Table F-2 (cont.) Comparison of MON 87701 to the Control for Dormancy and Germination Characteristics

Temp. Regime	Category	MS[1] Mean %[2]			SC[1] Mean %[2]			TX[1] Mean %[2]		
		MON 87701	Control	Reference Range[3]	MON 87701	Control	Reference Range[3]	MON 87701	Control	Reference Range[2]
10/20°C	Germinated	79.8*	90.8	63.7 – 96.0	69.5*	81.8	69.8 – 91.0	94.8	93.8	86.3 – 99.5
	Viable Hard	0.0	0.0	0.3 – 2.7	1.5	1.5	2.3 – 13.0	0.5	0.3	0.0 – 6.5
	Dead	20.3*	9.3	3.8 – 33.3	28.5*	16.8	6.0 – 16.5	4.8	6.0	0.5 – 7.3
	Viable Firm Swollen	0.0	0.0	0.0 – 0.3	0.5	0.0	0.0 – 1.8	0.0†	0.0	0.0 – 0.0
10/30°C	Germinated	83.3*	90.3	63.0 – 95.5	72.3	74.8	71.5 – 92.0	94.8	98.8	92.3 – 98.3
	Viable Hard	0.5*	0.0	0.0 – 2.3	0.8	1.0	1.3 – 8.3	0.8	0.5	0.0 – 3.5
	Dead	16.3*	9.8	4.5 – 34.3	27.0	24.3	5.5 – 20.3	4.5	0.8	1.8 – 4.0
	Viable Firm Swollen	0.0	0.0	0.0 – 0.3	0.0	0.0	0.0 – 0.5	0.0	0.0	0.0 – 0.3
20/30°C (AOSA)	Normal	62.7*	77.8	39.3 – 85.0	35.8*	47.8	44.8 – 68.0	72.3	67.3	52.3 – 84.5
	Abnormal	17.7*	11.0	8.3 – 19.5	24.8	20.3	16.5 – 22.3	25.7	30.0	14.0 – 39.0
	Viable Hard	0.0	0.0	0.0 – 2.0	1.8	2.0	2.0 – 6.5	0.7	0.8	0.0 – 4.3
	Dead	19.7*	11.3	6.8 – 39.0	37.3	29.8	13.5 – 26.0	1.3	1.8	1.3 – 4.3
	Viable Firm Swollen	0.0†	0.0	0.0 – 0.0	0.5	0.3	0.0 – 0.5	0.0	0.3	0.0 – 0.3

*Indicates a statistically significant difference between MON 87701 and the conventional soybean control ($p \leq 0.05$).
†No statistical comparison could be made due to lack of variability in the data.
[1] In some instances, the total percentage of both MON 87701 and the control did not equal exactly 100% due to numerical rounding of the means

Table F-3. Starting Seed of MON 87701, the Conventional Soybean Control, and Commercial Soybean Reference Varieties Used in the Disease Evaluation

Production Site[1]	Material Type[2]	Production Year	Material Name	Phenotype	Monsanto Source Lot
MS	Control	2007	A5547	Conventional	11217152
MS	Test	2007	MON 87701	Insect-Protected	11217153
MS	Reference	2007	A5427	Conventional	11217154
MS	Reference	2007	A5403	Conventional	11217155
MS	Reference	2007	A5560	Conventional	11217156
MS	Reference	2007	A5843	Conventional	11217157
SC	Control	2007	A5547	Conventional	11219208
SC	Test	2007	MON 87701	Insect-Protected	11219209
SC	Reference	2007	HBK C5894	Conventional	11219210
SC	Reference	2007	CMA5804A0C	Conventional	11219211
SC	Reference	2007	A5959	Conventional	11219212
SC	Reference	2007	DP 5634 RR	Glyphosate-tolerant[4]	11219213
TX	Control	2007	A5547	Conventional	11219214
TX	Test	2007	MON 87701	Insect-Protected	11219215
TX	Reference	2007	DP 5989	Conventional	11219216
TX	Reference	2007	DP 5414 RR	Glyphosate-tolerant[4]	11219217
TX	Reference	2007	C5215R	Glyphosate-tolerant[4]	11219218
TX	Reference	2007	Crows C5515R	Glyphosate-tolerant[4]	11219219
AL	Reference	2007	H6686	Conventional	11219225
AL	Control	2007	A5547	Conventional	11219554
AL	Reference	2007	CMA5804A0C	Conventional	11219224
AL	Reference	2007	A5959	Conventional	11219223
AL	Reference	2007	A5843	Conventional	11219222
AL	Test	2007	MON 87701	Insect-Protected	11219221
AR	Test	2007	MON 87701	Insect-Protected	11219227
AR	Reference	2007	Anand	Conventional	11219230
AR	Reference	2007	UA4805	Conventional	11219228
AR	Reference	2007	Hornbeck C5894	Conventional	11219231
AR	Control	2007	A5547	Conventional	11219226
AR	Reference	2007	Ozark	Conventional	11219229
GA	Reference	2007	A5560	Conventional	11219234
GA	Reference	2007	CMC5901C0C	Conventional	11219235
GA	Reference	2007	Lee74	Conventional	11219236
GA	Test	2007	MON 87701	Insect-Protected	11219233
GA	Reference	2007	A5403	Conventional	11219237
GA	Control	2007	A5547	Conventional	11219232
IL	Test	2007	MON 87701	Insect-Protected	11219406
IL	Control	2007	A5547	Conventional	11219407
IL	Reference	2007	A4922	Conventional	11219408
IL	Reference	2007	A5427	Conventional	11219416
IL	Reference	2007	H4994	Conventional	11219412
IL	Reference	2007	H5218	Conventional	11219415
NC	Test	2007	MON 87701	Insect-Protected	11219418
NC	Control	2007	A5547	Conventional	11219417
NC	Reference	2007	DP 5989	Conventional	11219419
NC	Reference	2007	USG 5601T	Conventional	11219421
NC	Reference	2007	Fowler	Conventional	11219427
NC	Reference	2007	Hutcheson	Conventional	11219420

Table F-3 (cont.). Starting Seed of the MON 87701, the Conventional Soybean Control, and Commercial Soybean Reference Varieties Used in Disease Evaulation

Production Sites[1]	Material Type[2]	Production Year	Material Name[3]	Phenotype	Monsanto Source Lot
ARNE	Control	2008	A5547	Conventional	11217134
ARNE	Test	2008	MON 87701	Insect-Protected	11217135
ARNE	Reference	2008	Anand	Conventional	11217136
ARNE	Reference	2008	A5403	Conventional	11217137
ARNE	Reference	2008	USG 5601T	Conventional	11217138
ARNE	Reference	2008	Crows C5515R	Glyphosate-tolerant[4]	11217139
GACH	Control	2008	A5547	Conventional	11217140
GACH	Test	2008	MON 87701	Insect-Protected	11217141
GACH	Reference	2008	Hutcheson	Conventional	11217142
GACH	Reference	2008	DP 5634 RR	Glyphosate-tolerant[4]	11217143
GACH	Reference	2008	Fowler	Conventional	11217144
GACH	Reference	2008	Jake	Conventional	11217145
SCEK	Control	2008	A5547	Conventional	11217146
SCEK	Test	2008	MON 87701	Insect-Protected	11217147
SCEK	Reference	2008	Anand	Conventional	11217148
SCEK	Reference	2008	A5403	Conventional	11217149
SCEK	Reference	2008	USG 5601T	Conventional	11217150
SCEK	Reference	2008	Crows C5515R	Glyphosate-tolerant[4]	11217151
MSLE	Test	2002 (May)	MON 87701	Insect-Protected	11220352
MSLE	(–) Isoline	2002 (May)	MON 87701(-)	Conventional	11220353
PR	Test	2002 (Nov.)	MON 87701	Insect-Protected	11220354
PR	(–) Isoline	2002 (Nov.)	MON 87701(-)	Conventional	11220355
PR	Control	2005	A5547	Conventional	11220363
PR	Test	2005	MON 87701	Insect-Protected	11220362
PR	Test	2005	MON 87701* (AG5602)	Insect-Protected	11220356
PR	Control	2005	AG5602	Glyphosate-tolerant[4]	11220358
PR	Control	2007	M-Soy 8329	Conventional	11220359
PR	(–) Isoline	2007	MON 87701(-)* (M-Soy 8329)	Conventional	11220360
PR	Test	2007	MON 87701* (M-Soy 8329)_	Insect-Protected	11220361

[1] MS = Washington County, Mississippi; SC = Barnwell County South Carolina; TX = Armstrong County, Texas; AL = Baldwin County, Alabama; AR = Jackson County, Arkansas; GA = Clarke County, Georgia; IL = Jackson County, Illinois; NC = Wayne County, North Carolina; ARNE = Jackson County, Arkansas; GACH = Tift County, Georgia; SCEK = Barnwell County, South Carolina; MSLE = Washington County, Mississippi; PR = Isabella, Puerto Rico.
[2] MON 87701 expresses the insect-protected trait. The control, reference varieties, and negative (-) isoline do not express the insect-protected trait.
[3] MON 87701 and conventional soybean control, with the exception of those materials followed by an asterisk (*) all have the A5547 genetic background. The genetic background of MON 87701 and conventional soybean control denoted by an asterisk are provided parenthetically.
[4] Commercially-available glyphosate-tolerant (Roundup Ready 40-3-2) soybean variety.

Table F-4. Comparison of MON 87701 to the Conventional Soybean Control and/or the Negative Isoline for Seed Infection by *Phomopsis* Complex

Sites[1]	Genetic Background	MON 87701 Means	MON 87701(-) Means	Control Means	Reference Range[2] Minimum	Reference Range[2] Maximum
MS	A5547	39.5	–	31.3	23.3	57.0
SC	A5547	14.8	–	17.5	3.5	10.0
TX	A5547	0.0†	–	0.0	0.0	0.0
AL	A5547	89.3	–	86.0	41.8	87.0
AR	A5547	2.5	–	3.5	0.0	4.0
GA	A5547	1.5	–	1.8	0.3	6.0
IL	A5547	0.0	–	0.0	0.0	2.0
NC	A5547	7.8*	–	0.8	4.8	10.8
ARNE	A5547	0.5	–	0.0	0.0	0.5
GACH	A5547	16.5	–	17.5	11.8	30.3
SCEK	A5547	7.3	–	7.8	10.8	20.8
MSLE (2002)	A5547	0.8	2.0	–	–	–
PR (2002)	A5547	0.0†	0.0	–	–	–
PR (2005)	A5547	0.3*	–	1.0	–	–
PR (2005)	A5602	0.3	–	0.3	–	–
PR (2007)	M-Soy 8329	1.8	2.3	0.3	–	–

Note: Four replications of 100 seed from each lot were evaluated for percent infection with Phomopsis complex.
* Indicates a significant difference was detected between MON 87701 and the control ($p \leq 0.05$).
† No statistical comparison could be made due to lack of variability in the data.
– Indicates seed was not available for evaluation.
MON 87701 (-) indicates negative isoline.
[1] MS = Washington County, Mississippi; SC = Barnwell County, South Carolina; TX = Armstrong County, Texas; AL = Baldwin County, Alabama; AR = Jackson County, Arkansas; GA = Clarke County, Georgia; IL = Jackson County, Illinois; NC = Wayne County, North Carolina; ARNE = Jackson County, Arkansas; GACH = Tift County, Georgia; SCEK = Barnwell County, South Carolina; MSLE = Washington County, Mississippi; PR = Isabella, Puerto Rico.
[2] Reference range = Minimum and maximum mean values from among the commercially-available reference soybean varieties.

Table F-5. Comparison of MON 87701 to the Conventional Soybean Control and/or the Negative Isoline for Seed Infection by *Cercospora kikuchii*

Sites[1]	Genetic Background	MON 87701 Means[2]	MON 87701(-) Means	Control Means	Reference Range[2] Minimum	Reference Range[2] Maximum
MS	A5547	5.5	–	1.8	1.0	6.5
SC	A5547	4.0	–	4.0	1.5	2.3
TX	A5547	0.8	–	0.5	0.0	0.0
AL	A5547	0.0	–	0.0	0.0	1.8
AR	A5547	10.8	–	6.8	0.0	0.8
GA	A5547	7.5	–	9.0	0.0	5.3
IL	A5547	17.5	–	12.3	1.8	14.0
NC	A5547	12.0*	–	21.3	2.0	13.3
ARNE	A5547	0.8	–	1.0	0.0	1.3
GACH	A5547	14.5*	–	8.8	2.3	5.5
SCEK	A5547	23.5*	–	15.8	2.8	17.3
MSLE (2002)	A5547	24.5	33.8	–	–	–
PR (2002)	A5547	0.0†	0.0	–	–	–
PR (2005)	A5547	4.5*	–	14.0	–	–
PR (2005)	A5602	2.0*	–	13.8	–	–
PR (2007)	M-Soy 8329	38.8	33.5	36.0	–	–

Note: Four replications of 100 seed from each lot were evaluated for percent infection with *Cercospora kikuchii*.
* Indicates a significant difference was detected between MON 87701 and the control ($p \leq 0.05$).
† No statistical comparison could be made due to lack of variability in the data.
– Indicates seed was not available for evaluation.
MON 87701 (-) indicates negative isoline.
[1] MS = Washington County, Mississippi; SC = Barnwell County, South Carolina; TX = Armstrong County, Texas; AL = Baldwin County, Alabama; AR = Jackson County, Arkansas; GA = Clarke County, Georgia; IL = Jackson County, Illinois; NC = Wayne County, North Carolina; ARNE = Jackson County, Arkansas; GACH = Tift County, Georgia; SCEK = Barnwell County, South Carolina; MSLE = Washington County, Mississippi; PR = Isabella, Puerto Rico.
[2] Reference range = Minimum and maximum mean values from among the commercially-available reference soybean varieties.

Appendix G. Material, Methods and Individual Site Results from Phenotypic, Agronomic and Ecological Interactions Analyses of MON 87701

Materials

The materials for phenotypic assessments include: MON 87701, a conventional soybean control (A5547), and 18 commercially-available soybean varieties as references. The references contain both conventional soybean and Roundup Ready soybean 40-3-2 varieties. The list of soybean varieties planted in each site is presented in **Table G-1**. The identities of MON 87701 and control (A5547) seed were confirmed by PCR analysis prior to use.

Field Sites and Plot Design

Data were collected from field trials conducted in 2007 at 16 sites within U.S. soybean production regions (**Section VIII**, **Table VIII-4**). The 16 sites provided a range of environmental and agronomic conditions representative of major U. S. soybean-growing regions. The field cooperators at each site were familiar with the growth, production, and evaluation of soybean characteristics.

The experiment was established at each of the 16 sites in a randomized complete block design with three replications. Each plot at the GA1, LA1, LA2, SC, and TX1 sites consisted of eight 30 ft long rows. Inter-row spacing was between 30 and 40 inches depending on normal agronomic practices at each site. Rows # 2 and 3 were designated for the collection of phenotypic, abiotic stress response, disease damage, and arthropod damage data. Rows # 5–7 were designated for the collection of arthropod samples. Rows # 1, 4, and 8 were used as buffer rows. Each plot was surrounded by an approximately 10 ft, four-row border of a commercially-available soybean variety to create a continuous soybean stand across the plot area to ensure collection of more robust arthropod abundance data within the test area.

Each plot at the AR1, AR2, IN, KS, MS, NC, and TX2 sites consisted of four 20 ft long rows. Inter-row spacing was between 30 and 40 inches depending on normal agronomic practices at each site. Rows # 2 and 3 were designated for the collection of phenotypic, abiotic stress response, disease damage, and arthropod damage data. Rows # 1 and 4 were used as buffer rows. The entire plot area was surrounded by an approximately 10 ft, four-row border of a commercially-available soybean variety.

Table G-1. Starting Seed for Phenotypic Assessments

Substance	Substance type	Relative Maturity Group	Phenotype[1]	Monsanto lot #	Sites[2]
MON 87701	Test	5.5	Insect-protected	GLP-0612-17898-S	All
A5547	Control	5.5	Conventional	GLP-0612-17895-S	All
A5843	Reference	5.8	Conventional	GLP-0702-18243-S	AL, AR2, LA1, MS
A5959	Reference	5.9	Conventional	GLP-0702-18245-S	AL, AR1, KS, SC
CMA 5804AOC	Reference	5.8	Conventional	GLP-0702-18244-S	AL, AR1, KS, SC
H6686	Reference	6.8	Glyphosate-tolerant[3]	GLP-0702-18247-S	AL
UA 4805	Reference	4.8	Conventional	GLP-0702-18123-S	AR3
Ozark	Reference	5.2	Conventional	GLP-0702-18124-S	AR3
Anand	Reference	5.0	Conventional	GLP-0702-18122-S	AR3
Hornbeck C5894	Reference	5.8	Conventional	GLP-0702-18125-S	AR2, AR3 , KS, SC
A5560	Reference	5.5	Conventional	GLP-0702-18242-S	GA2, AR2, LA1,MS, TX2
CMC 5901COC	Reference	5.9	Conventional	GLP-0702-18246-S	GA2
LEE 74	Reference	6.0	Conventional	GLP-0702-18248-S	GA2
A5403	Reference	5.4	Conventional	GLP-0702-18241-S	GA2, GA1, LA1, MS, TX2
A4922	Reference	4.9	Conventional	GLP-0702-18234-S	IL
H4994	Reference	4.9	Conventional	GLP-0702-18235-S	IL
H5218	Reference	5.2	Conventional	GLP-0702-18236-S	IL
H5218	Reference	5.2	Conventional	GLP-0702-18237-S	GA1, IN, LA2, TX2
A5427	Reference	5.4	Conventional	GLP-0702-18238-S	IL
A5427	Reference	5.4	Conventional	GLP-0702-18239-S	IL, GA1, IN, MS, TX2
DP 5989	Reference	5.9	Conventional	GLP-0702-18126-S	NC, AR1, KS, TX1

Table G-1 (continued). Starting Seed for Phenotypic Assessments

Substance	Substance type	Relative Maturity group	Phenotype[1]	Monsanto lot #	Sites[2]
Hutcheson	Reference	5.5	Conventional	GLP-0703-18396-S	NC
USG 5601T	Reference	5.6	Conventional	GLP-0703-18402-S	NC
Fowler	Reference	5.0	Conventional	GLP-0703-18395-S	NC
Delta and Pine 5414	Reference	5.4	Glyphosate-tolerant[3]	GLP-0703-18126-S	GA1, IN, LA2, TX1
Crows C5215 R	Reference	5.2	Glyphosate-tolerant[3]	GLP-0703-18428-S	AR1, LA2, TX1
Crows C5515 R	Reference	5.5	Glyphosate-tolerant[3]	GLP-0703-18429-S	IN, LA2, TX1
Delta & Pine 5634 RR	Reference	5.6	Glyphosate-tolerant[3]	GLP-0703-18358-S	AR2, LA1, SC

[1] MON 87701 expresses the insect-protected trait; whereas the conventional soybean control and reference varieties do not express the insect-protected trait.
[2] MON 87701 and the control were planted at all field sites; the reference varieties were site-specific. Site codes are as follows: AL = Baldwin County, AL; AR1 = Independence County, AR; AR2 = Crittenden County, AR; AR3 = Jackson County, AR; GA1 = Tift County, GA; GA2 = Clarke County, GA; IL = Jackson County, IL; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
[3]Commercially-available glyphosate-tolerant (Roundup Ready 40-3-2) soybean variety.

Table G-2. Field and Planting Information

Site[1]	Planting date (mm/dd/yr)	Seeding rate (seeds/ft)	Planting depth (in)	Plot length (ft)	Rows/ plot	Inter-row spacing[2] (in)	Soil series, organic matter, pH	Cropping history 2006	Cropping history 2005
AL	06/04/2007	8	0.70	20	6	36	Faceville fine sandy loam, 1.6, 6.1	Corn	Cotton
AR1	05/26/2007	8	0.75	20	6	30	Crowley silt loam, 2.5, 6.2	Soybean	Rice
AR2	05/18/2007	9	1.00	20	6	36	Commerce silt loam, 1.4, 5.6	Soybean	Sweet Corn
AR3	06/08/2007	8	0.80	20	6	30	Bosket silt loam, 1.3, 6.1	Grain Sorghum	Grain Sorghum
GA1	06/14/2007	9	0.50	30	8	38	Fuquay loamy sand, 2.0, 6.2	Peanut	Peanut
GA2	06/05/2007	8	1.50	20	6	36	Davidson sandy clay loam, 1.0, 6.1	Cotton	Cotton
IL	06/07/2007	8	1.50	20	6	30	Cairo silt clay, 2.6, 6.8	Corn	Corn
IN	05/31/2007	9	1.50	20	6	30	Alford silt loam, 1.9, 6.3	Corn	Soybean
KS	06/26/2007	9	1.00	20	6	30	Farnum loam, 2.6, 7.6	Wheat	Alfalfa
LA1	06/24/2007	9	1.00	30	8	36	Baldwin silt loam, 2.3, 5.2	Cotton	Cotton
LA2	05/26/2007	9	0.75	20	6	40	Norwood loam, 1.3, 7.0	Grain Sorghum	Grain Sorghum
MS	05/25/2007	9	1.75	20	6	38	Sandy loam, 8.1, 6.0	Cotton	Soybean
NC	06/06/2007	9	1.25	20	6	38	Kalmia sandy loam, 0.9, 6.0	Cucumber	Cotton
SC	05/19/2007	9	1.25	30	8	40	Varina loamy sand, 1.3, 5.7	Cotton	Soybean
TX1	05/25/2007	5-6[3]	0.75	30	8	40	Pullman silty clay, 2.9, 7.1	Fallow	Fallow
TX2	06/11/2007	9	1.00	20	6	40	Amarillo fine sandy loam, 0.6, 8.4	Fallow	Fallow

[1] Site codes are as follows: AL = Baldwin county, AL; AR1 = Independence County, AR; AR2 = Crittenden County, AR; AR3 = Jackson County, AR; GA1 = Tift County, GA; GA2 = Clarke County, GA; IL = Jackson county, IL; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.

[2] Inter-row spacing varied between sites due to variation in normal agronomic practices.

[3] Planting rate at the TX1 site may have varied slightly among the plots due to difficulty in calibrating the planter to 9 seeds/ft as specified in the protocol.

Each plot at the AL, AR3, GA2, IL, and NC sites consisted of six 20 ft long rows. Inter-row spacing was between 30 and 40 inches depending on normal agronomic practices at each site. Rows # 1 and 2 were designated for the collection of plant tissue and harvested seed samples. Rows # 4 and 5 were designated for the collection of the phenotypic data. Rows # 3 and 6 were used as buffer rows. The plots within each replicate were separated by an approximately 5 ft, two-row buffer of a commercially-available soybean variety, and the entire plot area was surrounded by an approximately 10 ft, four-row border of a commercially-available soybean variety.

Planting and Field Operations

Planting information is listed in **Table G-2**. Agronomic practices used to prepare and maintain each study site were characteristic of those used in each respective geographic region. Herbicides containing glyphosate were not used to avoid injury to the conventional soybean control or conventional soybean reference varieties and to ensure all plants were managed uniformly.

Phenotypic Observations

The description of the characteristics measured and the designated developmental stages where observations occurred are listed in **Section VIII**, **Table VIII-1**.

Ecological Observations

Ecological interactions (i.e., interactions between the crop plants and their receiving environment) were used to characterize MON 87701 by evaluating plant response to abiotic stressors, disease damage, arthropod damage, and pest and beneficial arthropod abundance in the plots using the following methods:

Abiotic Stress Response, Disease Damage, and Arthropod Damage

MON 87701 and the conventional soybean control were evaluated at each of 11 sites (AR1, AR2, GA1, IN, KS, LA1, LA2, MS, SC, TX1, and TX2) for differences in plant response to abiotic stressors, disease damage, and arthropod damage. Three abiotic stressors, three diseases, and three arthropod pests were evaluated four times during the growing season at the following intervals:

Observation 1: V2 – V4 growth stage
Observation 2: R1 – R2 growth stage
Observation 3: R3 – R5 growth stage
Observation 4: R6 – R8 growth stage

The principal investigator at each site chose abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in soybean during a given observation period. Therefore, abiotic stressors, diseases, and arthropod pests assessed often varied between observations at a site and between sites.

Abiotic stressors and disease damage were assessed in Rows # 2 and 3 of each plot using a continuous 0 – 9 rating scale of increasing symptomology. Data were collected numerically and then placed into one of the following categories for reporting purposes:

Rating	Severity of plant damage
0	none (no symptoms observed)
1 – 3	slight (symptoms not damaging to plant development)
4 – 6	moderate (intermediate between slight and severe)
7 – 9	severe (symptoms damaging to plant development)

Arthropod damage was assessed in Rows # 2 and 3 of each plot on the upper four nodes of 10 non-systematically selected plants using arthropod-specific 0 – 5 rating scales of increasing symptomology listed below. Data were collected numerically and then placed into one of the categories in the following rating scales for reporting purposes:

Defoliating arthropods (e.g., corn earworm, bean leaf beetle, Japanese beetle, soybean looper)		
Rating	**Defoliation (%)**	**Severity of plant damage**
0	none	none (no symptoms observed)
1	1 – 20 %	slight (symptoms not damaging to plant development)
2	21 – 40%	moderate (intermediate between slight and severe)
3	41 – 60%	
4	61 – 80%	severe (symptoms damaging to plant development)
5	> 80%	

Pod feeding arthropods (e.g., corn earworm, bean leaf beetle, stink bug, Lygus bug on reproductive plant parts)		
Rating	**Damaged pods (%)**	**Severity of plant damage**
0	none	none (no symptoms observed)
1	1 – 20 %	slight (symptoms not damaging to plant development)
2	21 – 40%	moderate (intermediate between slight and severe)
3	41 – 60%	
4	61 – 80%	severe (symptoms damaging to plant development)
5	> 80%	

Leafhoppers (e.g., potato leafhopper)		
Rating	**Foliar damage (%)**	**Severity of plant damage**
0	none	none (no symptoms observed)
1	1 – 50% of foliage with leaf yellowing; no leaf puckering or leaf margin necrosis	slight (symptoms not damaging to plant development)
2	1 – 50% of foliage with leaf yellowing, leaf puckering and/or leaf margin necrosis	moderate (intermediate between slight and severe)
3	> 50% of foliage with leaf yellowing; no leaf puckering or leaf margin necrosis	
4	> 50% of foliage with leaf yellowing, leaf puckering, and/or leaf margin necrosis	severe (symptoms damaging to plant development)
5	> 50% of foliage with necrotic leaves (leaves dead due to leafhopper damage)	

Aphids (e.g., soybean aphid)		
Rating	**Aphids present**	**Severity of plant damage**
0	none	none (no symptoms observed)
1	1 – 100 aphids per plant; no leaf puckering	slight (symptoms not damaging to plant development)
2	101 – 250 aphids per plant; no leaf puckering	moderate (intermediate between slight and severe)
3	≥ 250 aphids per plant with leaf puckering	
4	≥ 250 aphids per plant with leaf puckering and leaf yellowing and/or necrosis	severe (symptoms damaging to plant development)
5	≥ 250 aphids per plant with plant stunting	

For each abiotic stress response, disease damage, and arthropod damage observation at a site, the range of injury severity ratings observed across all three replications for each of MON 87701, the conventional soybean control and reference soybean varieties at the site was determined, and the numeric ranges were then converted to categorical ranges (e.g., none, slight, moderate, severe) for reporting purposes.

Arthropod Abundance

Pest and beneficial arthropods were collected at the GA1, LA1, SC, and TX1 sites three times during the growing season at the following intervals:

Collection 1: R1 – R2 growth stage
Collection 2: R3 – R5 growth stage
Collection 3: R6 – R8 growth stage

Arthropods were collected using a beat sheet sampling method (Kogan and Pitre, 1980). The beat sheet was an approximately 36 × 42 inch white, vinyl sheet spread between the

plants of two adjacent rows. Plants were shaken vigorously along the length of each side of the beat sheet to dislodge arthropods from the plants. A total of four sub-samples were collected in this way from each plot. Specifically, two sub-samples were collected from Rows # 5 and 6 of each plot (sub-samples 1 and 2) and two sub-samples were collected from Rows # 6 and 7 of each plot (sub-samples 3 and 4). The sub-samples collected from the same row were at least 10 ft apart and at least 3 ft from the edge of each plot. The four sub-samples were combined into one pre-labeled container and frozen on dry ice. The samples were then sent on dry ice to the Monsanto Regulatory Environmental Science Center for arthropod identification and enumeration.

A maximum of the six pre-selected or most abundant pest and six pre-selected or most abundant beneficial arthropods were determined for each collection interval from each individual site (e.g., Collection 1, AR1 site). These specific arthropods were then enumerated across all samples (i.e., one sample per plot) from a given collection interval at each individual site (e.g., Samples 1-18, Collection 1, AR1 site). The arthropods assessed often varied between collection intervals from a site and between sites due to differences in temporal activity and geographical distribution of the taxa.

Ecological Interactions Evaluation Criteria

For the assessments of abiotic stress response, disease damage, and arthropod damage, MON 87701 and the conventional soybean control were considered different in susceptibility or tolerance to an abiotic stressor, disease, or arthropod pest on a particular observation date if the range of injury severity to MON 87701 did not overlap with the range of injury severity to the control across all three replications. These data are categorical and, therefore, were not subjected to statistical analysis. For each observation at a site, the range of injury severity across the commercially-available reference soybean varieties provided data that are representative of commercially-available soybean varieties. Pest and beneficial arthropod abundance data were quantitatively evaluated and subjected to statistical analysis, as appropriate.

Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated for evidence of biologically-relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues that would impact the evaluation objectives were noted. Data were then subjected to statistical analysis as indicated below.

Statistical Analysis

Analysis of variance was conducted according to a randomized complete block design using SAS (Version 9.1.3). The level of significance was α=0.05. MON 87701 was compared to the control substance within each site (individual-site analysis) and pooled across all sites (combined-site analysis) for early stand count, seedling vigor, days to 50% flowering, plant height, lodging, shattering, final stand count, seed moisture, 100 seed weight, seed test weight, and yield. Yield data from the SC site were calculated using

standard soybean moisture of 13% as opposed to using measured seed moisture from the SC site (as was done for the other sites) due to measurements being taken by a moisture meter with poor resolution. Therefore, yield from the SC site was statistically analyzed within the site, but was not included in the combined-site analysis. Growth stage, flower color, plant pubescence, abiotic stress response, disease damage, and arthropod damage data were not statistically analyzed. Arthropod pest abundance and beneficial arthropod abundance data were statistically analyzed only within individual collection intervals and sites due to the variation in temporal activity and geographical distribution of the taxa.

No statistical comparisons were made between MON 87701 and reference soybean varieties. Instead, the reference range for each measured phenotypic characteristic was determined from the minimum and maximum mean values collected from the 24 reference soybean varieties planted among the sites. The reference range for the abundance of each arthropod evaluated from a given collection and site was determined from the minimum and maximum mean abundance values collected from the reference soybean varieties at the site.

Individual Field Site Plant Growth and Development Results and Discussion

For the individual-site analysis, a total of 20 (14.3%) statistically significant differences were detected out of 140 comparisons between MON 87701 and the conventional soybean control (**Table G-3**). These differences were distributed among nine out of the 13 phenotypic characteristics. Early stand counts were higher for MON 87701 than the control at the LA1 site (325.7 vs. 274.3 plants/plot). Seedlings of MON 87701 were more vigorous than the control at the IN site (5.0 vs. 5.7 rating). MON 87701 flowered earlier than the control at the AR3 (207.3 vs. 208.7 days), IL (217.3 vs. 218.7 days), and IN (213.7 vs. 217.3 days) sites, but later than the control at the GA1 site (210.0 vs. 209.3 days). Plants of MON 87701 were taller than the control at the NC site (38.0 vs. 34.6 inches). MON 87701 had less lodging than the control at the IL site (0.0 vs. 1.0 rating) and more lodging than the control at the NC site (6.7 vs. 3.3 rating). Pod shattering was higher for MON 87701 than the control at the NC site (1.7 vs.0.7 rating) and numerically higher for MON 87701 than the control at the SC site (3.0 vs. 1.0 rating), although statistical comparisons could not be made due to lack of variability in the data. Final stand counts were lower for MON 87701 than the control at the AL site (231.3 vs. 264.0 plants/plot) and higher for MON 87701 than the control at the LA1 site (322.3 vs. 273.0 plants/plot). Seed moisture was higher for MON 87701 than the control at the AR3 (11.6 vs. 10.6%), LA1 (17.5 vs. 16.5 %), LA2 (16.3 vs. 15.0%), and MS (14.3 vs. 13.2%) sites. Yield was lower for MON 87701 than the control at the GA2 (13.4 vs. 29.4 bu/ac), IN (30.4 vs. 34.4 bu/ac), and LA2 (36.1 vs. 46.5 bu/ac) sites, but higher for MON 87701 than the control at the TX1 site (62.9 vs. 58.8 bu/ac). Considering the statistical differences detected in the individual-site analysis were not detected in the combined-site analysis, this suggests these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of increased weed potential of MON 87701 compared to the conventional soybean control.

