felland et al., 1990; Leonard et al., 1990). Insecticides remain effective against velvetbean caterpillar (*A. gemmatalis*); however, infestations can quickly reach damaging levels and cause economic loss if insecticides are not applied promptly.

Biological insecticide formulations containing the Cry1Ac protein produced from *Bacillus thuringiensis* subsp. *kurstaki* for foliar application have been used widely on many crops, including soybean, since the 1960s. However, field efficacy has often been less than desired, because these materials are subject to weathering and deterioration by the elements and must be regularly reapplied or augmented by the use of other chemicals (Bohorova et al., 1997). One approach to utilize the efficacy of Cry1Ac, while avoiding issues related to field stability, has been the genetic engineering of plants (such as corn, cotton, and tomato) containing the *cry1Ac* gene. In contrast to a foliar application, these biotechnology-derived plants produce the insect control protein, Cry1Ac, within plant cells. This ensures that target insect pests are exposed to it whenever they feed on plants. As a result, control may be more effective, and applications of other insecticides to control the target lepidopteran species may be reduced or eliminated. Several insect-protected crops derived from biotechnology, including Bollgard cotton expressing the Cry1Ac protein, have been approved for commercial release in the U.S. since 1996 (EPA, 2008).

Monsanto Company has developed insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal (Cry) protein ( $\delta$ -endotoxin) derived from *Bacillus*

*thuringiensis* (Bt) subsp. *kurstaki*. The Cry1Ac protein provides protection from feeding damage caused by targeted lepidopteran pests. The Cry1Ac protein expressed in MON 87701 is greater than 99.1% identical to that produced by *Bacillus thuringiensis* subsp. *kurstaki* in nature and to that found in commercial formulations of Bt used in agriculture. The Cry1Ac protein has been shown to be active specifically against lepidopteran insects, and no biological activity against other insect species such as diptera, coleopteran, or nureopteran was observed. Results from field studies conducted from 2002-2003 in the U.S. and Argentina, as well as in subsequent trials, indicate that season-long Cry1Ac production in MON 87701 is highly efficacious in controlling target lepidopteran species, such as velvetbean caterpillar (*A. gemmatalis*), soybean looper (*P. includens*), soybean axil borer (*E. aporema*), and sunflower looper (*Rachiplusia nu*) in the field. As recommended by the EPA SAP panel (EPA, 1998a), this season-long, high-dose expression pattern in MON 87701 that is sufficient to control target insects that are heterozygous for any resistance genes, provides an effective tool in managing potential insect resistance to the Cry1Ac protein and thereby prolongs the durability of this product. MON 87701 would be efficacious in soybean production areas where insecticides are typically applied to control lepidopteran insects.

The southeastern states, which make up a relatively small portion of total U.S. soybean production, are consistently affected by the targeted lepidopteran pests that are usually controlled with insecticides. Due to this limited commercial potential in the U.S., the initial commercial production of MON 87701 is targeted for South America. In the U.S., MON 87701 plantings will be limited to breeding and seed multiplication activities unless and until a commercial planting registration is obtained from EPA (see **Section I.C** below).

Breeding and seed multiplication activities in the U.S. to support the commercial introduction of MON 87701 in South America could take place under APHIS notification or permit. However, Monsanto is seeking deregulation of MON 87701 for several reasons. First, the plant pest profile of MON 87701 supports a determination of nonregulated status. As this Petition demonstrates, MON 87701 does not pose a plant pest risk as that term is defined by the Plant Protection Act and APHIS (including no adverse impacts on non-target organisms and threatened and endangered species or habitat, no increased weediness, no adverse environmental impacts, etc.). Even if MON 87701 were planted in all the soybean producing areas within the U.S. that face economically significant lepidopteran pest pressure, it would not pose a plant pest risk. As mentioned above, if it were grown on a commercial scale in the U.S., it would be subject to all EPA commercial planting registration requirements.

Given the plant pest profile of MON 87701, a determination of nonregulated status enables breeding and seed multiplication activities within the U.S. without the expenditure of time, money and governmental resources that ongoing APHIS regulation of these activities would entail.

Finally, as mentioned above, MON 87701 is intended for commercial planting in the South American market. Although all countries that will plant MON 87701 have their own independent and functioning regulatory system to assess the health, safety and environmental impacts of the planting, use and consumption of MON 87701, some countries do take into consideration the evaluations conducted in the U.S. given the long

history and experience of APHIS in regulating products developed through biotechnology. Deregulation of biotechnology-derived products by APHIS informs other countries regarding the U.S. government's view of the safety of these products. For these reasons, Monsanto has chosen to seek full deregulation of MON 87701 at this time.

The major benefits of MON 87701 are:

1. Consistent and reliable control of lepidopteran pests: The Cry1Ac protein is expressed at consistently high levels in insect-protected soybean MON 87701 throughout the entire growing season providing nearly complete control of the targeted lepidopteran pests for the entire season (MacRae et al., 2005). Given the difficulty in controlling certain soybean lepidopteran pests, MON 87701 should provide protection that is superior to existing chemical and cultural control practices.
2. Reduced production costs and improved farming efficiency: Growers must work diligently to control lepidopteran pests at an early stage to prevent severe crop damage. Insect-protected soybean MON 87701 provides better control of key lepidopteran insect pests with less scouting and reduces risk of losses due to suboptimal timing of an insecticide application under traditional farm pest management, resulting in the prevention of potential damage to the crop later in the season. In addition, it will be safer and more convenient for growers to grow MON 87701 because no special equipment is required, and it reduces or eliminates the labor and time for growers to spray insecticides under traditional insect control practices, as well as reduces applicator exposure to chemical pesticides.
3. Control of target insects while maintaining beneficial species. The major lepidopteran pests causing significant soybean defoliation and yield loss across tropical and subtropical regions are the velvetbean caterpillar (*A. gemmatalis*), soybean looper (*P. includens*), soybean borer (*E. aporema*), and sunflower looper (*R. nu*) (Aragon et al., 1997). MON 87701 will provide efficacious control of these insect pests with reduced reliance on the insecticides currently used to control these lepidopteran pests. At the same time, MON 87701 does not impose any adverse impact on beneficial species compared to conventional insecticide-based programs.
4. Yield benefits and insecticide use reduction. In multi-year field tests in Argentina, MON 87701 was found to provide a significant yield increase of up to 4.5% relative to conventional soybean treated with insecticide under mild to moderate lepidopteran insect infestations. In addition to the benefits associated with its specificity for target pests, the reduced use of insecticides against lepidopteran pests will result in cost savings on insecticide and labor.

In summary, MON 87701 would improve upon current agricultural practices by eliminating or reducing insecticide use for targeted lepidopteran pests, reduce the risks posed to non-target species, and improve the efficiency of soybean production systems by increasing or maintaining yield potential while reducing insecticide costs.

