MONSANTO

Petition for the Determination of Nonregulated Status for Roundup RReady2Yield™ Soybean MON 89788

The undersigned submits this petition to request a determination of the nonregulated status of the article under 7 CFR Part 340.

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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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Summary

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for MON 89788 soybean and any progeny derived from crosses between MON 89788 and other soybeans, including conventional and other genetically-modified soybeans that have been granted nonregulated status under 7 CFR Part 340.

Roundup Ready® soybean 40-3-2 (herein referred to as Roundup Ready soybean) was the first soybean product containing a biotechnology trait commercialized in the U.S. Roundup Ready soybean was produced by incorporation of the *cp4 epsps* coding sequence derived from the common soil bacterium *Agrobacterium sp*. strain CP4. The *cp4 epsps* coding sequence directs the production of the 5-enolpyruvyl shikimate-3-phosphate synthase (termed CP4 EPSPS) that is less sensitive to inhibition by glyphosate compared to plant endogenous EPSPS. The CP4 EPSPS renders Roundup Ready soybean tolerant to glyphosate, which is the active ingredient in Roundup® agricultural herbicides. The utilization of Roundup agricultural herbicides plus Roundup Ready soybean, collectively referred to as the Roundup Ready soybean system, has provided significant convenience in weed control, encouraged the use of conservation-tillage, and provided positive economic impact to the farmers. In 2005, Roundup Ready soybean was planted on approximately 87% of the U.S. (USDA-NASS, 2005a) and 60% of the global soybean areas (James, 2005), which is the most cultivated biotechnology product to date.

Developments in biotechnology and molecular-assisted breeding have enabled Monsanto to develop a second-generation glyphosate-tolerant soybean product, Roundup RReady2Yield™ or MON 89788. MON 89788 will continue to provide growers flexibility, simplicity, and cost effective weed control options; in addition, MON 89788 and varieties containing the trait have the potential to enhance yield and thereby further benefit farmers and the soybean industry. MON 89788 was developed by introduction of the *cp4 epsps* gene cassette containing a promoter that has been used in other crops such as Roundup Ready Flex cotton (Fincher et al., 2003). In addition, the transformation was based on a new technique of *Agrobacterium*-mediated gene delivery to soybean meristem, where cells were induced directly to form shoots and give rise to transgenic plants (Martinell et al., 2002). This new technique allowed direct transformation of the gene cassette into elite soybean germplasm such as the Asgrow soybean variety A3244 (Paschal, 1997), which is known for its superior agronomic characteristics and high yielding property (Tylka and Marett, 1999). Using elite germplasm as the base genetics, the superior agronomic characteristic of A3244 can be introgressed to other soybean varieties through crosses with MON 89788 containing the *cp4 epsps* cassette. In general, MON 89788 has been found to have a 4 to 7% yield advantage compared to Roundup Ready soybeans in the same elite genetic background (A3244) while maintaining the weed control and crop safety benefits of the Roundup Ready soybean system. As a result, MON 89788 will be an excellent agronomic base trait for future breeding improvements and multi-trait products.

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The data and information presented in this summary demonstrate that MON 89788 is not likely to pose an increased plant pest potential or to have an increased environmental impact compared to conventional soybean. This conclusion is based on several lines of evidence. The first is the detailed molecular characterization of the inserted DNA. Results confirm the insertion of an intact *cp4 epsps* cassette integrated at a single locus within the genome. The second is a detailed biochemical characterization of the CP4 EPSPS protein produced in MON 89788. Data demonstrate that the CP4 EPSPS produced in MON 89788 is equivalent to the CP4 EPSPS proteins consumed in foods and feeds derived from other Roundup Ready crops such as Roundup Ready soybean that have an experience of safe use. The third is an updated assessment of the toxicity and allergenicity potential of the CP4 EPSPS protein produced in MON 89788 based on extensive information collected and studies performed on the protein. Results confirm the previous assessment and the safety of the CP4 EPSPS due to the lack of allergenic potential and the lack of acute toxicity when ingested. The fourth is the compositional and nutritional assessment of MON 89788 grain and forage, which confirms that MON 89788 is compositionally equivalent to and as safe as conventional soybeans. Finally, the extensive evaluation of the MON 89788 phenotypic characteristics and ecological interactions demonstrates that MON 89788 is not likely to increase plant pest potential or to have increased environmental impact compared to conventional soybean.

Molecular analyses indicate that MON 89788 contains a single intact *cp4 epsps* expression cassette integrated at a single locus within the soybean genome. DNA sequencing analyses of the MON 89788 insert confirm the expected coding region of the *cp4 epsps* gene cassette, which encodes a CP4 EPSPS protein identical to that in Roundup Ready soybean. No backbone sequences from the transformation plasmid were detected. In addition, no partial genetic elements, linked or unlinked to the inserted expression cassette were detected. Furthermore, the DNA insert and the glyphosate-tolerant trait in MON 89788 were shown to be stably inherited across multiple generations. Phenotypic segregation data confirmed the single chromosomal insertion, which resulted in the expected Mendelian segregation pattern.

The CP4 EPSPS protein expression levels were determined in MON 89788 tissues produced under commercially relevant field conditions. Results confirm that CP4 EPSPS is expressed in all tissues collected, including root, forage, seed, and leaf tissues at four developmental stages, and the CP4 EPSPS expression level in MON 89788 seed is lower than that in Roundup Ready soybean. Expression of CP4 EPSPS protein in MON 89788 confers its tolerance to Roundup agricultural herbicide applied at the commercial rate.

The protein characterization studies show that seed derived from MON 89788 contains the CP4 EPSPS protein of the expected molecular weight, amino acid sequence, immunological activity, and functional activity. The CP4 EPSPS protein in MON 89788 has the same functional and enzymatic activity as the CP4 EPSPS in other Roundup Ready crops, and is structurally homologous to EPSPSs naturally present in other crops. The amino acid sequence of the CP4 EPSPS protein in MON 89788 is identical to that in Roundup Ready soybean, Roundup Ready canola, and Roundup Ready Flex cotton, all of

which have been deregulated by USDA and have been commercialized. Taken together, these data and information demonstrate familiarity with respect to the family of EPSPS proteins, which naturally occur in crops and plants that have a long history of safe use.

Information and data from studies also support the safety of the CP4 EPSPS protein. These data have demonstrated that CP4 EPSPS is unlikely to be an allergen or toxin, the lack of acute oral toxicity in mice, and the history of safe consumption of similar EPSPS proteins from a variety of food sources. This conclusion is further supported by the lack of any documented reports of adverse effects from the consumption of other Roundup Ready crops since 1996.

Compositional assessment of the grain and forage demonstrated that MON 89788 is nutritionally and biologically equivalence to, and as safe and nutritious as its conventional counterpart, A3244. The A3244 has similar genetic background with MON 89788 but does not contain the *cp4 epsps* gene cassette. The composition analyses compared the levels of 63 components between MON 89788 and A3244, each of which were grown at five field sites in the U.S. during 2005. In addition, the same components were analyzed in 12 conventional soybean varieties to establish the 99% tolerance interval for each of the analytes. Results of the compositional analyses indicate that there were no statistically significant differences ($p \leq 0.05$) in 91% of the comparisons made between MON 89788 and A3244. Of the few analytes where statistical differences occurred, differences were not reproducible across sites and the trends of the differences were not consistent. Furthermore, the mean levels of all analytes from MON 89788 grain were within the 99% tolerance intervals for conventional soybeans. Therefore, the few statistically significant differences between MON 89788 and A3244 were not considered to be biologically relevant. These data support the conclusion that MON 89788 soybean grain and forage are compositionally and nutritionally equivalent to the conventional soybean, A3244. These data have been presented to the U.S. FDA for evaluation of food and feed safety for MON 89788 as part of the pre-market consultation process.

An important element in assessing plant pest potential and environmental impact of MON 89788 is to establish the familiarity of MON 89788 to conventional soybeans. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction of these factors. Familiarity provides a basis for comparative environmental risk assessment between a genetically-modified plant and its conventional counterpart through evaluating phenotypic, agronomic and ecological interaction characteristics. Data are used to assess whether a genetically-modified plant is likely to pose an increased plant pest potential or to have an increased environmental impact compared to conventional soybean.

Results of extensive plant characterization conclude that MON 89788 is not likely to pose an increased plant pest potential or to have increased environmental impact compared to conventional soybean. The evaluation was based on comparative assessments of the phenotypic characteristics between MON 89788 and A3244. The characteristics assessed include: seed dormancy and germination, pollen morphology, and symbiont interactions

conduced in the laboratory, and plant phenotypic observations and ecological interaction evaluations conducted in the field.

Seed dormancy and germination characterization indicated that MON 89788 seed had germination characteristics similar to that of A3244. For pollen characteristics and symbiont interactions, there were no statistically significant differences observed for all seven parameters measured, including pollen viability, nodule dry weight, and shoot total nitrogen. These results lead to the conclusion that MON 89788 is not likely to exhibit increased weed potential compared to conventional soybean.