Table G-3. Phenotypic Comparison of MON 87701 to the Conventional Soybean Control within Each Site

	Phenotypic Characteristic (units)									
	Early stand count (#/plot)		Seedling vigor (1-9 scale)		Days to 50% flowering		Flower color[2]		Plant pubescence[2]	
Site[1]	MON 87701	Control	MON 87701	Control	MON 87701	Control	MON 87701	Control	MON 87701	Control
AL	256.3	272.3	3.0	3.0	199.0†	199.0	White	White	Hairy	Hairy
AR1	182.7	179.7	4.0	4.7	198.0	198.7	White	White	Hairy	Hairy
AR2	211.3	248.0	4.3	5.0	195.0	195.0	White	White	Hairy	Hairy
AR3	166.0	190.0	4.3	4.0	207.3*	208.7	White	White	Hairy	Hairy
GA1	229.0	217.0	4.3	4.0	210.0*	209.3	White	White	Hairy	Hairy
GA2	167.3	203.7	3.7	3.3	209.7	207.7	White	White	Hairy	Hairy
IL	235.7	241.0	3.7	3.7	217.3*	218.7	White	White	Hairy	Hairy
IN	243.7	220.7	5.0*	5.7	213.7*	217.3	White	White	Hairy	Hairy
KS	272.7	269.0	5.0†	5.0	227.0	227.3	White	White	Hairy	Hairy
LA1	325.7*	274.3	4.0	3.0	217.0†	217.0	White	White	-	-
LA2	248.0	245.3	4.7	4.3	189.7	190.0	White	White	Hairy	Hairy
MS	206.3	185.7	3.3	5.0	185.3	187.7	White	White	Hairy	Hairy
NC	224.3	230.7	2.0	2.0	211.0†	211.0	White	White	Hairy	Hairy
SC	276.0	286.7	2.5	3.0	192.0†	192.0	White	White	Hairy	Hairy
TX1	—	—	1.3	1.0	208.0	208.0	White	White	Hairy	Hairy
TX2	—	—	3.0	3.3	219.0	219.0	White	White	Hairy	Hairy

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control ($p \leq 0.05$).
[1]AL = Baldwin County, AL; AR1 = Independence County, AR; AR2 = Crittenden County, AR; AR3 = Jackson County, AR; GA = Tift County, GA1; GA2 = Clarke County, GA; IL = Jackson County, IL; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
[2] Flower color and plant pubescence data were categorical and were not statistically analyzed.
† No statistical comparisons were made due to lack of variability in the data.
- Data not available or excluded from the data analysis.

Table G-3 (continued). Phenotypic Comparison of MON 87701 to the Conventional Soybean Control within Each Site

	Phenotypic Characteristic (units)									
	Plant height (in)		Lodging (0-9 scale)		Pod Shattering (0-9 scale)		Final stand count (#/plot)		Seed moisture (%)	
Site[1]	MON 87701	Control	MON 87701	Control	MON 87701	Control	MON 87701	Control	MON 87701	Control
AL	31.6	31.3	2.3	2.3	0.0†	0.0	231.3*	264.0	—	—
AR1	26.5	25.4	3.0	2.7	0.0†	0.0	171.3	172.7	12.7	12.9
AR2	37.2	35.8	0.3	1.3	0.0†	0.0	203.7	231.3	10.4	10.5
AR3	35.9	32.8	5.7	5.0	0.0†	0.0	157.0	183.7	11.6*	10.6
GA1	28.2	25.5	1.7	1.3	—	—	163.3	172.0	—	—
GA2	28.3	30.0	0.0†	0.0	0.0	0.3	153.3	172.7	13.8	13.5
IL	33.0	32.3	0.0*	1.0	0.0†	0.0	241.7	239.7	12.0	12.5
IN	36.7	35.8	2.7	3.0	1.0	1.0	197.0	185.7	12.5	12.0
KS	27.3	26.4	1.7	1.7	2.0	1.7	252.0	238.0	8.6	8.6
LA1	29.9	30.1	0.0†	0.0	0.0†	0.0	322.3*	273.0	17.5*	16.5
LA2	31.1	29.7	3.0	2.7	0.0	0.3	213.0	209.7	16.3*	15.0
MS	33.4	31.1	4.3	4.0	0.0†	0.0	135.3	127.7	14.3*	13.2
NC	38.0*	34.6	6.7*	3.3	1.7*	0.7	214.3	232.3	13.2	13.5
SC	32.7	28.8	1.0	0.3	3.0†	1.0	244.0	261.3	—	—
TX1	33.7	33.9	0.0†	0.0	1.7	0.7	—	—	13.6	14.1
TX2	26.9	28.1	0.0†	0.0	—	—	—	—	—	—

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control ($p \leq 0.05$).
[1]AL = Baldwin County, AL; AR1 = Independence County, AR; AR2 = Crittenden County, AR; AR3 = Jackson County, AR; GA1 = Tift County, GA; GA2 = Clarke County, GA; IL = Jackson County, IL; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
† No statistical comparisons were made due to lack of variability in the data.
- Data not available or excluded from the data analysis.

Table G-3 (continued). Phenotypic Comparison of MON 87701 to the Conventional Soybean Control within Each Site

	Phenotypic Characteristic (units)					
	100 seed weight (g)		Test weight (lb/bu)		Yield (bu/ac)	
Site[1]	MON 87701	Control	MON 87701	Control	MON 87701	Control
AL	—	—	—	—	—	—
AR1	18.5	18.3	53.3	53.5	74.6	74.7
AR2	15.8	15.8	51.7	51.7	74.2	75.1
AR3	20.7	20.2	54.2	53.9	73.0	71.3
GA1	—	—	—	—	—	—
GA2	17.2	17.2	53.6	55.7	13.4*	29.4
IL	15.7	15.3	59.7	59.0	43.3	40.9
IN	16.5	15.9	56.9	58.4	30.4*	34.4
KS	13.6	13.2	55.2	54.0	48.6	48.6
LA1	16.7	16.4	49.7	49.3	36.4	31.8
LA2	19.8	19.6	49.7	51.7	36.1*	46.5
MS	18.2	17.5	53.1	52.3	45.9	54.8
NC	13.6	14.2	53.3	54.3	60.1	62.4
SC	17.9	17.0	66.8	69.6	29.0	26.1
TX1	14.9	14.0	60.2	59.3	62.9*	58.8
TX2	—	—	—	—	—	—

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control ($p \leq 0.05$).
[1]AL = Baldwin County, AL; AR1 = Independence County, AR; AR2 = Crittenden County, AR; AR3 = Jackson County, AR; GA1 = Tift County, GA; GA2 = Clarke County, GA; IL = Jackson County, IL; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
† No statistical comparisons were made due to lack of variability in the data.
- Data not available or excluded from the data analysis.

Table G-4. Growth Stage Monitoring of MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed[2]									
Site[1]	**Substance**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
AL		06/21/2007	07/13/2007	08/03/2007	08/23/2007	09/13/2007	10/04/2007	10/29/2007	—	—	—
	MON 87701	V2	V9	R5	R6	R7	R8	R8	—	—	—
	Control	V2	V9	R4 – R5	R6	R7	R8	R8	—	—	—
	References	V2	V9	R3 – R4	R5 – R6	R6 – R7	R7 – R8	R8	—	—	—
AR1		06/20/2007	07/09/2007	07/27/2007	08/13/2007	09/04/2007	09/25/2007	10/13/2007	—	—	—
	MON 87701	V3	V7 – V9	R2 – R3	R5	R6	R7	R8	—	—	—
	Control	V3	V7 – V9	R2 – R3	R5	R6	R7	R8	—	—	—
	References	V3	V7 – V9	R2 – R3	R3 – R5	R6	R6 – R7	R8	—	—	—
AR2		06/04/2007	06/25/2007	07/24/2007	08/17/2007	09/11/2007	09/20/2007	10/22/2007	—	—	—
	MON 87701	V2	V9	R2	R5	R6	R7	R8	—	—	—
	Control	V2	V9	R2	R5	R6	R7	R8	—	—	—
	References	V2	V9	R2	R5	R6	R7	R8	—	—	—
AR3		06/27/2007	07/16/2007	08/01/2007	08/20/2007	09/07/2007	09/26/2007	10/13/2007	—	—	—
	MON 87701	V2	V8 – V9	R2	R4 – R5	R6	R7	R8	—	—	—
	Control	V2	V7 – V8	R2	R4 – R5	R6	R6 – R7	R8	—	—	—
	References	V2 – V3	V7 – V9	R2	R3 – R5	R5 – R6	R6 – R7	R8	—	—	—
		07/07/2007	07/23/2007	08/11/2007	09/03/2007	09/17/2007	10/06/2007	—	—	—	—
GA1	MON 87701	V3	V7–V8	R4	R5	R6	R8	—	—	—	—
	Control	V3	V7–V8	R4	R5	R6 – R7	R8	—	—	—	—
	References	V2 – V3	V7–V8	R3 – R4	R5	R6 – R7	R8	—	—	—	—

[1] Site codes are as follows: AL = Baldwin County, AL; AR1 = Independence County, AR; AR2 = Crittenden County, AR; AR3 = Jackson County, AR; GA1 = Tift County, GA.
[2] Obs. = Observation number; dates in month/day/year format.
- Indicates information not available.

Table G-4 (continued). Growth Stage Monitoring of MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed[2]									
Site[1]	**Substance**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
GA2		06/27/2007	07/18/2007	08/09/2007	08/28/2007	09/19/2007	10/09/2007	—	—	—	—
	MON 87701	V2	V8	R2	R4	R6	R7	—	—	—	—
	Control	V2–V3	V7–V8	R2	R4	R6	R7	—	—	—	—
	References	V2	V7–V8	R1–R3	R3–R6	R6–R8	R7–R8	—	—	—	—
IL		07/02/2007	07/07/2007	07/19/2007	08/03/2007	08/22/2007	09/07/2007	09/21/2007	10/19/2007	—	—
	MON 87701	V3	V5	V8	V11	R4	R5–R6	R6	R8	—	—
	Control	V3	V5	V8	V10–V11	R4	R5–R6	R6	R8	—	—
	References	V3	V4–V5	V7–R1	V10–V11	R4	R5–R6	R6–R7	R8	—	—
IN		06/12/2007	06/28/2007	07/25/2007	08/07/2007	09/10/2007	11/03/2007	—	—	—	—
	MON 87701	VE–VC	V3–V4	V10–V12	R2	R5–R6	R7	—	—	—	—
	Control	VE–VC	V3	V10–V12	R2	R5–R6	R7	—	—	—	—
	References	VE–VC	V2–V4	V9 – V13	R2	R5–R6	R7–R8	—	—	—	—
KS		07/19/2007	08/02/2007	08/13/2007	08/27/2007	09/11/2007	09/24/2007	10/08/2007	—	—	—
	MON 87701	V3	V7–V8	V11–V12	R2–R3	R5	R6	R8	—	—	—
	Control	V3	V7	V11	R2–R3	R5	R6	R8	—	—	—
	References	V3	V7–R1	V11–R2	R2–R5	R5–R6	R6–R8	R7–R8	—	—	—
LA1		7/17/2007	7/25/2007	08/06/2007	08/21/2007	09/21/2007	10/19/2007	11/01/2007	11/09/2007	—	—
	MON 87701	V3	V6	R1	R4	R5	R6	R7	R8	—	—
	Control	V3	V6	R1	R4	R5	R6	R7	R8	—	—
	References	V3	V6	R1	R4	R5	R6	R7	R8	—	—

[1] Site codes are as follows: GA2 = Clarke County, GA; IL = Jackson County, IL; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA

[2] Obs. = Observation number; dates in month/day/year format.

- Indicates information not available.

Table G-4 (continued). Growth Stage Monitoring of MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed[2]									
Site[1]	**Substance**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
LA2		06/14/2007	07/03/2007	07/23/2007	08/13/2007	08/27/2007	09/18/2007	10/11/2007	—	—	—
	MON 87701	V2–V3	V9	R3	R5	R5	R6	R8	—	—	—
	Control	V2–V3	V8–V9	R3	R5	R5	R6	R8	—	—	—
	References	V2–V3	V8–R1	R2–R3	R5	R5	R6	R8	—	—	—
MS		06/11/2007	06/25/2007	07/10/2007	07/31/2007	08/13/2007	08/30/2007	09/07/2007	09/28/2007	10/12/2007	11/02/2007
	MON 87701	VC–V2	V5–V6	R2	R3	R4	R5	R6	R6	R7	R8
	Control	VC–V2	V4–V5	R1–R2	R3	R4	R5	R6	R6	R7	R8
	References	V1–V2	V5–V6	R1–R2	R3	R4	R5	R6	R6	R7	R8
NC		06/28/2007	07/18/2007	07/31/2007	08/22/2007	10/01/2007	—	—	—	—	—
	MON 87701	V2	V7–V8	R2	R5	R8	—	—	—	—	—
	Control	V2	V7–V8	R1–R2	R5	R7–R8	—	—	—	—	—
	References	V2	V7–V8	R1–R2	R4–R5	R7–R8	—	—	—	—	—
SC		06/14/2007	06/26/2007	07/12/2007	07/27/2007	08/10/2007	08/24/2007	09/14/2007	10/05/2007	—	—
	MON 87701	V3	V6–V7	R2	R2–R3	R5	R6	R6	R7–R8	—	—
	Control	V3	V6–V7	R2	R2	R5	R6	R6	R7–R8	—	—
	References	V3–V4	V6–V7	R2	R2	R5	R6	R6	R7–R8	—	—

[1] Site codes are as follows: LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC.
[2] Obs. = Observation number; dates in month/day/year format
- Indicates information not available.

Table G-4 (continued). Growth Stage Monitoring of MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed[2]									
Site[1]	**Substance**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
TX1		06/7/2007	06/21/2007	07/07/2007	07/28/2007	08/13/2007	09/02/2007	09/22/2007	10/10/2007	10/30/2007	—
	MON 87701	V2	V5–V6	R1	R2	R3	R3	R4–R5	R6	R8	—
	Control	V2	V5–V6	R1	R2	R3	R3–R4	R4–R5	R6	R8	—
	References	V2	V5–V6	R1	R2	R3	R3–R4	R4–R5	R5–R6	R8	—
TX2		07/11/2007	07/25/2007	08/22/2007	09/12/207	09/26/2007	10/11/2007	10/17/2007	11/07/2007	—	—
	MON 87701	V3	V7–V8	R3–R4	R5	R6	R7	R7	R8	—	—
	Control	V3	V6–V8	R2–R4	R5	R6	R7	R7	R8	—	—
	References	V3	V6–V8	R2–R4	R5–R6	R6	R7	R7	R8	—	—

[1] Site codes are as follows: ; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
[2] Obs. = Observation number; dates in month/day/year format.
- Indicates information not available.

Table G-5. Abiotic Stressor Evaluation Using Observational Severity Scale for MON 87701 and the Conventional Soybean Control

Abiotic stressor	Number of observations across the sites[1] (AR1, AR2, GA1, IN, KS, LA1, LA2, MS, SC, TX1, TX2)	Number of observations where no differences were detected between MON 87701 and the control
Total	**109**	**109**
Chloride toxicity	2	2
Drought	18	18
Flood	11	11
Hail	14	14
Heat	22	22
Moisture stress	4	4
Nutrient deficiency	9	9
Soil compaction	5	5
Wind	24	24

Note: The experimental design was a randomized complete block with three replications. Observational data were collected at four crop development stages with the exception of the GA1 site where data were collected at only three developmental stages: Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8. No differences were observed between MON 87701 and the control during any observation. Subsequently, data were not subjected to statistical analysis.

[1] Site codes are as follows: AR1 = Independence County, AR; AR2 = Crittenden County, AR; GA1 = Tift County, GA; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.

Table G-6. Disease Damage Evaluations Using an Observational Severity Scale for MON 87701 and the Conventional Soybean Control

Disease	Number of observations across the sites[1] (AR1, AR2, GA1, IN, KS, LA1, LA2, MS, SC, TX1, TX2)	Number of observations where no differences were detected between MON 87701 and the control
Total	**131**	**131**
Alternaria leaf spot	5	5
Asian rust	12	12
Bacterial blight	11	11
Cercospora leaf blight	8	8
Downy mildew	13	13
Frogeye leaf spot	24	24
Phyophthora	1	1
Powdery mildew	7	7
Purple seed stain	1	1
Pythium	1	1
Rhizoctonia	7	7
Southern blight	4	4
Stem canker	2	2
Sudden death	8	8
Septoria (brown spot)	13	13
Soybean mosaic virus	5	5
Sooty mold	1	1
Soybean rust	7	7
White mold	1	1

Note: The experimental design was a randomized complete block with three replications. Observational data were collected at four crop development stages with the exception of the GA1 site where data were collected at only three developmental stages: Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8. No differences were observed between MON 87701 and the control during any observation. Data were not subjected to statistical analysis.
[1]Site codes are as follows: AR1 = Independence County, AR; AR2 = Crittenden County, AR; GA1 = Tift County, GA; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.

Table G-7. Arthropod Damage Evaluated Using an Observational Severity Scale for MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

Arthropod	Number of observations across the sites[1] (AR1, AR2, GA1, IN, KS, LA1, LA2, MS, SC, TX1, TX2)	Number of observations where no differences were detected between MON 87701 and the control
Total	**133**	**127**
Aphid[2]	15	15
Whitefly	6	6
Leafhopper	6	6
Stink bug[3]	18	18
Three-cornered alfalfa hopper	14	14
Grasshopper	9	9
Thrips	6	6
Armyworm[4]	3	3
Corn earworm	4	4
Cabbage looper	7	7
Green cloverworm	8	6*
Soybean looper	5	4*
Thistle caterpillar	4	4
Velvetbean caterpillar	2	1*
Yellow woolybear caterpillar	4	4
Blister beetle	1	1
Bean leaf beetle	15	13*
Japanese beetle	3	3
Spotted cucumber beetle	1	1
Spider mite	2	2

*Indicates a difference observed between MON 87701 and the control for green cloverworms at AR1 site (none vs. moderate; Observation 4) and the LA1 site (slight vs. moderate; Observation 4); soybean loopers at the LA1 site (slight vs. moderate; Observation 4); velvetbean caterpillars at the LA1 site (slight vs. moderate; Observation 4); bean leaf beetles at the AR1 site (none vs. moderate; Observation 4) and the LA2 site (none vs. slight; Observation 3). Data were not subjected to statistical analysis.
Note: The experimental design was a randomized complete block with three replications. Observational data were collected at four crop development stages with the exception of the GA1 site where data were collected at only the first three observations, Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8.
[1] Site codes are as follows: AR1 = Independence County, AR; AR2 = Crittenden County, AR; GA1 = Tift County, GA; IN = Posey County, IN; KS = Pawnee County, KS; LA1 = St. Landry Parish, LA; LA2 = Rapides Parish, LA; MS = Washington County, MS; SC = Barnwell County, SC; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
[2] Includes soybean aphids.
[3] Includes green stink bugs.
[4] Includes fall armyworms and beet armyworms.

Table G-8. Abundance of Pest Arthropods in Beat Sheet Samples Collected from MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Abundance of Pest Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	**Site[1]**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**
Bean leaf beetle	GA1	0.0†	0.0	0.0 – 0.0	0.0†	0.0	0.0 – 0.0	—	—	—
	LA1	2.0	1.3	0.0 – 1.3	0.3	0.3	0.3 – 3.3	2.0	3.0	2.0 – 7.5
	SC	0.0†	0.0	0.0 – 0.0	0.0†	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.3
	TX1	0.0†	0.0	0.0 – 0.0	0.0†	0.0	0.0 – 0.0	—	—	—
Corn earworm	SC	—	—	—	0.0*	4.3	0.0 – 5.3	0.7*	4.0	0.7 – 2.3
Green cloverworm	GA1	0.0†	0.0	0.0 – 0.0	0.0†	0.0	0.0 – 0.0	—	—	—
	LA1	0.3	2.0	0.0 – 1.3	0.0	1.0	1.3 – 2.7	0.0†	0.0	0.0 – 0.0
	SC	1.3	5.0	3.7 – 10.0	0.0	4.7	4.7 – 5.3	52.7	58.3	63.0 – 83.3
	TX1	0.0	0.0	0.0 – 1.0	0.3*	16.7	25.3 – 45.7	—	—	—
Potato leafhopper	SC	0.0	0.0	0.0 – 1.7	—	—	—	—	—	—
Soybean looper	GA1	—	—	—	0.0	2.0	2.0 – 16.0	—	—	—
	LA1	—	—	—	0.3	1.0	0.0 – 1.3	0.0	1.0	0.0 – 0.5
	SC	—	—	—	0.0	2.3	1.3 – 3.7	0.0*	8.7	7.3 – 14.7

* Indicates a statistically significant difference between MON 87701 and the control at $p \leq 0.05$.
Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection3 = R6-R8.
[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.
[2] MON 87701 and control values represent mean number of arthropods collected across three replications.
† No statistical comparisons were made due to lack of variability in the data.
- Indicates arthropod not evaluated.

Table G-8 (continued). Abundance of Pest Arthropods in Beat Sheet Samples Collected from MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Abundance of Pest Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	**Site[1]**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**
Stink bug	GA1	0.0	0.0	0.0 – 0.3	11.3	18.0	6.0 – 29.7	—	—	—
	LA1	0.3	0.3	0.0 – 3.3	2.0	6.3	2.3 – 7.7	3.5	3.7	1.5 – 5.7
	SC	4.3	5.3	0.7 – 10.3	12.3	14.3	6.7 – 16.3	38.0*	85.0	26.0 – 59.7
	TX1	0.0	0.0	0.0 – 0.7	1.3	0.0	1.3 – 1.3	—	—	—
Thrips	SC	231.3	116.0	114.7 – 220.0	-	-	-	—	—	—
Velvetbean caterpillar	GA1	—	—	—	0.0	0.3	1.0 – 1.7	—	—	—
Webworm	GA1	—	—	—	0.0*	6.7	4.0 – 16.0	—	—	—
	SC	—	—	—	0.3*	8.7	9.3 – 30.7	0.0*	4.7	1.7 – 4.7

* Indicates a statistically significant difference between MON 87701 and the control at $p \leq 0.05$.
Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection3 = R6-R8.
[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.
[2] MON 87701 and control values represent mean number of arthropods collected across three replications.
- Indicates arthropod not evaluated.

Table G-9. Abundance of Beneficial Arthropods in Beat Sheet Samples Collected from MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Abundance of Beneficial Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	**Site[1]**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**
Spiders	GA1	0.7	1.0	0.0 – 1.0	4.7	5.0	2.7 – 6.0	—	—	—
	LA1	4.3	4.7	3.0 – 4.0	1.3	0.3	0.3 – 3.3	0.0	0.3	0.0 – 1.0
	SC	6.3	6.7	4.3 – 5.3	2.3	2.7	3.3 – 9.7	1.0	1.0	0.0 – 3.3
	TX1	5.0	4.3	0.0 – 2.7	1.7	0.0	1.3 – 4.7	—	—	—
Big-eyed bug	GA1	0.3	1.3	0.3 – 2.0	30.7	53.0	11.7 – 70.7	—	—	—
	SC	10.7	7.3	3.3 – 10.7	3.7	4.3	3.0 – 7.7	6.0	8.7	6.0 – 8.7
	TX1	—	—	—	2.3	1.3	0.3 – 1.0	—	—	—
Carabids	LA1	1.0	0.0	0.0 – 1.0	0.7	0.0	0.0 – 2.0	—	—	—
Lacewing	GA1	0.3	0.3	0.0 – 1.0	-	-	-	—	—	—
Ladybird beetle	GA1	0.0	0.3	0.0 – 3.0	2.0	2.0	1.0 – 7.3	—	—	—
	LA1	—	—	—	—	—	—	0.0	0.7	0.5 – 1.3
	SC	1.7	0.3	0.0 – 1.3	0.0*	1.7	0.3 – 2.3	—	—	—
	TX1	0.3	0.0	0.0 – 0.7	0.7	0.3	0.3 – 2.3	—	—	—

* Indicates a statistically significant difference between MON 87701 and the control at $p \leq 0.05$.
Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection3 = R6-R8.
[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.
[2] MON 87701 and control values represent mean number of arthropods collected across three replications.
- Indicates arthropod not evaluated.

Table G-9 (continued). Abundance of Beneficial Arthropods in Beat Sheet Samples Collected from MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

		Abundance of Beneficial Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	**Sites[1]**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**	**MON 87701**	**Control**	**Reference Range**
Micro-parasitic parasitoid	LA1	2.7	0.7	0.0 – 0.3	—	—	—	0.0	0.0	0.0 – 9.5
	SC	1.7	1.7	0.0 – 1.7	—	—	—	—	—	—
Damsel bug	GA1	0.3	0.7	0.0 – 0.3	34.7	58.3	22.0 – 58.7	—	—	—
	LA1	0.3	0.7	0.0 – 1.0	0.7	0.3	0.3 – 1.0	0.0†	0.0	0.0 – 0.0
	SC	0.3	0.0	0.0 – 0.7	0.7	0.0	0.0 – 0.7	0.0	0.0	0.3 – 2.0
	TX1	1.3	2.3	1.0 – 3.7	1.3	1.3	1.0 – 2.0	—	—	—
Orius	GA1	0.0	0.0	0.0 – 0.3	0.3	0.0	0.0 – 1.0	—	—	—
	LA1	0.3	0.7	0.0 – 1.3	0.0	0.0	0.0 – 0.3	0.0†	0.0	0.0 – 0.0
	SC	9.3	8.0	7.3 – 10.7	0.3	0.3	0.0 – 1.7	0.0*	1.3	0.0 – 1.3
	TX1	0.0†	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.3	—	—	—

* Indicates a statistically significant difference between MON 87701 and the control at $p \leq 0.05$.
Note: The experimental design was a randomized complete block with three replications. Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection3 = R6-R8.
[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.
[2] MON 87701 and control values represent mean number of arthropods collected across three replications.
† No statistical comparisons were made due to lack of variability in the data.
- Indicates arthropod not evaluated.

Appendix H. Materials and Methods for Pollen Morphology and Viability Evaluation

Plant Production

MON 87701, a conventional soybean control (A5547), and four commercially available reference soybean varieties were grown in Jackson County, IL, in a randomized complete block design with three replications. Each plot consisted of six rows approximately 20 ft in length with inter-row spacing of approximately 30 in.

Flower Collection

When soybean plants were at flowering stage, whole flowers were collected from five non-systematically selected plants from the sixth row of each plot. The samples were identified by the plot number and the plant number (e.g., plot 101 plant 1, or simply 101-1). All flowers from all plots were collected on the same day. Four flowers were collected from each of the five plants per plot: one flower from the bottom, two flowers from the middle, and one flower from the top of each plant. Up to five additional flowers were collected from each plot to ensure a sufficient quantity of pollen for evaluation. All flowers selected from a plot were transferred into a single, clean container and labeled with the plot number from which the sample originated, the entry number, and the entry name. The containers were kept on wet ice or refrigerated for less than 24 hours until the pollen was prepared and stained.

Pollen Sample Preparation

Pollen samples were prepared in a laboratory. Clean microscope slides were labeled with the plot number. A circle of approximately 1 cm diameter was drawn in the center of the slide with a pap hydrophobic barrier pen. Tweezers were used to open each of the collected flowers from a plot and brush the pollen into the circle on the slide. The tweezers were cleaned between extractions. Approximately 20 µl of Alexander's stain (Alexander, 1980) was added to the center of the circle containing the pollen. The pollen was stained at ambient temperature for at least ten minutes prior to examination. Pollen samples from all plots within a replicate were stained and evaluated on the same day.

Data Collection

Pollen characteristics were assessed by viewing samples under an Olympus Provis AX70 light/fluorescence microscope equipped with an Olympus DP70 digital color camera. The microscope and camera were connected to a computer running Microsoft Windows 2000 Professional (© 1981-1999, Microsoft Corp.) and installed with associated camera software [DP Controller v1.2.1.108 and DP Manager v1.2.1.107(© 2001-2003, Olympus Optical Co., Ltd.)] and imaging software [Image-Pro Plus v4.5.1.27 (© 1993-2002, Media Cybernetics, Inc.)].

Pollen Viability: When exposed to the stain solution, viable pollen grains stained red to purple due to the presence of living cytoplasmic content. Non-viable pollen grains stained blue to green and may have appeared round to collapse in shape, depending on the degree of hydration. For each pollen sample, the number of viable and non-viable pollen grains was counted from a minimum of 75 pollen grains from a random field of

view under the microscope. Dense clusters of pollen or pollen grains adhering to flower parts were not counted because they may not have absorbed the stain solution uniformly.

Pollen Diameter: Micrographs (400X resolution) of ten representative pollen grains from each plot were taken and imported into the imaging software. The software was used to measure pollen grain diameter along two perpendicular axes for each selected pollen grain. Mean pollen diameter for each plot was calculated from the 20 total measurements.

General Pollen Morphology: General pollen morphology was observed from micrographs of MON 87701, the conventional soybean control, and commercial reference soybean varieties that were also used for pollen diameter measurements.

Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS (SAS, 2002-2003). The level of statistical significance was predetermined to be 5% ($p \leq 0.05$). MON 87701 was compared to the conventional soybean control for percent viable pollen and pollen diameter. No statistical comparisons were made between MON 87701 and the reference soybean varieties. Instead, a reference range for each measured characteristic was determined from the minimum and maximum mean values from among the reference soybean varieties. General pollen morphology was qualitative; therefore, no statistical analysis was conducted on these observations.

Reference:

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technol. 55(1):13-18.

Appendix I. Materials and Methods for Symbiont Evaluation

Materials

The starting seed of MON 87701 and the conventional soybean control were produced in Puerto Rico in 2006-2007 under Protocol IP036. The reference soybean varieties were obtained from commercial sources (see table below). Nodules, root tissue, and shoot tissue collected from MON 87701, the conventional soybean control, and reference soybean varieties were evaluated in the test.

Materials	Material Type	Phenotype
MON 87701	Test	Insect-Protected
A5547	Control	Conventional
3585N	Reference	Conventional
Hartz H5218	Reference	Conventional
A5427	Reference	Conventional
A5560	Reference	Conventional
5989	Reference	Conventional
H6686	Reference	Conventional

The presence or absence of MON 87701 in the test and control starting seed was verified by event-specific polymerase chain reaction (PCR) analyses. Results of PCR analyses were as expected.

Greenhouse Phase and Experimental Design

MON 87701, the conventional soybean control and reference soybean varieties starting seed were planted in 6-inch pots containing nitrogen-deficient potting medium (LB2 from Sun Gro Horticulture, Inc., Garland, TX) composed of primarily peat, vermiculite, and perlite. Plants from MON 87701, the conventional soybean control and reference soybean varieties starting seed were grown in a greenhouse with a 14-hour photoperiod and with a target day-time temperature of 27 ^{o}C and a target night-time temperature of 22 ^{o}C. Actual temperatures ranged from approximately 17 ^{o}C to approximately 31 ^{o}C. Eight replicate pots were planted with three seeds per pot for each of MON 87701, the conventional soybean control and reference soybean varieties. At planting, each seed was inoculated with approximately 1 x 10^{7} cells of *Bradyrhizobium. japonicum* (NOD+, Becker Underwood, Ames, IA) in phosphate-buffered saline. Pots were arranged in eight replicated blocks for the 6-week sampling period using a randomized complete block design.

The reference soybean varieties starting seed were planted on February 5-7, 2008, and MON 87701 and the conventional soybean control starting seed were planted on February 6, 2008. In all cases, replicate pots had a minimum of one plant emerge within

one week. A solution of nitrogen-free nutrient solution (~250 ml) was added weekly after plant emergence.

Plant Harvesting/Data Collection

Six weeks after emergence, plants were excised at the surface of the potting medium and shoot and root plus nodule material were removed from the pots. The shoot material was cut into smaller pieces and placed in labeled bags. The plant roots with nodules were separated from the potting medium by washing with water. Excess moisture was removed using absorbent paper towels and the roots plus nodules were placed in labeled bags. The same day that plants were harvested, nodules were removed by hand from the roots of each plant, enumerated, and the fresh weight (fwt) was determined. Nodules from each plant were then dried for at least 48 hours at approximately 65 °C, and dry weights were determined.

The remaining root and shoot mass (fresh weight) were determined for each plant. Root and shoot material from each plant was then dried for at least 48 hours at approximately 65 °C for dry weight determination. The shoot tissue was ground after drying with a mortar and pestle and sieved (1.7 mm) prior to analysis for total nitrogen. Shoot total nitrogen was determined by combustion using a nitrogen analyzer (Rapid N Cube, Elementar Americas, Inc.).

Statistical Analysis

The data consisted of six measurement endpoints taken at the six week sampling period: nodule number (NodN), nodule dwt (g) (NodDW), shoot dwt (g) (ShootDW), root dwt (g) (RootDW), and shoot total nitrogen (% and g/plant) (TotalN). Data obtained from MON 87701, the control (A5547), and 3538N, Hartz H5218, A5427, A5560, 5989, H6686 reference soybean varieties were analyzed.

An analysis of variance was conducted using a randomized complete block design with eight replications for each test, control and reference substance. Data were analyzed using the Statistical Analysis System (SAS Version 9.1.3, SAS Institute, Inc. 2002-2003) with the level of statistical significance predetermined to be 5% ($p \leq 0.05$).

Appendix J. Summary of Non-target Organism Evaluations

This addendum provides a short summary of a list of evaluations assessing potential effects of MON 87701 on non-target organisms. The Cry1Ac protein used in the earthworm, larval and adult honeybee, ladybird beetle, and parasitic wasp tests was *E. coli*-produced Cry1Ac protein that was shown to be equivalent to the Cry1Ac protein produced in MON 87701 (**Section VI**). The Cry1Ac protein used in the Collembola and *Orius* evaluations is described in their respective summaries. Each Cry1Ac test substance used for NTO testing of MON 87701 shares >98.9% amino acid identity to the Cry1Ac protein produced in MON 87701.