### **I.C. Submissions to Other Regulatory Agencies**

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived pesticide producing crops falls on three

federal agencies: FDA, EPA, and USDA (USDA, 1986). Deregulation of MON 87701 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87701 cannot be released and marketed until EPA, FDA, and USDA have completed their reviews and assessments under their respective jurisdictions.

### ***Submission to FDA***

MON 87701 falls within the scope of the 1992 U.S. Food and Drug Administration (FDA) policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). In compliance with this policy, Monsanto will initiate a consultation with the FDA on the food and feed safety and nutritional assessment summary for MON 87701.

### ***Submissions to EPA***

Substances that are pesticides, as defined under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) [7 U.S.C. §136(u)], are subject to regulation by the Environmental Protection Agency (EPA). Pesticides produced *in planta*, referred to as plant-incorporated protectants (PIPs), are also subject to regulation by the EPA under FIFRA.

Pursuant to §408(d) of the Federal Food Drug and Cosmetic Act [21 U.S.C. 346 a(d)] DEKALB Genetics Corporation (subsequently acquired by Monsanto) petitioned EPA for an exemption from the requirement of a tolerance for Cry1Ac protein in or on all raw agricultural commodities and the genetic material necessary for its production in or on all raw agricultural commodities in 1997. On April 11, 1997, the EPA established an exemption from the requirement of a tolerance for the plant-incorporated protectant Bt Cry1Ac protein and the genetic material necessary for its production in all raw agricultural commodities (40 CFR § 180.1155).

In September 2006, Monsanto filed an experimental use permit (EUP) application for MON 87701 and the genetic material necessary for its production with the U.S. EPA to facilitate MON 87701 field testing and safety evaluations. EUP (524-EUP-1) was granted in September 2007 by EPA. To support future breeding and seed multiplication activities in the U.S., Monsanto will file an application with the EPA for a Section 3 seed increase registration of the plant-incorporated protectant *Bacillus thuringiensis* Cry1Ac protein, and the genetic material (vector PV-GMIR9) necessary for its production in soybean. Under this type of seed increase registration, commercial sale of MON 87701 within the U.S. would be prohibited by law.

In the future, should Monsanto decide to commercially introduce MON 87701 in the U.S., Monsanto would be required by EPA to apply for a Section 3 commercial use registration of the plant-incorporated protectant *Bacillus thuringiensis* Cry1Ac protein, and the genetic material (vector PV-GMIR9) necessary for its production in soybean. As a condition of a Section 3 commercial use registration, the EPA would require that Monsanto develop, administer and oversee an EPA-approved insect resistance monitoring (IRM) program. Under the U.S. government's Coordinated Framework for Regulation of Biotechnology, the USDA and EPA have communicated the role of the EPA in

establishing the appropriate IRM plan for Bt crops (EPA, 1999; EPA, 2003). According to EPA's guidance for other Bt products, implementation of an IRM plan is not required if the seed multiplication covers less than 20,000 acres per county and up to a total of 250,000 acres per PIP active ingredient per registrant per year<sup>1</sup>. It is anticipated that EPA will not require IRM programs for MON 87701 with small acreages used under Section 3 seed increase registrations.

### ***Submissions to Foreign Government Agencies***

To support commercial introduction of MON 87701 in South America, regulatory submissions will be made to the appropriate authorities in those countries. As mentioned above, all countries that will plant MON 87701 commercially have their own independent and functioning regulatory system to assess the food, feed, and environmental safety for the planting, use and consumption of MON 87701.

Regulatory submissions will also be made to countries that import significant quantities of soybean or its processed fractions from the U.S. or South America and have established regulatory approval processes in place. These will include submissions to a number of foreign government regulatory authorities, including: GMO Office, Ministry of Agriculture, People's Republic of China; Japan's Ministry of Agriculture, Forestry, and Fisheries (MAFF) and the Ministry of Health, Labor, and Welfare (MHLW); the Canadian Food Inspection Agency (CFIA) and Health Canada; the European Food Safety Authority (EFSA); and the regulatory authorities in other soybean importing countries with functioning regulatory systems. As appropriate, notifications of importation will be made to importing countries that do not have a formal approval process.

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<sup>1</sup> MON 810 × MON 863 label  
[[http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5CND%5Cpesticide%5CProduct%20Label%5C524%5C524-545%5C524-545\\_YIELDGARD\\_PLUS\\_CORN\\_BORER\\_ROOTWORM\\_11\\_11\\_2008\\_5\\_17\\_22\\_PM.pdf](http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5CND%5Cpesticide%5CProduct%20Label%5C524%5C524-545%5C524-545_YIELDGARD_PLUS_CORN_BORER_ROOTWORM_11_11_2008_5_17_22_PM.pdf)]

## II. THE SOYBEAN FAMILY

This section summarizes the taxonomy, biology, and use of soybean based on: 1) the consensus document for *Glycine max* (L.) Merr. prepared by the Organization for Economic Co-operation and Development (OECD, 2000; OECD, 2001), 2) a summary prepared by USDA-APHIS (USDA-APHIS, 2006) and a biology document published by Canadian Food Inspection Agency-Plant Biosafety Office (CFIA, 1996), 3) information provided in the USDA petition for Roundup Ready 2 Yield soybean MON 89788 (petition# 06-178-01n), and 4) other published literature.

### II.A. Soybean as a Crop

Soybean is the most prevalently grown oilseed in the world, with approximately 222.1 million metric tons of harvested seed (MMT) produced in 2007, which represented 56% of world oilseed seed production that year (ASA, 2008; Soya and Oilseed Bluebook, 2008). Soybean is grown as a commercial crop in over 35 countries. The major producers of soybean are the U.S., Brazil, Argentina, China, and India, which accounted for approximately 91% of the global soybean production in 2007 (Soya and Oilseed Bluebook, 2008); also see **Table II-1**. Approximately one-third of the 2007 world soybean production was produced in the U.S. (Soya and Oilseed Bluebook, 2008). The soybean produced in China and India are primarily for domestic use, while a significant portion of that produced in U.S., Brazil, and Argentina is traded globally in the form of soybean harvested seed, soybean meal or soybean oil. Globally, the U.S. was the largest soybean seed export country, while Argentina led the soybean meal and soybean oil export markets in 2007 (ASA, 2008; Soya and Oilseed Bluebook, 2008).

**Table II-1. World Soybean Production in 2007/2008**

Country	Production (million metric tons)
U.S.	71.4
Brazil	61.0
Argentina	47.0
China	15.6
Other	8.9
India	7.9
Paraguay	6.2
Canada	3.1
EU	1.0

Source: Soya and Oilseed Bluebook (2008).

