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)

For:

Bayer CropScience LP
2 T.W. Alexander Dr.
Research Triangle Park, NC 27709
Telephone: 919-549-2599
FAX: 919-549-3929

and

M.S. Technologies LLC
103 Avenue D
West Point, Iowa 52656

Contributors (BCS):

(b)(6)

Contributors (MSTech):

(b)(6)

November 20, 2009

CONTAINS NO CONFIDENTIAL BUSINESS INFORMATION

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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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)

Bayer CropScience
2 T.W. Alexander Dr.
P.O. Box 12014
Research Triangle Park, NC 27709

Phone: 919-549-2599
FAX: 919-549-3929

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)

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
A	acre
ADF	Acid Detergent Fiber
ANOVA	Analysis Of Variance
APHIS	Animal and Plant Health Inspection Service
BCS	Bayer CropScience
BLASTP	Basic Local Alignment Search Tool
BLASTx	BLAST search of protein databases using a translated nucleotide query
BLOSUM	BLOcks SubstitUtion Matrix
bp	base pairs
bu/ac	bushels/acre
CAC	Codex Alimentarius Commission
DAD	DDBJ Amino acid sequence Database
DDBJ	DNA Data Bank of Japan
dw	Dry weight
DNA	DeoxyriboNucleic Acid
E. coli	*Escherichia coli*
ELISA	Enzyme Linked Immunosorbent Assay
EMBOSS	European Molecular Biology Open

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	United Nations
FDA	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
G. max	*Glycine max*
GM	Genetically Modified

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HRP	Horseradish Peroxidase
ID	identification

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kg	kilogram
L	liter
LB	Left Border
lb	pound (1 pound = 0,454 kg)
LC/MS	Liquid Chromatography/Mass Spectroscopy
LD_{50}	lethal dose for 50% of animals
LOQ	Limit of Quantitation
M	million
mg	milligram
mL	milliter
µg	microgram
NA	Not Applicable
ng	nanogram
ND	Not Detectable: Below the limit of detection
NDF	Neutral Detergent Fiber
nm	nanometer
nt	nucleotide
OECD	Organization for Economic Co operation and Development

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_1, T_2, etc	generations after T_0 (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
Z. mays	*Zea mays*, corn

TABLE OF CONTENTS

TITLE PAGE 1
COMPANY NAMES 2
RELEASE OF INFORMATION 3
CERTIFICATION 4
SUMMARY 5
ACRONYMS AND SCIENTIFIC TERMS 6
TABLE OF CONTENTS 7
LIST OF TABLES 11
LIST OF FIGURES 13

I. RATIONALE

I.A.Basis for the Request for Determination of Non-regulated status 15
I.B.Double-herbicide-tolerant soybean event FG72 15
I.C.Rationale for the development of event FG72 and benefits 15
I.D.Adoption of event FG72 16
I.E.Submissions to other regulatory agencies 17

II. THE BIOLOGY OF SOYBEAN

II.A.Biology of soybean 18
II.B.Characteristics of the recipient cultivar 18

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

III.A.Description of the transformation system 19
III.B.Parent line 19
III.C.Breeding Diagram 19
III.D.Generations Used for Analysis 19

IV. GENETIC MATERIAL USED FOR TRANSFORMATION OF EVENT FG72

IV.A.Construction of the plasmid used for transformation 21
IV.B.Donor 22
IV.B.1 [redacted] (b)(4) 22
IV.B.2 [redacted] 22
IV.C. Identit 23

V. GENETIC CHARACTERIZATION OF EVENT FG72

V.A.Overview 25
V.B.Copy number and Insertion 25
V.C.Absence of vector backbone 25
V.D. The flanking regions of the inserted sequence(s) 27
V.E.Mendelian inheritance 27
V.F.Stability across and within generations 28
V.G.Conclusion 29

VI. CHAR F THE INTRODUCED PROTEINS

VI.A.Th 30
V groun 30
VI.A.2. Characterization of the 30
VI.A.2.1. 30
..... 30
VI.B.Th 33
V d background 33
VI.B.2. The fun 33
VI.B.2.1 33
VI.B.2.2 35
VI.B.2.3 36
VI.B.2.4 38
VI.C.Expression of the 38
VI.C.1. 38
VI.C.2. 40
VI.D.Verificat 42
VI.D.1. 42
VI.D.2. 43
VI.E.Summary of the 44
VI.E.1. Familia 44
VI.E.2. Potential allergenicity 44
VI.E.2.1. Homology search to known allergens 44
VI.E.2.2. Potential N-glycosylation sites 45
VI.E.2.3. *In vitro* stability to human simulated gastric fluid 45
VI.E.2.4. *In vitro* stability to human simulated intestinal fluid 46
VI.E.2.5. *In vitro* stability to heat 46
VI.E.3. Homology search to known toxins 47
VI.E.4 47
VI.F.Summa 47
VI.F.1. Familia 47
VI.F.2. Potential allergenicity 48
VI.F.2.1. Homology search to known allergens 48
VI.F.2.2. Potential N-glycosylation sites 48
VI.F.2.3. *In vitro* stability to human simulated gastric fluid 49
VI.F.2.4. *In vitro* stability to human simulated intestinal fluid 49
VI.F.2.5. *In vitro* stability to heat 50
VI.F.3 51
VI.F.4 52

VII. AGRONOMIC AND PHENOTYPIC EVALUATION

VII.A.History of field activities 53
VII.B.Agronomic and phenotypic 55
VII.C.Agronomic performance of 57
VII.C.1. Agronomic evaluation of 57
VII.C.1.1.Yield evaluation
..... 57
VII.C.1.2 58
VII.C.2. Agrono 59
VII.C.2.1. Agronomic performance and yield evaluation o
..... 59
VII.C.2. 62

[redacted] 63
VII.D [redacted] 65
VII.E [redacted] 65
VII.F [redacted] 67
VII.G.Composition analysis 69
VII.G.1. Introduction 69
VII.G.2. Nutritional composition of soybean grain 69
VII. [redacted] 75
VII.I [redacted] 75

VIII. ENVIRONMENTAL SAFETY AND IMPACT ON AGRONOMIC PRACTICES
VIII.A.Environmental assessment of the introduced proteins 76
VIII.B.Potential for horizontal or vertical gene transfer 76
VIII.C.Weediness potential of double-herbicide-tolerant soybean event FG72 77
VIII.D.Current agronomic practices for soybean 77
VIII.E.Potential impact on agricultural practices for soybean 80
VIII.F.Weed resistance management 81
VIII.G.Potential impact on farming practices 82
VIII.H.Potential effects on non-target organisms, including beneficial organisms 82
VIII.I.Threatened and endangered species considerations 82
VIII.J Potential impact on biodiversity 83
VIII.K.Conclusion 83

IX. STATEMENT OF GROUNDS UNFAVORABLE 84

X. REFERENCES 85

Appendix 1.FIELD TRIAL TERMINATION REPORTS 2001-2008 92

Appendix 2. MATERIALS AND METHODS- PRODUCT CHARACTERIZATION
2.A. Materials and methods for molecular characterization 116
2.B. Materials and methods for protein characterization tests 118
2.C. Materials and methods for protein levels in seeds 122
2.D. Materials and methods for protein levels in plant parts and during the life cycle 127
2.E. Materials and methods for agronomic studies 131
2.F. Materials and methods for seed germination studies 133
2.G. Materials and methods for composition analysis 134

Appe[redacted]IZTION OF EVENT FG72
3.A [redacted] 140
3.B. [redacted] 154
3.C. [redacted] 159
3.D. [redacted] 171
[redacted] 171
3.D.2. [redacted] 176

Appendix 4.AGRONOMIC DATA 180

Appendix 5. HERBICIDE RESISTANCE AND STEWARDSHIP
5.A. Evolution of herbicide resistant weeds..... 186
5.B. Managing herbicide resistant weeds..... 187
5.C. Evolution of resistance to glyphosate and isoxaflutole herbicides 190
5.D. Characteristics of glyphosate and isoxaflutole herbicides 191
5.D.1. Glyphosate herbicide..... 191
5.D.2. Isoxaflutole herbicide..... 191
5.E. Stewardship of double-herbicide-tolerant soybean event FG72..... 192
5.E.1. Customer outreach 193
5.E.2. Additional customer support..... 194
5.E.3. Monitoring of effectiveness of the stewardship plan..... 195