1. Evaluation of Dietary Effects of Cry1Ac Protein in a Chronic Exposure Test with Collembola (*Folsomia candida* and *Xenylla grisea*).

Sims, S.R. and J.W. Martin. 1997. Effect of the Bacillus thuringiensis Insecticidal Proteins Cry1A(b), Cry1A(c), CryIIA, and CryIIIA on Folsomia candida and Xenylla grisea (Insecta: Collembola). Pedobiologia 41:412-416.

The objective of this evaluation was to determine the potential effect of chronic dietary exposure of Cry1Ac protein (and other Cry proteins) on survival and reproduction of two species of Collembola (*Folsomia candida* and *Xenylla grisea*). The test substance was a full-length Cry1Ac protein (*Btk* HD-73) produced in *Escherichia coli* that shares greater than 99% amino acid identity to the Cry1Ac protein expressed by MON 87701. Collembola were exposed for 21-days to a lyophilized yeast (*Sacchararomyces cerevisae*) diet containing 200 µg Cry1Ac/g diet or a negative control yeast diet. Additionally a positive control was included, which demonstrated the validity of the test system. For both species of Collembola evaluated, Cry1Ac had no adverse effect on survival or reproduction. Therefore, the NOEC of the Cry1Ac protein for Collembola was ≥200 µg/g diet.

2. Evaluation of Potential Effects of the Cry1Ac Protein on the Earthworm in an Acute Test Using an Artificial Soil Substrate.

Porch, J.R. and H.O. Krueger. 2009. Evaluation of Potential Effects of the Cry1Ac Protein on the Earthworm in an Acute Study using an Artificial Soil Substrate. Monsanto Study Number WL-2008-039.

The objective of this test was to evaluate the potential effects of acute exposure of the Cry1Ac protein administered to the earthworm, *Eisenia fetida*, during a 14-day exposure period when mixed in an artificial soil substrate. A single concentration of 250 mg Cry1Ac protein/kg soil dry weight was tested, which exceeded the maximum expected environmental concentration for the protein in the top 15 cm of soil. Appropriate negative and positive controls were also included in the study. The results indicate that there was no mortality in the assay control (soil only), control substance (soil with 6.6 mM carbonate-bicarbonate buffer containing reduced glutathione), and the Cry1Ac

protein treatments during the 14-day test. The positive control treatments of 15 mg and 30 mg chloroacetamide/kg soil resulted in 2.5% and 83% mortalities, respectively, demonstrating the validity of the test system. A slight loss in average individual earthworm body weight from test initiation to test termination was noted in all test groups, which was expected since the earthworms were not fed during the 14-day test period. There was no significant difference ($p>0.05$) in body weight losses between the Cry1Ac protein and the control substance treatments. The study concluded that the NOEC of the Cry1Ac protein for earthworms was ≥250 mg/kg dry soil.

3. Evaluation of the Dietary Effect(s) of a Cry1Ac Protein on Honeybee Larvae (*Apis mellifera* L.).

Richards, K.B. 2009. Evaluation of the Dietary Effect(s) of a Cry1Ac Protein on Honeybee Larvae (Apis mellifera L.). Monsanto Study CA-2007-062.

The objective of this test was to evaluate potential dietary effects of Cry1Ac protein when administered to honeybee larvae. The protein was tested at a concentration of 410 µg Cry1Ac/ml using a 50 mM CAPS buffer, which resulted in a safety factor of approximately 132× based on the maximum Cry1Ac protein expression level (3.1 µg/g fwt) in pollen from MON 87701. In addition, appropriate negative and positive controls were included in the study. Effects on honeybee larvae were determined at adult emergence after 17 days. The results revealed that the survival rate for the honeybee larvae in the assay control and the 50 mM CAPS buffer treatments were 98% and 94%, respectively. The Cry1Ac protein treatment yielded an 89% survival rate. There was no significant differences ($p>0.05$) in mean mortality between the Cry1Ac protein, buffer control, and assay control treatments. Behavioral observations at emergence indicated no adverse behavior or morphological effects. Based on statistical analyses and behavioral observations there were no significant effects on the development or survival of honeybees treated with either the Cry1Ac protein or the buffer/water controls. The survival rate for the positive control treatment was 0.0% at 2000 µg potassium arsenate/ml, confirming the validity of the test system. The NOEC of the Cry1Ac protein for honeybee larvae was ≥4.1 µg/cell or ≥410 µg/ml as a single dose.

4. Evaluation of the Dietary Effect(s) of a Cry1Ac Protein on Honeybee Adults (*Apis mellifera* L.).

Richards, K.B. 2009. Evaluation of the Dietary Effect(s) of a Cry1Ac Protein on Honeybee Adults (Apis mellifera L.). Monsanto Study CA-2007-063.

The objective of this test was to evaluate potential dietary effects of Cry1Ac protein on the adult honeybee during chronic feeding. The protein was tested at a concentration of 175 µg Cry1Ac/ml in a 30% sucrose solution, which resulted in a safety factor of approximately 56× based on the maximum Cry1Ac protein expression level (3.1 µg/g fwt) in pollen from MON 87701. In addition, appropriate negative controls (25 mM CAPS buffer in 30% sucrose, and 30% sucrose in water) and a positive control (100 µg/mL potassium arsenate in 30% sucrose) were included in the study. Adult honeybees (0 to 5 days old) were exposed to the test and control solutions continually for the test period. The number of dead bees was assessed on a daily basis. The assay acceptance

criteria stipulated that the assay be terminated at either 30 days or when the negative control mortality reached 30%. The 30% criterion was met sometime during the Day 9 (27.0%) to Day 10 (35.4%) time interval and thus the Day 9 and 10 data were used in the statistical analysis. The study was actually terminated on Day 10 so that all the bees in all cages could be counted to determine the exact number of bees present in each cage. The potassium arsenate positive control produced 100% mortality by Day 2, confirming the validity of the test system. Based on statistical analyses and behavioral observations there were no significant effects on the development or survival of honeybees treated with the Cry1Ac protein compared to the buffer control. The NOEC of the Cry1Ac protein for adult honeybees was ≥175 µg/ml.

5. Evaluation of Potential Dietary Effects of Cry1Ac on the Ladybird Beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae).

Paradise, M.S. 2009. Evaluation of Potential Dietary Effects of Cry1Ac on the Ladybird Beetle, Coleomegilla maculata (Coleoptera: Coccinellidae). Monsanto Study Number REG-08-337.

The objective of this evaluation was to examine the potential for dietary effects of the Cry1Ac protein on the mortality and development of the ladybird beetle, *Coleomegilla maculata* using an agar-based artificial diet. The test substance was incorporated at 60 µg Cry1Ac protein/g of diet, which resulted in a safety factor of approximately 19X based on the maximum Cry1Ac protein expression level (3.1 µg/g fwt) in pollen from MON 87701. In addition, appropriate negative controls (50 mM CAPS buffer, purified water) and a positive control (100 µg potassium arsenate/g diet) were included in the study. The results showed that there were no significant differences for the mean survival percentage of *C. maculata* among the Cry1Ac protein (97.5%), buffer control (92.5%), and the water control (92.5%) treatments. The positive control group (potassium arsenate) produced a survival rate of 7.5%, confirming the validity of the test system. Likewise, there were no significant differences for the mean percentage of *C. maculata* larvae that developed to adults among the Cry1Ac protein (97.5%), buffer control (92.5%), and the water control (92.5%) treatments. None of the insects in the potassium arsenate positive control group developed to the adult stage. In addition, there were no significant differences in the mean biomass of *C. maculata* adults among the Cry1Ac protein (9.94 mg), buffer control (9.90 mg), and assay control (10.07) treatments. The NOEC of the Cry1Ac protein for ladybird beetle was ≥60 µg/g of diet.

6. Evaluation of Potential Dietary Effects of Cry1Ac Protein on Minute Pirate Bugs, *Orius albidipennis* (Hemiptera: Anthocoridae).

Gonzalez-Zamora, J.E., S. Camunez and C. Avilla. 2007. Effects of Bacillus thuringiensis Cry Toxins on Developmental and Reproductive Characteristics of the Predator Orius albidipennis (Hemiptera: Anthocoridae) under Laboratory Conditions. Environ. Entolomol. 36(5):1246-1253.

This test examined the potential effects of the Cry1Ac protein on *Orius albidipennis* nymphs using a laboratory diet incorporation bioassay. The test substance was trypsinized Cry1Ac from *Bt* strain EG11070 that shares >98.9% amino acid identity to

the Cry1Ac produced in MON 87701. *O. albidipennis* nymphs were starved for two days without water or food, then provided a 4-µl drop of water containing blue stain and 1000 µg/ml Cry1Ac. Nymphs were allowed to feed on the drop *ad libitum* and then fed eggs of *Ephestia kuehniella* for 24 h. This feeding cycle was repeated one to three times until adult emergence. Nymphs were also allowed to feed on a drop of water containing blue stain, but lacking Cry1Ac, as a negative control. Dietary exposure to the Cry1Ac protein was confirmed by only using nymphs in the analysis that turned blue after drinking from the drop of water. Measurement endpoints included development time of nymphs and adults, percent survival of nymphs and adults, number of eggs per female per day (fecundity), and egg hatching. No adverse effects on development time, survival, fecundity, and egg hatching of *O. albidipennis* were observed when nymphs were exposed to a dose of 1000 µg/ml Cry1Ac protein in diet. The study concluded the NOEC of the Cry1Ac protein for *Orius* was ≥1000 µg/ml.

7. Evaluation of Potential Dietary Effects of the Cry1Ac Protein on the Parasitic Wasp, *Pediobius foveolatus* (Hymenoptera: Eulophidae).

Tan, J., M. Paradise and D. Carson. 2009. Evaluation of Potential Dietary Effects of the Cry1Ac Protein on the Parasitic Wasp, Pediobius foveolatus (Hymenoptera: Eulophidae). Monsanto Study Number REG-08-467.

The objective of this test was to examine the potential for dietary effects of the Cry1Ac protein on the survival of the adult parasitic wasp, *Pediobius foveolatus* (Hymenoptera: Eulophidae) using a 30% honey (v/v) diet. The test substance was incorporated into 30% honey diet at a concentration of 250 µg Cry1Ac protein/ml of diet, which resulted in a safety factor of approximately 81 based on the maximum Cry1Ac protein expression level (3.1 µg/g fwt) in pollen from MON 87701. In addition, appropriate negative controls (buffer in 30% honey solution and 30% honey alone) and positive controls (50 and 200 µg potassium arsenate/ml of diet) were included in the study. The results indicate there are no significant differences ($p>0.05$) in the mean survival of *P. foveolatus* adults among the Cry1Ac (98.8%), buffer control (98.8%), and assay control (97.7%) treatments. Percent mortalities for the positive controls of 50 and 200 µg/ml potassium arsenate in 30% honey were 16.7% and 100%, respectively, confirming the validity of the test system. The NOEC of the Cry1Ac protein for the parasitic wasp, *Pediobius foveolatus*, was ≥250 µg/ml.

8. Evaluation of Potential Dietary Effects of Harvested Seed from Insect-protected Soybean MON 87701 on the Northern Bobwhite in an Eight-day Dietary Test.

Hubbard, P.M. and J.B. Beavers. 2008a. Evaluation of Potential Effects of Grain from Insect-protected Soybean MON 87701 on the Northern Bobwhite in an Eight-day Study. Monsanto Study Number WL-2008-048.

Hubbard, P.M. and J.B. Beavers. 2008b. Effects of Conventional Raw Soybean in a Dietary Study with the Northern Bobwhite. Monsanto Study Number WL-2007-251.

The objective of this test was to examine the potential effects from a dietary exposure to harvested soybean seed from MON 87701 to bobwhite quail (*Colinus virginianus*).

Bobwhite quail are a commonly used surrogate species to develop data on dietary toxicity and are one of U.S. EPA's preferred test species. The test procedure followed the methodology of U.S. EPA ecological effects test guideline OPPTS Guideline Number 850.2200, which provides specific guidance for testing bobwhite quail. Groups of 30 bobwhite quail, 10 days of age, were fed diets for eight days containing 20% (w/w) raw ground soybean seed from MON 87701, a conventional soybean control variety, or three different conventional soybean varieties. A dietary level of 20% soybean seed in the diet was chosen because a previous study indicated the no-observed effect level for raw soybean was less than 25%, but greater than 20% raw soybean seed fed to quail (Hubbard and Beavers 2008b). No toxicity or adverse impact on behavior, body weight or food consumption was observed for quail fed diets containing 20% raw ground soybean seed from MON 87701. Therefore, the 8-day dietary LC_{50} was >20% soybean seed from MON 87701 and the NOEC was ≥20% soybean seed from MON 87701.

Taken together, the results from the short-term study with bobwhite quail demonstrate that no significant risk to wild avian species is anticipated from consumption of harvested seed from MON 87701. This evaluation is considered to be acceptable for assessing short-term risk to wild bird populations because: 1) the test followed accepted methodology for assessing short-term risk to wild avian populations, and 2) juvenile birds were tested at a high dietary level of soybean seed from MON 87701.

MONSANTO

Petition for the Determination of Nonregulated Status for Dicamba-Tolerant Soybean MON 87708

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 Part 340

July 6, 2010

OECD Unique Identifier: MON-877Ø8-9

Monsanto Petition Number: 10-SY-210U

Submitted by:

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Monsanto Company
800 North Lindbergh Blvd.

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RELEASE OF INFORMATION

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party. In the event the USDA receives a Freedom of Information Act request, pursuant to 5 U.S.C., § 552, and 7 CFR § 1, covering all or some of this information, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, *e.g.*, responsiveness, confidentiality, and/or competitive concerns. Monsanto understands that a copy of this information may be made available to the public in a reading room and upon individual request as part of a public comment period. Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

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CERTIFICATION

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

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EXECUTIVE SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status in whole for the new biotechnology-derived soybean product, MON 87708, any progeny derived from crosses between MON 87708 and conventional soybean, and any progeny derived from crosses of MON 87708 with other biotechnology-derived soybean that has been granted nonregulated status under 7 CFR Part 340.

Product Description

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® Roundup Ready and Roundup Ready 2 Yield are registered trademarks of Monsanto Technology LLC.

MON 87708 contains a gene from *Stenotrophomonas maltophilia* that expresses a mono-oxygenase enzyme that rapidly demethylates dicamba rendering it inactive, thereby conferring tolerance to dicamba. The demethylation of dicamba produces 3,6-dichlorosalicylic acid (DCSA), a known soybean, soil, and livestock metabolite whose safety has been evaluated by the Environmental Protection Agency (EPA). DCSA, in addition to dicamba, is included in the current 10 ppm pesticide residue tolerance for soybean seed that supports the existing uses of dicamba on commercial soybean (40 CFR § 180.227). Even with the expanded use of dicamba on MON 87708, compared to commercial soybean uses, the rapid metabolism of dicamba results in residues in dicamba-treated MON 87708 seed, including the DCSA metabolite, that are well below the established 10 ppm tolerance. Consequently, only approval for the expanded use pattern of dicamba on MON 87708 has been requested of EPA.

Data and Information Presented Confirm the Lack of Plant Pest Potential of MON 87708 Compared to Conventional Soybean

The data and information presented in this petition demonstrate MON 87708 is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its tolerance to dicamba. Moreover, the data presented demonstrate MON 87708 is unlikely to pose an increased plant pest risk, including weediness or adverse environmental impact, compared to conventional soybean. The food, feed, and environmental safety of MON 87708 was confirmed based on multiple, well-established lines of evidence:

1. Soybean is a familiar crop that does not possess any of the attributes commonly associated with weeds and has a history of safe consumption.

2. A detailed molecular characterization of the inserted DNA demonstrated a single, intact copy of the T-DNA insert in a single locus within the soybean genome.

3. Data confirmed that the dicamba mono-oxygenase (DMO) in MON 87708 (MON 87708 DMO) is unlikely to be a toxin or allergen based on extensive information collected.

4. A compositional assessment of seed and forage confirmed that MON 87708 is compositionally equivalent to conventional soybean.

5. An extensive evaluation on phenotypic and agronomic characteristics and environmental interactions of MON 87708 demonstrated no increased plant pest potential compared to conventional soybean.

6. An assessment of potential impact on non-target organisms (NTOs) and endangered species indicated that, under normal agricultural conditions, MON 87708 is unlikely to have adverse effects on these organisms compared to conventional soybean.

7. Evaluation of MON 87708 using current cultivation and management practices for soybean concluded that deregulation of MON 87708 will not significantly impact soybean agronomic practices or land use.

Soybean is a Familiar Crop Lacking Weedy Characteristics

There is a longstanding history of safe use and consumption of conventional soybean and processed products. Soybean is grown as a commercial crop in over 35 countries. Domestication occurred as early as 1000 B.C. and is now the most widely grown oilseed crop in the world, with approximately 211 million metric tons of harvested seed produced in 2008, which represented 56% of world oilseed seed production that year.

The commercial soybean species in the U.S. (*Glycine max* L. Merr.) does not exhibit weedy characteristics, does not invade established ecosystems, and does not outcross to weedy relatives. Soybean is not listed as a weed in major weed references, nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). During 2004 to 2008, U.S. growers planted between 64.7 and 75.7 million acres of soybean. Soybean does not possess any of the attributes commonly associated with weeds, such as long persistence of the seed in the soil, ability to disperse, invade, or become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. However, due to a pronounced lack of dormancy it is known that soybean seed can germinate quickly under adequate temperature and moisture conditions, and can potentially grow as a volunteer plant. However, a volunteer soybean plant likely would be killed by frost during the autumn or winter of the year it germinated. Furthermore, if a volunteer plant were to survive, it would not compete well with the succeeding crop, and would be controlled readily via mechanical or other chemical means. (b) (4) [redacted] (b) (4) Finally, since wild populations of *Glycine* species are not known to exist in the U.S., there is no potential for MON 87708 to outcross to wild or weedy relatives.

Conventional Soybean A3525 is an Appropriate Comparator to MON 87708

Soybean variety A3525 is the near isogenic line to MON 87708 and was used as the conventional soybean comparator to support the safety assessment of MON 87708. MON 87708 and the near isogenic conventional soybean control A3525 have similar genetic backgrounds with the exception of the *dmo* expression cassette, thus, the effect of the *dmo* expression cassette and the expressed MON 87708 DMO could be assessed in an unbiased manner.

Molecular Characterization Verifies the Integrity and Stability of the Inserted DNA in MON 87708

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Molecular characterization by Southern blot analyses determined that MON 87708 contains one copy of the T-DNA I at a single integration locus and all expression elements are present. These data also demonstrated that MON 87708 does not contain detectable backbone sequences from the plasmid vector or T-DNA II sequences. The complete DNA sequence of the insert and adjacent genomic DNA sequence in MON 87708 confirmed the integrity of the inserted *dmo* expression cassette within the inserted sequences and identified the 5′ and 3′ insert-to-genomic DNA junctions. Furthermore, Southern blot analysis demonstrated that the insert in MON 87708 has been maintained through at least five generations of breeding, thereby confirming the stability of the insert over multiple generations.

Data Confirm MON 87708 DMO Safety

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A multistep approach was used to characterize MON 87708 DMO. This detailed characterization and assessment confirmed that MON 87708 DMO is safe for human and animal consumption. The assessment involved: 1) characterization of the physicochemical and functional properties of MON 87708 DMO; 2) quantification of MON 87708 DMO levels in plant tissues; 3) comparison of the amino acid sequence of MON 87708 DMO to known allergens, gliadins, glutenins, toxins, and other biologically-active proteins known to have adverse effects on mammals; 4) evaluation of the digestibility of MON 87708 DMO in simulated gastric and intestinal fluids; 5) endogenous and exogenous substrate specificity of DMO; 6) documentation of the history of safe consumption of mono-oxygenases (the class of enzymes to which MON 87708 DMO belongs); and 7) investigation of the potential mammalian toxicity through an oral gavage assay.

DMO was found to be specific to dicamba when tested using structurally similar endogenous substrates and exogenous herbicide substrates representing a wide range of modes-of-action. MON 87708 DMO has no relevant amino acid sequence similarities with known allergens, gliadins, glutenins, toxins, and other biologically-active proteins that may have adverse effects on mammals. MON 87708 DMO was rapidly degraded in simulated gastric and intestinal fluids and a high dose of this protein in a mouse acute oral toxicity evaluation demonstrated that it is not acutely toxic, and does not cause any adverse effect. The safety assessment supports the conclusion that exposure to MON 87708 DMO poses no meaningful risk to the environment, or human and animal health.

MON 87708 is Compositionally Equivalent to Conventional Soybean

Detailed compositional analyses in accordance with OECD guidelines were conducted to assess whether levels of key nutrients and anti-nutrients in MON 87708 were comparable to levels present in the aforementioned near isogenic conventional soybean control A3525 and several commercial reference soybean varieties. Seed and forage were harvested from five individual sites in which MON 87708 (treated with dicamba herbicide at the V2-V3 growth stage), the conventional control, and a range of commercial reference varieties were grown concurrently in the same field trial. The commercial reference varieties used to establish a range of natural variability for the key nutrients and anti-nutrients in commercial soybean varieties have a history of safe consumption. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), fiber, amino acids (18 components), fatty acids (FA, C8-C22), and vitamin E (α-tochopherol) in seed, and proximates (ash, carbohydrates by calculation, moisture, protein, and fat) and fiber in forage. The anti-nutrients assessed in seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitors, and isoflavones (daidzein, genistein, and glycitein).

The combined-site analysis was conducted to determine statistically significant differences (5% level of significance) between MON 87708 and the near isogenic conventional control A3525. The results from the combined-site data were reviewed using considerations relevant to food and feed safety and nutritional quality. These considerations included assessments of: 1) the relative magnitudes of the difference in the mean values of nutrient and anti-nutrient components of MON 87708 and the conventional control, 2) whether the MON 87708 component mean value was within the range of natural variability of that component as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same field trial, 3) analyses of the reproducibility of the statistically significant combined-site component differences at individual sites, and 4) assessing the differences within the context of natural variability of commercial soybean composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

Assessment of the analytical results confirmed that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 87708 soybean. In addition, the levels of assessed components in MON 87708 were compositionally equivalent to the conventional control and within the range of variability of the commercial reference varieties that were grown concurrently in the same field trial.

MON 87708 Does Not Change Soybean Plant Pest Potential or Environmental Interactions

Assessing the plant pest potential of a biotechnology-derived crop includes the concept of familiarity that the USDA recognizes as an important consideration. Familiarity is based upon the fact that the new biotechnology-derived plant is developed from a conventional plant variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the receiving environment, and the interactions among these factors that provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Following this concept, the phenotypic, agronomic, and environmental interaction assessment of MON 87708 included the near isogenic conventional soybean control A3525 and the commercial reference varieties. Characteristics

assessed included: seed dormancy and germination, pollen morphology, and symbiont interactions conducted in the laboratory and greenhouse; and plant phenotypic and agronomic evaluations and environmental interaction observations conducted in the field. The commercial soybean reference varieties grown concurrently were used to establish a range of natural variability for each assessed characteristic in soybean. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 87708 is equivalent to the conventional control. Thus, MON 87708 is unlikely to have a changed plant pest potential compared to conventional soybean.

Seed dormancy and germination characterization demonstrated that MON 87708 seed had germination characteristics similar to seed of the conventional control. In particular, the lack of hard seed, a well-accepted characteristic of weediness affecting seed germination, supports a conclusion of no increased weediness of MON 87708 when compared to the conventional control. For pollen characteristics and symbiont interactions, there were no statistically significant differences (5% level of significance) observed between MON 87708 and the conventional control for any of the parameters measured, including pollen viability and diameter, nodule number and dry weight, shoot total nitrogen, and shoot and root dry weight. Collectively, these results support the conclusion that MON 87708 is not likely to exhibit increased plant pest potential compared to conventional soybean.

The field evaluation of phenotypic, agronomic, and environmental interaction characteristics of MON 87708 also support the conclusion that MON 87708 is not likely to have an increased plant pest potential compared to conventional soybean. The evaluations were conducted at 18 replicated field sites across North American soybean production regions. These assessments included plant growth and development characteristics, as well as observations for plant responses to abiotic stressors and plant-disease and plant-arthropod interactions. The observed phenotypic characteristics were similar between MON 87708 and the conventional control.

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In an assessment of arthropod-related damage, no statistically significant differences (5% level

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In summary, the phenotypic, agronomic, and environmental interaction data were collected to provide a detailed characterization of MON 87708 and to assess whether the introduction of the dicamba-tolerance trait in MON 87708 alters the plant pest potential compared to conventional soybean. The analysis considered the comparisons of MON 87708 to the conventional control, the reproducibility, magnitude, and direction of detected differences (trends), and comparison to the range of the commercial reference varieties. Results from the phenotypic, agronomic, and environmental interactions assessment indicated that MON 87708 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to conventional soybean.

MON 87708 Will Not Adversely Affect NTOs or Threatened and Endangered Species

Evaluation of the impacts of a biotechnology-derived crop on Non-Target Organisms (NTOs) and threatened and endangered species is a component of the plant pest risk assessment. Since MON 87708 does not possess pesticidal activity, all organisms that interact with MON 87708 are considered to be NTOs. The environmental assessment demonstrated that the presence of the dicamba-tolerance trait in MON 87708 did not alter plant-arthropod interactions, including beneficial arthropods, or alter disease susceptibility compared to the conventional control.

The biochemical information and experimental data for evaluation of MON 87708 included molecular characterization, MON 87708 DMO safety assessments, the history of environmental exposure to mono-oxygenases (the class of enzymes to which MON 87708 DMO belongs), information from the environmental interaction assessment, demonstration of compositional equivalence to conventional soybean, and demonstration of agronomic and phenotypic equivalence to conventional soybean. Taken together, these data support the conclusion that MON 87708 has no reasonable mechanism for harm to NTOs, or to pose an additional risk to threatened and endangered species compared to the cultivation of conventional soybean.

The potential for outcrossing and gene introgression from MON 87708 to sexually-compatible species in the U.S. is unlikely since no known wild *Glycine* species related to cultivated soybean are known to be present in North America. Furthermore, should cross-pollination occur, MON 87708 and its progeny are not expected to exhibit a significant environmental impact because, as described above, evaluations have shown that the presence of the dicamba-tolerance trait is not likely to enhance weediness or plant-pest potential. Therefore, the environmental consequence of pollen transfer from MON 87708 to other *Glycine* species is considered negligible.

Deregulation of MON 87708 Will Not Significantly Impact Soybean Agronomic Practices or Land Use

Soybean fields are typically highly managed agricultural areas that are dedicated to crop production for many years. Cultivation of MON 87708 would not be expected to differ from typical soybean cultivation, with the sole exception of an expanded window of dicamba applications due to the presence of the dicamba-tolerance trait in MON 87708. MON 87708 likely would be used in common rotations on land currently used for agricultural purposes. As demonstrated, MON 87708 is similar to conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics and has comparable levels of resistance to insects and diseases as compared to commercial soybean. Therefore, the introduction of MON 87708 into the Roundup Ready soybean system is not (b) (4) [redacted] Based on these considerations, there is no apparent potential for significant impacts on agronomic practices or land use.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 87708 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87708 and any progeny derived from crosses between MON 87708 and conventional soybean or previously deregulated biotechnology-derived soybean, be granted nonregulated status under 7 CFR Part 340.

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ABBREVIATION AND DEFINITIONS*

~	approximately
aadA	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 that confers spectinomycin and streptomycin resistance
AAPSE	American Association of Pesticide Safety Educators
ADF	acid detergent fiber
ALS	acetolactate synthase
AOSA	Association of Official Seed Analysts
aPAD	acute population adjusted dose
APHIS	Animal and Plant Health Inspection Service
B. japonicum	*Bradyrhizobium japonicum*
BIO	Biotechnology Industry Organization
bp	base pair
BRS	Biotechnology Regulatory Services
BSA	bovine serum albumin
bu/A	bushels per acre
CEQ	Council on Environmental Quality
CES	Cooperative Extension Service
CFR	Code of Federal Regulations
CHT	ceramic hydroxyapatite column
cp4 epsps	codon modified coding sequence of the *aroA* gene from *Agrobacterium species* strain CP4 encoding CP4 EPSPS
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase protein
cPAD	chronic population adjusted dose
CTAB	Hexadecyltrimethylammonium bromide
CTP2	Sequences encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS used to direct proteins into chloroplasts
CV	column volume
Da	Dalton
DAP	days after planting
dATP	Deoxyadenosine triphosphate
DCSA	3,6-dichlorosalicylic acid also known as 3,6-dichloro-2-hydroxybenzoic acid
(b) (4)	[redacted]
dCTP	Deoxycytidine triphosphate
DDI	daily dietary intake
DEEM	dietary exposure evaluation model
DGA	diglycolamine
DHB	dihydroxybenzoic acid
dicamba	3,6-dichloro-2-methoxybenzoic acid
dmo	Coding sequence of the dicamba mono-oxygenase gene from

(b) (4) [redacted]

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

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DnaK	5′ non-translated leader sequence from the *Petunia hybrid Hsp70* gene that is involved in regulating gene expression
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
DWCF	dry weight conversion factor
dwt	dry weight of tissue
E9	3′ non-translated region of the pea *RbcS2* gene that functions to direct polyadenylation of the mRNA
E. coli	*Escherichia coli*
EEC	estimated environmental exposure concentration
EFED	Environmental Fate and Effects Division
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ERS	Economic Research Service
ETA	Excellence Through Stewardship
Exo	exonuclease I
FA	fatty acid
FASTA	algorithm used to find local high scoring alignments between a pair of protein or nucleotide sequences
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FLt	*full-length transcript*
FMV	Promoter for the figwort mosaic virus 35S RNA
FQPA	Food Quality Protection Act
fwt	fresh weight of tissue
GLP	good laboratory practice
H.U.	hemagglutinating unit
HAL	health advisory level
HED	Health Effects Division
HPPD	4-hydroxyphenylpyruvate dioxygenase
HRP	horseradish peroxidase
IAA	indole acetic acid
ILSI	International Life Sciences Institute
IPM	integrated pest management
kb	kilobase
kDa	kiloDalton
LB	loading buffer
lb/bu	pounds per bushel
LC	lethal concentration
LD	lethal dose
Left Border	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA
LOAEL	lowest dosing level that produced an observable effect
LOC	level of concern
LOD	limit of detection
LOEC	lowest observed effect concentration
LOQ	limit of quantitation
MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass

	spectrometry
MMT	million metric tons
MOE	margin of exposure
(b) (4)	[redacted]
MSTA	Monsanto Technology Stewardship Agreement
MW	molecular weight
MWCO	molecular weight cut off
N/A	not applicable
NADH	nicotinamide adenine dinucleotide
NASS	National Agricultural Statistics Service, a branch of the USDA
NAWQA	National Water-Quality Assessment
NDF	neutral detergent fiber
NEPA	National Environmental Policy Act
NFDM	non-fat dried milk
NOAEL	no observable adverse effect level
NOEC	no observable effect concentration
NTOs	non-target organisms
OECD	Organization of Economic Co-Operation and Development
OPP	Office of Pesticide Programs
ORETF	Outdoor Residential Exposure Task Force
ori-pBR322	Origin of replication from pBR322 necessary for maintenance of plasmid in *E. coli*
ori V	Origin of replication from the broad host range plasmid RK2 necessary for maintenance of plasmid in *Agrobacterium*
OSL	over-season leaf
PAD	population adjusted dose
PBS	phosphate buffered saline solution
PBST	phosphate buffered saline solution containing 0.05% (v/v) Tween-20
PC1SV	Promoter for the full length transcript of peanut chlorotic streak virus that directs transcription in plant cells
PCR	polymerase chain reaction
pea	*Pisum sativum*
PHED	Pesticide Handler Exposure Database
RED	Reregistration Eligibility Decision
RfD	reference dose
PMSF	phenylmethylsulfonyl fluoride
PPA	Plant Protection Act
PPO	protoporphyrinogen oxidase
PRZM/EXAMS	Pesticide Root Zone Model/Exposure Analysis Modeling System
PVDF	polyvinylidene difluoride
RbcS	Sequences encoding the transit peptide region of the ribulose-1,5-bisphosphate carboxylase oxygenase gene from *Pisum sativum*
Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA
rop	Coding sequence for repressor of primer protein used for maintenance of plasmid copy number in *E. coli*
RQ	risk quotient
RT	room temperature
SAP	shrimp alkaline phosphate

SCI-GROW	Screening Concentration in Ground Water
SCN	soybean cyst nematode
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
S.E.	standard error
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
S. maltophilia	*Stenotrophomonas maltophilia*
soybean	*Glycine max* (L.) Merr.
T-DNA	transfer(ed) DNA
TBS	tris buffered saline
TES	threatened and endangered species
TEV	5′ non-translated region from the Tobacco Etch RNA virus genome that is involved in regulating gene expression
TFA	trifluoroacetic acid
TIU	trypsin inhibitor units
Tm	melting temperature
TMRC	theoretical maximum resiude concentration
TMB	3,3',5,5' tetramethyl-benzidine
TRED	tolerance reregistration eligibility decision
TSSP	tissue-specific pool
TUG	Technology Use Guide
U.S.	United States of America
USDA	U.S. Department of Agriculture
v/v	volume per volume
VMD	volume median diameter
w/v	weight per volume
WAT	weeks after treatment
WPS	Worker Protection standard
WSSA	Weed Science Society of America

I. RATIONALE FOR THE DEVELOPMENT OF MON 87708

I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR § 340.6

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for new biotechnology-derived soybean product, MON 87708, any progeny derived from crosses between MON 87708 and conventional soybean, and any progeny derived from crosses of MON 87708 with biotechnology-derived soybean that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Dicamba-Tolerant Soybean MON 87708

Total soybean (*Glycine max* (L.) Merr.) planted acreage in the U.S. has varied in the past decade from approximately 64.7 to 75.7 million acres, with the lowest acreage recorded in 2007 and the highest in 2008. During this period, the value of U.S. soybean reached $27.4 billion in 2008 (USDA-NASS, 2009b). The net value (production less operating cost) of soybean in the U.S. was reported to be $318.66 per acre (USDA-ERS, 2008). A major component of soybean crop production economics is managing input costs. Key decisions by growers on input costs include choosing the seed or soybean varieties to plant, and determining the appropriate pesticide management program to use, this includes weed control. There have been significant reductions in weed control costs in soybean production following the introduction of the Roundup Ready® soybean system in 1996. It is estimated that U.S. growers saved a net $216 million in weed control costs in 1999 compared to 1995, the year before Roundup Ready soybean was introduced (Carpenter, 2001). The Roundup Ready soybean system incorporates the application of Roundup® agricultural herbicides containing the active ingredient glyphosate for effective weed control. As with all herbicides used in agriculture, there is potential for weeds to develop resistance to the herbicide over time.[1] Glyphosate has had relatively few cases of weed resistance, particularly in relation to other herbicides, although a limited number of glyphosate-resistant weeds have developed. Glyphosate-resistant weeds are controlled by herbicides with other modes of action that are typically used sequentially or in combination with glyphosate.

® Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC. All other trademarks are the property of their respective owners.

[1] http://www.weedscience.org/In.asp

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MON 87708 contains a gene from *Stenotrophomonas maltophilia* that expresses a mono-oxygenase enzyme that rapidly demethylates dicamba rendering it inactive, thereby conferring tolerance to dicamba. The demethylation of dicamba produces 3,6-dichlorosalicylic acid (DCSA), a known soybean, soil, and livestock metabolite whose safety has been evaluated by the Environmental Protection Agency (EPA). DCSA, in addition to dicamba, is included in the current 10 ppm pesticide residue tolerance for soybean seed that supports the existing uses of dicamba on conventional soybean (40 CFR § 180.227). Even with the expanded use of dicamba on MON 87708, compared to conventional soybean uses, the rapid metabolism of dicamba results in residues in dicamba-treated MON 87708 seed, including the DCSA metabolite, that are well below the established 10 ppm tolerance. Consequently, only approval for the expanded use pattern of dicamba on MON 87708 has been requested of EPA.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived crops that do not include plant-incorporated protectants falls on two federal agencies: U.S. Food and Drug Administration (FDA) and United States Department of Agriculure (USDA).

Deregulation of MON 87708 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87708 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions. Additionally, EPA must complete its review and assessments prior to approving the use of dicamba on MON 87708.

I.C.1. Submission to FDA

MON 87708 falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (FDA, 1992). In compliance with this policy, Monsanto has initiated a consultation with the FDA (BNF No. 125) on the food and feed safety and compositional assessment of MON 87708. A safety and nutritional assessment summary document for MON 87708 will be submitted to FDA in the second half of 2010.

I.C.2. Submission to EPA

The EPA has authority over the use of pesticidal substances under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended (7 U.S.C. § 136 *et seq.*). Monsanto has submitted to the EPA an application to amend Registration Number 524-582 to register a new use pattern for dicamba on MON 87708. (b) (4) [redacted] EPA has reviewed the safety of dicamba and DCSA, the primary metabolite in MON 87708, during the reregistration of dicamba in 2006. EPA concluded in the 2006 dicamba Reregistration Eligibility Decision (RED) document that risks to human health and the environment associated with exposure to dicamba and its metabolites, including DCSA, were below the Agency's level of concern for all registered uses of dicamba including conventional soybean (EPA, 2009). Dicamba residues on soybean seed (less than 0.07 ppm average residue and less than 0.5 ppm maxiumum residue) resulting from its application on MON 87708 at the maximum labeled use rate are well below the established 10 ppm soybean seed pesticide residue tolerance. Therefore, a change to the current soybean seed tolerance is not needed to support the use of dicamba on MON 87708. However Monsanto has requested the establishment of new tolerances for soybean forage and hay, which will allow for the feeding of forage and hay to livestock. No other revisions to dicamba pesticide residue tolerances are needed including animal products such as meat or milk. Furthermore, the use of dicamba on MON 87708 does not present any new environmental exposure scenarios not previously evaluated and deemed acceptable by EPA. Additional details regarding dicamba and its use on MON 87708 are available in Appendix M).

I.C.3. Submissions to Foreign Government Agencies

To support commercial introduction of MON 87708 in the U.S., regulatory submissions will be made to countries that will eventually commercialize or import significant quantities of soybean or its processed fractions from the U.S. These will include submissions to a number of foreign government regulatory authorities, including:

Ministry of Agriculture, People's Republic of China; Japan's Ministry of Agriculture, Forestry, and Fisheries, Ministry of Environment, and the Ministry of Health, Labor, and Welfare; the Canadian Food Inspection Agency and Health Canada; the Intersectoral Commission for Biosafety of Genetically Modified Organisms, Mexico; the European Food Safety Authority, as well as to regulatory authorities in other soybean importing countries with functioning regulatory systems. As appropriate, notifications of importation will be made to importing countries that do not have a formal approval process.

II. THE BIOLOGY OF SOYBEAN

The Organization for Economic Co-Operation and Development (OECD) Consensus Document (OECD, 2000) on the biology of soybean provides key information on:

- a general description of soybean biology, including taxonomy and morphology as well as soybean use as a crop plant
- agronomic practices in soybean cultivation
- geographic centers of origin
- reproductive biology
- cultivated soybean as a volunteer weed
- inter-species/genus introgression into relatives and interactions with other organisms, and
- a summary of the ecology of soybean

The taxonomic information for soybean is available in the USDA's PLANTS Profile (USDA-NRCS, 2010).

To support the evaluation of the plant pest potential of MON 87708 relative to conventional soybean, additional information regarding several aspects of soybean biology can be found elsewhere in this petition. This includes: agronomic practices for soybean in Section VIII; volunteer management of soybean in Section VIII.J; and inter-species/genus introgression potential in Section VII.C.3.

II.A. Soybean as a Crop

Soybean is the most widely grown oilseed in the world, with approximately 211 million metric tons of harvested seed produced in 2008. This represents 56% of world oilseed seed production that year. Soybean is grown as a commercial crop in over 35 countries. The major producers are the U.S., Brazil, Argentina, China, India, and Paraguay, accounting for approximately 94% of the global soybean production in 2008. Approximately one-third of the 2008 world soybean production was in the U.S. (Soyatech, 2010). The U.S. was also the largest soybean seed exporting country in 2008 (ASA, 2009).

Soybean has a long history of planting and production in North America. Soybean was originally introduced into North America from China in 1765 and has since been reintroduced several times by scientists, seed dealers, merchants, military expeditions, and various individuals (Singh and Hymowitz, 1999). Conventional plant breeding is based on the interplay and combination of genes present in the particular crop genome, and soybean is limited with regard to genetic diversity (Chung and Singh, 2008).

II.B. Characteristics of the Recipient Plant

The conventional soybean variety A3525, used as the recipient for the *dmo* expression cassette insertion that produced MON 87708, was developed by Asgrow Seed Company. A3525 is a mid−maturity group III soybean variety with very high yield potential.

A3525 has superior yields relative to varieties of similar maturity and has excellent agronomic characteristics (Steffen, 2004).

II.C. Soybean as a Test System in Product Safety Assessment

Soybean variety A3525 is the near isogenic line to MON 87708 and was used as the conventional soybean comparator (hereafter referred to as the conventional control) in the safety assessment of MON 87708. MON 87708 and the conventional control have similar genetic backgrounds with the exception of the *dmo* expression cassette, thus, the effect of the *dmo* expression cassette and the expressed MON 87708 DMO could therefore be assessed in an unbiased manner. In addition, commercial soybean varieties that were derived through conventional methods and Roundup Ready soybean varieties (hereafter referred to as commercial reference varieties), were used as reference materials to establish ranges of natural variability or responses representative of commercial soybean varieties. The commercial reference varieties used at each location were selected based on their availability and agronomic fit for the respective geographic region.

III. DESCRIPTION OF THE GENETIC MODIFICATION

MON 87708 was developed through *Agrobacterium tumefaciens*-mediated transformation of conventional soybean A3525 meristem tissue utilizing transformation plasmid vector PV-GMHT4355. This section describes the plasmid vector, the donor genes, and the regulatory elements used in the development of MON 87708 and the deduced amino acid sequence of the MON 87708 DMO. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences.

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(b) (4) [redacted]

III.B. Description of the Transformation System

The *Agrobacterium*-mediated soybean transformation used to produce MON 87708 was based on the method described by Martinell et al. (2002), which allows for the generation of transformed plants without utilization of callus. Briefly, meristem tissues were excised from the embryos of germinated conventional seed. After co-culturing with the *Agrobacterium* carrying the vector, the meristems were placed on selection medium containing glyphosate, carbenicillin, cefotaxime, and ticarcillin/clavulanate acid mixture, to inhibit the growth of untransformed plant cells and excess *Agrobacterium*. The meristems were then placed in media conducive to shoot and root development. Rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R_0 plants generated through this transformation were self-pollinated to produce R_1 plants, and the unlinked insertions of T-DNA I and T-DNA II were segregated. A non-lethal dose of glyphosate was applied to R_1 plants and those plants with minor herbicide injury were selected for further analyses, whereas plants showing no injury, indicating that they contained the *cp4 epsps* coding sequence from T-DNA II, were eliminated from further development. Subsequently, plants that were homozygous for T-DNA I were identified by quantitative polymerase chain reaction (PCR) analysis. MON 87708 was selected as the lead event based on superior phenotypic characteristics, dicamba tolerance, and its molecular profile. The major development steps of MON 87708 are depicted in Figure III-2. The result of this process was the production of marker-free, dicamba-tolerant soybean MON 87708.

III.C. The *dmo* Coding Sequence and MON 87708 DMO (T-DNA I)

The *dmo* expression cassette (T-DNA I) present in MON 87708 encodes MON 87708 DMO (Figure III-3). The *dmo* expression cassette contains the coding region for the DMO from *Stenotrophomonas maltophilia* (Herman et al., 2005; Wang et al., 1997). The presence of MON 87708 DMO confers tolerance to dicamba (refer to Section V.A for more details).

III.D. The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein (T-DNA II)

The *cp4 epsps* expression cassette (T-DNA II), that is not present in MON 87708, encoded a 47.6 kDa CP4 EPSPS protein, consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996b). The *cp4 epsps* coding sequence is the codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS (Barry et al., 1997; Padgette et al., 1996a). CP4 EPSPS confers tolerance to glyphosate and was used as a selectable marker during the transformation selection process. Through conventional self-pollinated breeding methods and segregation, along with a combination of analytical techniques, plants that did not contain the *cp4 epsps* expression cassette were isolated.

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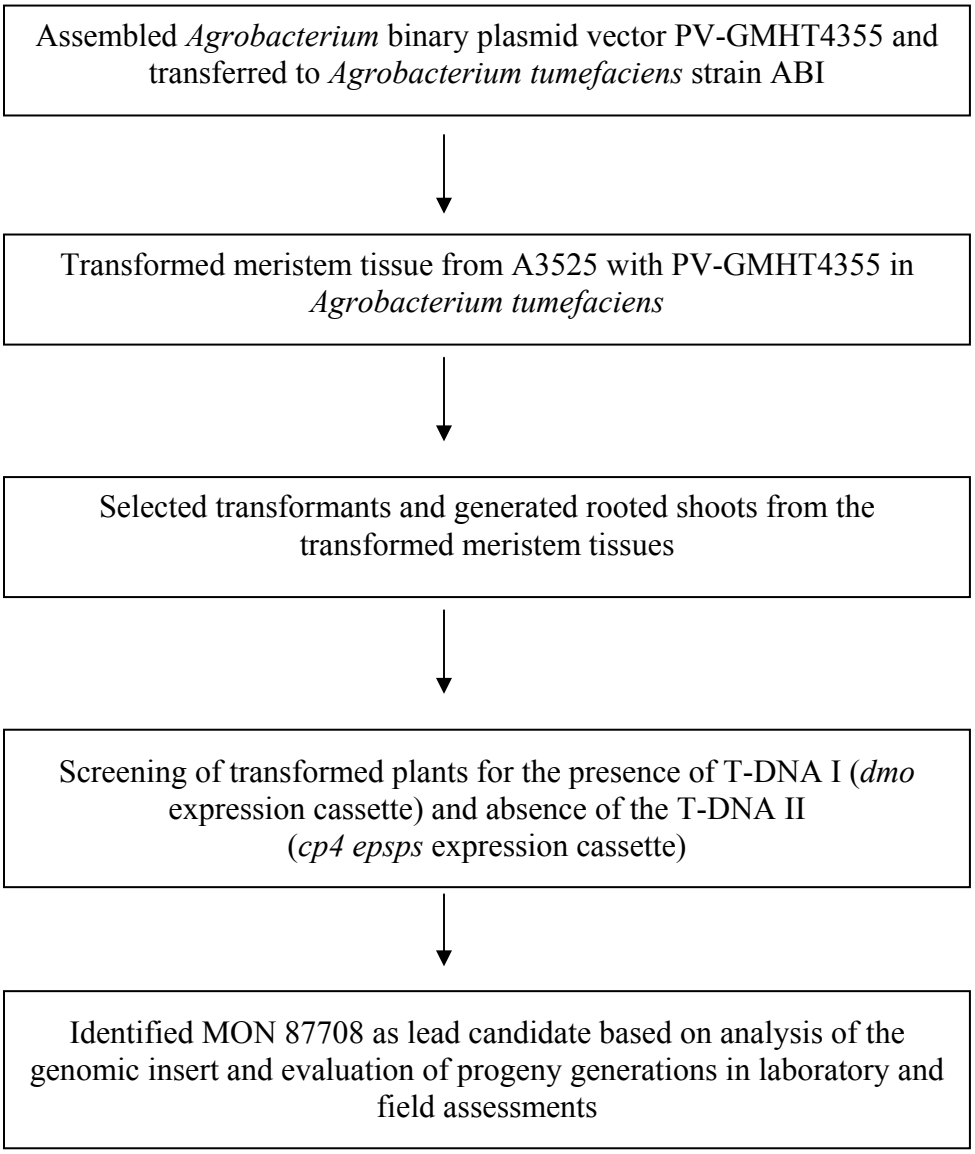

Figure III-2. Schematic of the Development of MON 87708

III.E. Regulatory Sequences

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III.F. T-DNA Borders

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III.G. Genetic Elements Outside of the T-DNA Borders

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IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

A multi-faceted approach was taken to characterize the genetic modification that produced MON 87708. The results confirmed that MON 87708 contains a single copy of the *dmo* expression cassette (T-DNA I) that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations (Section IV.G). The results confirmed that no T-DNA II or plasmid vector backbone sequences are detected in MON 87708. These conclusions are based on several lines of evidence: 1) Southern blot analyses to assay the entire soybean genome for the presence of DNA derived from PV-GMHT4355, and to confirm that a single copy of T-DNA I was inserted at a single site and that the insert is stably inherited; 2) DNA sequencing analyses to determine the exact sequence of the inserted DNA and allowed a comparison to the T-DNA I sequence in PV-GMHT4355 to confirm that only the expected sequences were integrated; and 3) a comparison of the DNA flanking T-DNA I to the sequence of the insertion site in conventional soybean to identify any rearrangements that occurred at the insertion site during transformation. Taken together, the characterization of the genetic modification demonstrates that a single copy of the T-DNA I was inserted at a single locus of the genome.

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The DNA sequencing analyses complement the Southern blot analyses. Southern blot results demonstrated that MON 87708 contains a single copy of T-DNA I at a single insertion site. Sequencing of the insert and the flanking DNA confirmed the organization of the elements within the insert, determined the 5′ and 3′ insert-to-plant junctions, as well as the complete DNA sequence of the insert and adjacent DNA. In addition, DNA sequencing analyses confirmed that each genetic element in the insert is intact and the sequence of the insert matches the corresponding sequence in PV-GMHT4355. Furthermore, genomic organization at the insertion site was assessed by comparing the insert and flanking sequence to the insertion site in conventional soybean.

The stability of the T-DNA I present in MON 87708 across multiple generations (R_2-R_6) was demonstrated by Southern blot analysis. Genomic DNA from five generations of MON 87708 was digested with one of the enzyme sets used for the insert and copy number analysis and was hybridized with a probe that detects restriction segments that encompass the entire T-DNA I. This fingerprint strategy consists of two border segments that assess not only the stability of T-DNA I, but also the stability of genomic DNA directly adjacent to T-DNA I.

The results of these analyses for MON 87708 demonstrated that a single copy of the T-DNA I was inserted at a single locus of the genome. Generational stability analysis demonstrated that an expected Southern blot fingerprint of MON 87708 was maintained through five generations of the breeding history, thereby confirming the stability of T-DNA I in MON 87708. Results from segregation analyses showed heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA I at a single chromosomal locus.

The Southern blot analysis confirmed that T-DNA I reported in Figure IV-1 represents the only detectable insert in MON 87708. Figure IV-1 is a linear map depicting restriction sites within the insert as well as within the known soybean genomic DNA immediately flanking the insert in MON 87708. The circular map of PV-GMHT4355 annotated with the probes used in the Southern blot analysis is presented in Figure III-1. Based on the linear map of the insert and the plasmid map, a table summarizing the expected DNA segments for Southern analyses is presented in Table IV-1. The genetic elements integrated in MON 87708 are summarized in Table IV-2. The generations used are depicted in the breeding history shown in Figure IV-9. Materials and methods used for characterization of T-DNA I in MON 87708 are found in Appendix B.

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IV.A. Insert and Copy Number of T-DNA I in MON 87708

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The results of this analysis are shown in Figures IV-2 through IV-4.

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Table IV-1). These results indicate that the probe is hybridizing to its target sequence.

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IV.B. Southern Blot Analysis to Determine the Presence or Absence of T-DNA II Sequences in MON 87708

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IV.C. Southern Blot Analysis to Determine the Presence or Absence of PV-GMHT4355 Backbone Sequences in MON 87708

To determine the presence or absence of PV-GMHT4355 backbone sequences,

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IV.D. Organization and Sequence of the Insert and Adjacent DNA in MON 87708

The organization of the elements within the T-DNA I was confirmed by DNA sequence

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IV.E. PCR and DNA Sequence Analyses to Examine the MON 87708 Insertion Site

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[redacted] during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

IV.F. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87708

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IV.G. Inheritance of the Genetic Insert in MON 87708

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IV.H. Genetic Modification Characterization Conclusion

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V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE MON 87708 DMO

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This section summarizes: 1) the functionality of DMO; 2) the characterization of MON 87708 DMO; 3) the levels of MON 87708 DMO in plant tissues; 4) assessment of the potential allergenicity of MON 87708 DMO and 5) the food, feed, and environmental safety assessment of MON 87708 DMO. The data support a conclusion that MON 87708 is safe for the environment and human or animal consumption based on several lines of evidence, all of which are summarized below.

V.A. Function of DMO and MON 87708 DMO

DMO was initially purified from *Stenotrophomonas maltophilia (S. maltophilia)* strain DI-6, isolated from soil at a dicamba manufacturing plant (Krueger et al., 1989). DMO is an enzyme that catalyzes the demethylation of dicamba to the non-herbicidal compound DCSA and formaldehyde (Chakraborty et al., 2005). DMO is a Rieske-type non-heme iron oxygenase, that is part of a three component system comprised of a reductase, a ferredoxin, and a terminal oxygenase, in this case the DMO. These three enzymes work together in a redox system similar to many other oxygenases to transport electrons from nicotinamide adenine dinucleotide (NADH) to oxygen and catalyze the demethylation (Behrens et al., 2007) as presented in Figure V-1.

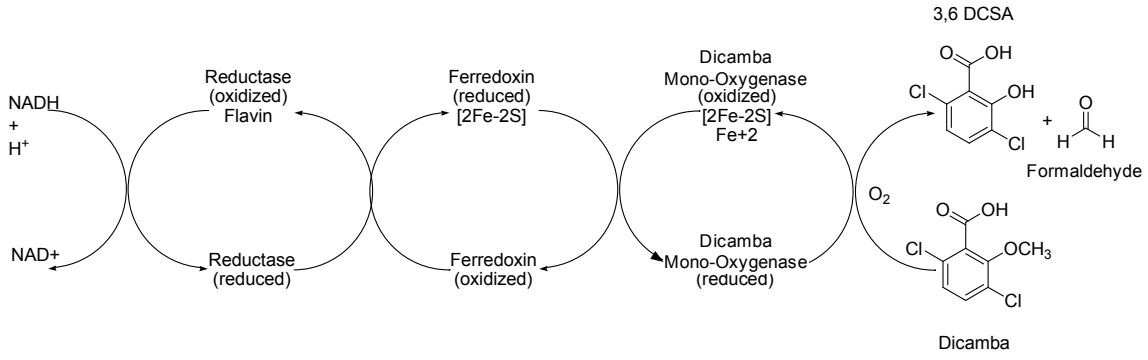

Figure V-1. Three Components of the DMO Oxygenase System

The crystal structure of a DMO has been solved (D'Ordine et al., 2009; Dumitru et al., 2009) and shows that the DMO monomers contain a Rieske [2Fe-2S] cluster domain and a non-heme iron center domain typical of all Rieske-type mono-oxygenases (Ferraro et al., 2005). To catalyze the demethylation of dicamba, electrons transferred from NADH are shuttled through an endogenous reductase and ferredoxin to the terminal DMO. The electrons are received by the Rieske [2Fe-2S] cluster on one DMO monomer and transferred to the non-heme iron center at the catalytic site of an adjacent monomer (D'Ordine et al., 2009; Dumitru et al., 2009), where it reductively activates oxygen to catalyze the final demethylation of dicamba. As a result of the reaction, 3.6 dichlorosalicylic acid (DCSA) and formaldehyde are formed. DCSA is a known metabolite, a known soybean, soil, and livestock metabolite whose safety has been evaluated by the EPA. Formaldehyde is found naturally in many plants at levels up to several thousand ppm (Adrian-Romero, et al.).

V.A.1. Formation of MON 87708 DMO

DMO is targeted to chloroplasts for co-localization with the endogenous reductase and ferredoxin enzymes that supply electrons for the DMO demethylation reaction as described by Behrens et al. (2007). (b) (4) [redacted]

V.A.2. Specificity of MON 87708 DMO

DMO has high specificity for its substrate dicamba (D'Ordine et al., 2009; Dumitru et al., 2009). The specificity of DMO for dicamba is likely due to the specific interactions that occur in the catalytic site between the dicamba substrate and DMO. Dicamba interacts with amino acids in the active site of DMO through both the carboxylate moiety and the chlorine atoms of dicamba, which are primarily involved in orienting the substrate in the

catalytic pocket. These chlorine atoms are required for catalysis (D'Ordine et al., 2009; Dumitru et al., 2009).

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and 2,4-DB (2,4-dichlorophenoxy butanoic acid). See Appendix C, Section C.2.

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V.C. Expression Levels of MON 87708 DMO

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O were less than the limit of quantitation (LOQ) of the assay in all tissue types.

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V.D. Assessment of Potential Allergenicity of MON 87708 DMO

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V.E.2. MON 87708 DMO Belongs to a Common Class of Mono-Oxygenases

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V.E.3. DMO is a Dicamba-Specific Mono-Oxygenase

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V.E.4. MON 87708 DMO is Not a Known Allergen or Toxin

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V.E.5. MON 87708 DMO is Labile in *in vitro* Digestion Assays

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V.E.6. MON 87708 DMO is Not a Toxin

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V.F. MON 87708 DMO Characterization and Safety Conclusion

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VI. COMPOSITIONAL ASSESSMENT OF MON 87708

Safety assessments of biotech crops typically include comparisons of the composition of forage and whole grain of the GM crop to that of conventional counterparts (Codex Alimentarius, 2003). Compositional assessments are performed using the principles and analytes outlined in the OECD consensus documents for soybean composition (OECD, 2001).

A recent review of compositional assessments conducted according to OECD guidelines that encompassed a total of seven GM crop varieties, nine countries and eleven growing seasons concluded that incorporation of biotechnology-derived agronomic traits has had little impact on natural variation in crop composition; most compositional variation is attributable to growing region, agronomic practices and genetic background (Harrigan et al., 2010). Numerous scientific publications have further documented the extensive variability in the concentrations of crop nutrients and anti-nutrients that reflect the influence of environmental and genetic factors as well as extensive conventional breeding efforts to improve nutrition, agronomics and yield. (Reynolds et al., 2005). Compositional equivalence between biotechnology-derived and conventional crops provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 2001). The OECD consensus documents emphasize quantitative measurements of essential nutrients and known anti-nutrients. This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and nutritional concerns. Levels of the components in seed and forage of the biotechnology-derived crop are compared to: 1) corresponding levels in a conventional comparator, the non-biotechnology near isogenic line, grown concurrently, under identical field conditions, and 2) natural ranges generated from an evaluation of commercial reference varieties grown concurrently and from data published in the scientific literature.

The latter comparison places any potential differences between the assessed crop and its comparator in the context of the well-documented variation in the concentrations of crop nutrients and anti-nutrients.

VI.A. Compositional Equivalence of MON 87708 Seed and Forage to Conventional Soybean

Seed and forage samples were collected from MON 87708 and the near isogenic conventional soybean control A3525 grown in a 2008 U.S. field production. Four different commercial reference varieties were included at each site of the field production to provide data on natural variability of each compositional component analyzed. The field production was conducted at five sites: Jefferson County, Iowa; Stark County, Illinois; Clinton County, Illinois; Parke County, Indiana; and Berks County, Pennsylvania. All soybean plants including MON 87708, the conventional control, and the commercial reference varieties were treated with maintenance pesticides as necessary throughout the growing season. In addition, MON 87708 plots were treated at the V2-V3 growth stage with dicamba herbicide at the maximum in-crop label rate (0.5 lb acid equivalence [a.e.]/acre).

Compositional analyses were conducted to assess whether levels of key nutrients and anti-nutrients in MON 87708 were equivalent to levels in the conventional control and to the composition of the commercial reference varieties. A description of nutrients and anti-nutrients present in soybean is provided in the OECD consensus document on compositional considerations for soybean (OECD, 2001). Nutrients assessed included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), fiber, amino acids (18 components), fatty acids (FA, C8-C22), and vitamin E (α-tochopherol) in seed, and proximates (ash, carbohydrates by calculation, moisture, protein, and fat) and fiber in forage. Anti-nutrients assessed in seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitors, and isoflavones (daidzein, genistein, and glycitein).

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VI.A.1. Nutrient Levels in Soybean Seed

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VI.A.2. Anti-Nutrient Levels in Soybean Seed

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VI.B. Compositional Assessment Conclusion

Analyses of nutrient and anti-nutrient levels in MON 87708 and the near isogenic conventional control A3525 were conducted to assess compositional equivalence. The tissues analyzed included seed and forage harvested from plants grown at five field sites in the U.S. during the 2008 field season. The composition analysis, conducted in accordance with OECD guidelines, also included measurement of nutrients and anti-nutrients in the commercial reference varieties concurrently grown with MON 87708 to provide data on natural variability of each compositional component. All soybean plants including MON 87708, the conventional control, and the commercial reference varieties were treated with maintenance pesticides as necessary throughout the growing season. In addition, MON 87708 plots were treated at the V2-V3 growth stage with dicamba herbicide at the maximum in-crop label rate (0.5 lb a.e./acre).

For MON 87708 the combined-site analysis of both seed and forage showed no

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from MON 87708 are compositionally equivalent to that of conventional soybean and that neither the dicamba-tolerance trait in MON 87708, nor the dicamba herbicide treatment, applied according to maximum in-crop label rates (including the associated dicamba residue levels) have a meaningful impact on the composition and therefore on the food and feed safety or the nutritional quality of MON 87708 compared to conventional soybean.

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides an assessment of the phenotypic, agronomic, and environmental interaction characteristics, including plant-symbiont associations of MON 87708 compared to the near isogenic conventional soybean control A3525. The data support a determination that MON 87708 is similar to conventional soybean with the exception of the dicamba-tolerance trait and, therefore, is no more likely to pose a plant pest risk or have a significant environmental impact than conventional soybean. These conclusions are based on the results of multiple evaluations.

Phenotypic, agronomic, and environmental interaction characteristics of MON 87708 were evaluated in a comparative manner to assess plant pest potential. These assessments included evaluation of seed germination characteristics, plant growth and development characteristics, observations for plant responses to abiotic stress, plant-disease and plant-arthropod interactions, pollen characteristics, and plant-symbiont interaction characteristics. Results from the phenotypic, agronomic, and environmental interactions assessment demonstrate that MON 87708 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to the conventional control.

VII.A. Characteristics Measured for Assessment

A detailed phenotypic description of the regulated article is requested as part of the petition for determination of nonregulated status in 7 CFR § 340.6 including differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived". As part of the characterization of MON 87708, data were collected to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 87708 and included an evaluation of specific characteristics related to altered weediness or plant pest potential.

The plant characterization and assessment of MON 87708 encompassed six general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) seed retention on the plant and lodging; 5) plant response to abiotic stress and interactions with diseases and arthropods; and 6) plant-symbiont interactions. An overview of the characteristics assessed is presented in Table VII-1.

The data were evaluated from a basis of familiarity (OECD, 1993) and were comprised of a combination of field, greenhouse, and laboratory assessments conducted by scientists who are familiar with the production and evaluation of soybean. In each of these assessments, MON 87708 was compared to the near isogenic conventional soybean control A3525 that has a genetic background similar to MON 87708 but does not possess the dicamba-tolerance trait. In addition, multiple commercial reference varieties (see Appendices F-I and Tables F-1, G-1, and I-1) were included to provide a range of

comparative values that are representative of existing commercial soybean varieties for each measured phenotypic, agronomic, and environmental interaction characteristic. Commercial reference soybean varieties are developed through a process of selecting and breeding for various desirable soybean characteristics and can provide a range of natural variability for characteristics and context for interpreting experimental results.

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in U.S. Field Trials, Laboratory, or Greenhouse Tests

Data category	Characteristics measured (associated section where discussed)	Evaluation timing (setting of evaluation)[1]	Evaluation description (measurement endpoints)
Seed germination, dormancy, and emergence	Normal germinated (VII.C.1)	Day 5 and 8 (20/30°C) (laboratory)	Percentage of seed producing seedlings exhibiting normal developmental characteristics
	Abnormal germinated (VII.C.1)	Day 8 (20/30°C) (laboratory)	Percentage of seed producing seedlings that could not be classified as normal germinated
	Germinated (VII.C.1)	Day 5, 8, and 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that had germinated normally and abnormally
	Dead (VII.C.1)	Day 5 and 8 (10, 20, 30, 10/20, 10/30, and 20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that had visibly deteriorated and become soft to the touch (also included non-viable hard and non-viable firm-swollen seed)
	Viable hard (VII.C.1)	Day 8 (20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that did not imbibe water and remained hard to the touch (viability determined by a tetrazolium test[2])
	Viable firm-swollen (VII.C.1)	Day 8 (20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that imbibed water and were firm to the touch but did not germinate (viability determined by a tetrazolium test[2])
	Early stand count (VII.C.2.1)	V2 - V4 (Field)	Number of emerged plants in two rows, standardized to 20 ft rows
	Final stand count (VII.C.2.1)	Maturity, R8 (Field)	Number of plants in two rows, standardized to 20 ft rows
Vegetative growth	Seedling vigor (VII.C.2.1)	V2 - V4 (Field)	Rated on a 1-9 scale, where 1 = excellent, 5 = average, and 9 = poor vigor
	Growth stage assessment (VII.C.2.1)	Every 2-3 weeks, V2-R8 (Field)	Average soybean plant growth stage per plot
	Flower color (VII.C.2.1)	Flowering, R2 (Field)	Color of flowers: purple, white, or mixed
	Plant pubescence (VII.C.2.1)	Maturity, R8 (Field)	Pubescence on plants in each plot categorized as hairy or hairless
	Plant height (VII.C.2.1)	Maturity, R8 (Field)	Distance (in) from the soil surface to the uppermost node on the main stem of five representative plants per plot
Reproductive development	Days to 50% flowering (VII.C.2.1)	Flowering, R1-R2 (Field)	Calendar day number (days from January 1) when approximately 50% of the plants in each plot were flowering
	Pollen viability (VII.C.3)	Flowering, R1-R2 (laboratory)	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen morphology (VII.C.3)	Flowering, R1-R2 (laboratory)	Diameter (µm) of viable pollen grains
	Seed moisture (VII.C.2.1)	Harvest (Field)	Percent moisture content of harvested seed
	100 seed weight (VII.C.2.1)	Harvest (Field)	Mass (g) of 100 harvested seed
	Test weight (VII.C.2.1)	Harvest (Field)	Mass (lb) of a bushel of harvested seed
	Yield (VII.C.2.1)	Harvest (Field)	Bushels of harvested seed per acre, adjusted to 13% moisture

Table VII-1 (continued). Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials, Laboratory or Greenhouse Tests

Data category	Characteristics measured (associated section where discussed)	Evaluation timing (setting of evaluation)[1]	Evaluation description (measurement endpoints)
Seed retention and lodging	Lodging (VII.C.2.1)	Maturity, R8 (Field)	Rated on 0-9 scale, where 0 = completely erect and 9 = completely flat or lodged
	Pod shattering (VII.C.2.1)	Maturity, R8 (Field)	Rated on 0-9 scale, where 0 = no shattering and 9 = completely shattered
Plant-environment interactions	Plant response to abiotic stress (VII.C.2.2)	Four times per growing season (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Disease damage (VII.C.2.2)	Four times per growing season (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Arthropod-related damage (VII.C.2.2)	Four times during growing season (Field)	Damage assessed on upper four nodes of 10 representative plants per plot using arthropod-specific 0-5 rating scales of increasing severity
	Arthropod abundance (VII.C.2.2)	Four times during growing season (Field)	Quantitative assessment of pest and beneficial arthropods
Plant-symbiont interactions	Biomass (VII.C.4)	6 weeks after emergence (Greenhouse)	Nodule, root, and shoot dry weight (g/plant)
	Nodule number (VII.C.4)	6 weeks after emergence (Greenhouse)	Nodule number
	Total nitrogen (VII.C.4)	6 weeks after emergence (Greenhouse)	Shoot total nitrogen (% and g/plant)

[1]Soybean plant growth stages were determined using descriptions and guidelines outlined in Soybean Growth and Development (ISU, 2004).