Approximately 50% of the world soybean seed supply was crushed to produce soybean meal and oil in 2007 (ASA, 2008; Soya and Oilseed Bluebook, 2008), and the majority was used to supply the feed industry for livestock use or the food industry for edible vegetable oil and soybean protein isolates. Another 34% of the world soybean seed supply was traded to other geographies, with China, EU, Japan, and Mexico being the top

soybean seed import geographies (ASA, 2008). The remainder of the soybean seed produced was used as certified seed, feed, or stocks.

Soybean is used in various food products, including tofu, soybean sauce, soymilk, energy bars, and meat products. A major food use for soybean is purified oil, for use in margarines, shortenings, cooking, and salad oils. Soybean oil generally has a smaller contribution to soybean's overall value compared to soybean meal because the oil constitutes just 18 to 19% of the soybean's weight. Nonetheless, soybean oil accounted for approximately 30% of all the vegetable oils consumed globally, and was the second largest source of vegetable oil worldwide, slightly behind palm oil at approximately 32% share (Soya and Oilseed Bluebook, 2008).

Soybean meal is used as a supplement in feed rations for livestock. Soybean meal is the most valuable component obtained from processing the soybean, accounting for roughly 50-75% of its overall value. By far, soybean meal is the world's most important protein feed, accounting for nearly 69% of world protein meal supplies (ASA, 2008). Industrial uses of soybean range from a carbon/nitrogen source in the production of yeasts via fermentation to the manufacture of soaps, inks, paints, disinfectants, and biodiesel. Industrial uses of soybean have been summarized by Cahoon (Cahoon, 2003) and the American Soybean Association (ASA, 2008).

Global soybean plantings reached 90.8 million hectares in 2007/08, an 8.9% increase over the previous four years with an average of 82.3 million hectares planted from 2002/03 – 2007/08 (Soya and Oilseed Bluebook, 2008). Soybean production has realized, on average, a 6.2% annual growth between 1995/96 to 2006/07. Increased planting flexibility, increased yield from narrow-row seeding practices, a higher rate of corn-soybean rotations, and low production costs favored expansion of soybean areas in the mid-1990s, and the expanded areas tended to be concentrated where soybean yields were highest.

## **II.B. History of Soybean**

Domestication of soybean is thought to have taken place in China during the Shang dynasty (approximately 1500 to 1027 B.C.) or earlier (Hymowitz, 1970). However, historical and geographical evidence could only be traced back to the Zhou dynasty (1027 to 221 B.C.) where the soybean was utilized as a domesticated crop in the northeastern part of China. By the first century A.D., the soybean probably reached central and southern China as well as peninsular Korea. The movement of soybean germplasms was probably associated with the development and consolidation of territories and the degeneration of Chinese dynasties (Ho, 1969; Hymowitz, 1970).

From the first century A.D. to approximately the 15th and 16th centuries, soybean was introduced into several countries, with land races eventually developing in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal, and northern India. The movement of soybean throughout this period was due to the establishment of sea and land trade routes, the migration of certain tribes from China, and the rapid acceptance of seeds as a staple food by other cultures (Hymowitz and Newell, 1981; Hymowitz et al., 1990).

Starting in the late 16th century and throughout the 17th century, soybean was used by the Europeans, and in the 17th century, soybean sauce was a common item of trade from the east to the west.

Soybean was introduced into North America in the 18th century. In 1851, soybean was introduced in Illinois and subsequently throughout the Corn Belt. In 1853, soybean seed were deposited at the New York State Agricultural Society, the Massachusetts Horticultural Society, and the Commissioner of Patents. The two societies and the Commissioner of Patents sent soybean seed to dozens of growers throughout the U.S. Soybean has been extensively cultivated and improved through conventional breeding program following its introduction in the U.S. and subsequently has become a key source of nutrients for food and feed use in the U.S. (Hymowitz and Singh, 1987).

### **II.C. Taxonomy and Phylogenetics of Soybean**

Cultivated soybean, *Glycine max* (L.) Merr., is a diploidized tetraploid ( $2n=40$ ), which belongs to the family Leguminosae, the subfamily Papilionoideae, the tribe Phaseoleae, the genus *Glycine* Willd., and the subgenus *Soja* (Moench) F.J. Herm.

Family: Leguminosae

Subfamily: Papilionoideae

Tribe: Phaseoleae

Genus: *Glycine*

Subgenus: *Soja* (Moench) F.J. Herm.

Species: *max*

The genus *Glycine* Willd. is of Asian and Australian origin and is divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* consists of 22 wild perennial species, which are indigenous to Australia, west, central and south Pacific Islands, China, Russia, Japan, Indonesia, Korea, Papua New Guinea, the Philippines, and Taiwan (Hymowitz, 2004). The subgenus *Soja* includes the cultivated soybean, *G. max* (L.) Merr. and its wild annual relatives from Asia, *G. soja* Sieb. and Zucc. The list of species in the genus *Glycine* Willd. is presented in **Table II-2**.

**Table II-2. List of Species in the Genus *Glycine* Willd., 2n Chromosome Number, Genome Symbol, and Distribution**

Genus	2n	Genome <sup>1</sup>	Distribution
<u>Subgenus <i>Glycine</i></u>			
1. <i>G. albicans</i> Tind. & Craven	40	I1	Australia
2. <i>G. aphyonota</i> B. Pfeil	40	-- <sup>2</sup>	Australia
3. <i>G. arenaria</i> Tind.	40	HH	Australia
4. <i>G. argyrea</i> Tind.	40	A2A2	Australia
5. <i>G. canescens</i> F.J. Herm.	40	AA	Australia
6. <i>G. clandestina</i> Wendl.	40	A1A1	Australia
7. <i>G. curvata</i> Tind.	40	C1C1	Australia
8. <i>G. cyrtoloba</i> Tind.	40	CC	Australia
9. <i>G. dolichocarpa</i> Tateishi and Ohashi	80	--	(Taiwan)
10. <i>G. falcate</i> Benth.	40	FF	Australia
11. <i>G. hirticaulis</i> Tind. & Craven	40	H1H1	Australia
	80	--	Australia
12. <i>G. lactovirens</i> Tind. & Craven.	40	I1I1	Australia
13. <i>G. latifolia</i> (Benth.) Newell & Hymowitz	40	B1B1	Australia
14. <i>G. latrobeana</i> (meissn.) Benth.	40	A3A3	Australia
15. <i>G. microphylla</i> (Benth.) Tind.	40	BB	Australia
16. <i>G. peratosa</i> B. Pfeil & Tind.	40	--	Australia
17. <i>G. pindanica</i> Tind. & Craven	40	H3H2	Australia
18. <i>G. pullenii</i> B. Pfeil, Tind. & Craven	40	--	Australia
19. <i>G. rubiginosa</i> Tind. & B. Pfeil	40	--	Australia
20. <i>G. stenophita</i> B. Pfeil & Tind.	40	B3B3	Australia
21. <i>G. tabacina</i> (Labill.) Benth.	40	B2B2	Australia
	80	Complex <sup>3</sup>	Australia, West Central and South Pacific Islands
22. <i>G. tomentella</i> Hayata	38	EE	Australia
	40	DD	Australia, Papua New Guinea
	78	Complex <sup>4</sup>	Australia, Papua New Guinea
	80	Complex <sup>5</sup>	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
<u>Subgenus <i>Soja</i> (Moench) F.J. Herm.</u>			
23. <i>G. soja</i> Sieb. & Zucc.	40	GG	China, Russia, Taiwan, Japan, Korea (Wild Soybean)
24. <i>G. max</i> (L.) Merr.	40	GG	Cultigen (Soybean)

<sup>1</sup> Genomically similar species carry the same letter symbols.