The field evaluation of phenotypic, agronomic and ecological characteristics of MON 89788 also support the conclusion that MON 89788 is not likely to pose an increased weed or plant pest potential compared to conventional soybean. These studies were conducted at 17 replicated field sites across the major soybean production areas. The assessments analyzed 11 phenotypic characteristics, 12 insect categories, 18 disease categories, and 10 abiotic stressor interactions.

The phenotypic characteristics were similar between MON 89788 and A3244. No statistically significant differences were observed for 10 of the 11 phenotypic characteristics measured, including early stand count, seedling vigor, days to 50% flowering, flower color, lodging, pod shattering, final stand count, seed moisture, seed test weight, and yield. The only statistically significant difference was in plant height at maturity, where MON 89788 was estimated to be 5% shorter than the control. The difference in plant height is not considered biologically meaningful as the magnitude of the difference is minimal, and the mean height of MON 89788 is well within the range observed for commercial soybeans. In addition, the interactions of MON 89788 with insect and disease, and its response to abiotic stressor were similar to that of A3244. No consistent qualitative differences between MON 89788 and A3244 were identified for any of the 40 categories evaluated. Taken together, these comparative assessments lead to the conclusion that MON 89788 is not likely to increase plant pest potential or to have increased environmental impact compared to conventional soybean.

The potential for MON 89788 outcrossing to sexually compatible species is unlikely since no known wild *Glycine* species related to cultivated soybean are known to be present in North America. In addition, soybean is considered a self-pollinated species where cross-pollination occurs at very low frequency (0.04 to 3.62%) in adjacent plants. Furthermore, in the rare event when cross-pollination does occur, MON 89788 and its progeny are not expected to exhibit significant environmental impact because studies conducted to date have shown that the glyphosate-tolerant trait in MON 89788 is not likely to enhance plant pest potential. Therefore, the environmental consequence of pollen transfer from MON 89788 to other *Glycine* species is considered negligible.

An assessment of the impact on agronomic practices indicates that MON 89788 will not alter cultivation and rotational practices, or the management of insects and diseases currently employed for conventional soybeans and Roundup Ready soybean system. In addition, MON 89788 will encourage the use of conservation-tillage and integrated weed

management practices that are facilitated by the use of the Roundup Ready soybean system. Based on the data and information presented in this submission, it is concluded that MON 89788 is not likely to pose an increased plant pest potential or to have increased environmental impact compared to conventional soybean. Furthermore, the successful adoption of MON 89788 is expected to increase economic benefits due to the enhanced yield potential, and maintain the environmental and weed control benefits afforded by the current product, Roundup Ready soybean. Therefore, Monsanto Company requests a determination from APHIS that MON 89788 and any progeny derived from crosses between MON 89788 and other soybeans be granted nonregulated status under 7 CFR Part 340.

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Abbreviations and Definitions

~	Approximately
AA	Amino acid
AACC	American Association for Clinical Chemistry
aadA	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme from the transposon Tn7
ACCase	Acetyl-CoA Carboxylase
ADF	Acid detergent fiber
ALS	Acetolacate synthase
AOAC	Association of Analytical Communities
AOCS	American Oil Chemists' Society
APS	Analytical protein standard
ASA	American Soybean Association
B-	Border region
BSA	Bovine serum albumin
CAPS	3-[cyclohexylamino]-1-propanesulfonic acid
CI	Confidence interval
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp.* strain CP4
cp4 epsps	Coding sequence for the CP4 EPSPS protein from *Agrobacterium sp.* strain CP4 present in plasmid PV-GMGOX20
CS-*rop*	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
CTAB	Cetyltrimethylammonium bromide
CTP2	Chloroplast transit peptide, isolated from *Arabidopsis thaliana* L. EPSPS
CV	Coefficient of variation
CVol	Column volume
dCTP	Deoxycytidine triphosphate
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
DWCF	Dry weight conversion factor
DW	Dry weight
ECL	Enhanced chemiluminescence
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase
FA	Fatty acid
FDA	United States Food and Drug Administration

FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FMV	Figwort mosaic virus
FW	Fresh weight
GLP	Good Laboratory Practice
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
ILDIS	International Legume Database and Information Service
ILSI-CCD	International Life Sciences Institute Crop Composition Database
IPM	Integrated pest management
I-*Tsf1*	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 alpha
IUPAC-IUB	International Union of Pure and Applied Chemistry - International Union of Biochemistry
kb	Kilo base pair
kDa	Kilo dalton
LOQ	Limit of quantitation
LOD	Limit of detection
L-*Tsf1*	Leader (exon 1) from the *Arabidopsis thaliana tsf1* gene encoding elongation factor EF-1 alpha
MAFF	Ministry of Agriculture, Forestry and Fisheries of Japan
MALDI-TOF MS	Matrix assisted laser desorption ionization time of flight mass spectrometry
MES	2-[N-Morpholino]ethanesulfonic acid
MHLW	Ministry of Health, Labor and Welfare of Japan
MOA	Ministry of Agriculture of China
MRL	Maximum residue level
MS	Mass spectrometry
MW	Molecular weight
NDF	Neutral detergent fiber
NFDM	Non-fat dried milk
NOEL	No observable effect level
OD	Optical density
OECD	Organization for Economic Co-operation and Development
OR	Origin of replication
OR-*ori*-PBR322	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*
OR-*ori*V	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2
OSL	Over-season leaf - leaf material collected from different time points during the growing season
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PCR	Polymerase chain reaction

PEP	Phosphoenolpyruvate
P-*FMV/Tsf1*	Chimeric promoter containing the *Arabidopsis thaliana Tsf1* gene promoter, encoding elongation factor EF-1 alpha, and enhancer sequences from the Figwort Mosaic virus 35S promoter
PMSF	Phenylmethylsulfonyl fluoride
PPO	Protoporphyrinogen oxidase
PSII	Photosystem II
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
RbcS2	Ribulose-1, 5-bisphosphate carboxylase small subunit
RCSB	Research Collaborator for Structural Bioinformatics
SAM	S-adenosyl methionine
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SE	Standard error
SGF	Simulated gastric fluid
sp	Species
STS	Sulfonylurea-tolerant soybean
T-DNA	Transfer(ed) DNA
TE	Tris-EDTA buffer
T-*E9*	DNA sequences derived from *Pisum sativum L.,* containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit E9 gene
TFA	Trifluoroacetic acid
TIU	Trypsin Inhibitor Unit
Tris	Tris(hydroxymethyl)aminomethane
TS-*CTP2*	Targeting sequence of chloroplast transit peptide, isolated from *Arabidopsis thaliana* L. EPSPS
TSSP	Tissue-specific site pool
U	Units
USB	United Soybean Board
USDA-APHIS	United States Department of Agriculture – Animal and Plant Health Inspection Service
USDA-ERS	United States Department of Agriculture – Economic Research Service
USDA-GRIN	United States Department of Agriculture – Germplasm Resources Information Network
USDA-NASS	United States Department of Agriculture – National Agricultural Statistics Service
v/v	Volume per volume
w/v	Weight per volume

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*.

I. Rationale for the Development of MON 89788

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

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772) and the Plant Quarantine Act (7 U.S.C. § 151-167), to prevent the introduction and dissemination of plant pests into the United States. The APHIS regulation 7 CFR § 430.6 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.

B. Rationale for the Development of RReady2Yield Soybean, MON 89788

Soybean is one of the largest crops produced in the U.S. in terms of the acreage planted and quantity harvested. In 2004, there were 85.5 million metric tons of soybeans produced domestically, which had a net value of greater than $[redacted] dollars (Soya and Oilseed Bluebook, 2005). Soybean yield can greatly impact the economic value of the crop, and increased yield can be achieved by proper weed management and by using soybean varieties that have enhanced yield potential.

MON 89788 is a second-generation glyphosate-tolerant soybean product, which provides enhanced yield potential relative to the current product, Roundup Ready soybean. MON 89788 produces the same CP4 EPSPS protein as in other Roundup Ready crops including Roundup Ready soybean.

Introduction of MON 89788 has the potential to enhance soybean yield. Field experiments with MON 89788 were conducted in 2001 to 2005 throughout the U.S. soybean growing regions under USDA notifications (Appendix A). Results from multi-site field trials during 2004-05 have demonstrated that MON 89788 could produce up to 7% more soybean than Roundup Ready soybean in similar genetic background and grown under similar environmental conditions and management. In comparison, the U.S. soybean yield increase averaged 0.4 bushels per acre per year according to regional yield trends from 1960 to 2004 (USDA-ERS, 2005). This increase in yield is equivalent to 1% yield gain per year based on national average of 40 bushels per acre. Therefore, using varieties containing MON 89788 is likely to enhance soybean yields and provide more economic benefits to the growers.