Appendix 6. REFERENCES - APPENDICES 1-5..... 196

LIST OF TABLES

Table 1. **Generations used for analysis of event FG72** 19
Table 2. **Genetic elements located on the insert** 23
Table 3. **Segregation data for progeny rows and individuals of self-pollinated event FG72** 28
Table 4. **Segregation data for individual F2 progeny of the FG72 x conventional line cross using zygosity PCR probes designed to identify the FG72 insert** 28
Table 5. **Risk group classification of *P. fluorescens*** 36
Table 6. **Protein expression levels in event FG72 soybean seeds** 39
Table 7. [redacted] 41
Table 8. [redacted] 41
Table 9. **Criteria and methodology of demonstrating protein equivalence** 42
Table 10. **Summary of field activities under USDA notifications for event FG72** 53
Table 11. **Summary of field activities under** [redacted] 54
Table 12. **Summary of field activities under** [redacted] 54
Table 13. **Summary of performance charact** [redacted] 56
Table 14. **Yield means for** [redacted] 57
Table 15. **Yield of event FG72 and Jack for selected herbicide treatments** 59
Table 16. **Trial site locations for the equivalence field tests** 60
Table 17. **Treatments for the 2008 season field studies** 60
Table 18. **Summary of agronomic performance for event FG72 and Jack** 61
Table 19. **General plant descriptors comparison for event FG72 and Jack** 63
Table 20. **Seed phenotype characteristics** 64
Table 21. **Paired comparison of variety characteristics** 64
Table 22. **Plant diseases and syndromes observed** 65
Table 23. **Summary of agronomic performance for event FG72 and commercial varieties** 66
Table 24. **Seed germination test results** 68
Table 25. **Proximate and fiber components** 71
Table 26. **Amino acids** 72
Table 27. **Fatty acids** 73
Table 28. **Anti-nutrients** 74
Table 29. **Isoflavones** 74
Table 30. **Sources of genetic based herbicide tolerance** 80
Table 31. **Validation of sample extraction with fortified non-transgenic soybean grain** 124
Table 32. **Limits of detection and quantification of 2mEPSPS and HPPD W336 proteins in fortified non-transgenic soybean grain** 125

LIST OF TABLES (continued)

Table 33. Amounts of 2mEPSPS and HPPD W336 proteins 126
Table 34. Limits of detection and quantification of 2mEPSPS protein in processed fractions derived from non-transgenic whole soybeans 129
Table 35. Limits of detection and quantification of HPPD W336 protein in processed fractions derived from non-transgenic whole soybeans 129
Table 36. Treatment schedule for agronomic field tests in 2008 132
Table 37. Sample identification 135
Table 38. Methods used for the analysis of soybean grain 136
Table 39. Methods used for the analysis of soybean hulls 136
Table 40. Methods used for the analysis of soybean meal and toasted meal 137
Table 41. Methods used for the analysis of soybean protein isolate 137
Table 42. Methods used for the analysis of soybean crude oil and RBD oil 138
Table 43. Methods used for the analysis of soybean lecithin 138
Table 44. Probes used in Southern hybridization of event FG72 140
Table 45. Expected and obtained hybridization fragments 142
Table 46. Probes used for demonstration of absence of vector backbone 154
Table 47. Expected and obtained hybridization fragments 156
Table 48. Overview of the tested seed lots of event FG72 160
Table 49. Probes used for stability studies 160
Table 50. Expected and obtained hybridization fragments 170
Table 51. Electrospray LC/MS peptide mapping of the 2mEPSPS protein 174
Table 52. Amino acid coverage of the 2mEPSPS protein 175
Table 53. Electrospray LC/MS peptide mapping of the HPPD W336 protein 178
Table 54. Amino acid coverage of the HPPD W336 protein 179
Table 55. Raw agronomic data for 2008 181

LIST OF FIGURES

Figure 1. Rise in Glyphosate resistance among different biotypes 17
Figure 2. Breeding Diagram for event FG72 20
Figure 3. [redacted] 21
Figure 4. Event FG72 insert diagram 26
Figure 5. [redacted] 34
Figure 6. [redacted] 35
Figure 7. [redacted] 50
Figure 8. Yield of event FG72 – Event FG72 and location means 58
Figure 9. Growth of herbicide tolerant traits 78
Figure 10. Comparison of herbicide use for the years 1996 and 2007 79
Figure 11. Schematic drawing of the event FG72 insert indicating restriction enzymes, probes, and expected fragment lengths 141
Figure 12 [redacted] .. 145
Figure 13 [redacted] .. 146
Figure 14 [redacted] .. 147
Figure 15 [redacted] .. 148
Figure 16 [redacted] .. 149
Figure 17 [redacted] .. 150
Figure 18 [redacted] .. 151
Figure 19 [redacted] .. 152
Figure 20 [redacted] .. 153
Figure 21 [redacted] .. 155
Figure 22. Southern blot analysis of event FG72 – Absence of vector backbone- PT056-1 probe and insert DNA probe 157
Figure 23. Southern blot analysis of event FG72 – Absence of vector backbone- PT057-1 probe and insert DNA probe 158
Figure 24. [redacted] 161
Figure 25. [redacted] 162
Figure 26. [redacted] 163
Figure 27. [redacted] 164
Figure 28. Background 3068115-48 x Jack 165
Figure 29. Background 3066617-48 x Jack 166
Figure 30. Generation T2 167
Figure 31. Generation T7 168

LIST OF FIGURES (continued)

Figure 32. Schematic drawing of hybridization strategy for the demonstration of structural stability 169
Figure 33 [redacted] 171
Figure 34. Standard curve of electrophoretic mobility versus molecular weight 172
Figure 35. [redacted] 73
Figure 36. [redacted] 76
Figure 37 [redacted] 177
Figure 38 [redacted] **of the development of herbicide resistant weeds** 187

I. RATIONALE

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

The United States Department of Agriculture (UDSA) 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

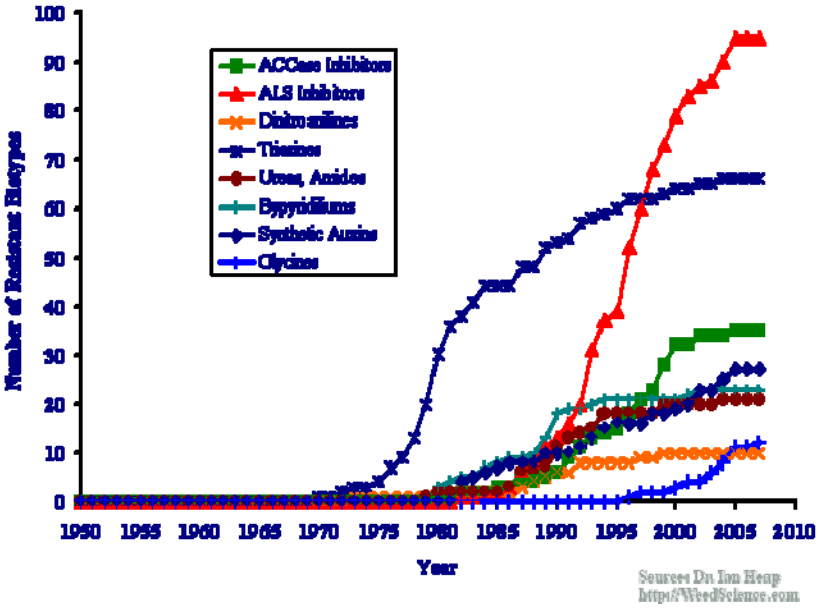
[redacted]

[redacted]

I.D. Adoption of event FG72

[redacted]

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.