<sup>2</sup> Genome designation has not been assigned to the species.

<sup>3</sup> Allopolyploids (A and B genomes) and segmental allopolyploids (B genomes).

<sup>4</sup> Allopolyploids (D and E, A and E, or any other unknown combination).

<sup>5</sup> Allopolyploids (A and D genomes, or any other unknown combination).

Note: Table is adapted from Hymowitz (2004).

*Glycine soja* grows wild in China, Japan, Korea, the Russian Far East, and Taiwan, and is commonly found in fields, hedgerows, roadsides, and riverbanks (Lu, 2004). The plant is an annual, slender in build with narrow trifoliolate leaves. The purple or very rarely white flowers are inserted on short, slender racemes. The pods are short and tawny with hirsute pubescence, producing oval-oblong seeds (Hermann, 1962).

*Glycine max* (L.) Merr., the cultivated soybean, is an annual that generally exhibits an erect, sparsely branched, bush-type growth habit with trifoliolate leaves. The leaflets are broadly ovate, and the purple, pink, or white flowers are borne on short axillary racemes or reduced peduncles. The pods are either straight or slightly curved, and one to three ovoid to sub-spherical seeds are produced per pod.

A third and unofficial species named *G. gracilis* is also described within the context of the *Soja* subgenus in addition to *G. soja* and *G. max*. The *G. gracilis* is known only from northeast China, is intermediate in morphology between *G. max* and *G. soja*, and is sometimes considered a variant of *G. max*. The three species in the *Soja* subgenus can cross pollinate, and the hybrid seed can germinate normally and subsequently produce fertile pollen and seed (Singh and Hymowitz, 1989). The taxonomic position of *G. gracilis* has been an area of debate, and neither ILDIS (International Legume Database and Information Service) nor USDA-GRIN (USDA Germplasm Resources Information Network) recognizes *G. gracilis* as a distinct species. The wild and weedy relatives (*G. soja* and *G. gracilis*) of soybean do not occur in the U.S., and, therefore, are not likely to contribute to the potential for outcrossing (USDA-APHIS, 2006).

#### **II.D. The Genetics of Soybean**

*Glycine* is the only genus in the tribe Phaseoleae where species have diploid chromosome numbers of 40 and 80, but not 20 (Lackey, 1981). The unique chromosome number of *Glycine* is probably derived from diploid ancestors with base number of 11. The ancestral species have undergone aneuploid reduction (loss of a specific chromosome), which is prevalent throughout the Papilionoideae, to a base number of 10 chromosomes (Lackey, 1981). Tetraploidization ( $2n = 2x = 40$ ) through autopolyploidy or allopolyploidy of the progenitor species occurred either prior to or after dissemination from the ancestral region. The path of migration from a common progenitor is assumed by Singh et al., (2001) as: wild perennial ( $2n = 4x = 40$ , unknown or extinct) to wild annual ( $2n = 4x = 40$ ; *G. soja*) to soybean ( $2n = 4x = 40$ ; *G. max*). Soybean should be regarded as a stable tetraploid with diploidized genome (Gurley, 1979; Lee and Verma, 1984; Skorupska, 1989).

#### **II.E. Pollination of Cultivated Soybean**

Soybean is a self-pollinated species, propagated by seed (OECD, 2000). The papilionaceous flower consists of a tubular calyx of five sepals, a corolla of five petals, one pistil, and nine fused stamens with a single separate posterior stamen. The stamens form a ring at the base of the stigma and elongate one day before pollination, at which time the elevated anthers form a ring around the stigma (OECD, 2000). The soybean flower stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive for 48 hours after anthesis. The anthers mature in the bud and directly pollinate

the stigma of the same flower. As a result, soybean is considered to be a highly self-pollinated species, with cross-pollination to other soybean varieties occurring at very low frequency (0.04 to 3.62%) in adjacent plants (Caviness, 1966). Pollination typically takes place on the day the flower opens. The pollen naturally comes in contact with the stigma during the process of anthesis. Anthesis normally occurs in late morning, depending on the environmental conditions. The pollen usually remains viable for two to four hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination can only take place during the short time when the pollen is viable.

#### **II.F. Cultivated Soybean as a Volunteer**

Cultivated soybean plants are annuals, and they reproduce solely by means of seeds. Mature soybean seeds have no innate dormancy (TeKrony, 1987), are sensitive to cold (Raper and Kramer, 1987), and are not likely to survive from one growing season to the next if left in the field over winter (Berglund, 2008). Due to the lack of dormancy (a trait that is indirectly selected for in commercial soybean seed), soybean seed can germinate quickly under adequate temperature and moisture and can potentially grow as a volunteer plant. However, volunteer plants likely would be killed by frost during autumn or winter of the year they were produced. If they did become established, volunteer plants would not compete well with the succeeding crop, and could be controlled readily by either mechanical or chemical means (OECD, 2000).

#### **II.G. Characteristics of the Recipient Plant**

The soybean variety used as the recipient for the DNA insertion to create MON 87701 was A5547, a non-transgenic conventional variety developed by Asgrow Seed Company. A5547 is an elite maturity group V soybean variety, which was developed and selected on the basis of its superior agronomic performance over other soybean lines (Rhodes, 1997). As a soybean variety in maturity group V, A5547 is a determinate variety adapted and most suitable for production in the Mid-South region.

#### **II.H. Soybean as a Test System in Product Safety Assessment**

In developing the data to support the safety assessment of insect-protected soybean MON 87701, A5547 was used as the non-transgenic comparator. In general, the genetic background of MON 87701 was matched with that of the control, so the effect of the genetic insertion and the presence of the Cry1Ac protein could be assessed in an unbiased manner. Since MON 87701 was derived from the A5547 conventional variety, it was deemed appropriate to use the non-transformed A5547 as the control variety as its use would minimize the potential bias in subsequent comparative assessments. In addition, commercial conventional and Roundup Ready soybean (40-3-2) varieties were used as reference materials to establish ranges of responses or values representative of commercial soybean varieties (see **Table F-1**). The reference varieties used at each location were selected based on their availability and agronomic fit (**Appendix E and Table F-1**).