In addition, MON 89788 is equivalent to Roundup Ready soybean in its tolerance to Roundup herbicide under the current label rate, which will provide the same weed control benefits as the Roundup Ready soybean.

Phenotypic characterization studies summarized in this submission demonstrate that MON 89788 is not different from a conventional soybean variety, A3244. There is no

evidence that growing MON 89788 soybean will result in any adverse effects to the environment. In addition, agronomic evaluations of plant vigor, growth habit characteristics, and general disease susceptibility have not shown biological meaningful differences in terms of plant pest potential between MON 89788 and the control. Use of a Roundup agricultural herbicide on MON 89788 is not expected to cause any adverse changes in the field environment outside of the current Roundup Ready soybean production system. The commercialization of MON 89788, following appropriate regulatory clearances, will represent an efficacious and environmentally compatible addition to the existing options of weed control in soybean.

The introduction of MON 89788 is expected to provide enhanced soybean yield potential, and continue to offer growers superb weed control options in addition to environmental benefits currently provided by the utilization of the Roundup Ready soybean system. These benefits include:

1. *Effective weed control:* The most critical period of weed control in soybean is the first month after planting, as early-season canopy closure gives soybean a competitive advantage over late-emerging weeds and increases herbicide effectiveness (Mickelson and Renner 1997; Wax et al., 1977; Yelverton and Coble 1991). The Roundup Ready soybean system provides growers improved efficacy in weed control compared to herbicide programs used in conventional soybeans (non-transgenic commercial soybean varieties), as specific preemergent herbicides that are used for prevention are replaced by a post-emergent herbicide that can be used on an as-needed basis (Roberts et al., 1999). Although soybean growers have many post-emergence herbicide options, none has the broad spectrum of weed control of glyphosate. Further, many conventional herbicides cause injury to the crop, while glyphosate may be applied over Roundup Ready varieties at any stage of growth without causing damage (Carpenter and Gianessi, 2001). Crop injury may not reduce yield, but it can delay canopy closure and increase weed competition with the crop.

2. *Convenience and simplicity:* The Roundup Ready soybean system increases simplicity and flexibility of a weed-control program that relies on glyphosate to control a broad spectrum of weeds without crop injury or crop rotation restrictions, which was a major driver for the adoption of Roundup Ready soybean (Carpenter and Gianessi, 1999). Additionally, the Roundup Ready soybean system has been recognized as affording outstanding flexibility of production system because it presents no herbicide carryover problems (Marra et al., 2002). The introduction of Roundup Ready soybeans in the U.S. has eliminated 19 million herbicide applications per year – a decrease of 12%, even though the total soybean acres increased by 18% from 1996-1999 (Carpenter, 2001). This decrease in herbicide applications means that growers make fewer trips over their fields to apply herbicides, which translates into ease of management and reduced fuel use.

3. *Increased adoption of reduced tillage practices:* Conservation tillage improves water quality and creates habitat for wildlife (CTIC, 2000; Fawcett and Towry, 2002), and control of existing weeds has been a major barrier to the success of conservation

tillage systems (Nowak, 1983; Wilson and Worsham, 1988). Success in adoption of conservation tillage has been enhanced with the introduction of Roundup Ready soybean and use of glyphosate in the cropping systems (Marra et al., 2004; Duffy, 2001; Swanton et al., 2000; Krausz et al., 1996). In an survey by the American Soybean Association (ASA), it was found that 48% of the growers have increased no-till soybean acres from 1996 to 2001 due to adoption of Roundup Ready soybeans, and 53% of the growers were making fewer tillage passes in soybean fields. Reduced tillage practices in Roundup Ready soybeans was estimated to save 247 million tons of irreplaceable topsoil and reduce fuel use by 234 million gallons in 2000 (ASA, 2001).

4. *Compatibility with Integrated Pest Management (IPM) and soil conservation techniques:* Roundup Ready soybean is highly compatible with integrated pest management and soil conservation techniques (Keeling et al., 1998; ASA, 2001; Fawcett and Towry, 2002), resulting in a number of important environmental benefits including reduced soil erosion and improved water quality as discussed above, improved soil structure with higher organic matter (Kay, 1995; CTIC, 2000), improved carbon sequestration (Reicosky, 1995; Reicosky and Lindstrom, 1995) and reduced CO_2 emissions (Kern and Johnson, 1993; CTIC, 2000).

5. *Increased income and enhanced value for the growers:* It has been estimated that U.S. soybean growers saved a net of $[redacted] in weed control costs in 1999 compared to 1995, the year before Roundup Ready soybean was introduced (Carpenter, 2001). In addition, there are nonpecuniary values that growers perceive in adopting Roundup Ready soybean according to a survey conducted of 610 growers in the Midwest and the South in 2003, where comparative assessments of all measurable costs and revenue were made between farms that grew conventional soybeans and those that produced Roundup Ready soybeans. This survey considered both financial and nonfinancial aspects of farm management practices, and assigned value judgments (in dollars) to assess costs and benefits of adopting Roundup Ready soybean. Results indicated that farmers perceived up to $[redacted] per acre benefit by adopting Roundup Ready soybean, and the most profound benefits came from reduced herbicide costs, overall convenience, and time saved from reduced tillage (Marra et al., 2004). Also, as noted above, use of MON 89788 is expected to increase soybean yield over use of Roundup Ready soybean. Therefore, adoption of MON 89788 will provide more income per acre and bring added values to the growers.

6. *Minimal environmental impact of glyphosate:* The Roundup Ready soybean system utilizes one main ingredient, glyphosate, to control a broad spectrum of weeds. Aside from being one of the most effective herbicides, glyphosate has been shown to have favorable environmental characteristics compared to other herbicides (Nelson and Bullock, 2003). In addition, glyphosate has been shown to have favorable safety profile as concluded by the U.S. EPA (1993) where it indicates that use of Roundup agricultural herbicides does not pose unreasonable risks to humans, birds, mammals, aquatic organisms, bees and invertebrates.

In summary, the Roundup Ready soybean system has become the preferred weed management system for soybean production in the U.S. Use of the current Roundup Ready soybean system has reduced the number of herbicide applications, which results in effective weed control and increased income for the soybean growers. The Roundup Ready soybean system also offers environmental benefits associated with the use of conservation tillage and integrated weed-management practices. MON 89788 provides all of the benefits afforded by the Roundup Ready soybean system, and in addition, provides enhanced yield benefits to the growers. Furthermore, use of MON 89788 will maintain effective and familiar weed control management practices that are fully compatible with conservation tillage practices.

C. Submissions to Other Regulatory Agencies

Submission to FDA

MON 89788 falls within the scope of the 1992 Food and Drug Administration's (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 submitted a food and feed safety and nutritional assessment summary for MON 89788 to FDA.

Submission to EPA

The United States Environmental Protection Agency 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.*). A proposed label for the use of Roundup WeatherMAX® herbicide (EPA Reg. No. 524-537) on MON 89788 will be submitted in 2007. The resulting glyphosate and plant metabolite residue levels using the proposed label will be consistent with national and international MRLs.

Submissions to Foreign Governments

Regulatory submissions will be made to countries that import significant soybean grain or food and feed products derived from U.S. soybeans and have functional regulatory review processes in place. These will include submissions to a number of additional governmental regulatory agencies including, but not limited to, Ministry of Agriculture (MOA) of China, Ministry of Health, Labor and Welfare (MHLW) and Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan, Canadian Food Inspection Agency (CFIA) and Health Canada, and the European Commission of the European Union. As appropriate, notifications will be made to countries that import significant quantities of U.S. soybeans and soybean products and do not have a formal regulatory review process for biotechnology-derived crops.

II. The Soybean Family

This section summarizes the biology of soybean based on the consensus document for *Glycine max* (L.) Merr. prepared by the Organization for Economic Co-operation and Development (OECD, 2000; OECD, 2001), a summary prepared by USDA-APHIS (USDA-APHIS, 2006), a biology document published by CFIA-PBO (CFIA, 1996), information provided in the USDA petition for Roundup Ready soybean (93-258-01P), as well as recent literature.

A. Soybean as a Crop

Soybean is grown as a commercial crop in over 35 countries. The major producers of soybeans were the U.S., Brazil, Argentina, China, and India, which accounted for approximately 90% of the global soybean production in 2004 (Soya and Oilseed Bluebook, 2005). The soybean produced in China and India are primarily for domestic use, while a significant portion of that produced in U.S., Brazil, and Argentina is traded globally in the form of soybean, soybean meal or soybean oil. Globally, the U.S. is the largest soybean export country, while Argentina led the soybean meal and soybean oil export markets in 2004 (Soy Stats, 2005).