[redacted]

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

[redacted]

III.B. Parent line

[redacted]

III.C. Breeding Diagram

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

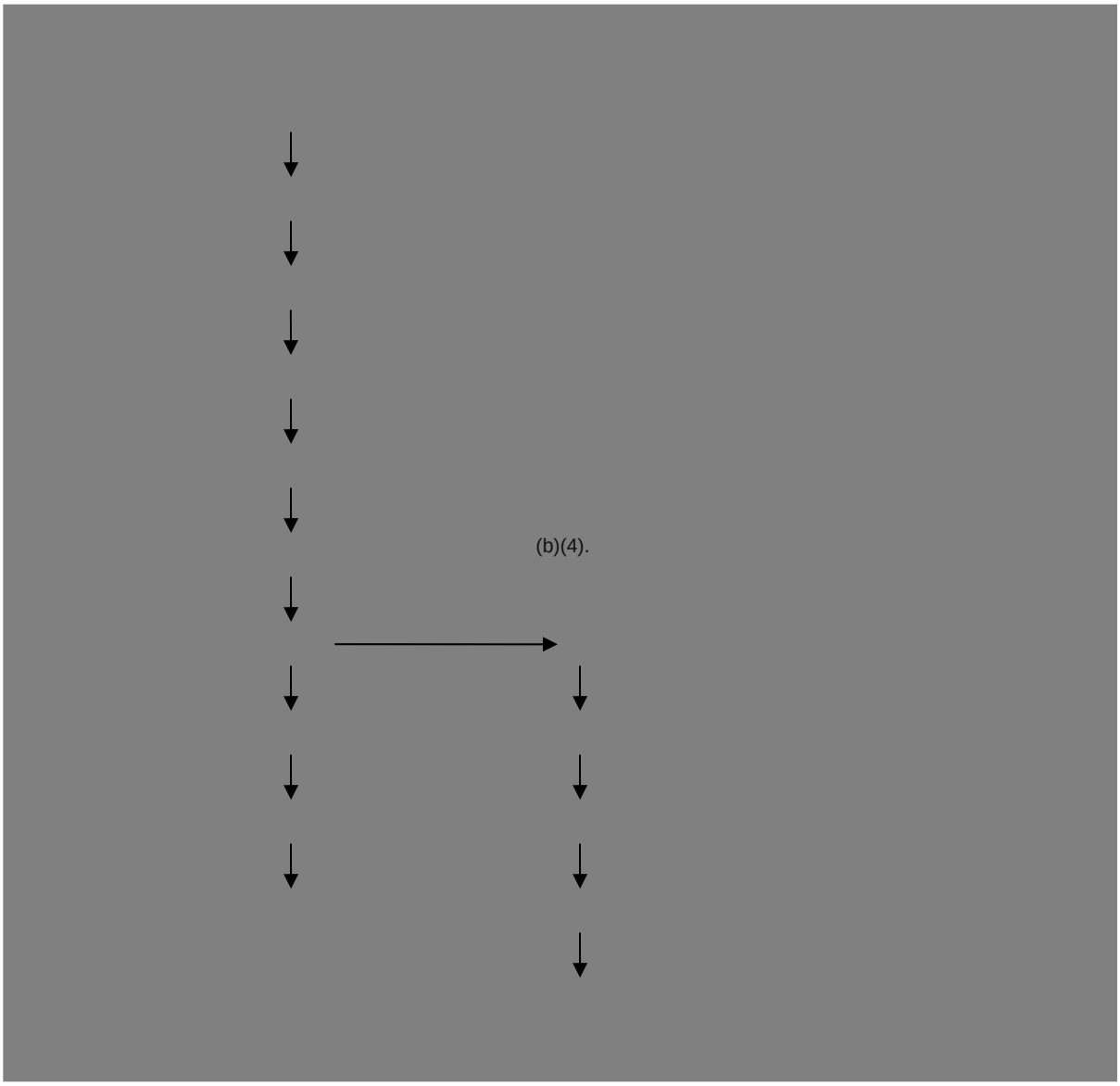
III.D. Generations Used for Analysis

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

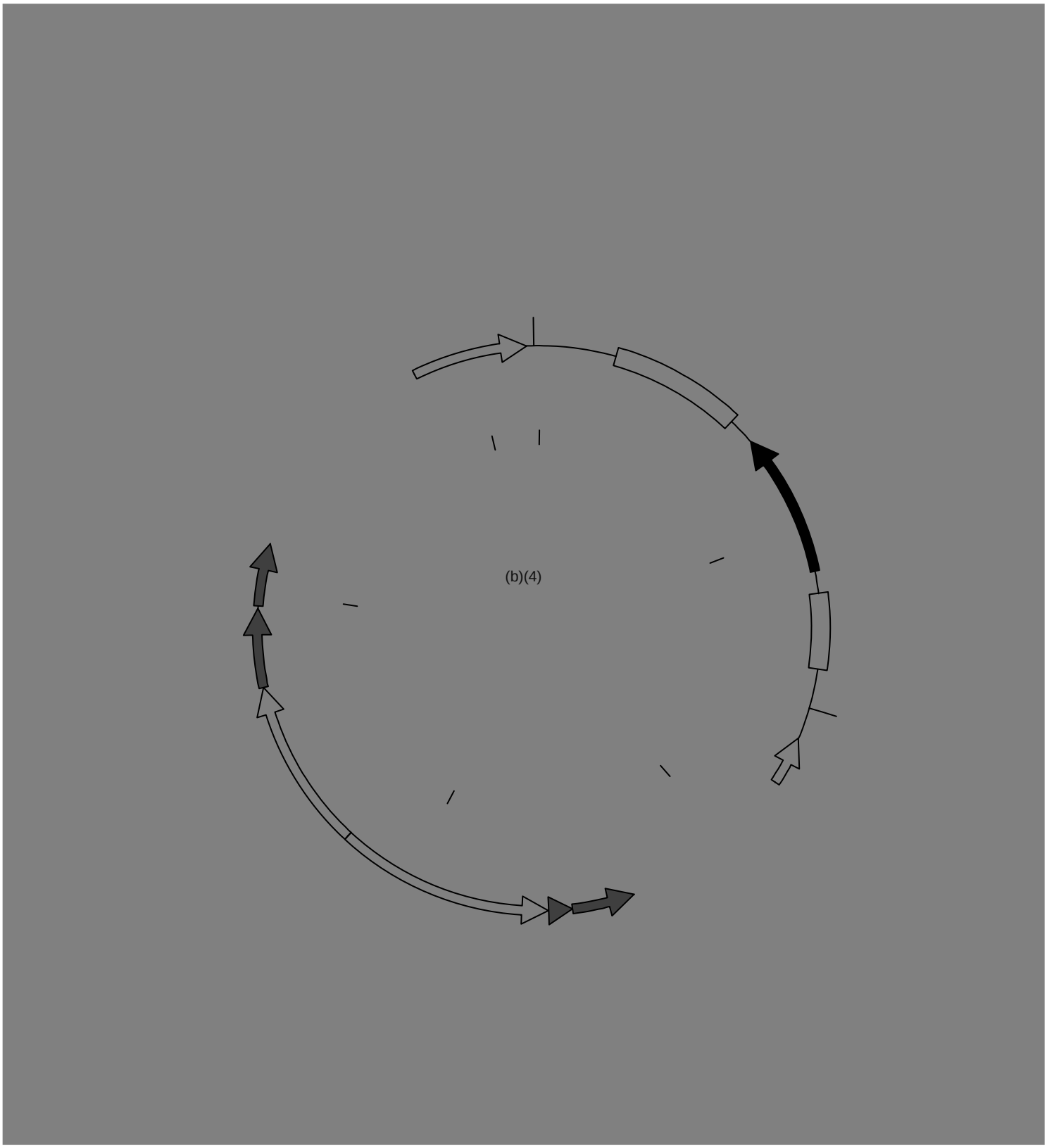
Table 1. Generations used for analysis of event FG72

Generation	Study
[redacted]	

Figure 2. Breeding Diagram of event FG72



IV. GENETIC MATERIAL USED FOR TRANSFORMATION OF EVENT FG72



IV.B. Donor genes and associated regulatory regions

(b)(4)

[redacted]

IV.C. Identity and source of the genetic material

[redacted]

Table 2. Genetic elements located on insert

Nt Positions	Orientation	Origin
[redacted]		