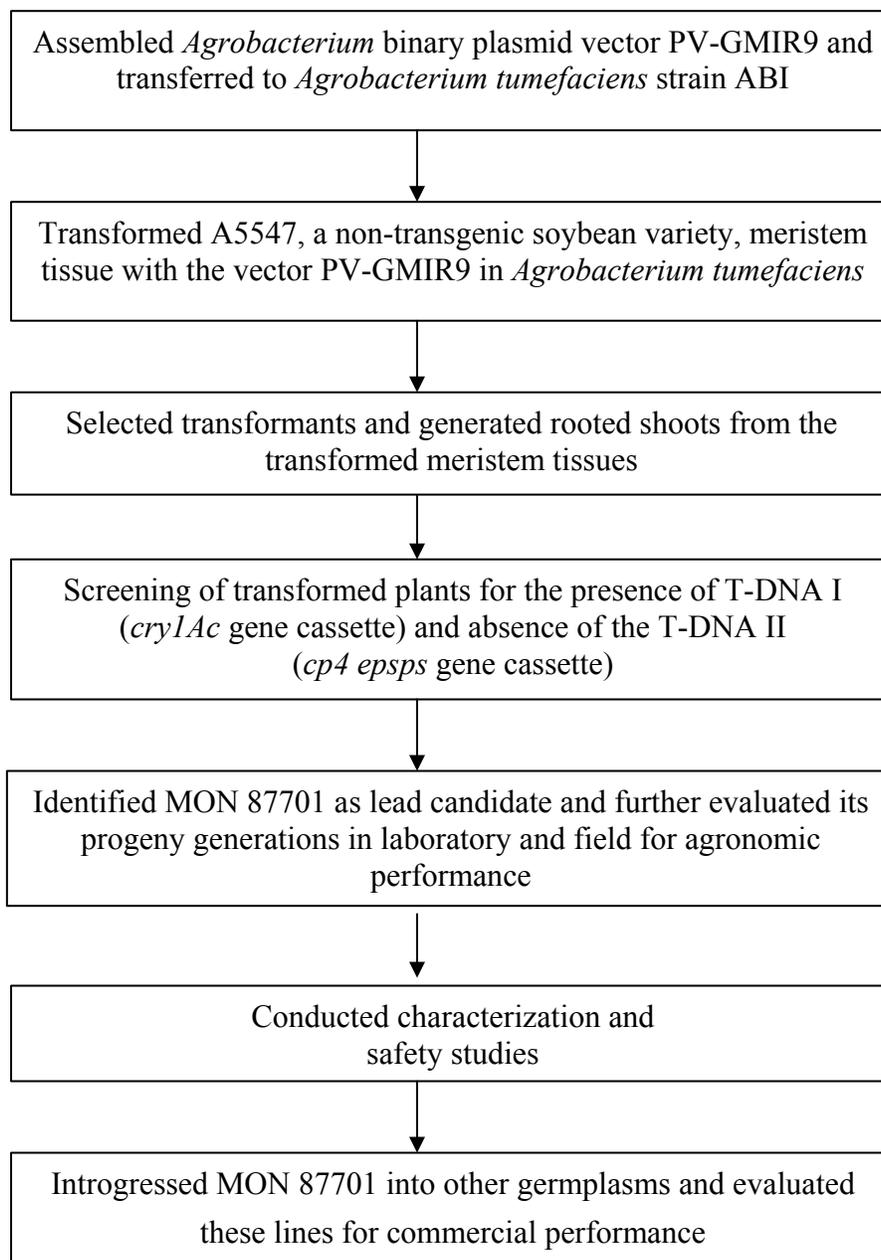
### III. DESCRIPTION OF THE TRANSFORMATION SYSTEM

MON 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue utilizing transformation vector, PV-GMIR9 (Section IV, Figure IV-1). PV-GMIR9 is a binary vector that contains well-characterized DNA segments required for selection and replication of the plasmid vector in bacteria and transfer of the T-DNAs into plant cells. Vector PV-GMIR9 contains two separate T-DNAs (hence the descriptor “2T-DNAs”) that can be effectively used to generate marker-free plants (Komari et al., 1996). The first T-DNA, designated as T-DNA I, contains the gene cassette bearing the gene of interest *cryIAc*, and the second T-DNA, designated as T-DNA II, contains the gene cassette of selectable marker gene *cp4 epsps*. During the process of *Agrobacterium*-mediated transformation, the distinct T-DNAs containing the *cryIAc* and *cp4 epsps* genes were integrated into the soybean genome at independent, unlinked loci, and the rest of the backbone of the vector PV-GMIR9 was not inserted into plant cells. Traditional breeding was then used to isolate plants that only contain the T-DNA I (*cryIAc* expression cassette) and do not contain the T-DNA II (*cp4 epsps* expression cassette). This resulted in the production of marker-free, insect-protected soybean MON 87701.

The *Agrobacterium*-mediated soybean transformation to produce MON 87701 was based on the method described by Martinell et al., (2002), which allows the generation of transformed plants without utilization of callus. Briefly, meristem tissues were excised from the embryos of germinated A5547 seed, after co-culturing with the *Agrobacterium* carrying the vector, the meristems were placed on selection medium containing glyphosate, spectinomycin, and chloramphenicol to inhibit the growth of untransformed plant cells and excess *Agrobacterium*, respectively, so that only cells containing T-DNA II and/or T-DNA I and T-DNA II survived. The absence of the *Agrobacterium* which was used for transformation was confirmed by PCR targeting backbone sequence of plasmid PV-GMIR9. The meristems were then placed in media conducive to shoot and root development. Rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R<sub>0</sub> plants generated through this process were self-pollinated to produce the R<sub>1</sub> seed. During subsequent selfing of the R<sub>0</sub> plants to produce the R<sub>1</sub> seed, the unlinked insertions of T-DNA I (*cryIAc* gene expression cassette) and T-DNA II (*cp4 epsps* gene expression cassette) were segregated. A non-lethal dose of glyphosate herbicide was applied to R<sub>1</sub> plants. The resulting plants with minor injury were selected for further analyses, whereas plants showing no injury, i.e., containing T-DNA II (*cp4 epsps* gene expression cassette), were eliminated from subsequent development. Subsequently, plants containing only a single T-DNA I (*cryIAc* gene cassette) were identified and selected by a combination of analytical techniques, including ELISA and TaqMan PCR analysis. Only R<sub>1</sub> plants that were homozygous for the T-DNA I cassette and not having the T-DNA II cassette were advanced for development. These R<sub>1</sub> plants were self-pollinated to generate a population of R<sub>2</sub> plants which were repeatedly self-pollinated through subsequent generations. These progeny were subjected to further molecular assessments to ensure the plants contained a single, intact insert and phenotypic assessments to ensure the plants met commercial specifications. MON 87701 was selected as the lead event based on its

superior phenotypic characteristics and molecular profile. Regulatory tests on MON 87701 were initiated to further characterize the genetic insertion and the expressed Cry1Ac protein, and to confirm the food, feed, and environmental safety relative to conventional soybean. The major steps involved in the development of MON 87701 are depicted in **Figure III-1**.