There were 85.5 million metric tons of soybeans produced in the U.S. in 2004, which contributed to greater than $[redacted] of total crop value (Soya and Oilseeds Bluebook, 2005). Approximately half the total soybean supply in the U.S. was crushed to produce soybean meal and oil, and the majority was used domestically, primarily supplying the feed industry for livestock use or the food industry for edible vegetable oil and soy protein isolates. Another one-third of the U.S. soybean supply was exported as grain to other geographies, with China, Japan, Mexico and EU being the top soybean import geographies (Soya and Oilseed Bluebook, 2005). The remainder of the soybean produced was used as seed, feed or stocks.

Soybeans are used in various food products, including tofu, soy sauce, soymilk, energy bars, and meat products. A major food use for soybean in North America is purified oil, for use in margarines, shortenings, and cooking and salad oils. Soybean oil generally has a smaller contribution to soybean's overall value compared to soybean meal because it constitutes just 18 to 19% of the soybean's weight. Nonetheless, soybean oil accounts for approximately two-thirds of all the vegetable oils and animal fats consumed in the U.S., and is still the largest source of vegetable oil worldwide (USDA, 2006).

Soybean meal is used as a supplement in feed rations for livestock. Soybean meal is the most valuable component obtained from processing the soybean, accounting for roughly 50-75% of its overall value. By far, soybean meal is the world's most important protein feed, accounting for nearly 65% of world supplies. Industrial uses of soybeans range from the production of yeasts via fermentation to the manufacture of soaps, inks, paints and disinfectants. Industrial uses of soybean have been summarized by Cahoon (2003), and United Soybean Board (2003).

U.S. soybean plantings reached 75.1 million acres in 2004, a 30% increase since 1990. Increased planting flexibility, rising yield improvements from narrow-row seeding practices, a higher rate of corn-soybean rotations, and low production costs favored expansion of soybean acreage in the 1990s. More than 80% of U.S. soybean acreage is concentrated in the upper Midwest, although significant amounts are still planted in historically important areas of the Delta and Southeast. Acreage tends to be concentrated where soybean yields are highest, and the top soybean producing states include Illinois, Iowa, Indiana, Minnesota, Missouri, Nebraska, and Ohio, which accounted for over 65% of U.S. soybean production in 2004 (USDA-NASS, 2005a and 2006a).

Convenience in weed management also has encouraged expansion of soybean acreage since the introduction of Roundup Ready soybean in 1996. Because glyphosate agricultural herbicides are highly effective against the majority of annual and perennial grasses and broadleaf weeds, growers planting Roundup Ready soybeans are able to reduce the number of herbicides used to control economically destructive weeds that grow in their fields. Farmers realize savings in weed control costs and enhancement in yield by reduction of crop-weed competition. The benefits of the Roundup Ready system (combining Roundup Ready soybean with Roundup herbicide use) was evidenced from the rapid adoption of Roundup Ready soybean. The U.S. soybean acreage planted with Roundup Ready soybean grew from less than 5% in 1996 to 87% in 2005 (USDA-NASS, 2006a). In 2004, Roundup Ready soybean was planted on 56% of the 86 million hectares of soybean grown globally (James, 2004).

B. History of Soybean

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

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

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

Soybean was introduced into North America in the 18th century. Samuel Bowen, a former seaman employed by the East India Company, brought soybean to Georgia from China, and Benjamin Franklin also brought soybean to North America in 1770 (Hymowitz and Harlan, 1983). In 1851, the soybean was introduced in Illinois and subsequently throughout the Corn Belt. In 1853, soybean seeds were deposited into the New York State Agricultural Society, the Massachusetts Horticultural Society, and the Commissioner of Patents. The two societies and the Commissioner of Patents sent soybean seeds to dozens of farmers throughout the U.S., and soybean has been cultivated ever since and subsequently has become a key source of nutrient for food and feed use in the U.S. (Hymowitz, 1987).

C. The Taxonomy of Soybean

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

Family: Leguminosae
- Subfamily: Papilionoideae
  - Tribe: Phaseoleae
  - Genus: *Glycine*
    - Subgenus: *Soja* (Moench) F.J. Herm.
      - Species: *max*

The genus *Glycine* Willd. is of Asian and Australian origin and is divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* consists of 22 wild perennial species, which are indigenous to Australia, west, central and south Pacific Islands, China, Russia, Japan, Indonesia, Korea, Papua New Guinea, the Philippines and Taiwan (Hymowitz, 2004). The subgenus *Soja* includes the cultivated soybean, *G. max* (L.) Merr. and its wild annual relatives from Asia, *G. soja* Sieb. and Zucc. The list of species in the genus *Glycine* Willd. is presented in Table II-1.

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

Table II-1. List of Species in the Genus *Glycine* Willd., 2n Chromosome Number, Genome Symbol, and Distribution

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

[1]Genomically similar species carry the same letter symbols.
[2] Genome designation has not been assigned to the species.
[3] Allopolyploids (A and B genomes) and segmental allopolyploids (B genomes).
[4] Allopolyploids (D and E, A and E, or any other unknown combination).
[5] Allopolyploids (A and D genomes, or any other unknown combination).
Note: Table is adapted from Hymowitz, 2004.

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

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

D. The Genetics of Soybean

Glycine is the only genus in the tribe Phaseoleae where species have diploid chromosome numbers of 40 and 80, but not 20 (Lackey, 1980). The unique chromosome number of *Glycine* is probably derived from diploid ancestors with base number of 11. The ancestral species have undergone aneuploid reduction, which is prevalent throughout the Papilionoideae, to a base number of 10 chromosomes (Lackey, 1980). Tetraploidization ($2n = 2x = 40$) through autopolyploidy or allopolyploidy of the progenitor species occurred either prior to or after dissemination from the ancestral region. The path of migration from a common progenitor is assumed by Singh et al. (2001) as: wild perennial ($2n = 4x = 40$, unknown or extinct) to wild annual ($2n = 4x = 40$; *G. soja*) to soybean ($2n = 4x = 40$; *G. max*). Soybean should be regarded as a stable tetraploid with diploidized genome (Gurley et al., 1979; Lee and Verma, 1984; Skorupska et al., 1989).

Crosses within the subgenus *Soja* indicated that the F1 hybrids of *G. soja* and *G. max* carried similar genomes, and their seeds were fertile (Newell and Hymowitz, 1983). However, inter-species cross-ability between *G. max* and the wild perennial *Glycine* species is extremely low, because they are genomically dissimilar (Table II-1), and pod abortion is common. From time to time, immature seeds of the crosses could be germinated aseptically *in vitro*, and the resulting F1 hybrids are slow-growing, morphologically weak, and completely sterile. Their sterility is due to poor chromosome pairing. Further, species distantly related usually produce nonviable F1 seeds, or premature death of germinating seedlings, and seedling and vegetative lethality (Singh and Hymowitz, 1989; Kollipara et al., 1993).

E. Pollination of Cultivated Soybean

Soybean is a self-pollinated species, propagated by seed (OECD, 2000). The papilionaceous flower consists of a tubular calyx of five sepals, a corolla of five petals, one pistil, and nine fused stamens with a single separate posterior stamen. The stamens form a ring at the base of the stigma and elongate one day before pollination, at which time the elevated anthers form a ring around the stigma (OECD, 2000). The soybean flower stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive for 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self-fertilization, and cross-pollination is usually less than one percent (Caviness, 1966). Pollination typically takes place on the day the flower opens. The pollen naturally comes in contact with the stigma during the process of anthesis. Anthesis normally occurs in late morning, depending on the environmental conditions. The pollen usually remains viable for two to four hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination only can take place during the short time when the pollen is viable.

F. Hybridization with Cultivated Soybean Varieties

In studies with cultivated traditional soybeans where conditions have been optimized to ensure close proximity and flowering synchrony, cross-pollination has been found to be generally very low. Outcrossing has been reported to range from 0.03 to 3.62% between adjacent rows (Beard and Knowles, 1971; Caviness, 1966). At distances of more than 4.5 meters from the pollen source, natural cross-pollination in soybean is very rare (less than 0.02%) and most often not detectable (Caviness, 1966). Consistent with earlier cross-pollination studies, recent data from Ray et al. (2003) found cross pollination rates ranging from 0.41% to 0.03% at distances of 0.9 m and 5.4 m from the pollen source, respectively. When plants are grown in very close proximity to each other (15 cm), average cross-pollination rates were 1.8% (Ray et al., 2003). Cross-pollination frequencies may vary due to growing season and genotype, and most outcrossing occurred with surrounding plants. Insect activity does increase the outcrossing rate, but soybeans generally are not the preferred plant for pollinators (Erickson, 1975; Erickson, 1984). The limited potential for cross-pollination is evident in certified seed regulations for Foundation seed, 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).