[2]Viability of hard and firm-swollen seed were determined by a tetrazolium test (AOSA, 2000).

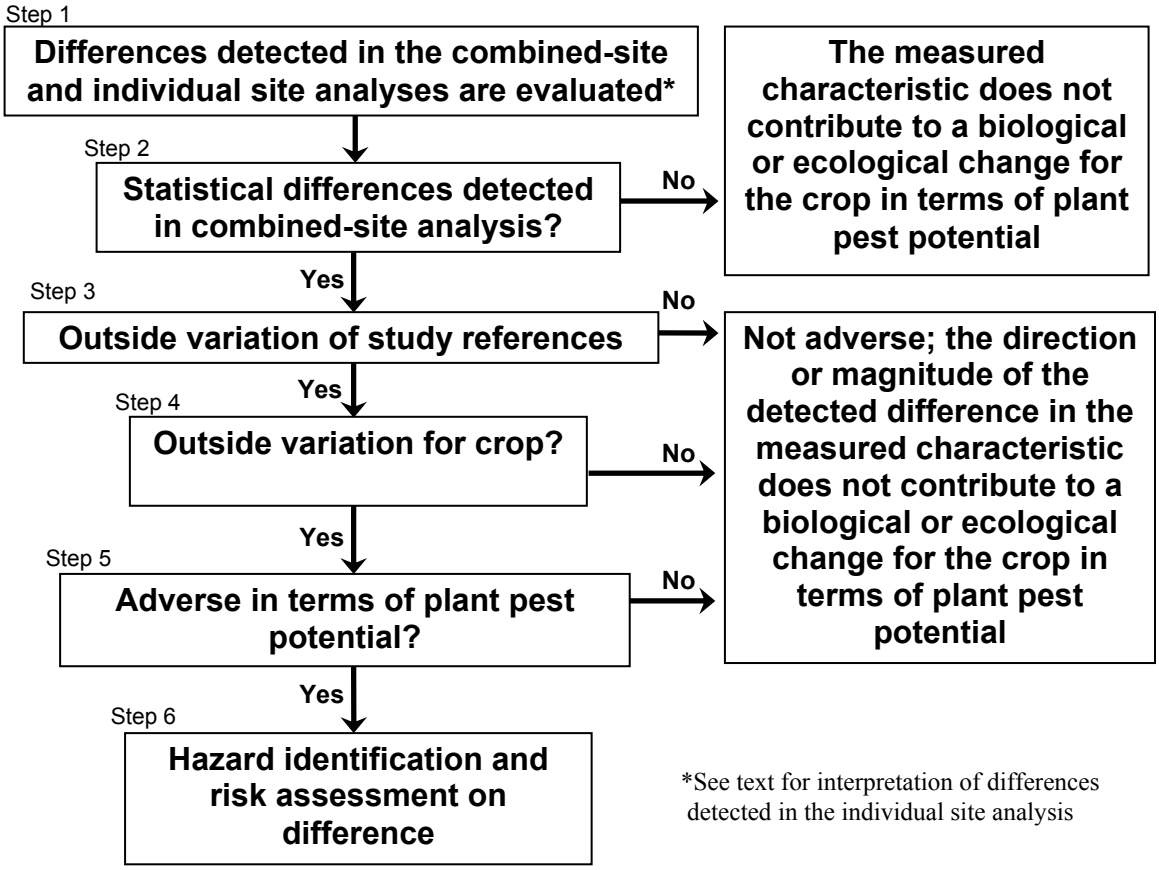
VII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are comparative assessments. Familiarity provides a basis from which the potential environmental impact of a biotechnology-derived plant can be evaluated. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a well-characterized conventional plant variety. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction of these factors, and provides a basis for comparative environmental risk assessment between a biotechnology-derived plant and its conventional counterpart.

Expert knowledge and experience with conventionally bred soybean was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for soybean. As such, MON 87708 was compared to the conventional control in the assessment of phenotypic, agronomic, and environmental interaction characteristics. An overview of the characteristics assessed is presented in Table VII-1. A subset of the data relating to well-understood weedy characteristics (*e.g.*, seed dormancy, pre-harvest seed loss characteristics, and lodging) was used to assess whether there is an increase in weediness of MON 87708, an element of APHIS's plant pest determination. Evaluation of environmental interaction characteristics (*e.g.*, plant-abiotic stress, plant-disease, and plant-arthropod interactions) was also considered in the plant pest assessment. Based on all of the data collected, an assessment was made to determine if MON 87708 is likely to pose an increased plant pest risk compared to conventional soybean. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an unexpected plant response. No unexpected observations or issues were identified.

VII.B.1. Interpretation of Detected Differences Criteria

Comparative plant characterization data from a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest potential as assessed by APHIS. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest potential of the biotechnology-derived crop compared to the conventional crop. Characteristics for which differences are detected are considered in a step-wise method (Figure VII-1). All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest potential of the biotechnology-derived crop. Ultimately, a weight of evidence approach considering all characteristics and data is used for the overall risk assessment of differences and their significance. Figure VII-1 illustrates the stepwise assessment process employed in detail:



Note: A "no" answer at any step indicates that the characteristic does not contribute to a biological or ecological change for the crop in terms of plant pest potential and subsequent steps are not considered. If the answer is "yes" or "uncertain", the subsequent step is considered.

Figure VII-1. Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods

Steps 1 and 2 - Evaluate Detected Statistically Significant Differences

Data on each measured characteristic are statistically analyzed, where appropriate, within each individual site and in a combined-site analysis, in which the data are pooled among sites. Differences detected in individual-site analyses must be observed in the combined-site analysis to be considered further for plant pest potential. Any difference detected in the combined-site analysis is further assessed.

Step 3 - Evaluate Differences Relative to Commercial Reference Varieties Range

If a difference for a characteristic is detected in the combined-site analysis across multiple environments, then the mean value of the biotechnology-derived crop for the characteristic is assessed relative to the commercial reference varieties.

Step 4 - Evaluate Differences in the Context of the Crop

If the mean value of the biotechnology-derived crop is outside the variation of the commercial reference varieties (*e.g.*, reference range), the mean value of the biotechnology-derived crop for the characteristic is assessed relative to known values common for the crop (*e.g.*, published values).

Step 5 - Plant Pest Potential

If the mean value of the biotechnology-derived crop is outside the range of values common for the crop, the detected difference for the characteristic is then assessed for whether or not it is adverse in terms of plant pest potential.

Step 6 - Conduct Risk Assessment on Identified Hazard

If an adverse effect (hazard) is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for and effects of trait transfer to feral populations of the crop or to a sexually-compatible species.

VII.C. Comparative Assessments of the Phenotypic, Agronomic, and Environmental Interaction Characteristics of MON 87708

This section provides the results of comparative assessments conducted in replicated laboratory, greenhouse, and/or multi-site field experiments to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 87708. The MON 87708 characteristics evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1), plant phenotypic and environmental interaction observations under field conditions (Section VII.C.2), pollen characteristics (Section VII.C.3), and symbiont interactions (Section VII.C.4). Additional details for each assessment are provided in Appendices F through I.

VII.C.1. Seed Dormancy and Germination Characteristics

USDA-APHIS considers the potential for weediness to constitute a plant pest factor (7 CFR § 340.6). Seed germination and dormancy mechanisms vary among species and their genetic basis tends to be complex. Seed dormancy (*e.g.*, hard seed) is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003); however, it is not uncommon to observe low levels of hard seed in conventional soybean (Mullin and Xu, 2001). Standardized germination assays are available and routinely used to measure the germination characteristics of soybean seed. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature range of 20/30°C as optimal for testing the germination characteristics of soybean seed (AOSA, 2007).

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 87708 and the conventional control. In addition, eight commercial reference varieties were included to provide a range of comparative values that are representative of existing commercial soybean varieties. The seed lots for MON 87708, conventional control, and the commercial reference varieties were produced in replicated field trials during 2008 in Iowa, Illinois, and Missouri, geographic areas which represent environmentally relevant conditions for soybean production for this product. In addition to the AOSA recommended temperature range of 20/30°C, seed was tested at five additional temperature regimes of 10, 20, 30, 10/20, and 10/30°C to assess seed germination properties. The details of the materials, experimental methods, and germination data from all individual production sites are presented in Appendix F.

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and the conventional control for percent germinated or percent dead seed in the 20, 30,

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VII.C.2. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated under Field Conditions

Plant growth, development, and yield characteristics were evaluated under field conditions as part of the plant characterization assessment of MON 87708. These data were developed to provide USDA-APHIS with a detailed description of MON 87708 relative to the conventional control and commercial reference varieties. According to 7 CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "a detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 87708 and the conventional control that may impact its pest potential. Certain growth, reproduction, and pre-harvest seed loss characteristics (*e.g.*, lodging, pod shattering) were used to assess whether there is an increase in weediness of MON 87708, an element of APHIS's plant pest determination. Environmental interactions were also assessed as an indirect indicator of phenotypic changes to MON 87708 compared to the same comparators described above and are also considered in the plant pest assessment.

Data were collected from field trials located at 16 field sites in the U.S. and two field sites in Canada during 2008 to evaluate phenotypic, agronomic, and environmental interaction characteristics. These 18 field sites provided a diverse range of environmental and agronomic conditions representative of commercial soybean production areas in North America (Table VII-3). The experiments were arranged as randomized complete block designs with three replications at each field site. All plots of MON 87708, the conventional control, and the commercial reference varieties at each site were uniformly managed in order to assess whether the introduction of the dicamba-tolerance trait altered the phenotypic and agronomic characteristics or the environmental interactions of MON 87708 compared to the conventional control. Therefore, dicamba herbicide was not applied to MON 87708 during the study. A description of the evaluated phenotypic and environmental interaction characteristics and the designated developmental stages when evaluations occurred are listed in Table VII-1. The methods and detailed results of the individual-site data comparisons are presented and discussed in Appendix G, while the combined-site analyses are summarized below. The results of this assessment demonstrated that the introduction of the dicamba-tolerance trait did not alter MON 87708 compared to the conventional control in terms of weediness. The lack of differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance further support the conclusion that the introduction of the dicamba-tolerance trait is not likely to result in increased plant pest potential or an altered environmental impact from MON 87708 compared to conventional soybean.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

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VII.C.2.2. Environmental Interaction Characteristics

USDA-APHIS considers the environmental interaction of the biotechnology-derived crop compared to its conventional counterpart to determine the potential for increased plant pest characteristics. Evaluations of environmental interactions were conducted as part of the plant characterization for MON 87708. In the 2008 North American field trials conducted for evaluation of phenotypic and agronomic characteristics of MON 87708, data were also collected on plant response to abiotic stress (drought, wind, nutrient deficiency, etc.), disease damage, arthropod-related damage, and arthropod abundance (Appendix G; Tables G-5, G-6, G-7, G-8, and G-9, respectively). These data were used as part of the environmental consequences (Section IX) to assess plant pest potential and provide an indication of potential effects of MON 87708 on non-target organisms (NTOs) and threatened and endangered species compared to the conventional control. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each assessed characteristic. The results of the field evaluations showed that the dicamba-tolerance trait did not unexpectedly alter the assessed environmental interactions of MON 87708 compared to the conventional control. The lack of significant biologically-meaningful differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance support the conclusion that the introduction of the dicamba-tolerance trait is unlikely to result in increased plant pest potential or an altered environmental impact from MON 87708 compared to conventional soybean.

In the 2008 field trials, the observations of plant response to abiotic stress, disease damage, and arthropod-related damage were performed four times during the growing season at all 18 sites, and arthropod abundance was assessed quantitatively from collections performed four times during the growing season at four of the 18 sites (*i.e.*, IL2, IN1, MI, and MO1 sites). The assessed stressors (abiotic, diseases, and arthropods) were at natural levels as no artificial infestation or imposed abiotic stress was used and, therefore, typically varied between observations at a site and among sites. Abiotic stress and disease damage data were collected from each plot using a 0 – 9 scale of increasing severity of observed damage. Damage data were collected numerically and then placed into one of the following categories: none, slight, moderate, or severe. These categorical data were not subjected to statistical analysis. The response of MON 87708 and the conventional control to an abiotic stress or disease were considered different on a particular observation date at a site if the range of injury severity to MON 87708 did not overlap with the range of injury severity to the control across all three replications (*e.g.*, "none" vs. "slight-moderate" rating). For each observation at a site, the range of injury severity across the commercial reference varieties provided assessment data that are representative of commercial soybean varieties. Arthropod-related damage was assessed from each plot on the upper four nodes of 10 representative plants using a 0 – 5 rating scale of increasing severity of observed damage. These numerical data along with the quantitative arthropod abundance data were subjected to statistical analysis.

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VII.C.3. Pollen Characteristics

USDA-APHIS considers the potential for gene flow and introgression of the biotechnology-derived trait into other soybean varieties and wild relatives to determine the potential for increased weedy or invasive characteristics of the receiving species. Pollen morphology and viability information are pertinent to this assessment and, therefore, were assessed for MON 87708. In addition, characterization of pollen produced by MON 87708 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87708 compared to the conventional control.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from MON 87708 compared to that of the conventional control. Pollen was collected from MON 87708, the conventional control, and four commercial reference varieties grown under similar agronomic conditions in a field trial in Illinois. The trial was arranged in a randomized complete block design with three replications. Twenty flowers were collected from each plot. Pollen was extracted, combined among flowers collected from the same plot, and stained with Alexander's stain (Alexander, 1980). Pollen viability was evaluated for each sample, and pollen grain diameter was measured for ten representative viable pollen grains per replication. General morphology of the pollen was observed for each of the three replications of MON 87708, the conventional control, and the commercial reference varieties (see Appendix H).

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Table VII-5. Pollen Characteristics of MON 87708 Compared to Conventional (b) (4)

VII.C.4. Symbiont Interactions

As part of the plant pest risk assessment, USDA-APHIS considers the impact of the biotechnology-derived crop on plant pest potential and the environment as well as on agricultural or cultivation practices compared to its conventional counterpart. Changes in the symbiotic relationship with rhizosphere-inhabiting bacteria *Rhizobiaceae* and *Bradyrhizobiaceae* could directly impact pest potential, the environment, or cultivation practices (*i.e.*, the need to add additional nitrogen to sustain soybean production). Thus, the purpose of this evaluation was to assess whether the introduction of the dicamba-tolerance trait altered the symbiotic interaction of MON 87708 with *Bradyrhizobium japonicum* (*B. japonicum*) compared to that of the conventional control.

Members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae* form a highly complex and specific symbiotic relationship with leguminous plants, including soybean (Gage, 2004). The nitrogen-fixing plant-microbe symbiosis results in the formation of root nodules, providing an environment in which differentiated bacteria called bacteroids are capable of reducing or fixing atmospheric nitrogen. The product of nitrogen fixation, ammonia, then can be utilized by the plant. In soybean, atmospheric nitrogen is fixed into ammonia through a symbiotic association with the bacterium *B. japonicum*. As a result of this relationship, nitrogen inputs are typically not necessary for agricultural production of soybean.

The relative effectiveness of the symbiotic association between a leguminous plant and its rhizobial symbiont can be assessed by various methods. Assessment of nodule number and mass along with plant growth and nitrogen status are commonly used to assess differences in the symbiotic association between a legume and its associated rhizobia (Israel et al., 1986). It should be noted, however, that nodule number relative to nodule dry weight may be variable in soybean experiments because nodules may be larger in diameter and less numerous, while others are not as developed (smaller) but more abundant (Appunu and Dhar, 2006; Israel et al., 1986).

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VII.D. Phenotypic, Agronomic, and Environmental Interactions Evaluation Conclusion

An extensive and robust set of information and data were used to assess whether the introduction of the dicamba-tolerance trait altered the plant pest potential of MON 87708 compared to the conventional control. Phenotypic, agronomic, and environmental interaction characteristics of MON 87708 were evaluated and compared to those of the conventional control and considered within the variation among commercial reference varieties. These assessments included plant growth and development characteristics; seed dormancy and germination characteristics; pollen characteristics; observations of abiotic stress response, disease damage, arthropod-related damage and arthropod abundance; and plant-symbiont interaction characteristics. Results from the phenotypic, agronomic, and environmental interactions assessment demonstrate that MON 87708 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or significant environmental impact compared to conventional soybean.

VIII. U.S. AGRONOMIC PRACTICES

VIII.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4), impacts to agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. for producing soybean and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 87708. Discussions include soybean production, seed production, plant growth and development, general management practices during the season, management of weeds, insects and diseases, soybean rotational crops, and volunteer soybean management. Information presented in Section VII.C.2 demonstrated that MON 87708 is no more susceptible to diseases or pests than conventional soybean. Additionally data presented in Section VII.C show that, with the exception of tolerance to the herbicide dicamba, MON 87708 is phenotypically equivalent to conventional soybean. Thus, there are no changes to the inputs needed for MON 87708, and no specific impacts to most of the agronomic practices employed for production of soybean. In the areas where there is potential for impact on agronomic practices from the deregulation of MON 87708, the scope and magnitude of those impacts will be discussed.

Soybean is planted in over 30 states, demonstrating its wide adaptation to varied soils and climate. The soil, moisture, and temperature requirements for producing soybean are generally similar to those for corn, and thus the two crops share a similar cultivation area. Proper seedbed preparation, appropriate variety selection, appropriate planting dates and plant population, and good integrated pest management practices are important for optimizing the yield potential and economic return for soybean.

Annual and perennial weeds are perceived to be the greatest pest problem in soybean production (Aref and Pike, 1998). Weeds compete with soybean for water, nutrients, and light resulting in substantial yield losses when left uncontrolled. Weed species in soybean vary from region to region and state to state. Economic thresholds for controlling weeds in soybean require some form of weed management practice on all soybean acreage. Weed management practices include mechanical tillage, crop rotations, cultural practices, and herbicide application. Numerous selective herbicides are available for preplant, preemergence, and postemergence control of annual and perennial weeds in soybean. Approximately 98% of the soybean acreage in the U.S. receives an herbicide application (USDA-NASS, 2006). Soybean insects and diseases generally are considered less problematic, although infestations can reach economic thresholds requiring treatment.

Volunteer soybean, *i.e.*, soybean plants that have germinated and emerged unintentionally in a subsequent crop, are not considered a significant concern in rotational crops primarily because of climatic conditions and adequate control of volunteer soybean from tillage practices. Additionally, mechanical and chemical control methods are available to manage the occasional volunteer soybean plant. Due to its lack of weediness potential, introduction of MON 87708 in the soybean production system would have a negligible impact on managing soybean volunteer plants in rotational crops such as corn, cotton,

and wheat. The numerous control measures that are effective on conventional and Roundup Ready soybean volunteer plants will continue to be effective on volunteer MON 87708 plants when they arise.

As shown in Sections VI and VII, with the exception of the dicamba tolerance trait, no phenotypic, compositional, or environmental differences between MON 87708 and conventional soybean have been observed. Moreover herbicide-tolerant soybean is currently grown on 91% of U.S. soybean acres (USDA-NASS, 2009c). Therefore, it is not anticipated that commercialization of MON 87708 in the U.S. would have a notable impact on current soybean cultivation practices, including the management of weeds, diseases, and insects.

VIII.B. Overview of U.S. Soybean Production

VIII.B.1. Soybean Production

Soybean first entered North America in the 18th century (Hoeft et al., 2000). During the 1930s, soybean started to be processed industrially in the U.S. for edible oil and protein meal. In 2008, soybean represented 56 percent of world oilseed production, and about a third of those soybean were produced in the U.S. (ASA, 2009). In 2008, the U.S. exported 1.16 billion bushels (31.6 million metric tons) of soybean, which accounted for 40 percent of the world's soybean exports (ASA, 2009). In total, the U.S. exported $20 billion worth of soybean and soybean products globally in 2008 (ASA, 2009). China was the largest export market for U.S. soybean with purchases totalling $7.2 billion. Mexico was the second largest export market with purchases of $1.7 billion. Other significant markets include the European Union ($1.6 billion) and Japan ($1.3 billion).

Approximately 94% of the world's soybean seed supply was crushed to produce soybean meal and oil in 2008 (Soyatech, 2010), and the majority was used to supply the feed industry for livestock use or the food industry for edible vegetable oil and soybean protein isolates.

The productivity of soybean is highly dependent upon soil and climatic conditions. In the U.S., the soil and climatic requirements for growing soybean are very similar to corn. The soils and climate in the Midwestern, Eastern, and portions of the Great Plains regions of the U.S. provide sufficient water under normal climatic conditions to produce a soybean crop. The general water requirement for a high-yielding soybean crop is approximately 20 inches of water during the growing season (Hoeft et al., 2000). Soil texture and structure are key components determining water availability in soils, where medium-textured soils hold more water, allowing soybean roots to penetrate deeper in medium-textured soils than in clay soils. Irrigation is used on approximately 9% of the soybean acreage to supplement the water supply during dry periods in the Western and Southern soybean growing regions (USDA-ERS, 2008).

Most of the soybean acreage is grown as a full-season crop. Approximately 8% of the soybean acres are planted in a double-crop system following winter wheat south of 35º North latitude (Boerma and Specht, 2004). However, this percentage can vary

significantly from year to year. The decision to plant double-crop soybean is influenced by both agronomic and economic factors. Agronomic factors include harvest date of the wheat crop, which determines the double-crop soybean planting date, and available soil moisture. Economic factors include expected soybean price and anticipated economic return (Boerma and Specht, 2004).

The U.S. soybean acreage in the past 10 years has varied from approximately 64.7 to 75.7 million acres, with the lowest acreage recorded in 2007 and the highest in 2008 (Table VIII-1). Average soybean yields have varied from 33.9 to 43.3 bushels per acre over this same time period. Annual soybean production ranged from 2.45 to 3.19 billion bushels over the past ten years. According to data from USDA-NASS (2009a), soybean was planted on approximately 75.7 million acres in the U.S. in 2008, producing 2.96 billion bushels of soybean (Table VIII-1). Soybean acreage and production in 2008 was up from 2007, mainly due to a decrease in corn acreage. The value of soybean reached $27.4 billion in the U.S. in 2008 (USDA-NASS, 2009b). In comparison, corn and wheat values in 2008 were $47.37 and $16.57 billion, respectively (USDA-NASS, 2009a, b).

For purposes of this agronomic practices discussion, soybean production is divided into three major soybean growing regions accounting for 99.1% of the 2008 U.S. soybean acreage: Midwest/Great Plains region (IL, IN, IA, KS, KY, MI, MN, MO, NE, ND, OH, SD, and WI), Southeast region (AL, AR, GA, LA, MS, NC, SC, and TN) and the Eastern Coastal region (DE, MD, NJ, NY, PA, and VA) (Table VIII-2). The vast majority of soybean was grown in the Midwest region, representing 82.1% of the total U.S. acreage. The Southeast and Eastern Coastal regions represented 14.3% and 2.7% of the acreage, respectively. Among the three regions, the Midwest region produced the highest average yield at 38.6 bushels per acre in 2008, and average state yields in this region ranged from 28.0 to 47.0 bushels per acre. The average yield in the Southeast region was 34.4 bushels per acre, with states within this region averaging from 30.0 to 40.0 bushels per acre. The average yield in the Eastern Coastal region was 34.1 bushels per acre, with individual state averages ranging from 27.5 to 46.0 bushels per acre.

Managing input costs is a major component to the economics of producing a soybean crop (Helsel and Minor, 1993). Key decisions on input costs include choosing what soybean varieties to plant, amounts of fertilizer to apply, and what herbicide program to use. The average operating cost for producing soybean in the U.S. in 2008 was $127.79 per acre, according to statistics compiled by the USDA-Economic Research Service (USDA-ERS, 2008). The value of the production less operating cost was reported to be $318.66 per acre. A summary of potential production costs and returns are presented in Table VIII-3.

Table VIII-1. Soybean Production in the U.S., 1999 – 2008[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
2008	75,718	74,641	39.6	2,959,174	27.40
2007	64,741	64,146	41.7	2,677,117	26.97
2006	75,522	74,602	42.7	3,188,247	20.42
2005	72,142	71,361	43.3	3,086,432	16.93
2004	75,208	73,958	42.2	3,123,686	17.89
2003	73,404	72,476	33.9	2,453,665	18.01
2002	73,963	72,497	38.0	2,756,147	15.25
2001	74,075	72,975	39.6	2,890,682	12.61
2000	74,266	72,408	38.1	2,757,810	12.47
1999	73,730	72,446	36.6	2,653,758	12.21
Ave.	73,277	72,151	39.6	2,854,672	18.02

[1]Source is USDA-NASS (2009a,b).

Table VIII-2. U.S. Soybean Production by Region and State in 2008[1]

Region/State	Acres Planted (thousands)	Acres Harvested (thousands)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
		Midwest Region			
Illinois	9,200	9,100	47.0	427,700	4.00
Indiana	5,450	5,430	45.0	244,350	2.27
Iowa	9,750	9,670	46.0	444,820	4.29
Kansas	3,300	3,250	37.0	120,250	1.03
Kentucky	1,390	1,380	34.0	46,920	0.42
Michigan	1,900	1,890	37.0	69,930	0.64
Minnesota	7,050	6,950	38.0	264,100	2.54
Missouri	5,200	5,030	38.0	191,140	1.72
Nebraska	4,900	4,860	46.5	225,990	2.12
North Dakota	3,800	3,760	28.0	105,280	0.96
Ohio	4,500	4,480	36.0	161,280	1.55
South Dakota	4,100	4,060	34.0	138,040	1.25
Wisconsin	1,610	1,590	35.0	55,650	0.51
Region Totals	**62,150**	**61,450**	**38.6**	**2,495,450**	**23.30**
		Southeast Region			
Alabama	360	350	35.0	12,250	0.12
Arkansas	3,300	3,250	38.0	123,500	1.09
Georgia	430	415	30.0	12,450	0.11
Louisiana	1,050	950	33.0	31,350	0.29
Mississippi	2,000	1,960	40.0	78,400	0.69
North Carolina	1,690	1,670	33.0	55,110	0.47
South Carolina	540	530	32.0	16,960	0.15
Tennessee	1,490	1,460	34.0	49,640	0.43
Region Totals	**10,860**	**10,585**	**34.4**	**379,660**	**3.35**
		Eastern Coastal Region			
Delaware	195	193	27.5	5,308	0.05
Maryland	495	485	30.0	14,550	0.13
New Jersey	92	90	29.0	2,610	0.02
New York	230	226	46.0	10,396	0.09
Pennsylvania	435	430	40.0	17,200	0.15
Virginia	580	570	32.0	18,240	0.16
Region Totals	**2027**	**1994**	**34.1**	**68,304**	**0.60**

Table VIII-3. U.S. Soybean Production Costs and Returns in 2008[1]

Production Cost or Return Category	Itemized Costs	Return per Planted Acre ($ USD)
Total Gross Value of Production		**446.45**
Operating Costs:	Seed	44.35
	Fertilizer	25.12
	Chemicals	15.73
	Custom operations	6.56
	Fuel, lube and electricity	20.20
	Repairs	12.91
	Purchased irrigation water	0.12
	Interest on operating capital	2.80
Total, operating costs		**127.79**
Allocated overhead:	Hired labor	2.07
	Opportunity cost of unpaid grower's labor	16.77
	Capital recovery of machinery and equipment	70.98
	Opportunity cost of land (rental rate)	94.58
	Taxes and insurance	9.64
	General farm overhead	14.29
Total, allocated overhead		**208.35**
Total cost listed		**336.13**
Value of production less total cost listed		**110.32**
Value of production less operating costs		**318.66**

Supporting Information: Yield = 43 bushels/acre, Price = $10.48/bushel, Enterprise size = 303 planted acres, Irrigated = 9%, Dry land = 91%.

[1]Source is USDA-ERS (2008).

VIII.B.2. Soybean Seed Production

Standardized seed production practices are responsible for maintaining high-quality seed stocks, an essential basis for U.S. agriculture. By the early 20th century, agronomists learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties that were distributed to growers for use. Seed was saved by growers and later sold to neighbors; however, the desirable traits of the varieties often were lost through random genetic changes and contamination with other crop and weed seed (Sundstrom et al., 2002). The value of seed quality (including genetic purity, vigor, and presence of weed seed, seed-borne diseases, and inert materials, such as dirt) was quickly identified as a major factor impacting crop yields. States developed seed laws and certification agencies to ensure that purchasers who received certified seed could be assured that the seed met established seed quality standards (Bradford, 2006). The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and the establishment of official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Under international agreements such as the Organization for Economic Co-Operation and Development (OECD) scheme, the U.S. and other countries mutually recognize minimum seed quality standards (Bradford, 2006). The Association of Official Seed Certifying Agencies (AOSCA) represents state and private seed certification organizations in the U.S., and includes international member countries in North and South America, Australia, and New Zealand.

Soybean seed is separated into four seed classes: 1) breeder, 2) foundation, 3) registered, and 4) certified (AOSCA, 2009). Breeder seed is seed directly controlled by the originating or sponsoring plant breeding organization or firm. Foundation seed is first-generation seed increased from breeder seed and is handled in a manner to maintain specific levels of varietal purity and identity. Registered seed is the progeny of foundation seed that is handled to maintain satisfactory varietal purity and identity. Certified seed is the progeny of breeder, foundation or registered seed, and is typically two generations removed from foundation seed. While not all soybean seed sold to growers is officially certified, commercial soybean seed sold and planted for typical soybean production is produced predominately to meet or exceed certified seed standards. This section of the petition will provide a broad overview of the practices used in producing certified seed.

Soybean seed breeders and producers have put in place practical measures to assure the quality and genetic purity of soybean varietal seed for commercial planting. The need for such systems arose from the recognition that the quality of improved soybean varieties quickly deteriorated in the absence of monitoring for quality and genetic purity (CAST, 2007). Seed certification programs were initiated in the early 1900s in the U.S. to preserve the genetic identity and variety purity of seed. There are special land requirements, seed stock eligibility requirements, field inspections and seed labeling standards for seed certification. Seed certification services are available through various state agencies affiliated with AOSCA. Large seed producers implement their own seed quality assurance programs. However, large seed producers often will utilize the services

of state certifying agencies as a third party source to perform certain field inspections and audits.

U.S soybean production for all purposes has varied from approximately 64.7 to 75.7 million acres in the past ten years (USDA-NASS, 2009a; Table VIII-1). To plant this area of soybean acreage requires 105 to 125 million units (50 lbs/unit) of soybean seed. This seed volume includes allowances for seed losses due to weather, poor yields, and quality issues. Additional allowances are included for distribution excess, seed returns, replants, and potential increases in soybean acreage. Assuming an average soybean yield of 45 bushels, or 54 units (50 lbs/unit) per acre, 1.9 to 2.3 million acres would be required to produce this volume of commercial certified soybean seed each year.

Certified soybean seed is produced throughout most of the U.S. soybean-growing regions. Soybean varieties are developed and adapted to certain geographical zones and are separated into ten maturity groups – Group 00 to Group VIII (see Section VIII.C). Seed production for these maturity groups is grown in the respective geographical zone for each maturity group. However, the production areas generally are on the northern edge of the respective zone to minimize incidences of disease.

Soybean seed is produced by a number of companies that produce and sell seed, such as Monsanto Company, Pioneer Hi-Bred International, Syngenta Seeds, Kruger Seed Co., and Becks Hybrids. In addition, certified seed is produced by toll seed producers, or tollers, which are companies that produce but do not directly sell certified seed, such as Remington Seeds LLC and Precision Soya. Seed companies and tollers in turn contract acreage with growers to produce the needed amount of soybean seed. Seed production or processing plants at these seed companies identify local soybean growers to produce the seed and also monitor and inspect seed fields throughout the growing season. The seed production plants also clean, condition, and bag the harvested soybean seed as well as monitor and inspect all the processes at the plant. Production plants typically produce between 100,000 units to 2,000,000 units of soybean seed. Production plants will produce the various soybean varieties in different climates or environments to spread production risks.