**Figure III-1. Schematic of the Development of MON 87701**

## IV. GENETIC ELEMENTS

This section describes the vector, the donor genes and the regulatory sequences used in the development of MON 87701 and the deduced amino acid sequence of the Cry1Ac protein produced in MON 87701 and the CP4 EPSPS protein selectable marker employed to produce MON 87701. In this section, T-DNA refers to DNA that is transferred to the plant during transformation. An expression cassette is composed of a coding sequence and the regulatory elements necessary for the expression of the coding sequence.

### IV.A. Vector PV-GMIR9

The PV-GMIR9 vector used for the transformation of soybean to produce MON 87701 is shown in **Figure IV-1** and its genetic elements described in **Table IV-1**. This vector is approximately 15.5 kb and contains two T-DNAs delineated by left and right border regions. Each of the two T-DNAs contains a single expression cassette. The first T-DNA (designated as T-DNA I) contains the *cry1Ac* expression cassette, which results in the expression of Cry1Ac protein. The *cry1Ac* expression cassette contains the *cry1Ac* coding sequence under the regulation of the *RbcS4* promoter and leader, *CTP1* chloroplast targeting sequence, and the *7S α' 3'* non-translated sequence. The second T-DNA (designated as T-DNA II) contains the *cp4 epsps* gene expression cassette. The *cp4 epsps* expression cassette contains the *cp4 epsps* coding sequence under the regulation of the *FMV* promoter, the *shkG* leader, the *CTP2* chloroplast targeting sequence and the *E9 3'* non-translated sequence. Utilizing a vector with two T-DNAs is the basis for an effective approach to generate marker-free plants. It allows for the T-DNA with the trait of interest (e.g., *cry1Ac*, T-DNA I) and the T-DNA encoding the selectable marker (e.g., *cp4 epsps*, T-DNA II) to insert into two independent loci within the genome of the plant. Following selection of the transformants, the inserted T-DNA encoding the selectable marker (e.g., T-DNA II) can be segregated from progeny through subsequent breeding and genetic selection processes, while the inserted T-DNA containing the trait(s) of interest is maintained (e.g., T-DNA I). The result is a marker free soybean containing only the *cry1Ac* expression cassette.

The backbone region outside of the T-DNAs contains two origins of replication for maintenance of plasmid in bacteria (OR-*ori V*, OR-*ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (*rop*). A description of the genetic elements and their prefixes (e.g., P-, L-, I-, TS-, OR-, B-, CS-, and T-) in PV-GMIR9 is provided in **Table IV-1**.

### IV.B. The *cry1Ac* Coding Sequence and the Cry1Ac Protein (T-DNA I)

MON 87701 expresses the Cry1Ac protein, an insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki*, which provides resistance to certain lepidopteran pests. The Cry1Ac protein expressed in MON 87701 shares >99% amino acid identity with Cry1Ac from *B. thuringiensis* (Bt) subsp. *kurstaki* and 100% amino acid sequence identity with the Cry1Ac protein present in Bollgard cotton, with the exception of four

additional amino acids at the N-terminus of the MON 87701-produced Cry1Ac protein (see **Figure VI-1**). These four amino acids are derived from the chloroplast targeting sequence. The deduced full-length amino acid sequence is shown in **Figure IV-2**.

#### **IV.C. The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein (T-DNA II)**

The *cp4 epsps* gene expression cassette is not present in MON 87701. The *cp4 epsps* gene expression cassette was used as a selectable marker during the transformation to produce MON 87701, but was segregated away by traditional breeding techniques at the R<sub>1</sub> generation. The CP4 EPSPS protein confers tolerance to glyphosate and has been used safely and successfully in many Roundup Ready crops such as canola, corn, cotton, soybean, and sugar beet. The deduced CP4 EPSPS full-length amino acid sequence is shown in **Figure IV-3**.

#### **IV.D. Regulatory Sequences**

Each expression cassette contains regulatory sequences involved in the expression of the respective coding sequences. T-DNA I contains the *cry1Ac* expression cassette, which consists of the *cry1Ac* coding sequence under the regulation of the *RbcS4* promoter and leader, *CTP1* targeting sequence, and the *7S α' 3'* non-translated sequence. The *RbcS4* promoter and leader are from the *Arabidopsis thaliana* ribulose 1,5-bisphosphate carboxylase small subunit 1A gene (Krebbers et al., 1988) and drives transcription of the *cry1Ac* gene in above-ground portions of the plant. The *CTP1* targeting sequence is the sequence encoding the transit peptide from the *Arabidopsis thaliana* small subunit 1A gene (Krebbers et al., 1988) and is present to direct the Cry1Ac protein to the chloroplast. The *7S α' 3'* non-translated region is from the *Glycine max* 7S seed storage protein gene (Schuler et al., 1982) and is present to terminate transcription and direct polyadenylation of the *CTP1-cry1Ac* transcript.

T-DNA II contains the *cp4 epsps* expression cassette, which consists of the *cp4 epsps* coding sequence under the regulation of the *FMV* promoter, the *shkG* leader, the *CTP2* targeting sequence and the *E9 3'* non-translated sequence. The *FMV* promoter is from the Figwort Mosaic Virus 35S RNA gene (Rogers, 2000) and drives transcription of *cp4 epsps* in most plant cell types. The *shkG* leader is the 5' untranslated region (UTR) from the *Arabidopsis thaliana shkG* gene (encoding EPSPS) (Klee et al., 1987) and acts to enhance expression. The *CTP2* targeting sequence is the sequence encoding the transit peptide from the *ShkG* gene of *Arabidopsis thaliana* (Klee et al., 1987) and is present to direct the CP4 EPSPS protein to the chloroplast. The *E9* non-translated region is the 3' non-translated sequence from the *RbcS2* gene of *Pisum sativum* (Coruzzi et al., 1984) and is present to direct polyadenylation of the *CTP2-cp4 epsps* transcript.