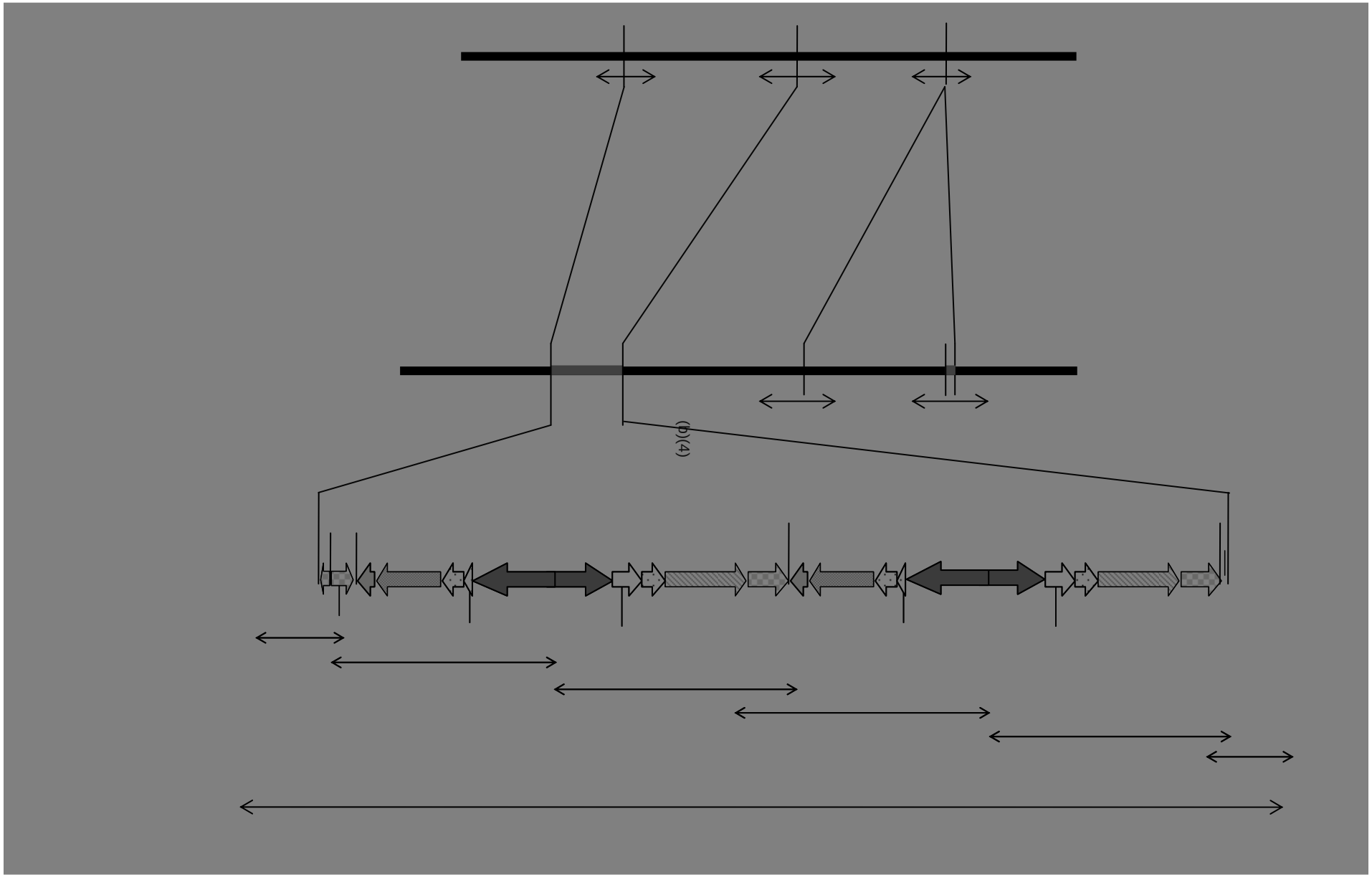
Table 2. [redacted]

[redacted]

V. GENETIC CHARACTERIZATION OF EVENT FG72

All redactions on this page are pursuant to (b)(4).

Figure 4. Event FG72 insert diagram



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

All redactions on this page are pursuant to (b)(4).

V.E. Mendelian inheritance

(b)(4)

Table 3

Table 4.

V.F. Stability across and within generations

V.G. Conclusion

VI. CHARACTERIZATION OF THE INTRODUCED PROTEINS

VI.A. [redacted]

VI.A.1. History and Background

[redacted]

VI.A.2. [redacted]

[redacted]

[redacted]

History of safe use

[redacted]

[redacted]

VI.B. [redacted]

VI.B.1. History and background

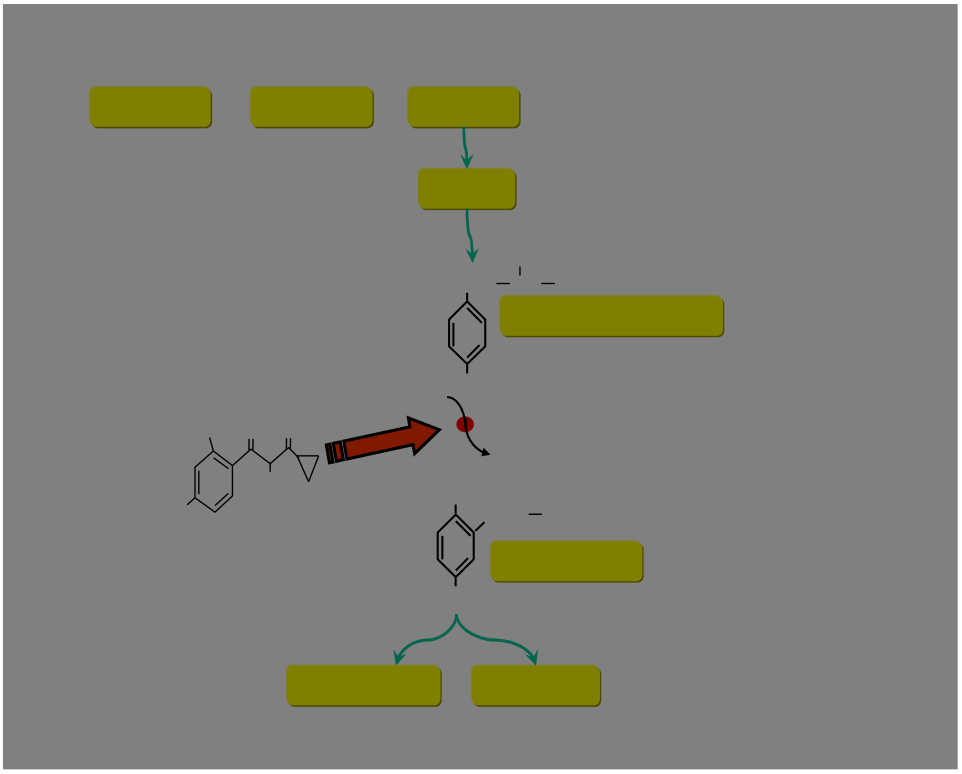
[redacted]

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

VI.B.2.1. [redacted]

[redacted]

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*

USA	Not classified. http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm, accessed on March 02, 2009.
Canada	Non-pathogenic organism. http://www.phac-aspc.gc.ca/ols-bsl/pathogen/organism-eng.php, accessed on March 02, 2009.
European Union	Not classified. Directive 2000/54/EC
Belgium	Risk Group 2 plant pathogen. Belgian Monitor 01.04.2004 18362-18442. 2004
Switzerland	1 + opportunistic pathogen. http://www.bafu.admin.ch/publikationen/index.html?action=show_publ&lang=de&id_thema=6&series=VU&nr_publ=4401; accessed on March 02, 2009.
France	Not classified. Commission de Genie Génétique
Germany	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 δ-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, phenathrene, 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

[redacted]

VI.C. [redacted]

VI.C.1. [redacted]

(b)(4)

Table 7.

Table 8.

VI.D. [redacted]

[redacted]

VI.D.1. [redacted]

[redacted]

VI.D.2.