G. Cultivated Soybean as a Volunteer

Soybean plants are annuals, and they reproduce solely by means of seeds. Mature soybean seeds have no innate dormancy (TeKrony et al., 1987), are sensitive to cold (Raper and Kramer, 1987), and are not likely to survive in the U.S. from one growing season to the next if left in the field over winter. Due to the lack of dormancy (a trait that is selected for in commercial soybean seed), soybean seeds can germinate quickly under adequate temperature and moisture and can potentially grow as a volunteers. However,

volunteers likely would be killed by frost during autumn or winter of the year they were produced. If they did establish, volunteers would not compete well with the succeeding crop, and could be controlled readily either mechanically or chemically (OECD, 2000).

H. Characteristics of the Recipient Plant

The soybean variety used as the recipient for the DNA insertion to create MON 89788 was A3244, a non-transgenic conventional variety developed by Asgrow Seed Company. The A3244 is an elite maturity group III soybean variety, which was developed and selected based on its superior agronomic performance over other soybean lines (Tylka and Marett, 1999).

I. Soybean as a Test System in this Petition

In developing the data to support this petition, MON 89788 and appropriate control materials (A3244 or *E. coli*-produced CP4 EPSPS) were used as comparator. In addition, conventional and commercial Roundup Ready soybean varieties were used as reference materials to establish a range of expected responses. In general, the genetic background of MON 89788 was matched with that of the control, so the effect of the genetic insertion and the presence of CP4 EPSPS protein could be assessed in an unbiased manner. Since the MON 89788 was derived from the A3244 conventional variety, it was deemed appropriate to use A3244 as the control variety as its use would minimize the potential bias in subsequent comparative assessments. On the other hand, reference varieties were selected based on prevalence and performance of the soybean varieties at each trial location. As a general principle, varieties that were well adapted to the local environments and were commonly used by the local producers would be considered for use as reference soybean varieties.

III. Description of the Transformation System

MON 89788 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using the double-border, binary vector PV-GMGOX20 (Section IV, Figure IV-1). *Agrobacterium tumefaciens* strain ABI contains a disarmed Ti plasmid that is incapable of inducing tumor formation due to the deletion of the phytohormone genes originally present in the *Agrobacterium* plasmid (Koncz and Schell, 1986). The vector, PV-GMGOX20, contains both the left and right border sequences flanking the transfer DNA (T-DNA) to facilitate transformation.

The *Agrobacterium*-mediated soybean transformation to produce MON 89788 was based on the method described by Martinell et al. (2002), which allows the generation of transformed plants without utilization of callus. Briefly, meristem tissues were excised from the embryos of germinated A3244 seed. After co-culturing with the *Agrobacterium* carrying the vector, the meristems were placed on selection medium containing glyphosate, and Carbenicillin and Claforan to inhibit the growth of untransformed plant cells and excess *Agrobacterium*, respectively. The meristems were then placed in media conducive to shoot and root development, and only rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R0 plants generated through the above transformation were screened for glyphosate tolerance, and subjected to numerous molecular and phenotypic assessments. MON 89788 was selected as the lead event based on superior phenotypic characteristics and the comprehensive molecular profile. Regulatory studies on MON 89788 were initiated to further characterize the genetic insertion and the expressed protein, and to establish the food, feed, and environmental safety relative to conventional soybean. The major steps involving the development of MON 89788 are depicted in Figure III-1.

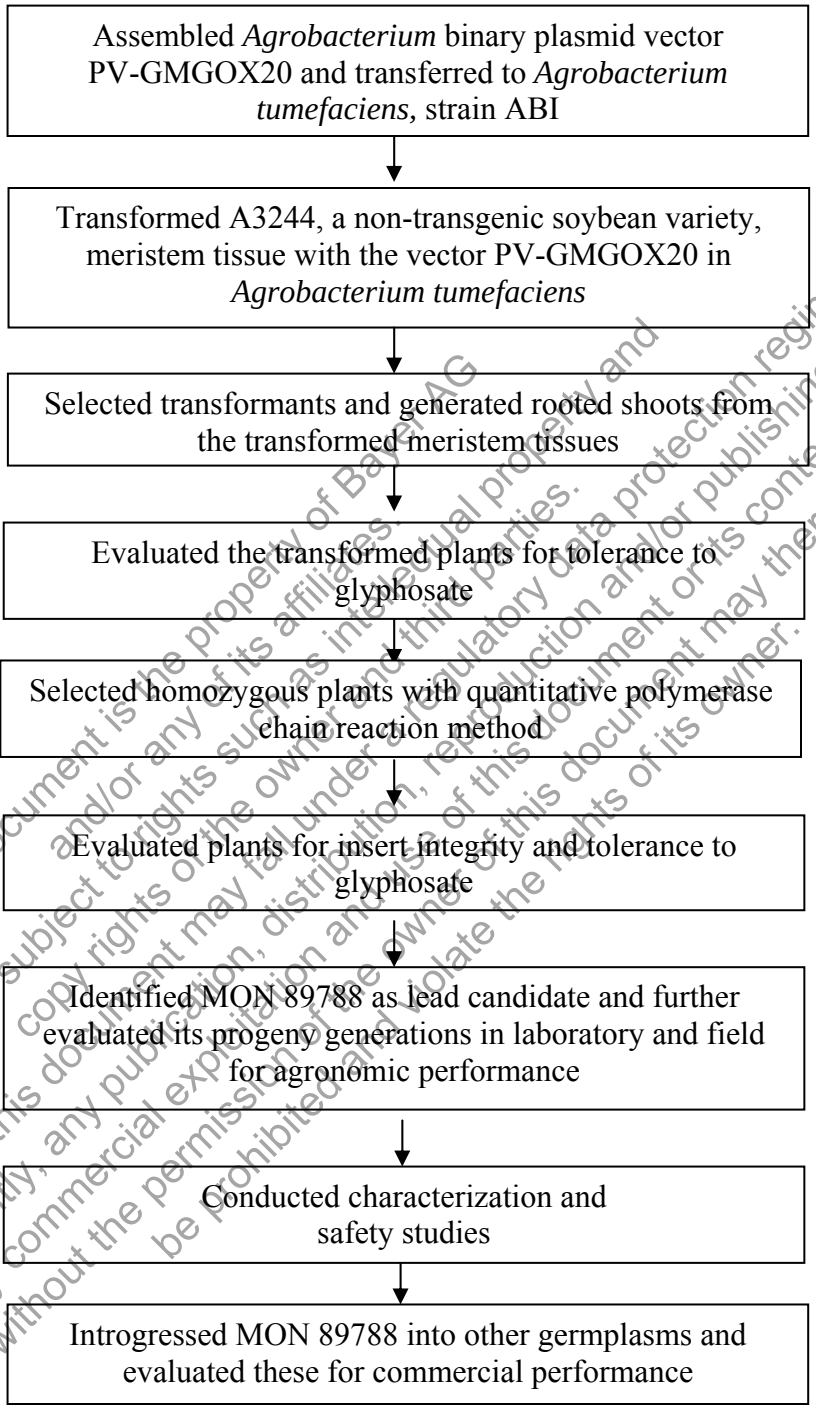


Figure III-1. Schematic of the Development of MON 89788

IV. Donor Genes and Regulatory Sequences

This section describes the donor genes and regulatory sequences used in the development of MON 89788, and the deduced amino acid sequence of the CP4 EPSPS protein produced in MON 89788.

A. Vector PV-GMGOX20

The PV-GMGOX20 vector used for the transformation of soybean meristem to produce MON 89788 is shown in Figure IV-1. This vector is approximately 9.7 kb and contains a *cp4 epsps* gene expression cassette delineated by left and right border regions. The T-DNA that is incorporated into the soybean genome is approximately 4.3 kb, and the DNA backbone region that is not incorporated into the soybean genome is approximately 5.4 kb.

The T-DNA contains, from the right border region, a chimeric transcriptional promoter (P-*FMV/Tsf1*), a leader and an intron sequence derived from *Tsf1* gene (L-*Tsf1* and I-*Tsf1*), a chloroplast transit peptide sequence (TS-*CTP2*), the *cp4 epsps* coding sequence (CS-*cp4 epsps*), and a polyadenylation sequence from *RbcS2* gene (T-*E9*). The *cp4 epsps* expression cassette used to generate MON 89788 is the same as one of the cassettes present in the current Roundup Ready Flex cotton product.

The backbone region outside of the T-DNA, which is not integrated into the soybean genome during transformation, contains two origins of replication for maintenance of plasmid in bacteria (OR-*oriV*, OR-*ori-PBR322*), as well as a bacterial selectable marker gene (*aadA*). A description of the genetic elements and their prefixes (e.g. P-, L-, I-, TS-, OR-, B-, CS-, and T-) in PV-GMGOX20 is provided in Table IV-1.