The entire seed production process at the majority of the seed companies and tollers operate using International Organization for Standardization (ISO) certification standards and; therefore, include internal and external audits (ISO, 2009). ISO standards ensure desirable characteristics of seeds and services, such as quality, safety, reliability, and efficiency. The ISO standards represent an international consensus on good management practices with the aim of ensuring that the organization can consistently deliver excellent product or services. The standards not only must meet the customer's requirements and applicable seed regulatory requirements, but must aim to enhance customer satisfaction and achieve continual improvement of its performance in pursuit of these objectives (ISO, 2009).

The field operations and management practices for producing soybean seed are similar to normal soybean production. However, special attention is needed in certain areas to produce seed with high quality, high germination rates, and high genetic purity (Helsel

and Minor, 1993). General guidelines specific for seed production are discussed below. Importantly, the seed production field should not have been planted with soybean in the previous crop season in order to avoid potential volunteer soybean plants (even though the risk of soybean volunteer plants is negligible) and to ensure genetic purity.

Very early planting is typically avoided because the seed produced from early planting often results in poorer quality seed (Helsel and Minor, 1993). Every effort must be made to eliminate weeds in a seed field through the use of herbicides and cultivation practices to prevent weed seed in the harvested soybean seed. Fields are scouted frequently for insect pests and insecticides are applied when insect pest infestations reach economical threshold levels. Foliar-applied fungicides should be considered when disease infestations are predicted in the area. Harvest should occur as soon as the mature soybean seed reaches 13% moisture content. Harvesting soybean seed with less than 13% moisture can cause damage to the seed coat and result in split soybean seed that can affect germination and viability. Harvesting equipment must be adjusted to minimize or avoid seed damage. Harvesting equipment must be cleaned before entering the seed fields to assure genetic purity. Certain seed handling equipment, such as auger elevators, should be avoided because they can increase seed damage.

Field inspections are vital to ensure the soybean seed meets seed certification requirements, ISO certification standards, regulatory standards, and trait licensing agreement standards. Field inspections are conducted on seed production fields throughout the soybean growing season to visually evaluate variety purity, ensure soybean plants are developing properly, and fields are maintained free of weeds, insects, and diseases. The fields are also mapped to ensure the seed field has the minimum federal isolation requirement of five feet as a physical barrier (AOSCA, 2009). Some states and seed producers have a stricter isolation requirement of 10 feet.

Production plant personnel make every effort to avoid mechanical damage to the harvested seed during the screening, cleaning, and bagging process. Specific methods are used to assure the genetic purity and the identity of the seed is maintained throughout the handling and storage operation. Bin inspections and sample collections are conducted at storage locations at the seed production plant to examine the physical characteristics of the soybean seed and to ensure proper bin cleanout. Seed is inspected for appearance, disease, discoloration, seed coat, mechanical damage, inert matter, and weed seed. Warm and cold germination tests are conducted on all seed lots to verify acceptable germination rates. Many seed companies will also conduct tetrazolium staining tests to assess seed viability.

Commercially certified soybean seed must meet state and federal seed standards and labeling requirements. AOSCA standards for certified soybean seed are as follows: 98% pure seed (minimum), 2% inert matter (maximum), 0.05% weed seed (maximum, not to exceed 10 per lb.), 0.60% total of other crop seeds (maximum), 0.5% other varieties (maximum, includes off-colored beans and off-type seeds), 0.10% other crop seeds (maximum, not to exceed three per lb.), and 80% germination and hard seed (minimum) (AOSCA, 2009). State seed certification standards vary slightly from state to state and can be more restrictive than the seed standards of AOSCA.

When deregulated, MON 87708 seed will be produced in the same manner as commercially certified soybean seed, such that it will meet all state and federal seed standards and labeling requirements.

VIII.C. Production Management Considerations

VIII.C.1. Pre-Season

Well in advance of planting a soybean crop, decisions are made regarding the planned crop rotation, the tillage system and row spacing that will be implemented, the planting equipment that will be used, the seed or variety that will be planted, and soil fertility management requirements. Many of the decisions in this area are made prior to or immediately after harvest of the previous crop. There are many benefits to crop rotation, with the majority of the soybean acreage planted in a two-year corn-soybean rotation (see Section VIII.I). Crop rotation is generally a long-term decision, but the rotation sequence can be modified to take advantage of a particular economic or market opportunity. The decision to plant soybean in a conservation tillage or no-till system may require special equipment and will be made long before planting. In addition, this decision on tillage system usually will be a long-term commitment, provided the system is successful. A decision to change row spacing is a similar long-term commitment that generally requires new equipment.

The benefits of conservation tillage or no-till systems are well documented and include reduced soil erosion, reduced fuel and labor costs, and conservation of soil moisture (CTIC, 2000). In 2004, approximately 27.5 million acres (39.6%) of soybean were planted in a no-till system (CTIC, 2007). Slow soybean emergence and growth leading to lower yields have been some of the concerns associated with adoption of conservation tillage systems in soybean, especially no-till. Research in Wisconsin and Minnesota shows that soil temperatures can be four to five degrees colder in no-till than conventional tillage systems, which can slow seedling emergence, but have little effect on soybean yield (Pedersen, 2008a). Improved planters for establishment of good soybean populations and planting Roundup Ready soybean allowing the use of glyphosate to effectively control weeds in no-till fields have made no-till a viable production system for soybean (Pedersen, 2008a). Extension specialists still recommend some spring tillage on fine-textured and poorly drained soils for proper seedbed preparation (Pedersen, 2008a).

Most field crops, including soybean, respond well to fertilizer when planted in soils with low fertility levels. Soybean requires 16 essential elements for growth and development. Deficiencies in any of these elements can reduce yields (Hoeft et al., 2000). The primary or major essential nutrients are nitrogen, phosphorus and potassium. The soybean plant is a member of the legume family, like alfalfa and clover, and fixes a significant portion of its own nitrogen through the symbiotic relationship with the nitrogen-fixing Bradyrhizobia bacteria (*Bradyrhizobium japonicum*) that live in the nodules on its roots. Bradyrhizobia are unicellar, microscopic bacteria that invade the soybean plant through its root hairs (Hoeft et al., 2000). The plant responds to this invasion by forming nodules which contain colonies of bacteria. Once established on the soybean root, bacteria in the nodule take gaseous nitrogen from the atmosphere and fix it in forms easily used by the

soybean plant. Since these bacteria are not native to U.S. soils and would not normally be found in these soils, inoculation of the soybean seed with these bacteria is recommended when soybean has not been grown in a field for three to five years. Nitrogen fertilizer applications at planting generally do not improve yield and decrease nodulation while increasing the plant's dependency on the soil for nitrogen (Pedersen, 2008a). Therefore, nitrogen fertilizer is seldom applied prior to planting a soybean crop.

Soil tests are the only reliable way to determine the pH, phosphorus, and potassium levels in the soil. Liming and fertilizer requirements subsequently are determined based on soil test results. Ideal soil test results for corn are also ideal for soybean (Scott and Aldrich, 1970). In corn-soybean rotations in the Midwest, phosphorus and potassium fertilizers are applied prior to a corn crop in accordance with soil test recommendations, but are seldom applied prior to a soybean crop. However, in some of the southern growing areas, differences in crop rotations and soil types may require a fertilizer application prior to planting soybean.

Although not common, deficiencies in soil can occur in secondary nutrients (calcium, magnesium, and sulfur) or micronutrients (boron, chloride, copper, iron, manganese, molybdenum, and zinc). The availability of soil nutrients is dependent on soil acidity or pH level. Because soybean is adversely affected when the pH is below approximately 5.8 (Hoeft et al., 2000), soil pH should be maintained at about 6.0 to 6.5 through the addition of limestone.

Soybean varieties are developed and adapted to certain geographical zones and are separated into ten maturity groups – Group 00 to Group VIII (Pimentel, 1991; Zhang et al., 2004). Groups 00 and 0 are the earliest maturity groups and are adapted best to the area north of latitude 46º North. Succeeding groups are adapted further south with Groups I and II within latitudes 41º and 46º North, and Group III within latitudes 38º and 41º North. Group 00 through Group IV soybean varieties are planted in the Midwest and Eastern Coastal regions. Groups II, III and IV account for approximately 76% (24%, 36%, and 16%, respectively) of the soybean planted in the U.S. (T. Schlueter, personal communication, August 2008). Groups IV through VIII are planted in the southern states with Groups V, VI and VII representing 7%, 2%, and 2% of the planted soybean, respectively (T. Schlueter, personal communication, August 2008).

Soybean variety selection is crucial for high yield and quality, and is the foundation of an effective management plan (Pedersen, 2008a). Characteristics to consider in selecting a variety include maturity, yield potential, disease and pest resistance, iron deficiency tolerance (chlorosis), lodging score, height, and specific soybean quality traits, such as protein and oil content. If a field has a history of a particular disease or pest, planting soybean varieties that have resistance or tolerance to these pests and diseases can be an effective and economical method of control.

VIII.C.2. Planting and Early Season

An understanding of the growth stages of soybean is also important for the proper timing of certain management practices, such as herbicide and insecticide applications. In

addition, the impact of certain weather conditions, insect pests, and diseases on soybean yield is dependent on growth stage. The system of soybean growth stages divides plant development into vegetative (V) and reproductive (R) stages (Pedersen, 2008a). The vegetative stages begin with VE, which designates emergence. V stages continue and are numbered according to how many fully developed trifoliate leaves are present (*i.e.*, V1, V2, etc.). The reproductive (R) stages begin at flowering (R1) and include pod development and plant maturation. Full maturity is designated as R8.

Adequate soil moisture and warm temperatures facilitate rapid seed germination and emergence. The ideal soil temperature for soybean germination and emergence is 77ºF (Pedersen, 2008a). However, waiting for soils to reach this soil temperature will delay planting beyond the optimum planting date that will maximize yield. Soybean can germinate at a soil temperature of 50ºF when planted at a depth of two inches. However, emergence is slow and can take up to three weeks in northern climates. Because of fluctuations in soil temperature in early spring, soil temperature should not be the only criteria for optimum planting time. Planting into a good seedbed is the most important consideration. Planting into soil that is too wet will reduce emergence and plant population, and can lead to reduced yield.

Planting date has the greatest impact on yield, according to research conducted in the Northern states (Hoeft et al., 2000). Highest yields are generally obtained when planting in early to mid May. Yields begin to decline quite rapidly when planting is delayed until late May. For example, the optimum planting dates for soybean in Iowa are the last week of April in the southern two-thirds of the state and the first week of May in the northern one-third of the state (Pedersen, 2008a). In the Southern U.S., planting adapted varieties before late April results in shorter plants and, in many cases, lower yields than when the same varieties are planted in May or early June. Planting after early June generally decreases plant height and yield due to water shortages in July and August.

Variations in plant spacing through row spacing and plant population have a significant effect on canopy development and soybean yield. Row spacing is important to maximize soybean yield. Research in the Midwest over the past 20 years consistently shows that row spacing of less than 20 inches is preferred for soybean regardless of tillage system, rotation sequence or planting date (Pedersen, 2008a). In the Southern states, the advantage from narrow rows is less consistent and less beneficial. In 2000, approximately 40% of soybean was planted in row spacing of 10 inches or less, 27% in 10.1 to 28.5 inches, and 33% in rows wider than 28.5 inches (Hoeft et al., 2000).

Soybean has the ability to produce good yield over a wide range of plant populations. Most soybean varieties have the ability to branch and adjust the number of pods on branches to compensate for large differences in seeding rate. Maximum yields generally require planting rates that result in about 2.5 to 5 plants per square foot (Hoeft et al., 2000). Therefore, a full stand of soybean is approximately eight to ten plants per foot of row at harvest for 40-inch rows, six to eight plants per foot of row in 30-inch rows, four to six plants in 20-inch rows, and two to three plants in 10-inch rows. This translates to 109,000 to 218,000 plants per acre at harvest. Higher populations are recommended in narrow rows for maximum yields because plants are more uniformly spaced in narrow

rows. Seeding rates are generally 10 to 25% higher than the desired harvest population, especially in no-till fields, to account for the losses in germination, emergence, and seedling diseases. The accuracy of the planting equipment also can impact the decision on seeding rate. Soybean seed traditionally has been sold by weight. Therefore, the grower must know the number of seeds per pound for the particular soybean varieties being planted for accurate seeding rates.

Treating soybean seed with a fungicide (*e.g.*, metalaxyl or mefenoxam) to prevent damping-off diseases may be beneficial when planting in cold, wet soils, using reduced till and no-till planting systems, and when planting seed with a low germination rate (<80%) or low seed vigor (Pedersen, 2008a).

Annual and perennial weeds are considered to be the greatest pest problem in soybean production (Aref and Pike, 1998). In order to maximize yields, weeds must be controlled during the early growth stages of soybean because weeds compete with soybean for water, nutrients, and light. A combination of tillage and herbicides are used to control weeds throughout the growing season (Section VIII.F).

VIII.C.3. Mid to Late Season

Ideal daytime temperatures for soybean growth are between 75ºF and 85ºF (Hoeft et al., 2000). Warmer temperatures result in larger plants and earlier flowering. Sustained temperatures below 75ºF will delay the beginning of flowering significantly. Seed set also is affected by temperature. Seed set is generally good when pollination follows night temperatures around 70ºF. Soybean varieties differ in their response and tolerance to temperatures.

Soybean is photoperiod sensitive, which means that it transitions from vegetative to flowering stage in direct response to length of daylight (Scott and Aldrich, 1970). Most soybean varieties begin flowering soon after the day length begins to shorten. Flowering of southern varieties is initiated by a shorter day than that of varieties adapted to the north. The extent of vegetative growth occurring after the initiation of flowering depends not only on environmental factors but also the growth habit. Soybean varieties are described as either indeterminate or determinate in their growth habit (Scott and Aldrich, 1970). Indeterminate varieties increase their height by two to four times after flowering begins. Indeterminate varieties are typically grown in the northern and central U.S. Determinate varieties increase their height very little after flowering and generally are grown in the southern U.S. Indeterminate and determinate varieties also differ in flowering characteristics. Indeterminate plants generally bloom first at the fourth or fifth node and progress upward. Flowering on determinate plants begins at the eight or tenth node and progresses both downward and upward.

The first appearance of flowers signals the beginning of the reproductive stage, namely the R1 stage (Hoeft et al., 2000). The reproductive period consists of flowering, pod set, and seed formation. Climatic conditions such as temperature and moisture supply during the flowering period will affect the number of flowers. The soybean plant does not form a pod from each flower. It is common for the soybean plant to have 75% of the flowers

fail to develop a pod (Scott and Aldrich, 1970). This characteristic makes soybean less susceptible than corn to short periods of adverse weather during flowering. Under normal conditions, pod set occurs over about a three week period. Good soil moisture is most critical during the pod-filling stages to prevent pod abortion and to ensure high yields (Hoeft et al., 2000). Another critical requirement during the seed-filling stages is a high rate of photosynthesis to maximize yield. High humidity and temperatures during seed development and maturity can result in poor seed quality because these conditions promote the development of reproductive-stage diseases.

VIII.C.4. Harvest Season

When dry matter accumulation ends, the plant is considered to be physiologically mature. The seed moisture content is approximately 55 to 60% at this stage (Hoeft et al., 2000). At this stage, namely R7, at least one normal pod on the plant reaches the mature pod color. Under warm and dry weather conditions, seed moisture content will drop to 13 to 14% in 10 to 14 days from physiological maturity (Hoeft et al., 2000). Soybean can be harvested when the moisture content drops below 15%. However, soybean should be at 13% moisture to be stored without artificial drying (Scott and Aldrich, 1970). Moisture content below 12% may increase seed cracking and seed coat damage.

Pre-harvest losses are influenced by soybean variety, weather, and timeliness of harvest (Scott and Aldrich, 1970). Timely harvest when the moisture content is 13 to 14% also will minimize losses. Proper operation and adjustment of the combine is essential to minimizing harvest losses in the field.

VIII.D. Management of Insects

Although insects are rated as less problematic than weeds in U.S. soybean production, management of insect pests during the growth and development of soybean is important for protecting the yield of soybean (Aref and Pike, 1998). Understanding the impact of insects on soybean growth is essential for proper management (Higley and Boethel, 1994). It is important to understand the way that insects injure soybean as well as how the soybean plant responds to insect injury. Insect injury can impact yield, plant maturity, and seed quality. Insect injury in soybean seldom reaches levels to cause an economic loss, as indicated by the low percentage (16%) of soybean acreage that receives an insecticide treatment (USDA-NASS, 2007b).

Characterizing soybean responses to insect injury is essential in establishing economic injury levels (Higley and Boethel, 1994). Most often, soybean insects are categorized or defined by the plant parts they injure, namely root-feeding, stem-feeding, leaf-feeding, or pod-feeding insects. The root- and stem-feeding insect groups are often the hardest to scout and typically are not detected until after they have caused their damage. The leaf-feeding insects comprise the biggest group of soybean insect pests, but not necessarily the most economically damaging insects. Research on defoliation has determined that a major effect of leaf injury is to reduce light interception by the soybean canopy which in turn can have a significant effect on yield (Higley and Boethel, 1994). Soybean has an extraordinary capacity to withstand considerable defoliation early in the season without

significant yield loss. By contrast, defoliation during the flowering and pod filling stages poses a greater threat to yield because the soybean plant has less time to compensate for injury compared to other growth stages. Research indicates that the soybean plant can sustain a 35% leaf loss prior to the pre-bloom period without lowering yield (NDSU, 2002). However, from pod-set to maturity, the plant can tolerate only a 20% defoliation level before yield is impacted.

VIII.E. Management of Diseases and Other Pests

More than 100 pathogens are known to affect soybean, of which 35 are considered to be of economic importance (Heatherly and Hodges, 1999). The estimated yield losses to soybean diseases in the U.S. were 10.9, 11.9, and 14.0 million metric tons in 1996, 1997, and 1998, respectively (Wrather et al., 2000), which equated to 16.7%, 16.0% and 18.6% of total soybean production, respectively. Pathogens can affect all parts of the soybean plant, resulting in reduced quality and yield. The extent of losses depends upon the pathogen, the state of plant development and health when infection occurs, the severity of the disease on individual plants, and the number of plants affected (Heatherly and Hodges, 1999).

One or more diseases can generally be found in fields wherever soybean is grown (Heatherly and Hodges, 1999). However, a pathogen may be very destructive one season and difficult or impossible to find the next season. The extent and severity of soybean diseases depend on the degree of compatibility between the host and the pathogen and the influence of the environment.

According to field surveys conducted in fifteen soybean-producing states during 1996 to 1998, soybean cyst nematode (SCN), *Heterodera gylcines*, caused the greatest soybean yield losses (Wrather et al., 2000). *Phytophthora* root and stem rot (*Phytophthora sojae*), brown stem rot (*Phialophora gregata*), *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*), and seedling diseases followed in economical importance. As expected, yield losses varied by region. *Sclerotinia* stem rot caused yield losses in several Northern states, but not in other states. *Rhizoctonia* foliar blight losses were greatest in Arkansas, Louisiana, and Texas where humidity and temperature conditions are suitable for disease development.

Selecting resistant varieties is the primary tool growers have for disease control (Heatherly and Hodges, 1999). Resistant varieties may have morphological or physiological characteristics that provide immunity, resistance, tolerance or avoidance to certain pathogens. Cultural practices can also play an important role in disease management by reducing initial inoculums or reducing the rate of disease development (Heatherly and Hodges, 1999). Preplant tillage can bury crop residue, which encourages the decomposition of fungal-resting structures. Crop rotation is routinely recommended as a disease-management strategy. Rotating crops interrupts the disease cycle and allows time for the decomposition of inoculums. One exception is *Rhizontonia sp*., a soil-inhabitant pathogen that grows on a wide variety of crops and can survive sufficiently in the soil to make crop rotation as a means of controlling this pest impractical. Row

spacing, plant population, and planting date also can be changed to manage soybean diseases.

Soybean cyst nematode is one of the most damaging pathogens of soybean throughout the soybean growing regions of the U.S. (Pedersen, 2008b). Losses have been estimated to be at about $1.5 billion in the U.S. (Pedersen, 2008a). SCN can cause yield losses up to 50%, where this pest in 2004 alone caused an estimated loss of 50 million bushels of soybean in Iowa (Pedersen, 2008c). Soybean cyst nematodes feed on the roots, causing severely stunted and yellow plants. The simplest, least expensive method to reduce populations of this pest is to rotate soybean with a non-host crop such as corn, small grains, or sorghum. Planting resistant varieties is regarded as the best and most effective management practice to prevent losses from this pest. Several public and private soybean varieties offer sources of resistance to certain races of nematode. Alternating varieties with different sources of resistance also is beneficial.

High-quality seed is essential for controlling seedling diseases. The most important seedling diseases in soybean are *Phytophthora sp*., *Pythium sp*., *Rhizoctonia sp*., and *Fusarium sp*. (Pedersen, 2008a). Many soybean varieties demonstrate resistance to specific taxonomic races of *Phytophthora*. Treating soybean seed with a fungicide (*e.g.*, metalaxyl or mefenoxam) is effective against damping-off disease (seedling blight) caused by common soil fungi, such as *Phytophthora sp.* and *Pythium sp*. Fungicide seed treatments are recommended where there is a history of these seedling diseases.

Asian soybean rust is a foliar fungal disease that typically infests soybean during reproductive stages of development and can cause defoliation and reduce yields significantly in geographies such as Brazil (Dorrance et al., 2007). Soybean rust is caused by the fungus *Phakopsora pachyrhizi*. This disease in the U.S. was first detected in Louisiana in 2004 (LSU, 2009). At this time, foliar application of fungicides is the standard disease-management practice to limit yield losses due to soybean rust.

Foliar fungicide applications can effectively reduce the incidence of many fungal diseases (Heatherly and Hodges, 1999). However, the economic return from a fungicide application may be limited to select soybean production systems; for example, high-yield environments or when producing soybean seed. According to USDA-NASS (2007b) statistics, fungicides were applied on approximately 4% of the soybean acreage in 2006.

VIII.F. Weed Management

Annual weeds are perceived to be the greatest pest problem in soybean production, followed by perennial weeds (Aref and Pike, 1998). Soybean insects and diseases are rated less problematic but may reach economic thresholds requiring treatment. Weed control in soybean is essential to optimizing yields. Weeds compete with soybean for light, nutrients, and soil moisture. Weeds can harbor insects and diseases, and also can interfere with harvest, causing extra wear on harvest equipment (Pedersen, 2008a). The primary factors affecting soybean yield loss from weed competition are the weed species, weed density, and the duration of the competition. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001).

Generally, the competition between crops and weeds increases with higher levels of weed density. The time period that weeds compete with the soybean crop influences the level of yield loss. In general, early season weed competition will have the greatest negative impact on yield (Dalley et al., 2001). Although, soybean plants withstand early-season weed competition longer than corn without affecting yield, and the canopy closes earlier in soybean than corn. In addition, canopy closure is much sooner when soybean is drilled or planted in narrow rows. The most common weeds in soybean for each of the three major U.S. growing regions are presented in Tables VIII-4, VIII-5 and VIII-6.

Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in soybean. Foxtail spp. (*Setaria spp.*), pigweed (*Amaranthus spp.*), velvetleaf (*Abutilon theophrasti*), lambsquarters (*Chenopodium album*), and cocklebur (*Xanthium strumarium*) are common weeds in Midwest corn and soybean fields. However, growers consider giant ragweed (*Ambrosia artemisiifolia*), lambsquarters, Canada thistle (*Cirsium arvense)*, cocklebur, and velvetleaf to be the top five most problematic weeds in corn and soybean because of difficulty controlling these weeds (Nice and Johnson, 2005). In a recent survey of growers utilizing glyphosate-tolerant crops, pigweed, morningglory (*Ipomoea spp.*), Johnsongrass (*Sorghum halepense*), ragweed spp. (*Ambrosia spp.*), foxtail, and velvetleaf were mentioned as the most problematic weeds, depending on the state and cropping system (Kruger et al., 2009). With the exception of morningglory and pigweed, the weed species identified as problematic were present and problematic before glyphosate-tolerant crops were introduced, but then were to a reduced degree after implementing glyphosate-tolerant cropping systems (Kruger et al., 2009). Common waterhemp (*Amaranthus rudis*) and ragweed were the most frequently mentioned problematic weeds in glyphosate-tolerant crops in Illinois, Indiana and Iowa.

The most frequently reported common weeds in the Southeast region were morningglory (*Ipomoea spp.*), prickly sida (*Sida spinosa*), johnsongrass (*Sorghum halepense*), sicklepod (*Cassia obtusifolia*), and broadleaf signalgrass (*Brachiaria platyphylla*) (Webster et al., 2005, 2009). Morningglory, sicklepod, and pigweed are the most frequently mentioned problematic weeds in glyphosate-tolerant crops in Mississippi and North Carolina (Kruger et al., 2009).

Cultural and mechanical weed control practices can be important components of an effective weed management program (Loux et al., 2009). Crop rotation, narrow row spacing and planting date are a few of the crop management practices that are implemented to provide the crop with a competitive edge over weeds. Although the primary purpose of tillage is for seedbed preparation, tillage is still used to supplement weed control with selective herbicides in soybean production. Approximately 98% of the soybean acreage received an herbicide application in 2006, indicating the importance of excellent weed control in maximizing soybean yield (USDA-NASS, 2007b).

Herbicide-tolerant soybean was introduced to provide growers with additional options to improve crop safety and/or improve weed control. The Roundup Ready soybean system (planting Roundup Ready soybean and applying glyphosate in crop to provide primary weed control) was introduced in 1996 and has become the standard weed control program

in U.S. soybean production and is utilized on 91% of U.S. soybean acreage (USDA-NASS, 2009c).

Herbicides provide effective and economical control of weeds in soybean. The risk of weeds developing resistance to herbicides and the potential impact of resistance on the usefulness of an herbicide vary greatly across different mechanisms of action and are dependent on a combination of factors, such as selection pressure, herbicide soil residual activity, herbicide chemistry, prolific seed production and high genetic variation in plants (see Appendix K for a more detailed discussion of herbicide resistance in weeds). Weed-resistance management programs that integrate the use of herbicides with different mechanisms of action and short residual activity times in soil reduce selection pressure exerted on weed species (Prather et al., 2000). Crop rotation can also be beneficial in managing resistance because it may allow the grower to manipulate planting times to avoid early-season weed germination and to use mechanical as well as chemical weed control methods (Jordan et al., 1995). As described in Appendix K, when utilized in an integrated manner, these management practices can be used to impede the development of herbicide resistance in weeds.

Table VIII-4. Common Weeds in Soybean Production: Midwest Region

Foxtail spp. (12)[1]	Ragweed, giant (3)	Dandelion (1)
Pigweed spp. (11)	Shattercane (3)	Johnson grass (1)
Velvetleaf (11)	Quackgrass (3)	Milkweed, honeyvine (1)
Lambsquarters (10)	Buckwheat, wild (2)	Nightshade, hairy (1)
Cocklebur (9)	Crabgrass spp. (2)	Oats, wild (1)
Ragweed, common (7)	Kochia (2)	Pokeweed, common (1)
Smartweed spp. (6)	Mustard, wild (2)	Prickly sida (1)
Morningglory spp. (5)	Nightshade, Eastern black (2)	Proso millet, wild (1)
Sunflower, spp. (5)	Palmer pigweed (2)	Sandbur, field (1)
Waterhemp spp. (5)	Canada thistle (1)	Venice mallow (1)
Horseweed (marestail) (3)	Chickweed (1)	Volunteer cereal (1)
Panicum, fall (3)	Cupgrass, woolly (1)	Volunteer corn (1)

[1]Number provided in parenthesis is the number of states out of the thirteen total states in the Midwest region reporting each weed as a common weed.

Sources:

IL: University of Illinois (2002) and Aaron Hager, Extension Weed Specialist, University of Illinois - Personal Communication (2006).

IN: 2003-2005 Statewide Purdue Horseweed Weed Survey, Special database query and personal communication (2006), Bill Johnson, Extension Weed Specialist, Purdue University.

IA, MN, OH, WI: WSSA, 1992.

KS: Dallas Perterson, Extension Weed Specialist, Kansas State - Personal communication (2006).

KY, MO: Webster et al., 2005.

MI: Davis et al., 2005.

NE: Alex Martin, Extension Weed Specialist, University of Nebraska – Personal communication (2006).

ND: Zollinger, 2000.

SD: Michael Moechnig, Extension Weed Specialist, South Dakota State University – Personal communication (2006).

Table VIII-5. Common Weeds in Soybean Production: Southeast Region

Morningglory spp. (8)[1]	Goosegrass (3)	Cutleaf evening-primrose (1)
Crabgrass spp. (6)	Johnsongrass (3)	Groundcherry (1)
Prickly sida (6)	Ragweed, common (3)	Henbit (1)
Nutsedge spp. (6)	Cocklebur (2)	Lambsquarters (1)
Sicklepod (5)	Florida beggarweed (2)	Ragweed, giant (1)
Signalgrass, broadleaf (5)	Hemp sesbania (2)	Smartweed (1)
Palmer pigweed (4)	Horseweed (marestail) (2)	Spurge, nodding/hyssop (1)
Pigweed spp. (4)	Texas millet (2)	Spurge, Prostrate (1)
Barnyard grass (3)	Browntop millet (1)	Tropic croton (1)
Florida pusely (3)	Copperleaf, hophorn (1)	

[1] Number provided in parenthesis is the number of states out of the eight total states in the Southeast region reporting each weed as a common weed.
Sources:
AL, AR, GA, LA, NC, SC: Webster et al., 2009.
MS, TN: Webster et al., 2005.

Table VIII-6. Common Weeds in Soybean Production: Eastern Coastal Region

Foxtail spp. (6)[1]	Morningglory spp. (4)	Dandelion (1)
Ragweed, common (6)	Panicum, fall (4)	Goosegrass (1)
Velvetleaf (6)	Crabgrass spp. (3)	Johnson grass (1)
Lambsquarters (5)	Nutsedge spp. (3)	Nightshade, Eastern black (1)
Pigweed spp. (5)	Quackgrass (2)	Prickly sida (1)
Cocklebur (4)	Canada thistle (1)	Shattercane (1)
Jimson weed (4)	Burcucumber (1)	Smartweed spp. (1)

[1]Number provided in parenthesis is the number of states out of the six total states in the Eastern Coastal region reporting each weed as a common weed. Data were not available for DE in soybean.
Sources:
DE, MD, NJ, PA: WSSA, 1992.
NY: Russell Hahn, Extension Weed Specialist, Cornell University – Personal Communication (2006).
VA: Webster et al., 2009.

VIII.F.1. Methods of Weed Control in Soybean

Mechanical methods of weed control including tillage have been used for centuries to control weeds in crop production. Spring or fall preplant tillage and in-crop shallow cultivation can effectively reduce the competitive ability of weeds by burying the plants, disturbing or weakening their root systems, or causing sufficient physical injury to kill the weeds. Research in the early 1900s centered on determining the economic benefits of removing weeds with the use of cultivation (Klingman et al., 1975). A consequence of in-crop cultivation for weed control can be injury to crop roots and moisture loss. Selective herbicides have proved more efficacious and reduced the need for in-crop tillage or cultivation to control weeds in soybean production. The development of selective herbicides has progressed rapidly since the introduction of the first synthetic

herbicide (2,4-D) for weed control in corn in the early 1940s. Although the primary purpose of tillage is for seedbed preparation, tillage still is used to supplement weed control with selective herbicides in soybean production.

Alanap (1949), amiben (1958), trifluralin (1959), linuron (1960), and alachlor (1966) led the way for numerous selective herbicides in soybean (Agranova, 2010). Bentazone (1968) was one of the early selective postemergence herbicides used in soybean production. By the early 1990s, there were over 70 registered herbicides or premix herbicides for weed control in soybean (Gianessi et al., 2002). Table VIII-7 provides a summary of herbicide use in soybean production in the U.S. from 1995 through 2001. Weed control programs in soybean production during this time period consisted of preemergence herbicides used alone or in a tank mixture with other preemergence herbicides. Applications were made as preplant incorporated or preemergence surface applications prior to or at planting. Tank mixtures of two preemergence herbicides were used to broaden the spectrum of control to both grasses and broadleaf weed species. Preemergence herbicides are followed by postemergence applications to control weed escapes that emerge later in the crop. Total postemergence programs were seldom used in soybean production prior to 1995. For soybean planted in a no-till system, an additional preplant burndown herbicide application for broad-spectrum control of existing weeds at time of planting was also applied. Therefore, multiple herbicides and/or multiple applications were generally made in soybean production. The average number of herbicide applications per acre in soybean rose from 1.5 in 1990 to 1.7 applications in 1995 reflecting the use of at-plant and postemergence applications or two postemergence applications (Gianessi et al., 2002).

It is important to understand herbicide use in 1995, as this was prior to the introduction of Roundup Ready soybean system. The most widely used herbicides in 1995 were the sulfonylurea (chlorimuron, thifensulfuron) and imidazonlinone (imazethapyr, imazaquin) herbicide classes that are applied preemergence and postemergence in a soybean crop. These two classes of herbicides, both acetolactate synthase (ALS) inhibitors, were applied on approximately 87% of the soybean treated acres in 1995 (Table VIII-7). The dinitroanaline herbicides (trifluralin and pendimethalin) were the second most widely used preemergence herbicides. Selective postemergence herbicides were used on 52% of the treated acres and were generally either effective on grass species or broadleaf species. Sethoxydim, clethodim, quizalofop, and fluazifop were among the postemergence grass herbicides. Acifluorfen and bentazon were the main postemergence broadleaf herbicides. Glyphosate was used on 20% of the treated acres, mainly as a preplant burndown treatment, but it also was used in spot treatments or ropewick applications to control weed escapes or volunteer corn in soybean.