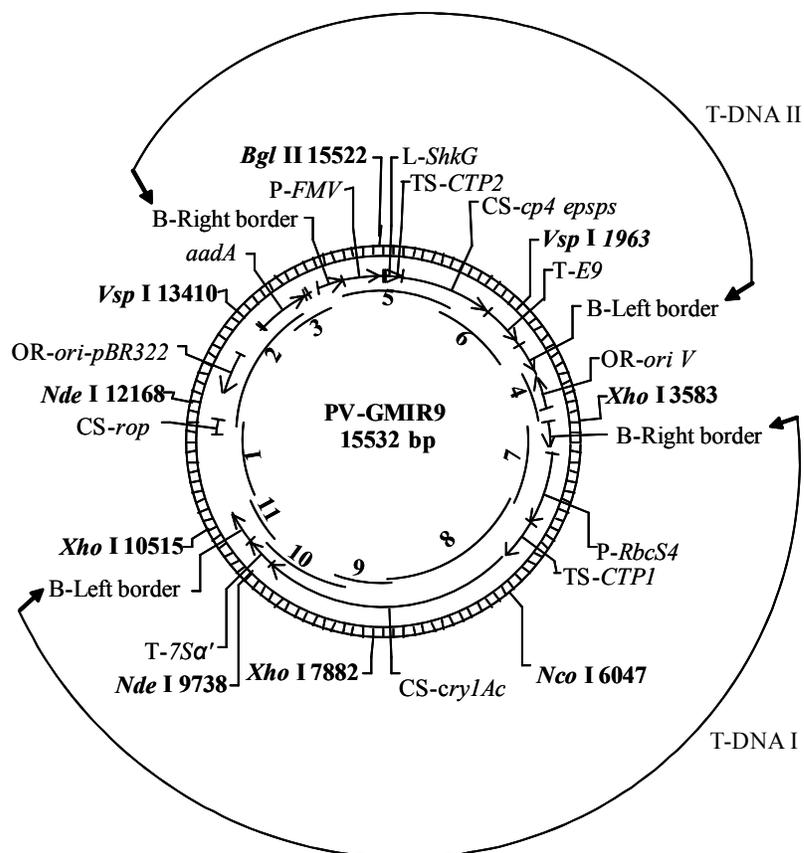
#### **IV.E. T-DNA Borders**

Plasmid PV-GMIR9 contains right border and left border regions (**Figure IV-1** and **Table IV-1**) that were derived from *Agrobacterium tumefaciens* plasmids (Barker et al., 1983; Depicker et al., 1982). The border regions each contain a 24-25 bp sequence, called the “nick” site, which is the site of DNA exchange during transformation. The border regions delineate the T-DNA and are involved in their efficient transfer into the

soybean genome. Because PV-GMIR9 is a two T-DNA vector, it contains two right border regions and two left border regions, where one set is for T-DNA I and the other set is for T-DNA II (see description above).

#### **IV.F. Genetic Elements Outside of the T-DNA Borders**

Genetic elements that exist outside of the T-DNA borders are those that are essential for the maintenance and selection of the vector PV-GMIR9 in bacteria. The origin of replication *OR-ori V* is required for the maintenance of the plasmid in *Agrobacterium* (Stalker et al., 1981b) and is derived from the broad host plasmid RK2. The origin of replication OR-pBR322 is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid pBR322 (Sutcliffe, 1978). *CS-rop* is the coding sequence of the repressor of primer (ROP) protein and is necessary for the maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989). The selectable marker *aadA* is a bacterial promoter and coding sequence for an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance (Fling et al., 1985) in *E. coli* and *Agrobacterium* during molecular cloning. As these elements are outside of the border regions, they are not expected to be transferred into the soybean genome. The absence of the backbone sequence in MON 87701 has been confirmed by Southern blot analyses (see **Section V.B.**).



**Figure IV-1. Plasmid Map of Vector PV-GMIR9 Showing Probes 1-11**

Probe	DNA Probe	Start Position (bp)	End Position (bp)	Total Length (~kb)
1	Backbone Probe 1	10513	12013	1.5
2	Backbone Probe 2	11813	13640	1.8
3	Backbone Probe 3	13440	14549	1.1
4	Backbone Probe 4	2852	3595	0.74
5	T-DNA II Probe 5	14907	1375	2.0
6	T-DNA II Probe 6	1225	2409	1.2
7	T-DNA I Probe 7	3596	5596	2.0
8	T-DNA I Probe 8	5471	6971	1.5
9	T-DNA I Probe 9	6846	8046	1.2
10	T-DNA I Probe 10	7846	9650	1.8
11	T-DNA I Probe 11	9450	10512*	1.1

A circular map of the plasmid vector PV-GMIR9 used to develop MON 87701 is shown. Genetic elements and restriction sites used in Southern blot analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The probes used in the Southern blot analyses are shown on the interior of the map. PV-GMIR9 contains two T-DNA regions designated as T-DNA I and T-DNA II. The left and right border regions of T-DNA II share 100% homology with those of T-DNA I and thus were not included in the T-DNA II analysis.

\* Nucleotide 10512 is vector backbone sequence

**Table IV-1. Summary of Genetic Elements in Plasmid Vector PV-GMIR9**

<b>Genetic Element</b>	<b>Location in Plasmid</b>	<b>Function (Reference)</b>
<b>TDNA II (Continued from bp 15532)</b>		
Intervening Sequence	1-14	Sequences used in DNA cloning
<b>L<sup>1</sup>-ShkG</b>	15-81	5' non-translated leader sequence from the <i>Arabidopsis ShkG</i> gene encoding EPSPS (Klee et al., 1987) that is involved in regulating gene expression
<b>TS<sup>2</sup>-CTP2</b>	82-309	Targeting sequence encoding the chloroplast transit peptide from the <i>ShkG</i> gene of <i>Arabidopsis thaliana</i> encoding EPSPS (Klee et al., 1987)
<b>CS<sup>3</sup>-cp4-epsps</b>	310-1677	Codon modified coding sequence of the <i>aroA</i> gene from <i>Agrobacterium sp.</i> strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgett et al., 1996)
Intervening Sequence	1678-1719	Sequences used in DNA cloning
<b>T<sup>4</sup>-E9</b>	1720-2362	3' non-translated sequence from <i>RbcS2</i> gene of <i>Pisum sativum</i> encoding the Rubisco small subunit, which functions to direct polyadenylation of the mRNA (Coruzzi et al., 1984)
Intervening Sequence	2363-2409	Sequences used in DNA cloning
<b>B<sup>5</sup>-Left Border</b>	2410-2851	DNA region from <i>Agrobacterium tumefaciens</i> containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
<b>Vector Backbone</b>		
Intervening Sequence	2852-2937	Sequences used in DNA cloning
<b>OR<sup>6</sup>-ori V</b>	2938-3334	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in <i>Agrobacterium</i> (Stalker et al., 1981a)
Intervening Sequence	3335-3595	Sequences used in DNA cloning
<b>TDNA I</b>		
<b>B-Right Border</b>	3596-3952	DNA region from <i>Agrobacterium tumefaciens</i> containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982)
Intervening Sequence	3953-4061	Sequences used in DNA cloning