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

VI.E.1. Familiarity to the protein

EPSPS is the 6th 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; 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 allergenic 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_{50} 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

[redacted]

VI.F.2. Potential allergenicity

VI.F.2.1. Homology search to known allergens

[redacted]

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

[redacted]

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

[redacted]

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

[redacted]

Figure 7. [redacted]

[redacted]

[redacted]

VI.F.3. Homology search to known toxins

[redacted]

[redacted]

VI.F.4. [redacted]

VII. AGRONOMIC AND PHENOTYPIC EVALUATION

VII.A. History of field activities

[redacted]

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

USDA Notification #	Planting / Harvest Dates	Number of Locations	Type of Trial	Locations
[redacted]				

Table 11. Summary of field activities under [redacted]

[redacted]

Table 12. Summary of field activities under [redacted]

[redacted]

VII.B. Agronomic and phenotypic evaluation

[redacted]

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

(b)(4)

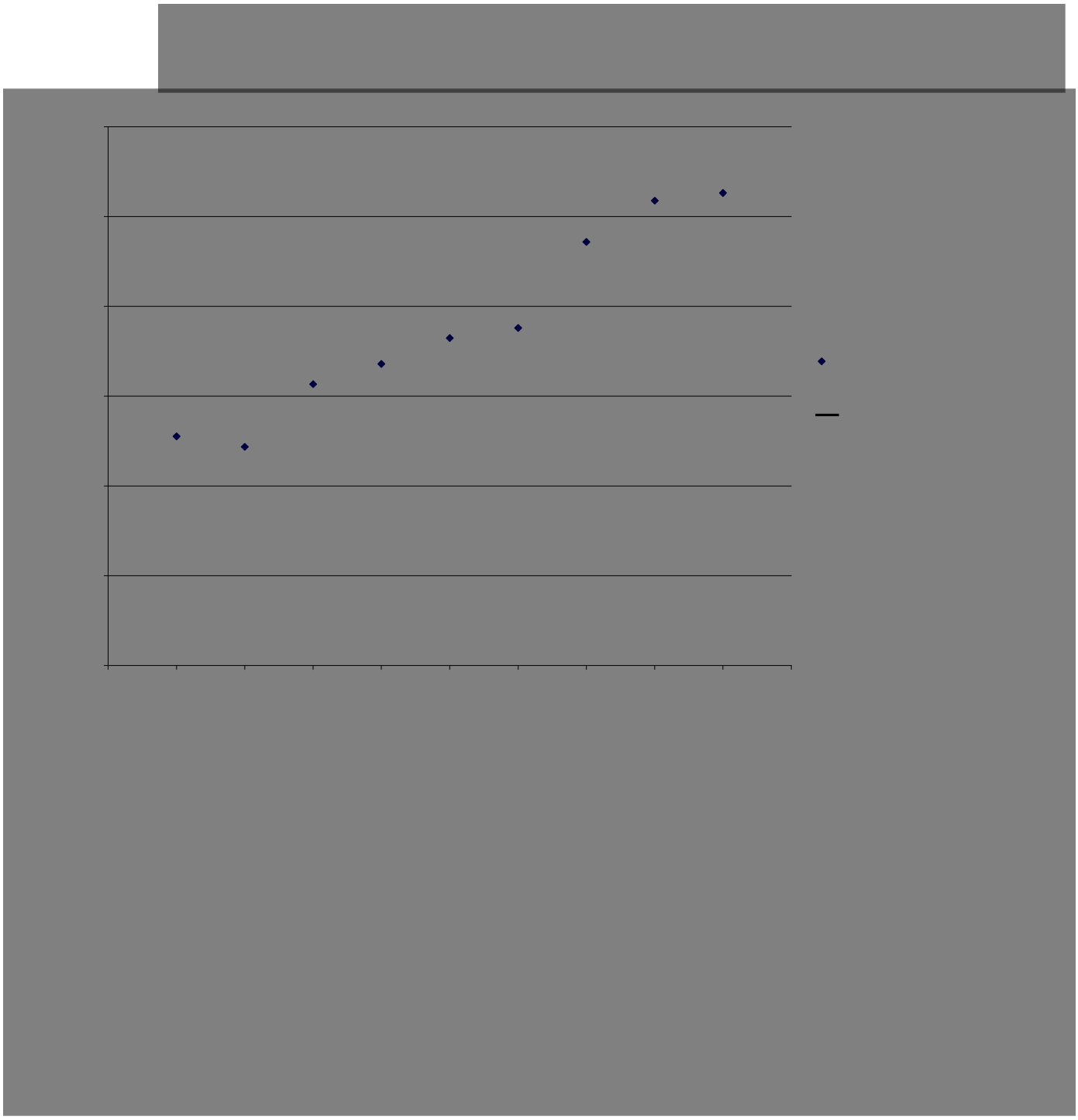
VII.C. Agronomic performance of event FG72

VII.C.1.

VII.C.1.1.

Table 14.

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

[redacted]

[redacted]

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

[redacted]

VII.C.2.2. Objective variety description

[redacted]

(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 ± std)[a]	Jack (mean ± std)[a]
Seed size [b]	13.1 ± 1.4	12.3 ± 1.0
Hilum color	Yellow	Yellow
Mottling [c]	1.9% ± 1.6	1.5% ±1.7
Seed coat color	Yellow	Yellow
Seed coat luster	Dull	Dull
Seed quality[d]	7.3 ± 0.9	7.0 ± 0.9
Seed shape[e]	Spherical	Spherical
L/W	1.1	1.2
L/T	1.0	1.1
T/W	1.1	1.1

[a] Mean and standard deviation for the replicate measurements from 10 locations (2008).
[b] Seed size was recorded as the weight in grams of 100 seed.
[c] Mottling is the number of mottled seeds in each 100 seed reported as %
[d] Seed quality rating is a numerical score of 1-9 based on visual appearance of the seed (9 = best quality, 1 = worst quality)
[e] 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	*Peronospora manshurica*
Bacterial blight	*Pseudomonas syringae pv. Glycinea*
Cercosopora leaf blight	*Cercospora kikuchii*
Brown spot	*Septoria glycines*
Frogeye leafspot	*Cercospora sojina*
Powdery mildew	*Microsphaera diffusa*
Top die back	cause unknown, syndrome described by Iowa State University as plants dying from the top down
Sudden death syndrome	*Fusarium virguliforme*

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

[redacted]

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[a] (%)	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
	Mean	**93.7**	**1.4**	**0**	**95.1**	**4.2**	**0.7**	
	StDev[b]	**5.4**	**2.0**	**0.0**	**4.0**	**3.6**	**1.1**	
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
	Mean	**96.2**	**0.3**	**0**	**96.5**	**3**	**0.5**	
	StDev[b]	**2.1**	**0.9**	**0.0**	**1.8**	**1.7**	**0.7**	

[a] Warm germination – 8 reps of 50 seed

[b] 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_8 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 [b]	Literature Reference[c]
Moisture % fw	Mean [a] Range	9.51 6.57-10.50	9.65 7.90-11.50	9.45 6.51-10.90	8.00 – 10.60	5.6-12
Protein % dw	Mean Range	38.2 36.2-40.3	38.2 36.8-39.8	38.1 36.5-39.6	35.8 – 40.1	32 – 45.5
Fat % dw	Mean Range	19.3 17.9-21.4	18.9 16.6-21.0	19.2 17.1-21.6	15.1 – 21.4	8.1 – 24.7
Ash % dw	Mean Range	5.24 4.38-6.07	5.07 4.17-5.56	5.06 4.50-5.68	4.89 – 5.73	3.9 – 7.0
Carb. % dw	Mean Range	37.3 34.3-39.3	37.9 35.6-39.7	37.6 35.3-40.0	34.8 – 41.6	29.6- 50.2
ADF % dw	Mean Range	17.8 14.2-22.4	18.1 14.1-23.5	17.9 15.2-21.4	13.6 – 23.5	7.8 – 18.6
NDF % dw	Mean Range	19.8 16.8-24.5	20.3 16.9-25.4	20.0 17.4-23.0	16.1 – 24.8	5.0 – 21.3

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

Table 26. Amino acids

Amino acid % dw		Jack	FG72	FG72 Treated	Commercial Lines [b]	Literature Reference[c]
Alanine	Mean [a]	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

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

Table 27. Fatty acids

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

[a] Least mean square
[b] Reference ranges of the 3 analyzed commercial soybean lines
[c] Reference ranges from OECD (2001) and ILSI (2007)

Table 28. Anti-nutrients

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

[a] Least mean square
[b] Reference ranges of the 3 analyzed commercial soybean lines
[c] Reference ranges from OECD (2001) and ILSI (2007)

Table 29. Isoflavones

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

[a] Least mean square
[b] Reference ranges of the 3 analyzed commercial soybean lines
[c] Reference ranges from ILSI (2007)
[d] some or all values reported below the limit of quantification
[e] 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

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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[i]; 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.