Herbicide programs used in conventional soybean have not changed significantly since 1995, with many of the traditional herbicides still in use. Although, new active ingredients have been introduced, including carfentrazone, sulfentrazone, flufenacet, flumetsulam, flumiclorac, flumioxazin, cloransulam, and imazamox. These new active ingredients improve the level or spectrum of weed control. Numerous products have been introduced that are a pre-mixture product of two active ingredients for broad spectrum weed control. Some of the new active ingredients and pre-mixtures are more

effective in controlling waterhemp, ALS-resistant weeds, and other hard-to-control weeds.

Table VIII-8 provides a summary of the herbicide use in soybean in the U.S. in 2006. In 2006, herbicide-tolerant soybean (glyphosate-tolerant) was planted on 89% of the 64.1 million acres of soybean (USDA-NASS, 2007a). With the high percentage of glyphosate-tolerant soybean and the additional use of glyphosate for preplant burndown applications on both glyphosate-tolerant and conventional soybean, it is not surprising that glyphosate was used on 97% of the total soybean acres in 2006. The percentage of herbicide-tolerant soybean has subsequently increased to 91% in 2009 (USDA-NASS, 2009c). The remaining preemergence and postemergence herbicides are utilized in conventional soybean as well as glyphosate-tolerant soybean. A grower survey conducted in 2006 showed that 15 to 21% of growers applied non-glyphosate herbicides as another mode-of-action in addition to glyphosate for weed control in glyphosate-tolerant soybean (Givens et al., 2009). These non-glyphosate herbicides were applied prior to planting, at planting and postemergence in soybean. The non-glyphosate herbicides mainly included applications of chlorimuron, flumiclorac, pendimethalin, imazethapyr, and 2,4-D, which were commonly used herbicides in weed management programs prior to the introduction of glyphosate-tolerant soybean. Although these non-glyphosate herbicides were applied to supplement the weed control provided by glyphosate, researchers report that approximately 40 to 55% of the growers utilizing glyphosate-tolerant crops indicate that rotating herbicides or tank mixing glyphosate with other herbicides is an effective management practice to minimize glyphosate resistance development (Johnson et al., 2009). It should be noted in Table VIII-8 that it is estimated that approximately 16,000 lbs of dicamba is currently used in soybean production which is a sufficient amount of dicamba to treat 64,000 acres at the average application rate of 0.25 lb dicamba acid equivalent (a.e.) per acre. Dicamba is currently labeled only for preplant and preharvest applications in soybean, where restrictions on days after preplant treatment are required due to insufficient crop tolerance. Similarly, dicamba currently cannot be used in-crop postemergence applications on soybean due to a lack of crop tolerance.

Tables VIII-9 and VIII-10 provide a summary of the crop tolerance to herbicides applied in soybean production and the efficacy of these herbicides on 25 of the common weed species identified in Section VIII.F. These tables list only the most commonly used herbicides in soybean production. Glyphosate applied postemergence (as part of the Roundup Ready soybean system) and four other herbicides applied either preemergence or postemergence have the highest crop tolerance rating of excellent. The other herbicides are rated only good to poor. Seldom would one field or farm have all 25 weed species, but they generally have a mixture of grass and broadleaf weed species. These ratings can be used by growers to facilitate the selection of an herbicide program for a soybean crop, which offers the best overall control of the weed species. Based on Tables VIII-9 and VIII-10, glyphosate is considered to have better control (>80%) on more grass and broadleaf weed species than any other herbicide. Glyphosate/imazethapyr has the next highest overall rating, but it is rated only good on crop tolerance. S-Metolachlor and pendimethalin are rated high on many grass species, but are rated low on most of the broadleaf weed species. Chlorimuron/tribenuron, fomesafen, and

flumioxazin/cloransulam are rated high on the broadleaf species, but are rated low on grass species.

Table VIII-7. Herbicide Use in Soybean in the U.S. from 1995 through 2001[1]

	Percent-Treated Acres						
Active Ingredient	1995	1996	1997	1998	1999	2000	2001
2,4-D	10	13	8	7	5	5	4
2,4-DB	1	<1	1	<1	<1	NA	NA
Acifluorfen	12	11	12	7	3	3	3
Alachlor	4	5	3	2	2	1	<1
Bentazon	12	11	11	7	4	2	1
Chlorimuron	16	14	13	12	12	10	5
Clethodim	5	7	4	4	5	4	4
Clomazone	4	3	5	4	1	<1	<1
Cloransulam	NA	NA	NA	1	5	4	5
Dimethenamid	1	1	1	1	<1	<1	NA
Ethalfluralin	1	1	<1	NA	<1	<1	NA
Fenoxaprop	6	4	6	4	4	4	3
Fluazifop	10	7	7	5	4	5	3
Flumetsulam	2	2	4	2	2	2	<1
Flumiclorac	NA	2	1	<1	<1	<1	<1
Fomesafen	4	5	6	6	4	7	7
Glyphosate	20	25	29	47	62	66	76
Imazamox	NA	NA	NA	7	3	6	5
Imazaquin	15	15	13	8	5	4	2
Imazethapyr	44	43	38	17	16	12	9
Lactofen	5	8	4	2	2	2	1
Linuron	2	1	1	<1	<1	<1	NA
Metolachlor	7	5	7	4	4	2	NA
Metribuzin	11	9	10	6	5	4	2
Paraquat	2	1	2	1	1	<1	NA
Pendimethalin	26	27	25	18	14	11	10
Quizalofop	6	7	4	3	1	<1	<1
S-Metolachlor	NA	NA	NA	NA	NA	NA	<1
Sethoxydim	7	9	7	5	3	2	1
Sulfentrazone	NA	NA	NA	3	4	4	5
Thifensulfuron	12	10	9	5	5	6	2
Trifluralin	20	22	21	16	14	14	7

[1]Source is Gianessi et al. (2002).

Table VIII-8. Agricultural Chemical Applications Registered for Soybean Use in AR, IA, IL, IN, KS, KY, LA, MI, MN, MS, MO, NE, NC, ND, OH, SD, TN, VA, and WI in 2006[1]

Herbicide	Chemical Family	Mode-of-Action (MOA)	Percent-Treated Acres	Total Area Applied (Percent/MOA)	Quantity Applied (1000 lbs)	Total Quantity Applied (1000 lbs/MOA)
Glyphosate	glycine	EPSPS inhibitor	4	97	2,841	92,856
Glyphosate, amm. Salt	glycine		*		142	
Glyphosate, iso. salt	glycine		92		88,903	
Sulfosate	glycine		1		970	
Pendimethalin	dinitroanaline	Tubulin inhibitor	3	5	1,894	3,348
Trifluralin	dinitroanaline		2		1,454	
Bentazon	benzothiadiazinone	PSII inhibitor	*	3	70	577
Metribuzin	triazinone		2		437	
Sulfentrazone	triazolinone		1		70	
Chlorimuron-ethyl	sulfonylurea	ALS inhibitor	4	11	52	265
Cloransulam-methyl	triazolopyrimidine		1		17	
Flumetsulam	triazolopyrimidine		*		8	
Imazamox	imidazolinone		*		9	
Imazaquin	imidazolinone		1		66	
Imazethapyr	imidazolinone		3		100	
Imazethapyr, ammon.	Imidazolinone		*		5	
Thifensulfuron	sulfonylurea		1		3	
Tribenuron-methyl	sulfonylurea		1		5	
Alachlor	chloroacetamide	Cell division inhibitor	*	1	485	1,402
S-Metolachlor	chloroacetamide		1		837	
Flufenacet	oxyacetamide		*		80	
Paraquat	bipyridilium	PSI disruption	1	1	335	335

Table VIII-8 (continued). Agricultural Chemical Applications Registered for Soybean Use in AR, IA, IL, IN, KS, KY, LA, MI, MN, MS, MO, NE, NC, ND, OH, SD, TN, VA, and WI in 2006[1]

Herbicide	Chemical Family	Mode-of-Action (MOA)	Percent-Treated Acres	Total Area Applied (Percent/MOA)	Quantity Applied (1000 lbs)	Total Quantity Applied (1000 lbs/MOA)
Clethodim	cyclohexenone	ACCase inhibitor	3	4	190	266
Fenoxaprop	aryloxyphenoxy propionate		*		9	
Fluazifop-P-butyl	aryloxyphenoxy propionate		1		43	
Quizalofop-P-ethyl	aryloxyphenoxy propionate		*		14	
Sethoxydim	cyclohexenone		*		10	
Acifluorfen	diphenyl ether	PPO inhibitor	*	6	47	565
Carfentrazone-ethyl	triazolinones		*		10	
Flumiclorac-pentyl	N-phenylphthalimide		1		17	
Flumioxazin	N-phenylphthalimide		3		138	
Fomesafen	diphenyl ether		2		330	
Lactofen	diphenyl ether		*		23	
2,4-D, 2-EHE	phenoxy	Synthetic auxin	7	10	2,505	3,542
2,4-D, dimeth. salt	phenoxy		3		953	
2,4-D (butoxy ester)	phenoxy		*		68	
Dicamba, digly salt	benzoic acid		*		16	
					Total	**103,156**

* Area receiving application is less than 0.5 percent.

[1]Data derived from USDA-NASS (2007b). Planted acreage for the nineteen primary soybean production states was 72.9 million acres, which represented 96.5% of total planted acres.

Table VIII-9. Crop Tolerance and Common Grass Weed Responses to Herbicides Applied in Soybean Production

		Common Grass Weeds[1,2]										
Herbicide/Application	CT[3]	BY	BS	CG	FP	FT	GG	SC	JGs	JGr	RR	NSy
Preplant Incorporated												
Trifluralin	1	9	9	9	9	9	9	7	7	3	9	-
Preplant or Preemergence												
Chlorimuron/tribenuron	2	-	8	-	-	-	7	-	-	2	8	-
Cloransulam	0	-	NA	-	-	-	NA	-	-	NA	NA	-
Flumioxazin	2	-	5	-	-	-	5	-	-	0	8	-
Flumioxazin/cloransulam	2	-	5	-	-	-	5	-	-	0	8	-
Imazaquin	1	-	7	-	-	-	5	-	-	2	5	6
Imazethapyr	1	6	NA	7	7	7	NA	6	6	NA	NA	-
Metribuzin	2	2	6	6	5	6	7	6	-	0	4	-
Pendimethalin	2	8	9	9	9	8	9	7	7	3	4	-
s-Metolachlor	1	8	8	9	8+	8+	9	-	-	0	3	8+
Postemergence												
Bentazon	1	-	0	-	-	-	0	-	-	0	NA	8+
Chlorimuron	2	-	0	-	-	-	0	-	-	0	0	8
Clethodim	0	9	9	8+	9	9	9	9	9	9	8	-
Cloransulam	1	-	0	-	-	-	0	-	-	0	0	6
Clorimuron/thifensulfuron	0	-	NA	-	-	-	NA	-	-	NA	NA	8
Fluazifop/fenoxaprop	0	9	8	8+	9	9	9	9	9	9	7	-
Flumiclorac	2	-	NA	-	-	-	NA	-	-	NA	NA	-
Fomesafen	2	-	3	-	-	-	3	-	-	3	0	-
Glyphosate	0*	8+	9	8+	8+	9	8	8	9	9	8	7
Glyphosate/imazethapyr	1*	9	NA	8+	9	9	NA	9	9	NA	NA	7
Imazamox	2	6	NA	7	7	7-8+	NA	-	-	NA	NA	-
Imazethapyr	1	6	7	7	7	7-8	5	8	8	6	4	-
Lactofen	3	-	4	-	-	-	4	-	-	2	0	-
Thifensulfuron	2	-	NA	-	-	-	NA	-	-	NA	NA	-

[1]All weed control ratings except for BS, GG, JGr and RR are from the 2009 Weed Control Guide for Ohio and Indiana, Ohio State University and Purdue University (Loux et al., 2009). Ratings for BS, GG, JGr and RR are from the 2009 Weed Control Guidelines for Mississippi, Mississippi State University (MSU, 2009). Weed control rating for weeds, except BS, GG, and RR, are: 9 = 90% to 100%, 8 = 80% to 90%, 7 = 70% to 80%, 6 = 60% to 70%, - = less than 60% control, not recommended. Weed control ratings for BS, GG, and RR are: 9-10 = excellent, 7-8 = good, 4-6 = fair, 0-3 = none to slight. Ratings assume the herbicides are applied in the manner suggested in the guidelines and according to the label under optimum growing conditions.

[2]Weed species: BY = barnyardgrass, BS = broadleaf signalgrass, CG = crabgrass, FP = fall panicum, FT = giant and yellow foxtail, GG = goosegrass, SC = shattercane, JGs = seedling Johnsongrass, JGr = rhizome Johnsongrass, RR = red rice, and NSy = yellow nutsedge.

[3]Crop tolerance (CT) rating: 0 = excellent, 1 = good, 2 = fair, 3 = poor.

NA denotes not available. *Rating based on application to Roundup Ready soybean.

Table VIII-10. Common Broadleaf Weed Responses to Herbicides Applied in Soybean Production

	Common Broadleaf Weeds[1,2]													
Herbicide/Application	BN	CB	CR	GR	HS	LQ	MG	PA	PW	PS	SP	SW	VL	WH
Preplant Incorporated Only														
Trifluralin	-	-	-	-	0	8+	2	7	9	0	4	-	-	8
Preplant or Preemergence														
Chlorimuron/tribenuron	-	8	9	7	9	9	8	8	9	7	NA	9	8+	-
Cloransulam	-	8	9	7	NA	9	NA	NA	9	NA	NA	8	8+	-
Flumioxazin	9	-	7	-	9	9	6-8	9	9	8	7	7	7	8
Flumioxazin/cloransulam	9	8	9	7	9	9	7-8	9	9	8	7	9	8+	8
Imazaquin	9	8	8	7	0	9	6-8	9	9	9	5	9	7	-
Imazethapyr	9	7	6	-	NA	9	NA	NA	9	NA	NA	9	8	-
Metribuzin	-	-	-	7	9	7	2-8	9	8	9	8	9	9	7
Pendimethalin	-	-	-	-	0	8+	2	7	9	4	2	-	-	7
s-Metolachlor	8	-	-	-	0	6	0	8	8	4	2	-	-	7
Postemergence														
Bentazon	-	9	7	6	4	7	2-9	4	-	8	0	9	8+	-
Chlorimuron	-	9	8	7+	8	-	8-9	6	9	2	7	8	8	-
Cloransulam	-	9	9	9	3	-	8-9	2	-	2	7	8	9	-
Clorimuron/thifensulfuron	-	9	8	7+	NA	8	NA	NA	9	NA	NA	9	9	-
Flumiclorac	-	7	7	-	NA	7	NA	NA	7	NA	NA	-	9	7
Fomesafen	8	7	8+	8	9	-	8-9	8	9	2	3	7	6	9
Glyphosate	8	9	8+	8	7	8	7-9	9	9	7	8	8	8	8
Glyphosate/imazethapyr	9	9	8+	8+	NA	8+	NA	NA	9	NA	NA	9	9	8
Imazamox	9	8	7	8	NA	8	NA	NA	9	NA	NA	8	9	-
Imazethapyr	9	9	6	7	0	6	7-9	6	9	6	0	9	9	-
Lactofen	8+	8	9	8	9	-	8-9	8	9	8	5	6	7	9
Thifensulfuron	-	6	-	-	NA	8	NA	NA	9	NA	NA	8	9	-

[1]All weed control ratings except for HS, MG, PA, PS, and SP are from the 2009 Weed Control Guide for Ohio and Indiana, Ohio State University and Purdue University (Loux et al., 2009). Ratings for HS, MG, PA, PS, and SP are from the 2009 Weed Control Guidelines for Mississippi, Mississippi State University (MSU, 2009). Weed control ratings for weeds, except HS, MG, PA, PS, and SP, are: 9 = 90% to 100%, 8 = 80% to 90%, 7 = 70% to 80%, 6 = 60% to 70%, - = less than 60% control, not recommended. Weed control ratings for HS, MG, PA, PS, and SP are: 9-10 = excellent, 7-8 = good, 4-6 = fair, 0-3 = none to slight. Ratings assume the herbicides are applied in the manner suggested in the guidelines and according to the label under optimum growing conditions.
[2]Weed species: BN = black nightshade, CB = cocklebur, CR = common ragweed, GR = giant ragweed, LQ = lambsquarters, MG = morningglory spp., HS = hemp sesbania, PA = palmer and spiny pigweed, PW = pigweed, PS= prickly sida, SP = sicklepod, SW = smartweed, VL = velvetleaf, and WH = waterhemp.
NA denotes not available.

VIII.G. Dicamba Herbicide Use in the U.S.

(b) (4)

[redacted]

[redacted]

[redacted]

(b) (4)

(b) (4)

(b) (4)

[2] Clarity product label can be found at: http://www.cdms.net/LDat/ld797002.pdf.

[3] Clarity label specifies that preharvest application requires that soybean pods must have reached mature brown color and at least 75% leaf drop has occurred. Harvest of soybean is allowed 14 or more days after preharvest application.

(b) (4)

(b) (4)

[redacted]

(b) (4)

(b) (4)

(b) (4)

(b) (4)

[redacted]

(b) (4)

(b) (4)

[4] Clarity product label can be found at: http://www.cdms.net/LDat/ld797002.pdf.

(b) (4)

[redacted]

(b) (4)

VIII.H. Dicamba-Tolerant Soybean MON 87708

(b) (4)

[redacted] 1.

(b) (4)

[redacted]

[redacted]

[redacted]

(b) (4)

[redacted]

[redacted]

(b) (4)

(b) (4)

(b) (4)

(b) (4)

[6] Clarity product label can be found at: http://www.cdms.net/LDat/ld797002.pdf. Label specifies when applying to corn not to apply Clarity when soybean are growing nearby if any of the following conditions exist: 1) corn is more than 24" tall, 2) soybean is more than 10" tall, and 3) soybean has begun to bloom.

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4) [redacted]

[redacted]

VIII.H.3. Conclusions on Dicamba-Tolerant Soybean MON 87708

(b) (4) [redacted] growers will have the ability to continue use of established soybean production practices including tillage systems; the same planting and harvesting machinery; traditional management of insects, diseases, and other pests; and many of the current herbicides used for weed control, including glyphosate with its established environmental benefits. Similarly, certified seed production will continue to use well-established industry practices to deliver high quality seed to growers. Due to the excellent crop safety of MON 87708 to dicamba, growers will have a new herbicide mode-of-action for in-crop control of glyphosate's hard-to-control and resistant broadleaf weeds that are present in U.S. soybean production. As expected with a new use of dicamba in U.S. soybean production, the number of dicamba-treated soybean acres and the total dicamba use will increase. (b) (4) [redacted] .

VIII.I. Crop Rotation Practices in Soybean

The well-established farming practice of crop rotation is still a key management tool for growers. The purpose of growing soybean in rotation is to improve yield and profitability of one or both crops over time, decrease the need for nitrogen fertilizer on the crop following soybean, increase residue cover, mitigate or break disease, insect, and weed cycles, reduce soil erosion, increase soil organic matter, improve soil tilth and soil physical properties, and reduce runoff of nutrients, herbicides, and insecticides (Boerma and Specht, 2004; Al-Kaisi et al., 2003). According to USDA Economic Research Service, 95% of the soybean-planted

acreage has been in some form of a crop rotation system since 1991 (USDA-ERS, 2001). Corn- and wheat-planted acreage has been rotated at a slightly lower level of 75% and 70%, respectively. Although the benefits of crop rotations can be substantial, the grower must make cropping decisions by evaluating both the agronomic and economic returns of various cropping systems. Crop rotations also afford growers the opportunity to diversify farm production in order to minimize market risks.

Continuous soybean production is not a common practice in the Midwest and is discouraged by most extension soybean specialists to reduce the risk of damage from diseases and nematodes (Hoeft et al., 2000; Al-Kaisi et al., 2003). Corn and soybean occupy more than 80% of the farmland in many of the Midwestern states, and the two-year cropping sequence of soybean-corn is used most extensively in this region. However, a soybean crop sometimes is grown after soybean and then rotated to corn in a 3-year rotation sequence (soybean-soybean-corn) in the Midwest. Compared to corn, soybean shows a greater yield response to being grown after a number of years without soybean. The yield of both corn and soybean is approximately 10% higher when grown in rotation than when either crop is grown continuously (Hoeft et al., 2000).

A combination of conservation tillage practices and crop rotation has been shown to be very effective in improving soil physical properties. Long-term studies in the Midwest indicate that the corn-soybean rotation improves yield potential of no-till systems compared to continuous corn production (Al-Kaisi, 2001). The reduction in yield of continuous corn production in no-till systems is attributed to low soil temperature during seed germination, which is evident on poorly drained soils under no-till practices.

Unique to the southern portion of the Midwest and the Southeast regions, soybean is grown in a double-cropping system. Double-cropping refers to the practice of growing two crops in one year. This practice can improve income and reduce soil and water losses by having the soil covered with a plant canopy most of the year (Hoeft et al., 2000). In the Midwest, winter wheat is harvested in late June or July, and then soybean is planted into the wheat residue in a no-till system to conserve moisture. Due to the uncertainty of double-cropping yields, growers sometimes do not plant if soils are too dry at the time of wheat harvest. Soybean typically is grown in a corn-wheat-soybean rotation sequence when soybean is grown in a double-cropping system.

Rotation practices for soybean vary from state to state. However, there are similarities among states within certain growing regions. This section provides a detailed description and quantitative assessment of the rotational cropping practices immediately following soybean production, by region and state. This assessment accounts for about 99% of total U.S. soybean acreage. This assessment is based on current agronomic practices following soybean production and current dicamba herbicide usage in labeled crops. USDA-NASS (2004, 2006, 2007b, 2008) and AgroTrak (2009) data on dicamba herbicide usage for corn, sorghum, cotton, wheat, barley, and oats were utilized for this assessment. For the purpose of this assessment, a 50% adoption rate in U.S. soybean production was assumed for MON 87708. These data on rotational patterns are presented in Tables VIII-22 through VIII-25.

The majority of the U.S. soybean acreage (68.6%) is rotated to corn (Table VIII-22). The second largest rotational crop following soybean is soybean. Approximately 14.5% of the soybean acreage is rotated back to soybean the following year. Wheat follows soybean on approximately 11.2% of the U.S. soybean acreage, with cotton, rice, and sorghum the next largest rotational crops. However, these three crops were planted on only 4.6% of the soybean acreage. Other minor rotational crops that follow soybean production are listed in Table VIII-22.

Column J of each table provides the percentage of soybean acreage as a function of the total rotational crop acreage to indicate the level that soybean is the primary crop preceding the rotational crops. For the entire country (Table VIII-22), this percentage is 35.3% indicating that soybean is a major crop preceding these rotational crops. The percentage of soybean as a preceding crop varies widely in different states, which ranges from 16.8% (KS, Table VIII-23) to 95.2% (NJ, Table VIII-25). In the Midwest region where 82% of the soybean is grown, 34.6% of the rotational crop area was planted with soybean during the previous growing season.

(b) (4) [redacted]

(b) (4)

[redacted]

(b) (4)

[redacted]

(b) (4)

(b) (4)

(b) (4)

(b) (4)

[redacted]

(b) (4)

(b) (4)

(b) (4) [redacted]

(b) (4)

[redacted]

(b) (4)

[redacted]

VIII.J. Soybean Volunteer Management

Volunteer soybean is defined as a plant that has germinated and emerged unintentionally in a subsequent crop. Soybean seeds can remain in a field after soybean harvest as a result of pods splitting before or during harvest. Soybean seeds also can remain in a field when pod placement on the plants is too close to the ground for the combine head to collect all the pods or when the combine is improperly adjusted for efficient harvesting. Volunteer soybean in rotational crops is not a concern in the Midwest region because the soybean seed is typically not viable after the winter period (Carpenter et al., 2002; OECD, 2000). In southern soybean growing areas of the U.S. where the winter temperatures are milder, it is possible for soybean seed to remain viable over the winter and germinate the following spring.

Volunteer soybean normally is not a concern in rotational crops, such as corn, cotton, rice, and small grains (*e.g.*, wheat, barley, sorghum, and oats), that are the significant rotational crops following soybean due to control measures that are available for volunteer soybean when they arise (Carpenter et al., 2002; OECD, 2000). Preplant tillage is the first management tool for control of emerging volunteer soybean in the spring. If volunteer soybean should emerge after planting, shallow cultivation will control most of the plants and effectively reduce competition with the crop. Several postemergence herbicides also are available to control volunteer soybean (conventional or glyphosate-tolerant soybean, and by extension dicamba-tolerant soybean) in each of the major soybean rotational crops. Table VIII-26 provides control ratings on volunteer soybean for several herbicides used in the major rotational crops. (b) (4) [redacted]

To provide control of volunteer soybean in corn, postemergence applications of AAtrex® (atrazine), Hornet® (flumetsulam + clopyralid) and Widematch® (clopyralid + fluroxypyr) provide excellent control (Zollinger, 2009). AAtrex and Permit® (halosufuron) provide excellent volunteer control in sorghum. In small grains (wheat, barley, oats), Bronate® Advanced (bromoxynil/MCPA), and Widematch® applied postemergence provide excellent control of volunteer soybean (Zollinger, 2009).

Volunteer soybean in cotton is normally not a concern. However, hurricanes or other extreme weather conditions can damage a soybean crop preceding cotton production in the Mid-South region states, where the unharvested soybean seed can produce volunteer plants. Preplant applications of paraquat or herbicide mixtures containing paraquat will effectively control volunteer glyphosate-tolerant soybean (Montgomery et al., 2002; Murdock et al., 2002). Recent research in North Carolina indicates Envoke (trifloxysulfuron) will provide excellent postemergence control of (volunteer) soybean containing traits for glyphosate and sulfonylurea herbicide tolerance in Roundup Ready cotton (York et al., 2005).

Volunteer soybean in rice is rarely a concern due to the combination of preplant tillage, flooding practices, and herbicides used in producing rice. If volunteer plants should emerge in rice, postemergence applications of Grandstand (triclopyr), Regiment (bispyribac), Grasp (penoxsulam), and Permit (halosulfuron) typically used for weed control in rice will effectively alleviate competition from volunteer soybean (Dillon et al., 2006; Bond and Walker, 2009).

Given the low potential for soybean to volunteer in subsequent crops, the availability of multiple herbicidal and cultivation methods for controlling volunteers, as well as the demonstrated lack of difference in germination of MON 87708 compared to conventional soybean (see Section VII.C.1), (b) (4) [redacted] ot expected to impact the management of soybean volunteer plants.

Table VIII-26. Ratings for Postemergence Control of Volunteer Soybean in Labeled Rotational Crops[1]

Product	Rate (Product/Acre)	Soybean V2 – V3	Soybean V4- V6
Corn[2]			
AAtrex 4L (atrazine)	0.38 qts	E	P
	0.50 qts	E	F
Hornet WDG (flumetsulam/clopyralid)	1 – 2 oz	E	F-G
Widematch (clopyralid/fluroxypyr)	0.25 pt	E	G
Sorghum[2,4]			
AAtrex 4L (atrazine)	0.38 qts	E	P
	0.50 qts	E	F
Permit (halosulfuron)	2/3 oz	E	E
Buctril® (bromoxynil)	1 pt		
Wheat, Barley & Oats[2]			
Buctril (bromoxynil)	1 pt	E	E
Widematch (clopyralid/fluroxypyr)	0.25 pt	E	G
Cotton[3]			
Envoke® (trifloxysulfuron)	0.1 oz	E	E
Rice[4]			
Grandstand® CA (triclopyr)	0.5 pint	E	E
Regiment® (bispyribac)	0.4 oz	E	E
Grasp® SC (penoxsulam)	2 oz	E	E
Permit (halosulfuron)	2/3 oz	E	E

NA denotes "not applicable."
[1]Weed control ratings: E = Excellent (90 to 99% control), G = Good (80 to 90% control), F = Fair (65 to 80 control), and P = Poor (40 to 65% control).
[2]Source is Zollinger (2009).
[3]Source is York et al. (2005).
[4]Sources are Dillon et al. (2006); Bond and Walker (2009).

VIII.K. Weed Resistance to Dicamba Herbicide

The risk of weeds developing resistance and the potential impact of resistance on the usefulness of an herbicide vary greatly across different herbicide modes-of-action and is dependent on a combination of different factors. Monsanto considers product stewardship to be a fundamental component of customer service and business practices, and invests considerably in research to understand the proper uses and stewardship of our herbicide-tolerant soybean systems. This research includes an evaluation of the factors that can contribute to the development of weed resistance. Detailed information regarding dicamba stewardship is presented in Appendix K.

VIII.L. Stewardship of MON 87708

Monsanto Company develops effective products and technologies and is committed to assuring that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship[SM] (ETS) Program[7]. These policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides[8] that are distributed annually to growers who utilize Monsanto branded traits.

As an integral action of fulfilling this commitment, Monsanto will seek biotechnology regulatory approvals for MON 87708 in all key soybean import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. These actions will be consistent with the Biotechnology Industry Organization (BIO) Policy on Product Launch[9] Monsanto continues to monitor other countries that are key importers of soybean from the U.S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions.

Monsanto also commits to best industry practices on seed quality assurance and control to ensure the purity and integrity of MON 87708 seed. As with all of Monsanto's products, before commercializing MON 87708 in any country, a MON 87708 detection method will be made available to soybean producers, processors, and buyers.

Dicamba is a selective herbicide registered with the U.S. EPA for the preemergent and postemergent control of certain broadleaf weeds in agriculture. Dicamba has a long history of effective use in U.S. crop production, including corn, soybean, cotton, sorghum, wheat, barley, oats, millet, pasture, rangeland, asparagus, sugarcane, turf, grass

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[7] Excellence Through Stewardship Program can be found at: http://www.excellencethroughstewardship.org/.

[8] Monsanto Technology Use Guides can be found at: http://www.monsanto.com/monsanto/ag_products/pdf/stewardship/technology_use_guide.pdf.

[9] BIO's Product Launch guidelines can be found at: http://www.excellencethroughstewardship.org/facts/documents/Guide%20for%20Product%20Launch%20Stewardship.pdf.

grown for seed, conservation reserve programs, and fallow croplands (as described in Section VIII.G). Although herbicide resistance may eventually occur in a weed species when an herbicide is widely used, resistance can be delayed, contained and managed through research, education and good management practices. Stewardship of dicamba to preserve its usefulness for growers is an important aspect of Monsanto's stewardship commitment, as is discussed in Appendix K.

(b) (4) [redacted] Monsanto is seeking regulatory approvals with the U.S. EPA for the expanded application of dicamba herbicide, as a weed control tool, as well as establishing appropriate dicamba Maximum Residue Levels (MRLs) for key soybean import countries, including the EU, Canada, and Japan, and CODEX, to support importing countries that do not have an established regulatory system to set MRLs including China.

As with all U.S. EPA registered herbicides for agricultural use, it is possible that offsite movement during and/or following application can occur such that non-target plants may be exposed to direct spray or to spray drift. Research has demonstrated that herbicide formulation, application equipment and application procedures can be optimized to significantly reduce spray drift potential in most circumstances, as discussed in Section VIII.G.3.

(b) (4) [redacted]

VIII.M. Impact of the Introduction of MON 87708 on Agricultural Practices

Introduction of MON 87708 is expected to have no impact on current cultivation and management practices for soybean, with the exception of expanded dicamba application timings. Dicamba has been used in corn, soybean and small grain cropping systems since 1967. (b) (4) [redacted]

((b) (4) [redacted] tillage and crop rotation) from deregulation of MON 87708, see Appendix J.

MON 87708 has been shown to be no different from conventional soybean in its agronomic, phenotypic, and compositional characteristics (refer to Sections VI, VII, and VIII), and has the same levels of susceptibility to insects and diseases as commercial soybean. Like other herbicide-tolerant soybean, such as Roundup Ready and Roundup Ready 2 Yield that have been cultivated and consumed in the U.S. since 1996, dicamba-tolerant soybean MON 87708 will improve the current agricultural practices for U.S. soybean growers by providing another in-crop herbicide mode-of-action for the control of glyphosate's hard-to-control and resistant broadleaf weeds, thereby improving the efficiency in the U.S. soybean production system to maximize or maintain soybean yield potential.

IX. ENVIRONMENTAL CONSEQUENCES

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 87708 and its impact on current agronomic practices. USDA-APHIS has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition may be granted, thereby allowing unrestricted introduction of the article.

The definition of "plant pest" in the Plant Protection Act (PPA) includes living organisms that could directly or indirectly injure, damage, or cause disease in any plant or plant product (7 U.S.C. § 7702[14]).

The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the regulated article is not likely to pose a plant pest risk. The approach used to assess the plant pest potential of MON 87708 is a weight of the evidence approach based primarily on eight lines of evidence: 1) insertion of a single functional copy of the *dmo* expression cassette, 2) characterization of MON 87708 DMO expressed in MON 87708, 3) safety of MON 87708 DMO, 4) compositional equivalence of harvested MON 87708 seed and forage to conventional soybean, 5) phenotypic, agronomic, and environmental interaction characteristics demonstrating no increased plant pest potential compared to conventional soybean, 6) negligible risk to NTOs and threatened or endangered species, 7) familiarity with soybean as a cultivated crop and the inherently low plant pest potential of soybean, and (8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds (other than the intended benefit of dicamba weed control), diseases, and insects than conventional soybean.