B. The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein

The *cp4 epsps* gene from *Agrobacterium* sp. strain CP4, a common soil-borne bacterium, has been sequenced and shown to encode a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). In plants, the endogenous EPSPS enzyme is located within the chloroplast. The CP4 EPSPS protein produced in Roundup Ready plants is functionally identical to endogenous plant EPSPS enzymes with the exception that CP4 EPSPS naturally displays reduced affinity for glyphosate relative to endogenous plant EPSPSs (Padgette et al., 1996). The amino acid sequence of the mature CP4 EPSPS protein in MON 89788 is identical to that in Roundup Ready soybean. The deduced full-length amino acid sequence is shown in Figure IV-2.

In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of the 5-enolpyruvyl shikimate-3-phosphate, thereby depriving plants of essential amino acids that are necessary for growth and development (Steinrücken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, the presence of CP4 EPSPS

reconstitutes the shikimic acid pathway, and is able to continuously synthesize aromatic amino acids even in the presence of glyphosate (Padgette et al., 1996).

C. The *Arabidopsis thaliana* EPSPS Transit Peptide

The *cp4 epsps* coding sequence is preceded by a chloroplast transit peptide sequence, *CTP2*, derived from the *Arabidopsis thaliana epsps* gene (Klee et al., 1987). This transit peptide directs the transport of the CP4 EPSPS protein to the chloroplast, which is where the plant EPSPS resides and the site of aromatic amino acid biosynthesis (Klee et al., 1987; Kishore et al., 1988). Transit peptides are typically cleaved from the translated polypeptide following delivery to the plastid (Della-Cioppa et al., 1986). The *CTP2* present in PV-GMGOX20 is identical to the *CTP2* transit peptide sequence in Roundup Ready Flex cotton.

D. Regulatory Sequences

From the right border region of plasmid PV-GMGOX20, the *CTP2/cp4 epsps* coding sequence is under the regulatory control of the P-*FMV/Tsf1* transcriptional promoter. P-*FMV/Tsf1* is a chimeric promoter containing the *Arabidopsis thaliana Tsf1* gene promoter (Axelos et al., 1989) and enhancer sequences from the figwort mosaic virus 35S promoter (Richins et al., 1987). Located between the P-*FMV/Tsf1* promoter and the *CTP2/cp4 epsps* coding sequence are the nontranslated L-*Tsf1* leader sequence (exon 1) and the I-*Tsf1* nontranslated intron (Axelos et al., 1989). The *CTP2/cp4 epsps* coding sequence is linked at the 3' end to the T-*E9* DNA sequence derived from *Pisum sativum*, containing the 3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase, small subunit (*RbcS2*) E9 gene (Coruzzi et al., 1984) for transcriptional termination and polyadenylation of the *CTP2/cp4 epsps* mRNA.

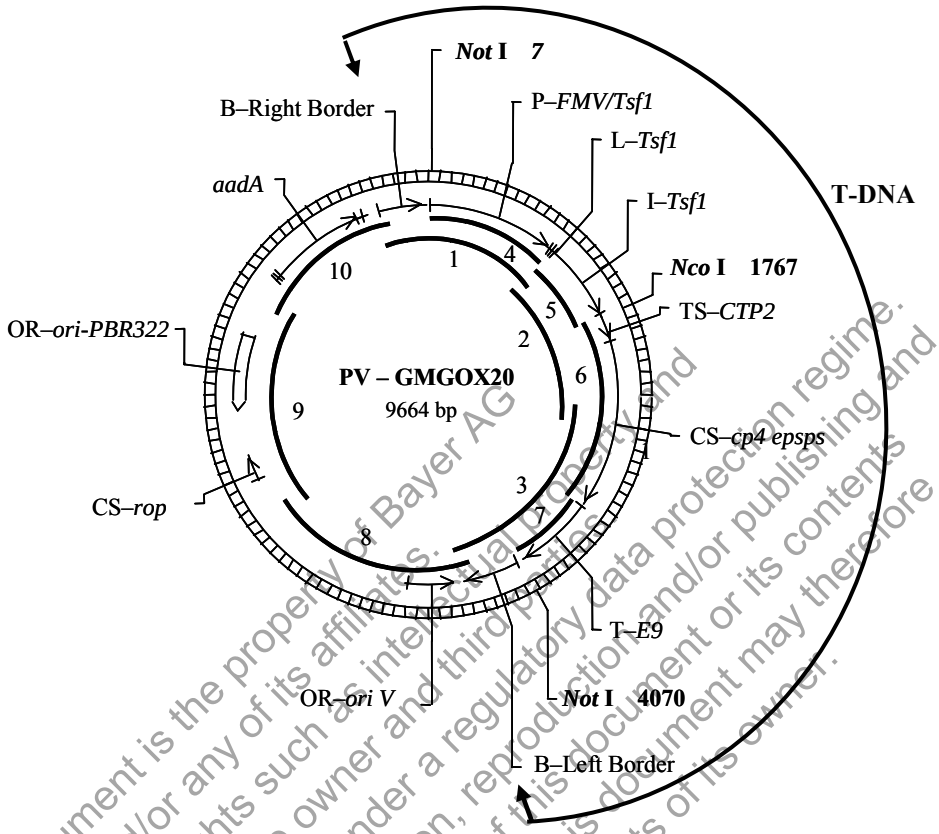
E. T-DNA Borders

Plasmid PV-GMGOX20 contains right border and left border regions that delineate the T-DNA to be transferred into soybean and are necessary for the efficient transfer of the T-DNA into the soybean genome. These border regions (Figure IV-1 and Table IV-1) were derived from *Agrobacterium tumefaciens* plasmids (Depicker et al., 1982; Barker et al., 1983).

F. Genetic Elements outside of the T-DNA Borders

Four genetic elements exist outside of the T-DNA borders that are essential for the maintenance and selection of the vector PV-GMGOX20 in bacteria. They include: *OR-ori V,* origin of replication for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981); CS-*rop,* coding sequence for repressor of primer (ROP) protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989); *OR-ori-pBR322,* origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978); and *aadA*, a bacterial promoter and coding sequence of an enzyme from transposon Tn7 that confers spectinomycin and streptomycin resistance for molecular cloning and selection purposes (Fling et al., 1985). As these elements are outside of the border regions, they

are not expected to be transferred into the soybean genome. The absence of the backbone sequence in MON 89788 has been confirmed by Southern blot analyses, which are presented in the following section.



Probe	DNA Probe	Start Position	End Position	Total Length (~kb)
1	T–DNA Probe 1	9271	1164	1.6
2	T–DNA Probe 2	1071	2916	1.8
3	T–DNA Probe 3	2784	4583	1.8
4	P–*FMV/Tsf1*/ L–*Tsf1*	28	1153	1.1
5	I–*Tsf1* Probe	1131	1764	0.6
6	TS–*CTP2*/CS–*cp4 epsps* Probe	1769	3364	1.6
7	T–*E9* Probe	3407	4060	0.7
8	Backbone Probe 1	4508	6178	1.7
9	Backbone Probe 2	6041	8187	2.1
10	Backbone Probe 3	8056	9322	1.3

Figure IV-1. Circular Map of Plasmid PV-GMGOX20

Plasmid PV-GMGOX20 containing the T-DNA was used in *Agrobacterium*-mediated transformation to generate MON 89788. Genetic elements and restriction sites for enzymes used in the Southern analyses (with positions relative to the plasmid vector) are shown on the exterior of the map. Probes used in the Southern analyses are detailed in the accompanying table.

Table IV-1. Summary of Genetic Elements in the Plasmid PV-GMGOX20

Genetic Element[1, 2]	Position in Plasmid	Function and Source (Reference)
	T-DNA	
Intervening Sequence	1-51	Sequences used in DNA cloning
P – *FMV/Tsf1*	52-1091	Chimeric promoter consisting of enhancer sequences from the 35S promoter of the Figwort Mosaic virus (Richins et al., 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
L – *Tsf1*	1092-1137	5' nontranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
I – *Tsf1*	1138-1759	Intron from the *Tsf1* gene of *Arabidopsis thaliana* encoding elongation factor EF-1 alpha (Axelos et al., 1989)
Intervening Sequence	1760-1768	Sequences used in DNA cloning
TS – *CTP2*	1769-1996	Sequences encoding the chloroplast transit peptide from the *ShkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee et al., 1987)
CS – *cp4 epsps*	1997-3364	Codon optimized coding sequence of the *aroA* (epsps) gene from the *Agrobacterium sp.* strain CP4 encoding the CP4 EPSPS protein (Padgette et al., 1996; Barry et al., 1997)
Intervening Sequence	3365-3406	Sequences used in DNA cloning
T – *E9*	3407-4049	3' nontranslated sequence from the ribulose-1, 5-bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene of pea (*Pisum sativum*)(Coruzzi et al., 1984)
Intervening Sequence	4050-4092	Sequences used in DNA cloning
B – Left Border	4093-4534	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
	Vector Backbone	
Intervening Sequence	4535-4620	Sequences used in DNA cloning
OR – *ori V*	4621-5017	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	5018-6525	Sequences used in DNA cloning
CS – *rop*	6526-6717	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)

Table IV-1 (continued). Summary of Genetic Elements in the Plasmid PV-GMGOX20

Intervening Sequence	6718-7134	Sequences used in DNA cloning
OR – *ori-PBR322*	7135-7763	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1978)
Intervening Sequence	7764-8263	Sequences used in DNA cloning
aadA	8264-9152	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3' (9)-O-nucleotidyltransferase from the transposon Tn7 (Fling et al., 1985)
Intervening Sequence	9153-9288	Sequences used in DNA cloning
T-DNA		
B – Right Border	9289-9645	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982)
Intervening Sequence	9646-9664	Sequences used in DNA cloning

[1] Intervening sequences are not regarded as genetic elements.