<b>Genetic Element</b>	<b>Location in Plasmid</b>	<b>Function (Reference)</b>
<b>P<sup>7</sup>-RbcS4</b>	4062-5784	Promoter, leader, and 5' non-translated region of the <i>Arabidopsis thaliana</i> RbcS4 gene encoding ribulose 1, 5-bisphosphate carboxylase small subunit 1A, (Krebbers et al., 1988). Promoter expresses in above ground tissues
<b>TS-CTPI</b>	5785-6048	Targeting sequence encoding the transit peptide of the <i>Arabidopsis</i> RbcS4 encoding small subunit 1A transit peptide, from <i>Arabidopsis thaliana</i> , present to direct the cry1Ac protein to the chloroplast (Krebbers et al., 1988)
<b>CS-cry1Ac</b>	6049-9585	Codon-modified coding sequence of the Cry1Ac protein of <i>Bacillus thuringiensis</i> (Fischhoff and Perlak, 1995)
Intervening Sequence	9586-9594	Sequences used in DNA cloning
<b>T-7S α'</b>	9595-10033	3' region of the <i>Sphas1</i> gene of <i>Glycine max</i> encoding the 7S α' seed storage protein, β-conglycinin, including 35 nucleotides of the carboxyl terminal β-conglycinin coding region with the termination codon and the polyadenylation sequence (Schuler et al., 1982). The element functions to terminate transcription and direct polyadenylation of the mRNA.
Intervening Sequence	10034-10069	Sequences used in DNA cloning
<b>B-Left Border</b>	10070-10511	DNA region from <i>Agrobacterium tumefaciens</i> containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
<b>Vector Backbone (Continued from bp 3595 )</b>		
Intervening Sequence	10512-11786	Sequences used in DNA cloning
<b>CS-rop</b>	11787-11978	Coding sequence for repressor of primer protein derived from ColE1 plasmid for maintenance of plasmid copy number in <i>E. coli</i> (Giza and Huang, 1989)
Intervening Sequence	11979-12405	Sequences used in DNA cloning
<b>OR-ori-pBR322</b>	12406-12994	Origin of replication from pBR322 for maintenance of plasmid in <i>Escherichia coli</i> (Sutcliffe, 1978)
Intervening Sequence	12995-13524	Sequences used in DNA cloning

<b>Genetic Element</b>	<b>Location in Plasmid</b>	<b>Function (Reference)</b>
<i>aadA</i>	13525-14413	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3' (9)-O-nucleotidyltransferase from the transposon Tn7 (Fling et al., 1985) (GenBank accession) that confers spectinomycin and streptomycin resistance
Intervening Sequence	14414-14549	Sequences used in DNA cloning
<b>TDNA II (Continued from bp 2851)</b>		
<b>B-Right Border</b>	14550-14906	DNA region from <i>Agrobacterium tumefaciens</i> containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982)
Intervening Sequence	14907-14939	Sequences used in DNA cloning
<b>P-FMV</b>	14940-15503	Promoter for the 35S RNA from figwort mosaic virus (FMV) (Rogers, 2000) that directs transcription in most plant cells
Intervening Sequence	15504-15532	Sequences used in DNA cloning

**L**<sup>1</sup> -Leader; **TS**<sup>2</sup> - Targeting Sequence; **CS**<sup>3</sup> - Coding Sequence; **T**<sup>4</sup> - Transcription Termination Sequence; **B**<sup>5</sup> - Border; **OR**<sup>6</sup> - Origin of Replication; **P**<sup>7</sup> - Promoter

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1  MASSMLSSAT MVASPAQATM VAPFNGLKSS AAFPATRKAN NDITSITSNG GRVNCMQVWP
61  PIGKKKFETL SYLPDLTDSG GRVNCMQAMD NNPININECIP YNCLSNPEVE VLGGERIETG
121 YTPIDISLSL TQFLLSEFVP GAGFVLGLVD IIWGIFGPSQ WDAFLVQIEQ LINQRIEEFA
181 RNQAISRLEG LSNLYQIYAE SFREWEADPT NPALREEMRI QFNDMNSALT TAIPLFAVQN
241 YQVPLLSVYV QAANLHLSVL RDVSVFGQRW GFDAATINSR YNDLTRLIGN YTDHAVRWYN
301 TGLERVWGPD SRDWIRYNQF RRELTLTVLD IVSLFPNYDS RTYPIRTVSQ LTREIYTNPV
361 LENFDGSFRG SAQGIEGSIR SPHLMDILNS ITIYTDHARG EYYWSGHQIM ASPVGFSGPE
421 FTFPLYGTMG NAAQQRIVA QLGQGVYRTL SSTLYRRPFN IGINNQQLSV LDGTEFAYGT
481 SSNLPSAVYR KSGTVDSLDE IPPQNNNVPP RQGFSHRLSH VSMFRSGFSN SSVSIIRAPM
541 FSWIHRSAEF NNIIASDSIT QIPAVKGNFL FNGSVISGPG FTGGDLVRLN SSGNNIQNRG
601 YIEVPIHFPS TSTRYRVRVR YASVTPIHLN VNWGNSSIFS NTVPATATSL DNLQSSDFGY
661 FESANAFTSS LGNIVGVRNF SGTAGVIIDR FEFIPVTATL EAEYNLERAQ KAVNALFTST
721 NQLGLKTNVT DYHIDQVSNL VTYLSDEFCL DEKRELSEKV KHAKRLSDER NLLQDSNFKD
781 INRQPERGWG GSTGITIQQG DDVFKENYVT LSGTFDECYP TYLYQKIDES KLKAFTRYQL
841 RGYIEDSQDL EIYSIRYNAK HETVNVPGTG SLWPLSAQSP IGKCGEPNRC APHLEWNPDL
901 DCSCRDGEKC AHSHHFSLD IDVGCTDLNE DLGVWVIFKI KTQDGHARLG NLEFLEEKPL
961 VGEALARVKR AEKKWRDKRE KLEWETNIVY KEAKESVDAL FVNSQYDQLQ ADTNIAMIHA
1021 ADKRVHSIRE AYLPELSVIP GVNAAIFEEL EGRIFTAFSL YDARNVIKNG DFNGLSCWN
1081 VKGHVDVEEQ NNQRSVLVVP EWEAEVSQEV RVCPCGRGYIL RVTAYKEGYG EGCVTIHEIE
1141 NNTDELKFSN CVEEEIYPNN TVTCNDYTVN QEEYGGAYTS RNRGYNEAPS VPADYASVYE
1201 EKSQYDGRRE NPCEFNRGYR DYTPLVGYV TKELEYFPET DKVWIEIGET EGTFIIVDSVE
1261 LLLMEE.

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**Figure IV-2. Deduced Amino Acid Sequence of the CTP1 Targeting Sequence and the Full Length Cry1Ac Protein in MON 87701**

The amino acid sequence of the Cry1Ac protein was deduced from the full-length *cry1Ac* coding sequence present in PV-GMIR9. The underlined sequence represents the CTP1 targeting sequence. The amino acids in bold are amino acids that remains after cleavage of CTP1.