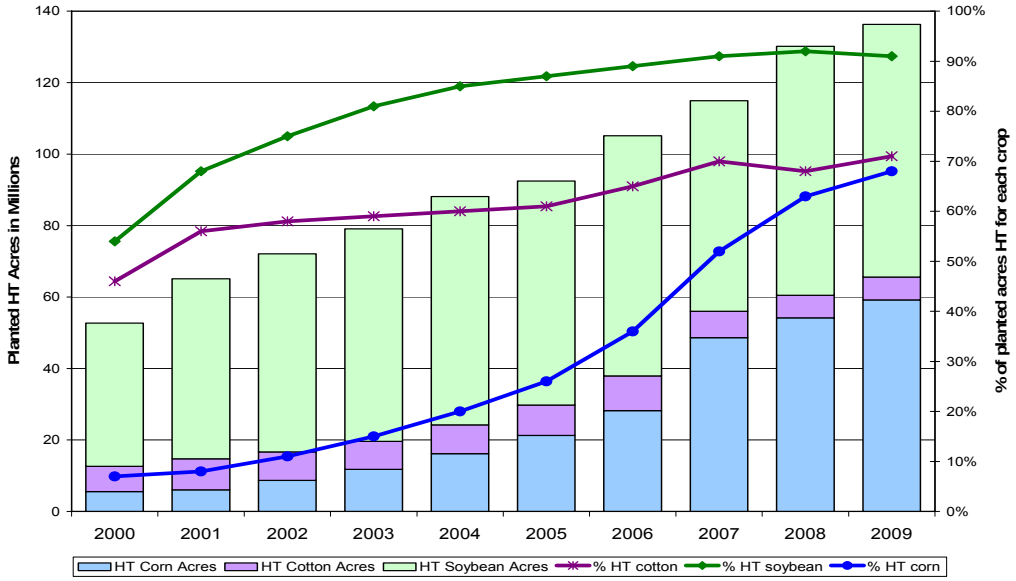
Glyphosate herbicide is exceptionally effective for controlling a broad spectrum of weeds, including many difficult to control weeds, in glyphosate tolerant soybeans with virtually no crop damage. Weed management with glyphosate is also exceptionally simple as the application technique (spray nozzles, 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

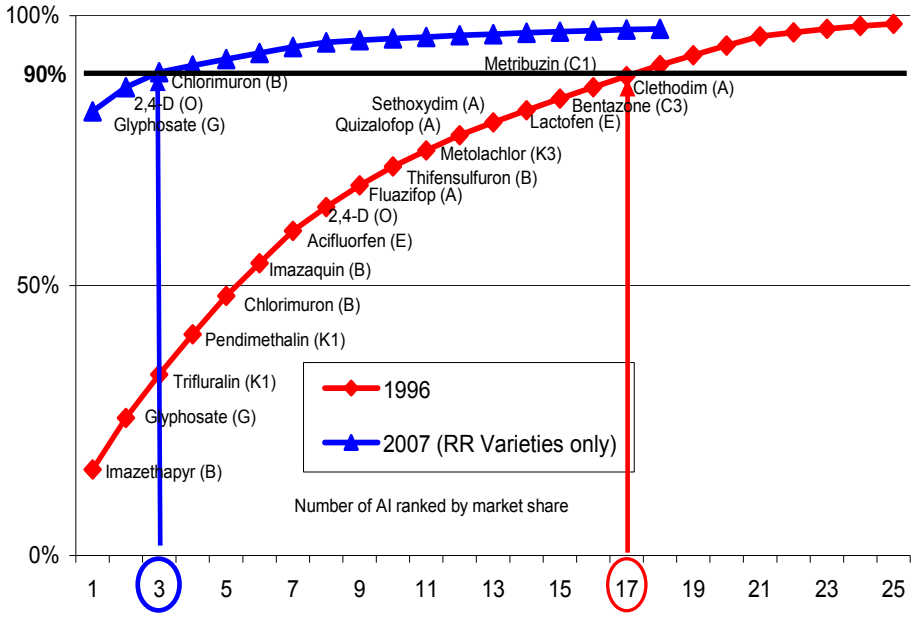
The extensive use of glyphosate was encouraged by the availability of glyphosate tolerance in other crops. Glyphosate tolerant corn is grown on 68% of the 87 million acres of corn in the US. Glyphosate tolerant cotton is grown on 71% of the 9.5 million acres of cotton (Figure 9, USDA 2009). Also available 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

Applicant(s)	Event / Trade Name	Trait Description(s)
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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Appendix 1

FIELD TRIAL TERMINATION REPORTS 2001-2008

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USDA Field Termination Report

Notification No.:

Applicant No.:

Permittee:

Regulated Article:

Site Release Information:

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Information on each release follows:

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Observations:

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Results:

Plant Disposition:

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Volunteer Monitoring:

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Field Management:

Non-Target Organisms:

Weather Synopsis:

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Containment Measures:

USDA Field Termination Report

Notification No.:

Applicant No.:

Permittee:

Regulated Article:

Site Release Information:

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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

Notification No.:

Applicant No.:

Permittee:

Regulated Article:

Site Release Information:

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Purpose of Release:

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Observations:

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Results:

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Plant Disposition: See below:

[redacted]

[redacted]

*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# [redacted]

Applicant: [redacted]

Regulated Article: [redacted]

Release Site Information:

[redacted]

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(b)(6) Date 7/14/09

USDA Termination Report

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USDA Termination Report for Herbicide Tolerant Soybean

Permit #: [redacted]

Permittor: (b)(4)

Permittee: [redacted]

Regulated Article: (b)(4)

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

Identity of the materials

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2.B. Materials and methods for protein characterization tests

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All redactions on this page are pursuant to (b)(4).

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

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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 (β-carotene)	BCLC	AOAC 941.15
Vitamin B_1 (Thiamin)	BIDE	AOAC 942.23, 953.17 and 957.17
Vitamin B_2 (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 (β-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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his page are pursuant to (b)(4).