[2] P – Promoter; L– Leader; I – Intron; TS– Targeting Sequence; CS – Coding Sequence; T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences; B – Border; OR – Origin of Replication.

```
  1  MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL
 51  LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA
101  TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
151  RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR
201  DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF
251  PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301  VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL
351  RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT
401  HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS
451  DTKAA
```

Figure IV-2. Deduced Amino Acid Sequence of the CP4 EPSPS Protein Present in MON 89788

The amino acid sequence of the plant-produced CP4 EPSPS protein in MON 89788 was deduced from the full-length *cp4 epsps* coding sequence present in PV-GMGOX20.

V. Genetic Analysis

This section details the molecular analyses that characterized the integrated DNA insert in MON 89788. The results confirmed the presence of each genetic element at the insertion site and not at any region outside of the insert, confirmed the lack of plasmid backbone elements, and confirmed the insert stability across generations. In addition, DNA sequencing analyses were performed, and results confirmed the expected nucleotide sequence of the insert in MON 89788 as well as the organization of the genetic elements. Furthermore, insert segregation analysis also confirmed that the expected and the observed segregation ratios were identical. This result is consistent with the finding of a single chromosomal insertion of the *cp4 epsps* gene cassette that segregates according to Mendel's laws of genetics.

Genomic DNA from MON 89788 was digested with restriction enzymes and subjected to Southern blot analyses to characterize the DNA that was integrated into the soybean genome. Genomic DNA samples from conventional soybean (A3244) were used as the negative controls on the blots to determine potential nonspecific hybridization signals. The positive controls for Southern blots were generated by digestions of plasmid DNA with different restriction enzymes or enzyme combinations to produce the DNA banding patterns that were most relevant to the molecular assessment of MON 89788. In addition, DNA markers were included to provide size estimation of the hybridized bands on Southern blots. The genetic elements within the T-DNA that are expected to be present in MON 89788 are listed in Table IV-1 (Section IV) starting at Right Border and ending at Left Border. The probes used in the Southern analyses and the map of the plasmid (PV-GMGOX20) used in the transformation to generate MON 89788 are presented in Figure IV-1 of Section IV. The information and results derived from the molecular analyses were used to construct a linear map of the insert in MON 89788. This linear map depicts restriction sites identified in the T-DNA insert and the flanking soybean genome, and provides information on the expected banding patterns and sizes of the DNA fragments after restriction enzyme digestions. The liner map is shown in Figure V-1. Based on these two figures and the probes used in the analyses, a table summarizing the expected DNA fragments for Southern analyses is presented in Table V-1. The materials and methods used in the analyses are presented in Appendix B.

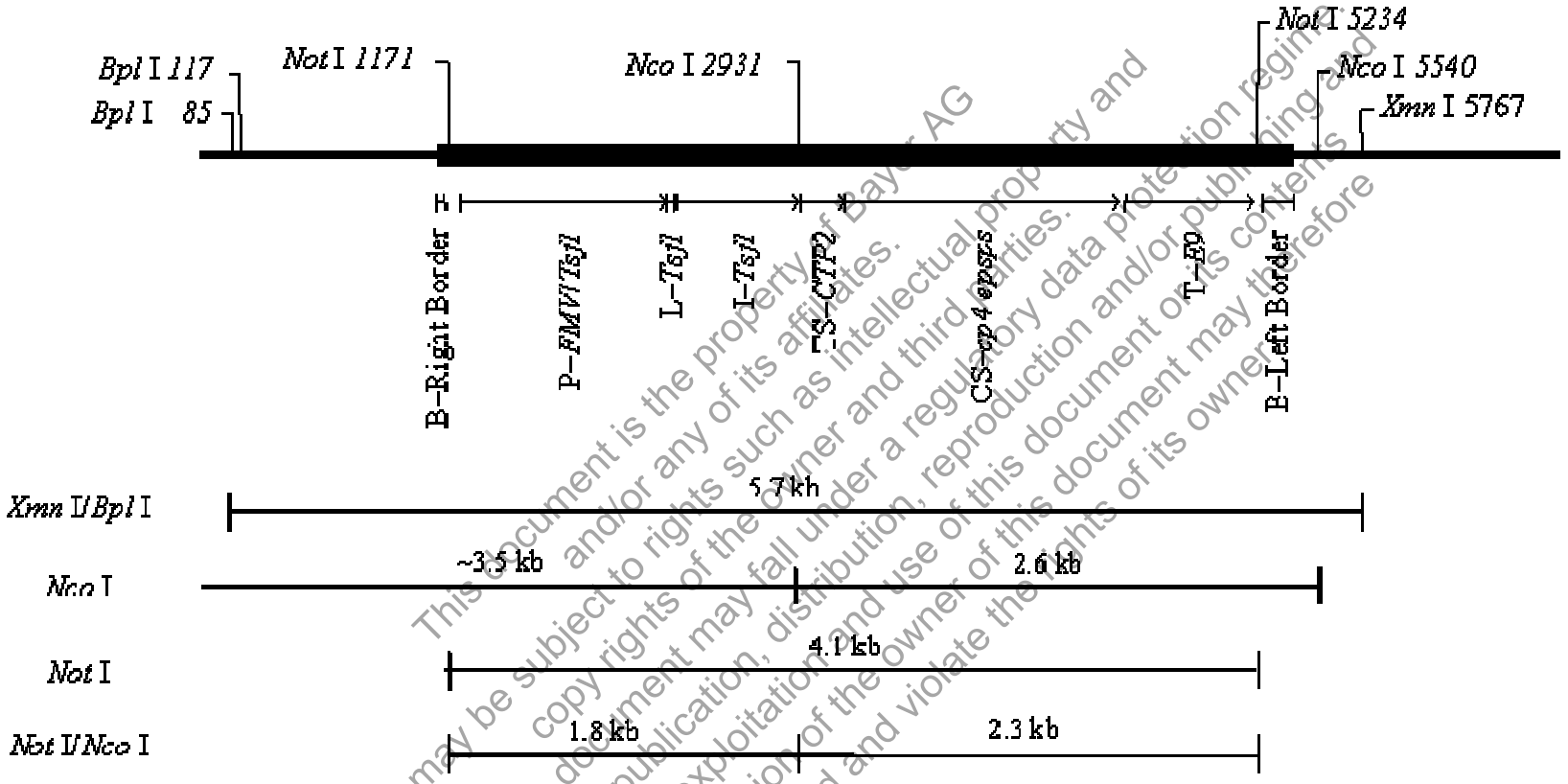


Figure V-1. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 89788
A linear map of the insert and genomic DNA flanking the insert in MON 89788 is shown. The upper portion of the figure displays genetic elements within the insert (thick rectangular bar), as well as restriction sites used in Southern blot analyses. The positions of the restriction sites are consistent with the information presented in the plasmid map (Section IV, Figure IV-1). Arrows underneath the designated insert indicate the direction of transcription. Shown on the lower portion of the map are the expected sizes of the DNA fragments after digestions with respective restriction enzyme or combination of enzymes.

Table V-1. Summary Chart of the Expected DNA Fragments Using Combinations of Restriction Enzymes and Probes

	Expected Size of the DNA Fragment (kb)					
Probes used	**1, 2, 3**	**8, 9, 10**	**4**	**5**	**6**	**7**
Southern blot in Figure	V-2; V-9[1]	V-3; V-10[1]	V-4	V-5	V-6	V-7
Plasmid						
Not I	5.6 + 4.1	5.6	4.1	--	4.1	4.1
Not I + *Nco* I	--[2]	--	--	1.8	--	--
MON 89788						
Xmn I/*Bpl* I	5.7	ND[3]	--	--	--	--
Nco I	~3.5 + 2.6	ND	--	--	--	--
Not I	--	--	4.1	4.1	4.1	4.1
Not I + *Nco* I	--	--	1.8	1.8	2.3	2.3

[1] In Figures V-9 and V-10, MON 89788 DNA samples were only digested with *Nco* I and not with *Xmn* I/*Bpl* I.
[2] '--' indicates that the particular restriction enzyme or the combination of the enzymes was not used in the analysis.
[3] 'ND' indicates that no DNA band was detected.