Using the assessment above, the data and analysis presented in this petition lead to a conclusion that MON 87708 is unlikely to be a plant pest and, therefore, should no longer be subject to regulation under 7 CFR § 340.

In 2008, APHIS proposed amendments to 7 CFR Part 340 that included provisions to utilize its noxious weed authority in regulating genetically engineered plants (73 FR 60008). Because the data presented in this petition demonstrate that MON 87708 has no potential to cause injury or damage to protected interests under the noxious weed authority, MON 87708 would not be considered a "noxious weed" as defined by the Plant Protection Act.

IX.B. Plant Pest Assessment of the MON 87708 Insert and Expressed Protein

IX.B.1. Characteristics of the Genetic Insert and the Expressed Protein

This section summarizes the details of the genetic insert, characteristics of the genetic modification, and safety and expression of the MON 87708 DMO used to evaluate the food, feed, and environmental safety of MON 87708.

IX.B.1.1. Genetic Insert

(b) (4)

[redacted]

Molecular analyses demonstrated that MON 87708 contains one copy of the inserted T-DNA I at a single integration locus. No T-DNA II or backbone sequences from PV-GMHT4355 were detected in the genome of MON 87708. Additionally, the data confirmed the organization and sequence of the insert and demonstrated the stability of the insert over several generations. These data demonstrated that there are no unintended changes in the MON 87708 genome as a result of the insertion of the *dmo* expression cassette, and supports the overall conclusion that MON 87708 is unlikely to be a plant pest.

IX.B.1.2. Protein Safety

MON 87708 exhibits tolerance to the herbicide dicamba through the insertion of a demethylase gene from *Stenotrophomonas maltophilia* that encodes for DMO. *S. maltophilia* is an environmentally ubiquitous bacterium. Infections caused by *S. maltophilia* are extremely uncommon and it can be found in healthy individuals without any harmful effects. DMO is a Rieske type non-heme iron oxygenase that catalyzes the demethylation of dicamba to the non-herbicidal compound DCSA (Section V.A). DMO is specific for dicamba (Dumitru et al., 2009; Section V.A).

(b) (4)

[redacted]

(b) (4)

[redacted]

The low level of MON 87708 DMO expressed in MON 87708 tissues taken together with the safety of MON 87708 DMO support the conclusion that food and feed products containing or derived from MON 87708 are as safe for human and animal consumption as soybean currently on the market. Therefore, unintended environmental effects are not anticipated from dietary exposure to MON 87708 DMO, and support the overall conclusion that MON 87708 is unlikely to be a plant pest.

IX.B.2. Compositional Characteristics

Detailed compositional analyses in accordance with OECD guidelines were conducted to assess whether levels of key nutrients and anti-nutrients in MON 87708 were comparable to levels present in the near isogenic conventional soybean control A3525 and several commercial reference varieties. Seed and forage were harvested from five individual sites in which MON 87708, the conventional control, and a range of commercial reference varieties were grown concurrently in the same field trial. The commercial reference varieties were used to establish a range of natural variability for the key nutrients and anti-nutrients in commercial soybean varieties that have a history of safe consumption.

The combined-site analysis was conducted to determine statistically significant differences (5% level of significance) between MON 87708 and the conventional control A3525. The results from the combined-site data were reviewed using considerations relevant to food and feed safety and nutritional quality including the relative magnitudes of the difference in the mean values of nutrient and anti-nutrient components of MON 87708 and the conventional control, whether the MON 87708 component mean value was within the range of natural variability of that component as represented by the 99% tolerance interval of the commercial reference varieties grown concurrently in the same field trial, and analyses of the reproducibility of the statistically significant combined-site component differences at individual sites.

Assessment of the analytical results confirmed that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 87708 soybeans. In addition, the levels of assessed components in

MON 87708 were compositionally equivalent to the conventional control and within the range of variability of commerical soybeans that were grown concurrently in the same field trial. These results support the overall conclusion that MON 87708 is unlikely to be a plant pest.

IX.B 3. Phenotypic and Agronomic and Environmental Interaction Characteristics

An extensive set of comparative plant characterization data were used to assess whether the introduction of the dicamba-tolerance trait altered the plant pest potential of MON 87708 compared to the conventional control (Section VII). Phenotypic, agronomic, and environmental interaction characteristics of MON 87708 were evaluated and compared to those of the conventional control (Section VII.B). As described below, these assessments included: seed dormancy and germination characteristics; plant growth and development characteristics; observations for abiotic stress response, disease damage, arthropod-related damage; pollen characteristics; and arthropod abundance; and plant-symbiont interaction characteristics. Results from the phenotypic, agronomic, and environmental interaction assessments demonstrated that MON 87708 does not possess weedy characteristics, or increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors, or altered symbiont interactions compared to conventional soybean. Taken together, the results of the analysis support a determination that MON 87708 is no more likely to pose a plant pest risk or have a biologically meaningful change in environmental impact than conventional soybean.

IX.B.3.1. Seed Dormancy and Germination

Seed dormancy and germination characterization demonstrated that MON 87708 seed had germination characteristics similar to those of the conventional control (Section VII.C.1). In particular, the lack of hard seed, a well-accepted characteristic often associated with plants that are weeds, supports a conclusion of no increased weediness or plant pest potential of MON 87708 compared to conventional soybean.

IX.B.3.2. Plant Growth and Development

Evaluations of plant growth and development characteristics in the field are useful for assessing potential weediness characteristics such as lodging and pod shattering (Section VII.C.2.1). Of the growth and development characteristics assessed between MON 87708 and the conventional control, no statistically significant differences were detected (5% level of significance) with the exception of plant height and 100 seed weight in a combined-site analysis of the data. The differences in these parameters were relatively small in magnitude, and the mean values of MON 87708 were within the range of the commercial reference varieties for these characteristics. Thus, the differences in these parameters are not considered to be biologically meaningful in terms of increased weediness or plant pest potential of MON 87708 compared to conventional soybean.

IX.B.3.3. Response to Abiotic Stressors

No biologically meaningful differences were observed during comparative field observations between MON 87708 and the conventional control and their response to

abiotic stressors, such as drought, heat stress, high winds, nutrient deficiency, etc. (Section VII.C.2.2). The lack of significant biologically-meaningful differences in the MON 87708 response to abiotic stress support the conclusion that the introduction of the dicamba-tolerance trait is unlikely to result in increased weediness or plant pest potential compared to conventional soybean.

IX.B.3.4. Pollen Morphology and Viability

Evaluations of pollen morphology and viability from field-grown plants provide information useful in a plant pest assessment as it relates to the potential for gene flow to, and introgression of, the biotechnology-derived trait into other soybean varieties and wild relatives (Section VII.C.3). Pollen morphology and viability evaluations demonstrated no statistically significant differences between MON 87708 and the conventional control. Taken together, these comparative assessments indicate that MON 87708 is not likely to have increased weediness or plant pest potential compared to conventional soybean.

IX.B.3.5. Interactions with Non-Target Organisms Including Threatened and Endangered Species

Evaluation of MON 87708 for potential adverse impacts on NTOs is a component of the plant pest risk assessment. Since MON 87708 is a product with no pesticidal activity, all organisms that interact with MON 87708 are considered to be NTOs. In a 2008 U.S. phenotypic and agronomic assessment, observational data on environmental interactions were collected for MON 87708 and the conventional control. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each characteristic. The environmental interactions assessment (Section VII.C.2.2) included data collected on plant-arthropod and plant-disease interactions. The results of this assessment indicated that the presence of the dicamba-tolerance trait did not alter plant-arthropod interactions, including beneficial arthropods and arthropod pests, nor did it alter disease susceptibility of MON 87708 compared to conventional soybean. The lack of differences in disease damage, arthropod-related damage, and pest and beneficial arthropod abundance demonstrate that the introduction of the dicamba-tolerance trait is unlikely to be biologically meaningful in terms of increased plant pest potential.

In the field, soybean forms a complex symbiotic relationship with members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae*. This symbiosis results in the formation of root nodules in which the bacteria reduce or fix atmospheric nitrogen-producing ammonia that can be used by the plant. MON 87708 was assessed for changes in the symbiotic relationship with *B. japonicum* relative to the conventional control by evaluating shoot total nitrogen, nodule number, and nodule, root, and shoot dry weights (Section VII.C.4). No statistically significant differences were detected between MON 87708 and the conventional control for the parameters measured, indicating no impact on either the symbiotic relationship or the symbiotic nitrogen-fixing bacteria. These data support a conclusion of no change in plant pest potential and no expected impact to cultivation practices relative to nitrogen inputs for MON 87708 compared to conventional soybean.

The potential for MON 87708 to harm NTOs was evaluated using a combination of biochemical information and experimental data. The biochemical information and experimental data included molecular characterization, the MON 87708 DMO safety assessments, the history of environmental exposure to mono-oxygenases (the class of enzymes to which DMO belongs), results from the environmental assessment described above, and the demonstration of compositional, agronomic and phenotypic equivalence to conventional soybean. Taken together, these data support the conclusion that MON 87708 is unlikely to adversely affect NTOs, or pose an additional risk to threatened and endangered species above those posed by the cultivation of conventional soybean.

Furthermore, according to APHIS, the only listed threatened or endangered animal that occupies a habitat where it is likely to include soybean fields, and that might feed on soybean, is the federally endangered Delmarva Peninsula Fox Squirrel, (*Sciurus niger cinereus*), found in areas of the mid-Atlantic Eastern seaboard (USDA-APHIS, 2007). It is known to utilize certain agricultural lands readily, but its diet includes acorns; nuts/seeds of hickory, beech, walnut, and loblolly pine; buds and flowers of trees; fungi; insects; fruit; and an occasional bird egg (NatureServe, 2007). The safety of the MON 87708 DMO, the compositional, agronomic and phenotypic equivalence of MON 87708 to conventional soybean, and the diversity of the Fox Squirrel diet, support a conclusion that no biologically significant changes to the habitat or diet of the Delmarva Peninsula Fox Squirrel are expected. Consequently, the planting of MON 87708 is not expected to affect the Delmarva Peninsula Fox Squirrel.

IX.C. Weediness Potential of MON 87708

The commercial *Glycine* species in the U.S. (*Glycine max L.*) does not exhibit weedy characteristics and is not effective in invading established ecosystems. Soybean is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Holm et al., 1997), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Soybean does not possess any of the attributes commonly associated with weeds (Baker, 1965), such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes or the ability to compete well with native vegetation. Due to the lack of dormancy, which is a trait that has been removed from soybean through commercial breeding, soybean seed can germinate quickly under adequate temperature and moisture conditions, and potentially grow as volunteer plants. However, volunteer plants likely would be killed by frost during autumn or winter of the year they were produced. If they did become established, volunteer plants would not compete well with the succeeding crop, and could be controlled readily by either mechanical or chemical means (OECD, 2000). In addition, since wild populations of *Glycine* species are not known to exist in the U.S., the potential does not exist for MON 87708 to outcross to wild or weedy relatives and to alter their weediness potential.

In comparative studies between MON 87708 and the conventional control, phenotypic, agronomic, and environmental interaction data were evaluated (Section VII) for changes that would impact the plant pest potential and, in particular, plant weediness potential. Results of these evaluations show that there is no biologically meaningful difference

between MON 87708 and the conventional control for characteristics potentially associated with weediness. Furthermore, comparative field observations between MON 87708 and its conventional control in their response to abiotic stressors, such as drought, heat stress, and high winds, indicated no biologically meaningful differences and, therefore, no increased weediness potential. Data on environmental interactions also indicate that MON 87708 does not confer any biologically meaningful increased susceptibility or tolerance to specific diseases or insect pests. Collectively, these findings support the conclusion that MON 87708 has no increased weediness potential compared to conventional soybean.

IX.D. Potential for Pollen Mediated Gene Flow

Gene introgression is a process whereby one or more genes successfully integrate into the genome of a recipient plant population. Introgression is affected by many factors, including the frequency of the initial pollination event, environmental factors, sexual compatibility of pollen donor and recipient plants, pollination biology, flowering phenology, hybrid stability and fertility, selection, and the ability to backcross repeatedly. Because gene introgression is a natural biological process, it does not constitute an environmental risk in and of itself (Sutherland and Poppy, 2005). Gene introgression must be considered in the context of the transgene(s) inserted into the biotechnology-derived plant, and the likelihood that the presence of the transgene(s) and their subsequent transfer to recipient plants will result in increased plant pest potential. The potential for gene introgression from MON 87708 is discussed below.

The assessment for gene introgression from MON 87708 with other cultivated or wild relatives of soybean, discussed in detail below, indicates that MON 87708 is no more likely to become a weed than conventional soybean, and MON 87708 is expected to be similar to conventional soybean regarding its potential for and impacts from gene flow. Soybean lacks sexually-compatible relatives in the U.S.; therefore, the only pollen-mediated gene flow would be within cultivated soybean.

IX.D.1. Hybridization with Cultivated Soybean

Although soybean is largely a self-pollinated species, low levels of natural cross-pollination can occur (Caviness, 1966; OECD, 2000; Ray et al., 2003; Yoshimura et al., 2006). In studies with cultivated soybean, where conditions have been optimized to ensure close proximity and flowering synchrony, natural cross-pollination generally has been found to be very low. Most outcrossing occurred with surrounding plants, and cross-pollination frequencies varied depending on growing season and genotype. Insect activity does increase the outcrossing rate, but soybean generally is not a preferred plant for pollinators (Abrams, 1978; Erickson 1975; Jaycox 1970a; Jaycox 1970b).

Numerous studies on soybean cross-pollination have been conducted, and the published results, with and without supplemental pollinators, are summarized in Table IX-1. Under natural conditions, cross-pollination among adjacent plants in a row or among plants in adjacent rows ranged from 0 to 6.3%. In experiments where supplemental pollinators (usually bees) were added to the experimental area, cross-pollination ranged from 0.5 to

7.74% in adjacent plants or adjacent rows. However, cross-pollination does not occur at these levels over long distances. Cross-pollination rates decrease to less than 1.5% beyond one meter from the pollen source, and rapidly decrease with greater distances from the source. The following cross-pollination rates at extended distances have been reported: 0.05% at 5.4 meters (Ray et al., 2003), 0% at 6.5 meters (Abud et al., 2003b), 0% at 10.5 m (Yoshimura et al., 2006), and 0.004% at 13.7 meters of separation (Caviness, 1966).

The potential for cross-pollination in soybean is limited. This is recognized in certified seed regulations for foundation seed in the U.S., which permit any distance between different soybean cultivars in the field as long as the distance is adequate to prevent mechanical mixing (USDA-APHIS, 2006).

The consequence of introgression of the dicamba-tolerance trait from MON 87708 into other soybean is negligible since soybean gene flow is naturally low; therefore the dicamba-tolerance trait confers no increased plant pest potential to cultivated soybean.

IX.D.2. Hybridization with Wild Annual Species within Subgenus *Soja*

The subgenus *Soja* includes the cultivated soybean *Glycine max* and the wild annual species *Glycine soja*. *Glycine soja* is found in China, Taiwan, Japan, Korea, and Russia and can hybridize naturally with the cultivated soybean, *G. max* (Hymowitz, 2004; Lu, 2004). Hybridization between female *G. soja* and male *G. max* was less successful than hybridization in the opposite direction (Dorokhov et al., 2004), where frequency of spontaneous cross pollination in reciprocal combinations of *G. max* and *G. soja* varied from 0.73 (♀ *G. soja* × ♂ *G. max*) to 12.8% (♀ *G. max* × ♂ *G. soja*). Species relationships in the subgenus *soja* indicated that F_1 hybrids of *G. max* and *G. soja* carry similar genomes and are fertile (Singh and Hymowitz, 1989). Abe et al. (1999) note that "natural hybrids between *G. max* and *G. soja* are rare and hybrid swarms involving both species have never been reported." This is also supported by work from Kuroda et al. (2008) in which molecular markers were used and no gene flow from *G. max* to *G. soja* was detected. Many barriers to natural hybridization exist between soybean and wild relatives, including the highly selfing nature of both plants, required proximity of wild soybean to cultivated soybean, synchrony of flowering, and presence of pollinators. As such, it is highly unlikely that naturally occurring, pollen-mediated gene flow and transgene introgression into wild soybean relatives from incidentally released biotechnology-derived soybean will occur at any meaningful frequency.

The subgenus *Soja* also contains an unofficial species, *G. gracilis* (Hymowitz, 2004). *Glycine gracilis* is known only from Northeast China, and is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *Glycine gracilis* may be a hybrid between *G. soja* and *G. max* (Hymowitz, 1970; Lu, 2004). Interspecific fertile hybrids formed by intentional crosses between *G. max* and *G. soja* and between *G. max* and *G. gracilis* have been easily obtained (Dorokhov et al., 2004; Singh and Hymowitz, 1989). Although hybridization between *G. max* and members of the subgenus *Soja* can take place, *G. soja* is not found in North or South America, and it is highly unlikely that gene transfer will occur.

IX.D.3. Hybridization with the Wild Perennial Species of *Glycine* Subgenus

Wild perennial species of the *Glycine* subgenus occur in Australia; West, Central and South Pacific Islands; China; Papua New Guinea; Philippines; and Taiwan (Hymowitz et al., 1992). Therefore, the only opportunities for inter-subgeneric hybridization would occur in areas where those species are endemic. Nonetheless, the likelihood of interspecific hybridization between *G. max* and the wild perennial *Glycine* species is extremely low because they are genomically dissimilar (Hymowitz, 2004; Lu, 2004) and pod abortion is common. From time to time, immature seeds of the crosses have been germinated aseptically *in vitro*, but the resulting F1 hybrids are slow-growing, morphologically weak, and completely sterile. Their sterility is due to poor chromosome pairing. Furthermore, species distantly related usually produce nonviable F1 seeds that either have premature death of the germinating seedlings or suffer from seedling and vegetative lethality (Kollipara et al., 1993). In North and South America, it is not possible for gene transfer to occur between cultivated soybean and wild perennial species of *Glycine* subgenera because these wild species do not exist in these regions.

IX.D.4. Transfer of Genetic Information to Species with Which Soybean Cannot Interbreed (Horizontal Gene Flow)

Monsanto is unaware of any reports regarding the unaided transfer of genetic material from soybean species to other sexually-incompatible plant species. The likelihood for horizontal gene flow to occur is exceedingly small. Therefore, potential ecological risk associated with horizontal gene flow from MON 87708 due to the presence of the dicamba-tolerance trait is not expected. The consequence of horizontal gene flow of the dicamba-tolerance trait into other plants that are sexually-incompatible is negligible since, as data presented in this petition confirm, the gene and trait confer no increased plant pest potential to soybean. Thus in the highly unlikely event that horizontal gene transfer were to occur, the presence of the dicamba-tolerance trait would not be expected to increase pest potential in the recipient species.

IX.E. Potential Impact on Soybean Agronomic Practices

An assessment of current soybean agronomic practices was conducted to determine whether the cultivation of MON 87708 has the potential to impact current soybean and weed management practices (Section VIII). Soybean fields are typically highly managed agricultural areas that are dedicated to crop production. MON 87708 is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 87708 to growers. Cultivation of MON 87708 is not expected to differ from typical soybean cultivation, with the exception of an expanded window of dicamba applications. (b) (4) [redacted]

(b) (4)

[redacted]

MON 87708 is similar to conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics and has levels of resistance to insects and diseases comparable to conventional soybean. Therefore, no significant impacts on current cultivation and management practices for soybean are expected following the introduction of MON 87708. Based on this assessment, the introduction of MON 87708 will not impact current U.S. soybean cultivation practices or weed management practices, other than intended weed control benefits

IX.F. Summary of Plant Pest Assessments

Plant pests are defined in the Plant Protection Act as certain living organisms that can directly or indirectly injure, cause damage to, or cause disease to any plant or plant product (7 U.S.C. § 7702[14]). Characterization data presented in Sections III through VII of this petition confirm that although MON 87708 contains the dicamba-tolerant trait, it is not different from conventional soybean in terms of pest potential in its phenotypic, agronomic, and environmental interaction characteristics. Monsanto is not aware of any study results or observations associated with MON 87708 that would suggest an increased plant pest risk would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 87708 compared to conventional soybean, followed by a risk assessment on detected differences. The risk assessment considered various factors including: 1) insertion of a single functional copy of the *dmo* expression cassette, 2) characterization of the MON 87708 DMO, 3) safety of the MON 87708 DMO, 4) compositional equivalence of harvested MON 87708 soybean seed and forage as compared to conventional soybean, 5) phenotypic, agronomic, and environmental interaction characteristics demonstrating no increased plant pest potential compared to conventional soybean, 6) negligible risk to NTOs and threatened or endangered species, 7) familiarity with soybean as a cultivated crop and the inherently low plant pest potential of soybean, and 8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than conventional soybean.

Based on the data and information presented in this petition, it is concluded that, like conventional soybean and currently deregulated biotechnology-derived soybean, MON 87708 is highly unlikely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87708 and any progeny derived from crosses between MON 87708 and other commercial soybean be granted nonregulated status under 7 CFR Part 340.

Table IX-1. Summary of Published Literature on Soybean Cross Pollination

Distance from Pollen Source (meters)	Cross-Pollination (%)	Comments	Reference
0.3	0.04 (estimated per pod)	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties. Percent outcrossing calculated per pod rather than per seed.	(Woodworth, 1922)
0.8	0.07 to 0.18	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	(Garber and Odland, 1926)
0.1	0.38 to 2.43	Adjacent plants within a row. Experiment conducted in a single year. Several male and female parental varieties.	(Cutler, 1934)
0.1	0.2 to 1.2	Adjacent plants within a row. Experiment conducted in single year at two locations. Several male and female parental varieties.	(Weber and Hanson, 1961)
0.9 2.7–4.6 6.4–8.2 10–15.5	0.03 to 0.44 0.007 to 0.06 0 to 0.02 0 to 0.01	Frequency by distance was investigated. Experiment conducted over three years. Single male and female parental varieties.	(Caviness, 1966)
0.8 m	0.3 to 3.62	Various arrangements within and among adjacent rows. Experiment conducted over three years. Several male and female parental varieties.	(Beard and Knowles, 1971)
One row (undefined)	1.15 to 7.74	Bee pollination of single-row, small-plots of pollen receptor surrounded by large fields (several acres) of pollen donor soybean. Soybean is not a preferred flower for alfalfa leafcutting bees.	(Abrams et al., 1978)
0.1–0.6	0.5 to 1.03 (depending on planting design)	Bee pollination of soybean grown in various spatial arrangements. Experiment conducted over four years. Several soybean cultivars.	(Chiang and Kiang, 1987)
1.0	0.09 to 1.63	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	(Ahrent and Caviness, 1994)
0.5 1.0 6.5	0.44 to 0.45 0.04 to 0.14 none detected	Frequency by distance was investigated. Experiment conducted in a single year. Single male and female parental varieties.	(Abud et al., 2003a)
0.9 5.4	0.29 to 0.41 0.03 to 0.05	Frequency by distance was investigated. Experiment conducted in a single year. Single male and female parental varieties.	(Ray et al., 2003)
0.15	0.65 to 6.32 (avg. 1.8)	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties.	(Ray et al., 2003)
0.7 1.4 2.1 2.8 3.5 7.0 10.5	0 to 0.19 0 to 0.04 0 to 0.05 0 to 0.08 0 to 0.04 0 to 0.04 0	Interspaced plants within a row arranged in small plots. Experiment conducted in a four year period. Single male and two female parental varieties.	(Yoshimura et al., 2006)

X. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto knows of no results or observations associated with MON 87708 or the MON 87708 DMO indicating that there would be an adverse environmental consequence from the introduction of MON 87708. MON 87708 contains DMO that renders the soybean plant tolerant to the herbicide dicamba. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 87708 and conventional soybean is tolerance to dicamba.

The data and information presented in this petition demonstrate that MON 87708 is unlikely to pose an increased plant pest risk or to have an adverse environmental consequence compared to conventional soybean. This conclusion is based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional soybean, followed by risk assessment on detected differences: 1) characterization evaluations included molecular analyses, which confirmed the insertion of a single functional copy of the *dmo* expression cassette at a single locus within the soybean genome; 2) measurement of the MON 87708 DMO levels in various soybean tissues; 3) characterization of the MON 87708 DMO confirming it is not novel and is structurally and functionally similar to oxygenase homologs widely present in bacteria and plants, where a history of safe use is established; and 4) extensive characterization of the plant phenotype, including compositional analysis of key nutrients and antinutrients, and environmental interactions. Therefore, based on the lack of increased pest potential or adverse environmental consequences compared to conventional soybean, the risks for humans, animals, and other NTOs from MON 87708 are negligible under the conditions of use. Additionally the introduction of MON 87708 will not adversely impact cultivation practices or the management of weeds, diseases, and insects in soybean production systems, other than the use of dicamba postemergence in soybean.

Successful integration of MON 87708 into the Roundup Ready soybean system will provide growers with an opportunity for an efficient, effective weed management system for the management of glyphosate's hard-to-control and resistant broadleaf weeds; provide an easy system for inclusion of a second herbicide mode-of-action in soybean production practices as recommended by weed science experts to manage weed resistance development; and continue to provide soybean growers with effective weed control systems necessary for production yields to meet the growing needs of the food, feed and industrial markets.

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APPENDICES

Appendix A: USDA Notifications

Field trials of MON 87708 have been conducted in the U.S. since 2005. The protocols for these trials included field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to MON 87708 phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2008-2009 seasons, are still in preparation. A list of trials conducted under USDA notifications and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications Approved for MON 87708 and Status of Trials Conducted under These Notifications and Permits

USDA Number	Effective Date	Release Site (State)	Trial Status
2005			
05-269-02n	11/16/2005	PR	Submitted to USDA
2006			
06-045-15n	5/18/2006	HI(5)	Submitted to USDA
06-045-17n	5/18/2006	PR(3)	Submitted to USDA
06-052-01n	3/20/2006	IL(7), KS(5)	Submitted to USDA
06-052-02n	4/24/2006	IA(7), IL(5), IN(2)	Submitted to USDA
06-052-09n	4/24/2006	IA(2), IL(6), IN(2)	Submitted to USDA
06-067-05n	4/24/2006	IL(2)	Submitted to USDA
06-090-03n	5/5/2006	IL(2)	Submitted to USDA
06-275-102n	11/14/2006	PR	Submitted to USDA
06-345-101n	1/10/2007	PR(3)	Submitted to USDA
2007			
07-018-103n	2/17/2007	IL(10), IN(3), MO, PR	Submitted to USDA
07-018-106n	2/17/2007	IA(7), KS(6)	Submitted to USDA
07-018-109n	2/17/2007	IA, IL(10), IN(3), MO	Submitted to USDA
07-024-101n	3/18/2007	IA(7), KS(6)	Submitted to USDA
07-039-101n	3/18/2007	IA(4), IL(5), IN(3), KS(3)	Submitted to USDA
07-043-102n	4/10/2007	IA, IL(2), KS, MD, WI	Submitted to USDA
07-050-107n	4/9/2007	IA, IL(2), IN, KS, KY, MN, NE, SD	Submitted to USDA
07-057-109n	4/6/2007	AL, IA(3), IL, IN, LA, MN, MO(2), MS, NE, SD, TN	Submitted to USDA
07-094-104n	5/4/2007	IA(2)	Submitted to USDA
07-094-116n	5/4/2007	MN	Submitted to USDA
07-113-103n	6/4/2007	PR(2)	Submitted to USDA
07-241-103n	9/28/2007	PR	Submitted to USDA
07-250-102n	10/7/2007	PR(2)	Submitted to USDA
07-261-101n	10/18/2007	PR	Submitted to USDA
07-271-101n	10/28/2007	PR(2)	Submitted to USDA
07-312-101n	12/5/2007	PR	Submitted to USDA

Table A-1 (continued). USDA Notifications Approved for MON 87708 and Status of Trials Conducted under These Notifications and Permits

USDA Number	Effective Date	Release Site (State)	Trial Status
2008			
07-352-101rm	3/26/2008	IA(8), IL(16), IN(4), KS(7), MO	Submitted to USDA
08-030-103n	2/29/2008	PR(2)	Submitted to USDA
08-031-105n	3/13/2008	IA(5), IL(4), KS(5)	Submitted to USDA
08-031-106n	3/1/2008	IA(2), IL(5), IN(3)	Submitted to USDA
08-039-107n	3/9/2008	IA(5), IL, IN(3), KS(5), MO	Submitted to USDA
08-043-107n	3/13/2008	IA(3), IL(10), IN, OH	Submitted to USDA
08-049-101n	3/19/2008	IL, MD, WI	Submitted to USDA
08-058-101n	3/28/2008	IA(3), IL(2), IN, MO, PA, WI(2)	Submitted to USDA
08-059-109n	3/29/2008	IA	Submitted to USDA
08-059-110n	3/29/2008	IL	Submitted to USDA
08-059-112n	3/29/2008	IN	Submitted to USDA
08-060-103n	4/2/2008	MN	Submitted to USDA
08-063-112n	4/2/2008	IA(4), IL(2), IN, MI, MO, NE(2)	Submitted to USDA
08-063-113n	4/4/2008	MN(2), ND, SD(5), WI(5)	Submitted to USDA
08-065-101n	4/4/2008	IL(2), IN	Submitted to USDA
08-064-102n	4/3/2008	PA	Submitted to USDA
08-064-103n	4/3/2008	IL	Submitted to USDA
08-064-104n	4/3/2008	AR, GA, KS(5), LA, MO, SC	Submitted to USDA
08-064-105n	4/3/2008	AR, IL(2), IN, KS(3), MD, MN(3), NC, SD, WI, ND	Submitted to USDA
08-072-110n	4/25/2008	AR, IA, IN(3), KS, MI, MO(2), NE	Submitted to USDA
08-079-101n	4/17/2008	IA(3)	Submitted to USDA
08-084-102n	4/24/2008	IA, NE	Submitted to USDA
08-182-101n	8/1/2008	PR(2)	In Progress
08-253-101n	10/7/2008	PR(3)	In Progress
08-263-101n	10/19/2008	AR, IA, IL, IN, MO	In Progress
08-266-105n	10/22/2008	PR	In Progress
08-316-101n	12/16/2008	PR(2)	In Progress
08-316-103n	12/11/2008	PR	In Progress
08-323-101n	12/18/2008	PR(3)	In Progress
08-340-104n	2/25/2009	PR(2)	In Progress
08-357-101rm	3/17/2009	IA(11), IL(15), IN(3), KS(5), MO, NE	In Progress

Table A-1 (continued). USDA Notifications Approved for MON 87708 and Status of Trials Conducted under These Notifications and Permits

USDA Number	Effective Date	Release Site (State)	Trial Status
2009			
09-007-106n	2/25/2009	PR(2)	In Progress
09-030-105n	3/1/2009	IA(3), IL(4), IN, KS, NE	In Progress
09-036-103n	3/7/2009	IA(2), IL(2), IN, MS, NE(2)	In Progress
09-039-101n	3/10/2009	IA(10), IL(2), IN(3)	In Progress
09-040-101n	3/10/2009	IL(12), MO, NE	In Progress
09-049-110n	3/20/2009	IA, IL	In Progress
09-050-133n	3/31/2009	HI, IA(2), IL(3), IN(2), MD, MN, NC, OH, PR, SD	In Progress
09-050-136n	4/3/2009	HI, IA(2), IL(3), IN(2), MD, MN, NC, OH, PR, SD	In Progress
09-050-140n	3/23/2009	GA(4), IA, IL, KS(5), LA, MS(2)	In Progress
09-061-108n	4/1/2009	AR, IA, IL(5), IN(2), KS, MI, MO, NE, WI(2)	In Progress
09-061-117n	4/1/2009	IL, IN(2), MI, MO(5), WI(3)	In Progress
09-075-112n	4/15/2009	GA(4), IA, IL, KS(4), LA, MS(2)	In Progress
09-075-114n	4/15/2009	AR, MD, MN(4), NC, ND(3), SD, WI	In Progress
09-077-109n	4/17/2009	IL(12), MO, NE	In Progress
09-082-103n	4/22/2009	AR, IA, IL(3), IN(2), KS, NE, PA	In Progress
09-091-103n	5/1/2009	AR	In Progress
09-093-120n	5/3/2009	AR	In Progress
09-124-102n	6/3/2009	PR(2)	In Progress
09-124-105n	5/13/2009	IA	In Progress
09-135-104n	6/14/2009	IL	In Progress
09-162-105n	7/11/2009	PR	In Progress
09-162-106n	7/11/2009	PR	In Progress
09-222-101n	9/9/2009	PR(3)	In Progress
09-237-104n	9/24/2009	HI, PR	In Progress

Appendix B: Materials, Methods, and Results for Molecular Analyses of MON 87708

(b) (4)

[redacted]

(b) (4)

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Appendix C: Materials, Methods, and Results for the Characterization of MON 87708 DMO and Substrate Specificity of DMO

(b) (4)

(b) (4)

[redacted]

(b) (4)

twice over two days and the dialysis was conducted at 4°C. The dialysate was aliquoted,
(b) (4)

(b) (4)

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(b) (4)

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