Figure 11

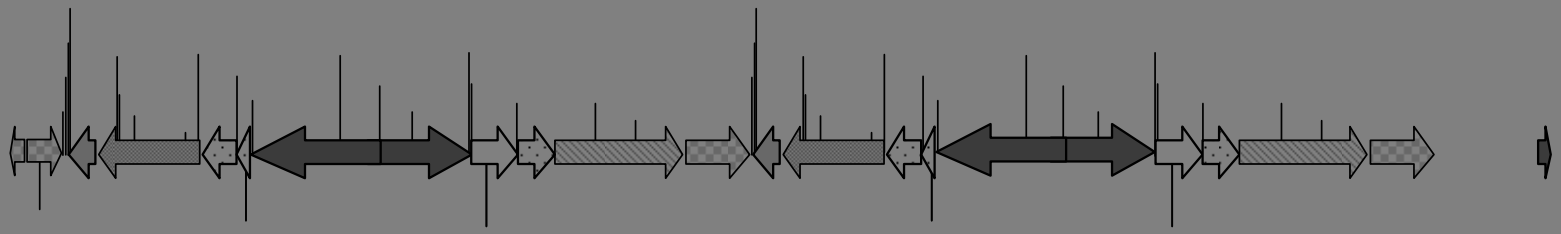

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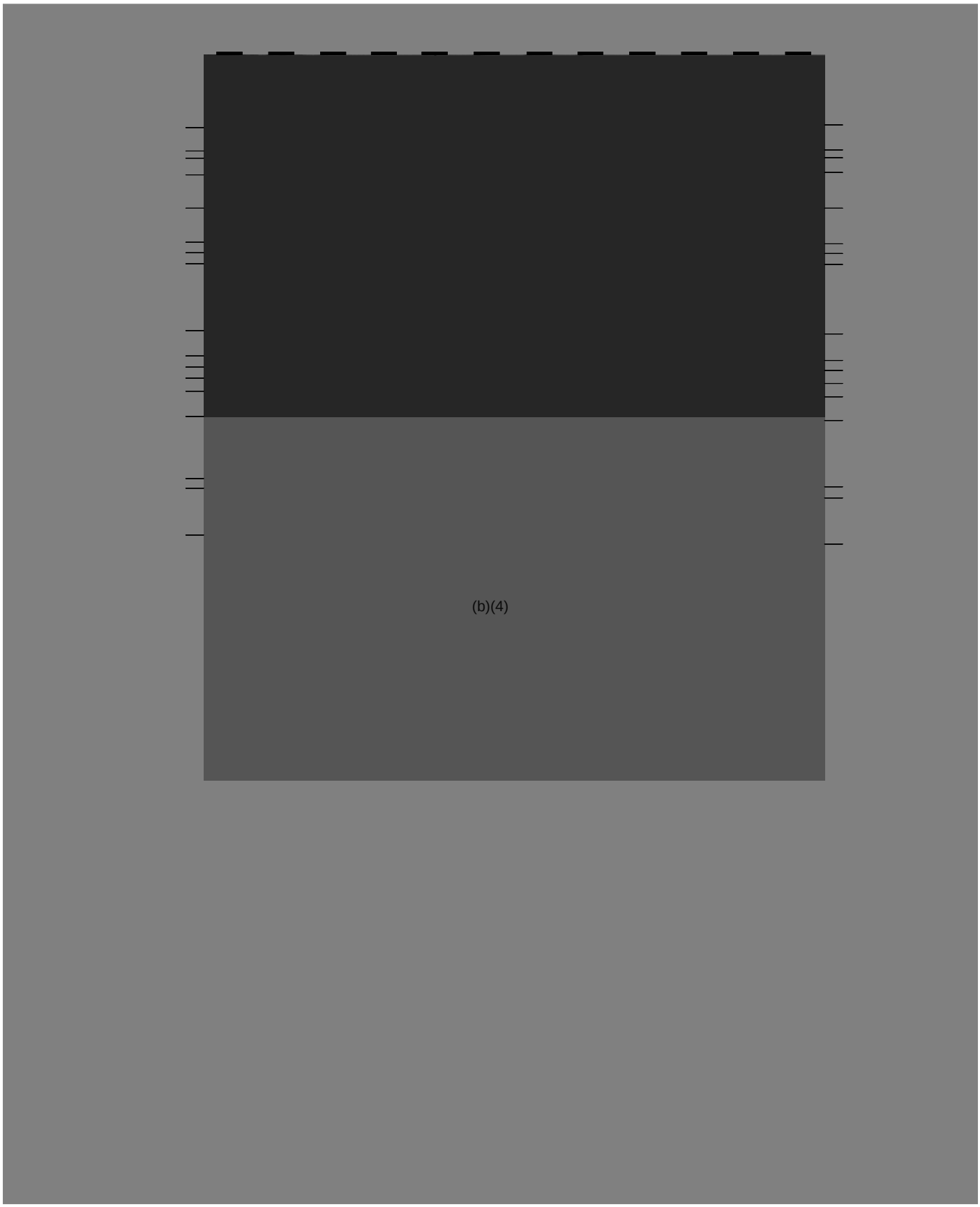
Figure 20. Southern blot analysis of event FG72 – insert-DNA probe



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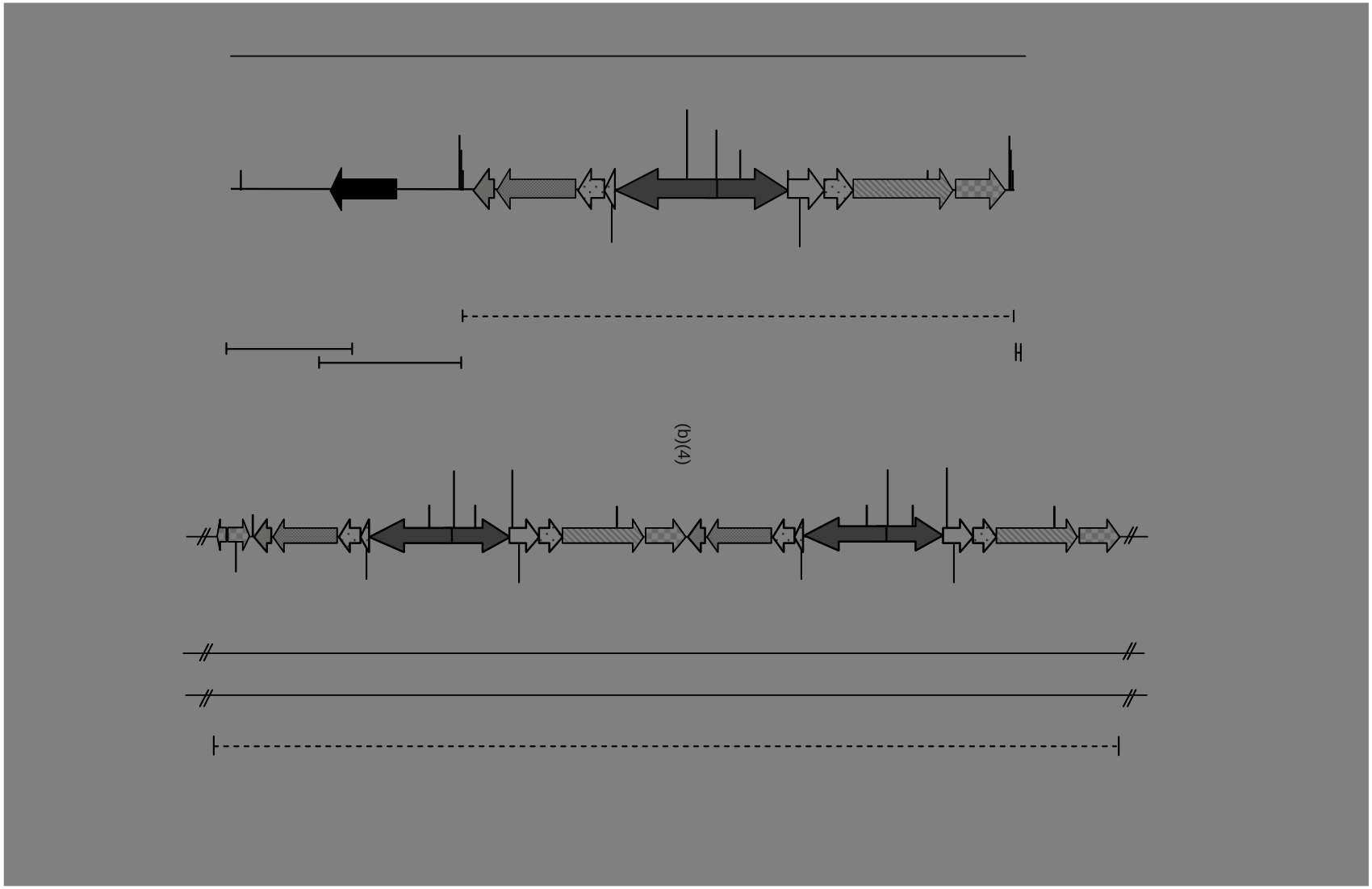
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(b)(4)

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 –

(b)(4)

3.C. Stability across and within generations

(b)(4)

(b)(4)

Figure 24. Environment Adel

[redacted]

[redacted]

[redacted]

[redacted]

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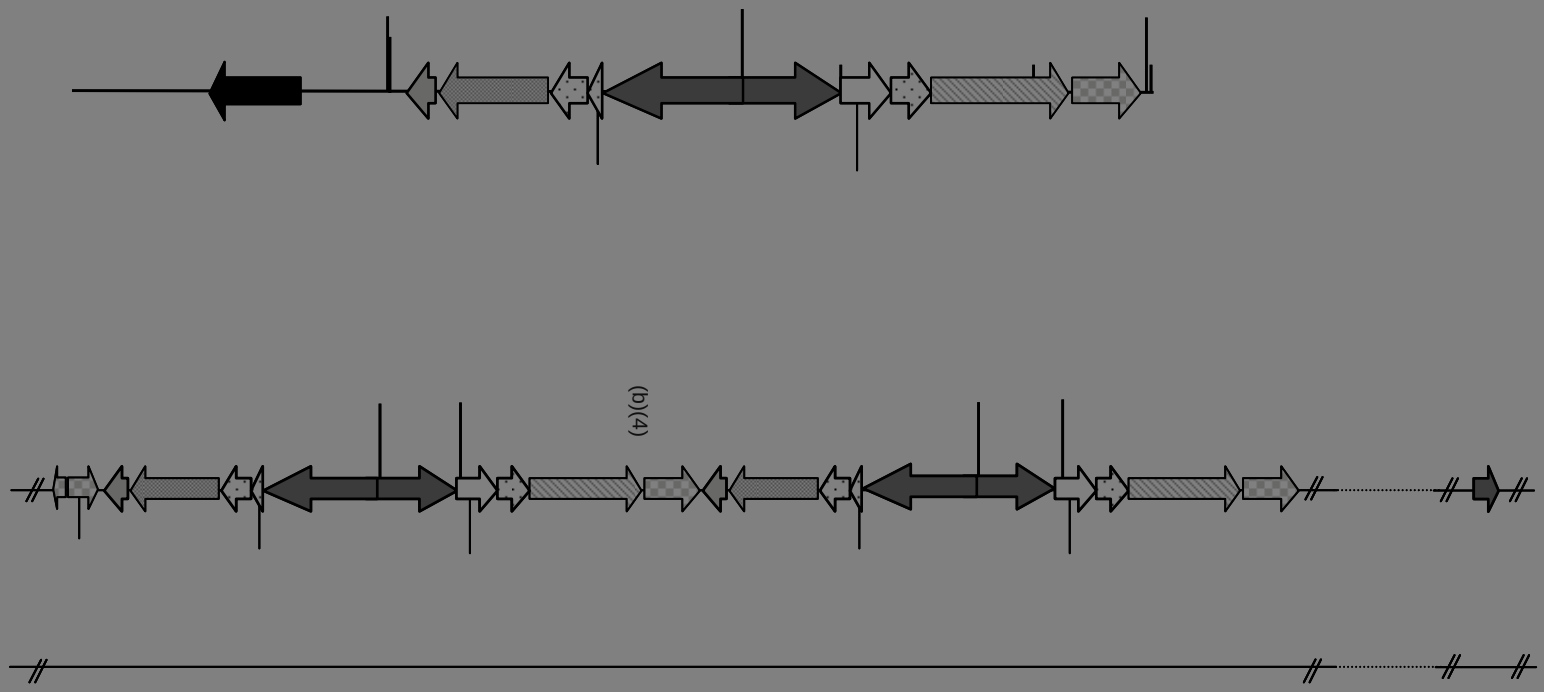
[redacted]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3.D. Demonstration of protein equivalence

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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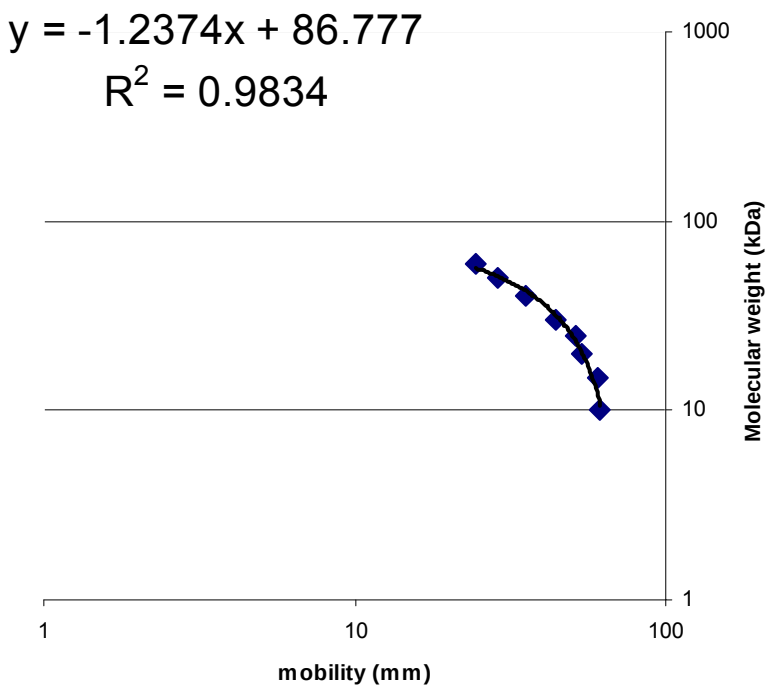
Figure 34. Standard curve of electrophoretic mobility versus molecular weight



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.

(b)(4)

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

2mEPSPS Residue	Sequence	Theoretical [M+H]	98% cov. *E. coli* 2mEPSPS protein*	71% cov. FG72 Soybean Leaf*	Non-transgenic Jack Soybean Leaf*
1 to 13	MAGAEEIVLQPIK		700.9 [M+2H]	ND	ND
2 to 13	AGAEEIVLQPI	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	MAGAEEIVLQPIKEISGTV	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	ILLLAALSEGTTVVDNLLNSEDV 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	QLGADVDCFLGTDCPPVR	1907	1011.7 [M+2H]	1011.7 [M+2H]	ND
161 to 171	VNGIGGLPGGK	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 to243	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	NAYVEGDASSASYFLAGAAITG 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	MPDVAMTLAVVALFADGPTAIR	2260.7	ND**	ND	ND
352 to 357	DVASWR	733.8	733.9 [M+H]	734.3 [M+H]	ND
330 to 357	MPDVAMTLAVVALFADGPTAIR 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	LGASVEEK PDYCIITPPEK	2019	1039.2 [M+2H]	1039.2 [M+2H]	ND
393 to 405	LNVTAIDTYDDHR	1533.6	767.9 [M+2H]	768.1 [M+2H]	767.4 [M+2H]
406 to 423	MAMAFSLAACAEVPVTIR	1882	ND**	ND**	876.3 [M+3H]
424 to 429	DPGCTR	648.7	ND**	ND**	ND
406 to 429	MAMAFSLAACAEVPVTIRDPGC 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	TFPDYFDVLSTFVK	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.

Table 52. Amino acid coverage of the 2mEPSPS protein

Calculation of % Amino Acid coverage	Number of Amino Acids Not Detected		Residue Number
	E. coli 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[a]
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 *E. coli.*	100	72	

[a] NA = Not Applicable

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

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).

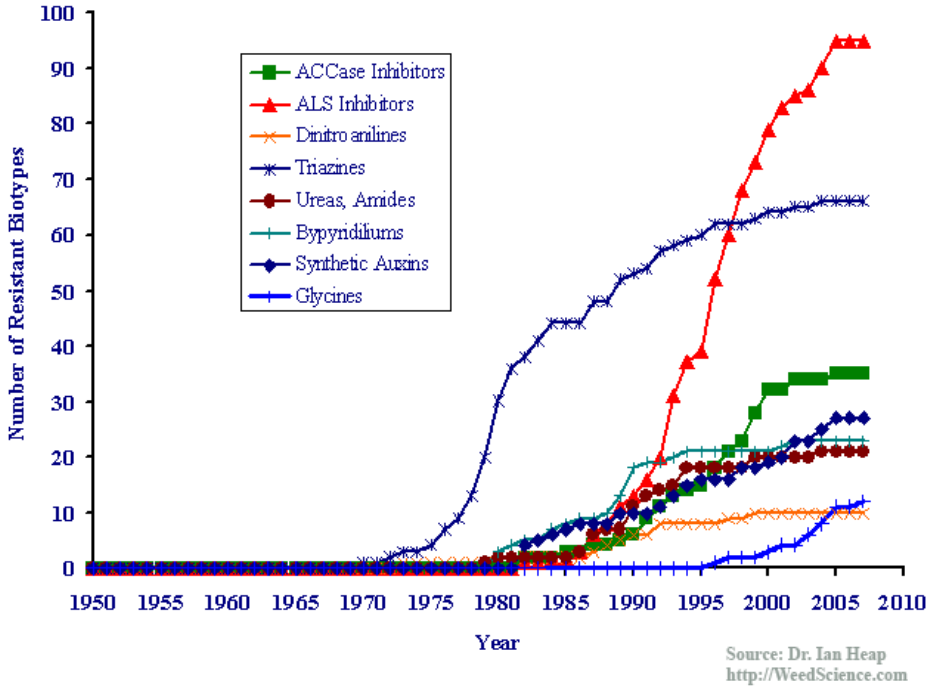
Weed control in the absence of complementary cultural control practices has resulted in the use of herbicides as the only weed control tactic. With this decline in use of alternative weed control methods, extensive use of herbicides with a single MOA has not only resulted in weed shifts but also high 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:

<u>Know your weeds, know your fields</u>
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.

<u>Soil-applied residual herbicides</u>
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®), trifluralin (Treflan®), and ethalfluralin (Sonalan®) 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®), s-metolachlor + fomesafen, dimethenamid-P (Outlook®) and alachlor (Intrro®, Micro-tech®). Another pre-emergence herbicide for the control of waterhemp is metribuzin (Sencor®), 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®, Phoenix™), fomesafen (Flexstar®, Reflex®) and s-metolachlor + fomesafen (Prefix™) 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, there 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®), glyphosate trimethylsulfonium salt (Touchdown®), or glyphosate diammonium salt (Touchdown® 4 or Touchdown Pro®). Glyphosate is the only member of the glycine herbicide family. Glyphosate inhibits the biosysnthesis 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® 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.

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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®, 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® industry stewardship initiative. Further information relating to this initiative can be found at 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 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® FLEXX Herbicide is also recommended as the first herbicide applied in an integrated weed control program that includes sequential post-emergence herbicide applications.
>
> CORVUS™ 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™ Herbicide, or IGNITE® 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.

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 feed back 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

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