A. Insert and Copy Number Determination

The insert number (the number of integration sites of the T-DNA in the soybean genome) was determined by digesting the MON 89788 and A3244 DNA with the combination of restriction enzymes *Bpl* I and *Xmn* I, which do not cleave within the T-DNA. Therefore, these enzymes should release a restriction fragment containing the entire T-DNA and adjacent plant genomic DNA (Figure V-1). The number of restriction fragments detected should indicate the number of inserts present in MON 89788. The number of copies of the T-DNA integrated at a single locus was determined by digesting the MON 89788 DNA with the restriction enzyme *Nco* I, which cleaves once within the T-DNA (Figure V-1). If MON 89788 contains one copy of the T-DNA, Southern blot probed with the entire T-DNA will result in two bands, each representing a portion of the T-DNA along with adjacent plant genomic DNA.

The blot was hybridized with three overlapping ^{32}P-labeled T-DNA probes (probes 1, 2, and 3, Figure IV-1; Section IV). The results of this analysis are presented in Figure V-2, and the expected DNA fragments are summarized in Table V-1. As shown in the figure, the A3244 DNA digested with a combination of *Bpl* I and *Xmn* I (lanes 1 and 7) or *Nco* I alone (lanes 3 and 9) produced no hybridization signal. Plasmid PV-GMGOX20 DNA that was mixed with A3244 DNA and digested with *Not* I (lanes 5 and 6) produced the expected size bands of 4.1 kb and 5.6 kb (refer to Table V-1). MON 89788 DNA digested with a combination of *Bpl* I and *Xmn* I (lanes 2 and 8) produced a single band of 5.7 kb, indicating that MON 89788 contains one insert located within a 5.7 kb *Bpl* I/*Xmn* I restriction fragment. MON 89788 DNA digested with *Nco* I (lanes 4 and 10) produced two unique bands of 2.6 and ~3.5 kb representing the two expected fragments. This banding pattern indicates that only one single copy of the T-DNA is present in MON 89788.

B. Confirmation of the Absence of Plasmid PV-GMGOX20 Backbone

To confirm the absence of PV-GMGOX20 backbone sequence, MON 89788 and A3244 DNA were digested with either a combination of the restriction enzymes *Bpl* I and *Xmn* I or the restriction enzyme *Nco* I. Plasmid PV-GMGOX20 DNA digested with *Not* I was used as a positive hybridization control. The blot was hybridized simultaneously with three overlapping probes (probes 8, 9, and 10, Figure IV-1; Section IV) that spanned the backbone sequence of PV-GMGOX20. The results are shown in Figure V-3, and the expected DNA fragments are summarized in Table V-1.

A3244 control DNA digested with a combination of *Bpl* I and *Xmn* I (lanes 1 and 7) or *Nco* I (lanes 3 and 9) showed no detectable hybridization bands, as expected for the negative control. Plasmid PV-GMGOX20 *Not* I restriction fragments mixed with control DNA (lanes 5 and 6) produced the expected size band at 5.6 kb. MON 89788 DNA digested with either a combination of *Bpl* I and *Xmn* I (lanes 2 and 8) or *Nco* I (lanes 4 and 10) showed no detectable hybridization signal. This result indicates that MON 89788 does not contain any detectable backbone sequence from the transformation vector PV-GMGOX20.

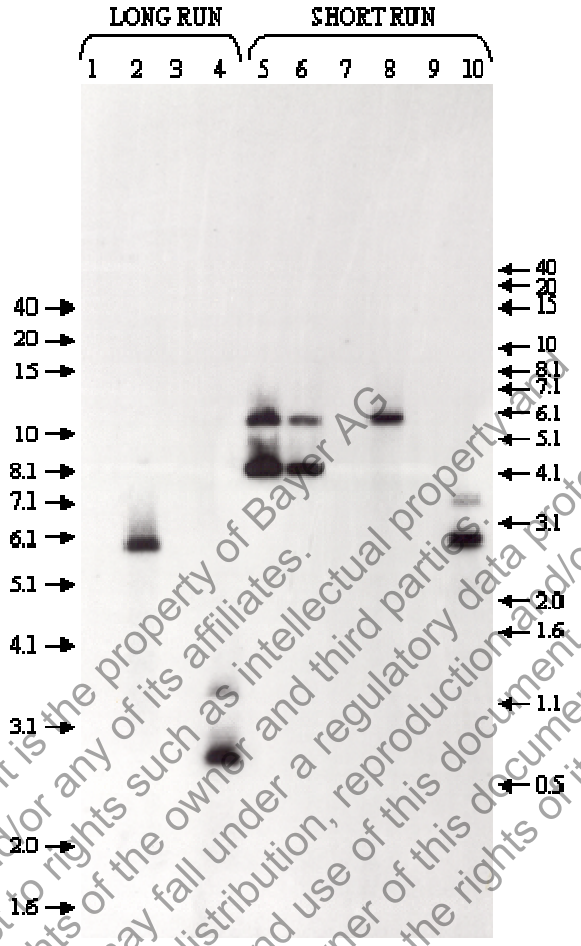


Figure V-2. Southern Blot Analysis of MON 89788: Insert and Copy Number
The blot was hybridized simultaneously with three overlapping ^{32}P-labeled T-DNA probes (probes 1, 2, and 3, Figure IV-1 in Section IV). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Bpl* I/*Xmn* I)
2: MON 89788 (*Bpl* I/*Xmn* I)
3: Conventional (*Nco* I)
4: MON 89788 (*Nco* I)
5: Conventional mixed with PV-GMGOX20 (*Not* I) [2 copies]
6: Conventional mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Bpl* I/*Xmn* I)
8: MON 89788 (*Bpl* I/*Xmn* I)
9: Conventional (*Nco* I)
10: MON 89788 (*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

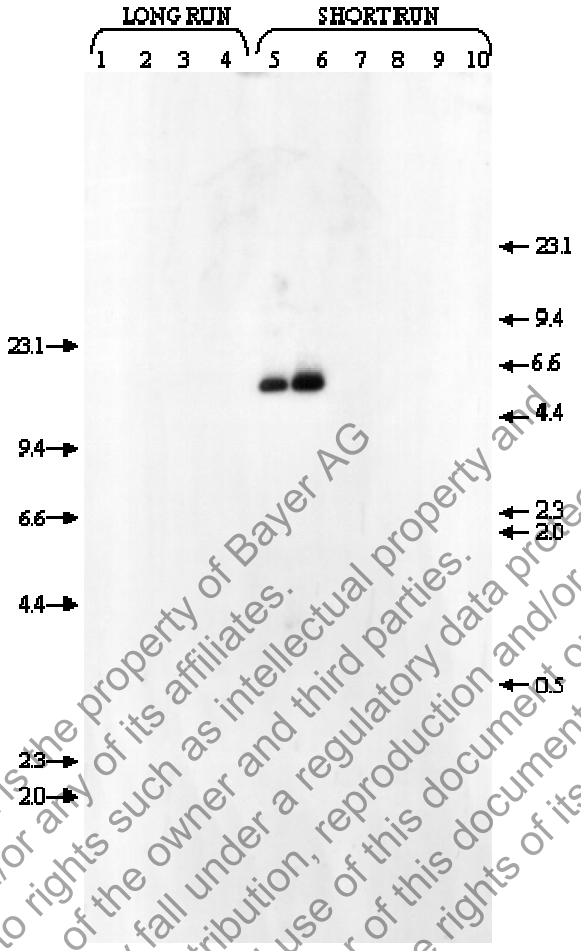


Figure V-3. Southern Blot Analysis of MON 89788: PV-GMGOX20 Backbone
The blot was hybridized simultaneously with three ^{32}P-labeled probes that span the entire backbone sequence (probes 8, 9, and 10, Figure IV-1 in Section IV) of plasmid PV-GMGOX20. Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Bpl* I/*Xmn* I)
2: MON 89788 (*Bpl* I/*Xmn* I)
3: Conventional (*Nco* I)
4: MON 89788 (*Nco* I)
5: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Bpl* I/*Xmn* I)
8: MON 89788 (*Bpl* I/*Xmn* I)
9: Conventional (*Nco* I)
10: MON 89788 (*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

C. *cp4 epsps* Cassette Integrity

The intactness of the inserted *cp4 epsps* coding sequence and the associated genetic elements was assessed by digesting MON 89788 DNA with *Not* I or a combination of *Not* I and *Nco* I and probing the Southern blots with individual genetic elements in the *cp4 epsps* cassette. Digestion with *Not* I was expected to generate a single 4.1 kb restriction fragment containing the *cp4 epsps* gene cassette, and digestion with the combination of *Not* I and *Nco* I was expected to generate two restriction fragments of 1.8 kb and 2.3 kb (Figure V-1). The 1.8 kb fragment contains the *FMV/Tsf1* promoter, *Tsf1* leader, and *Tsf1* intron, whereas the 2.3 kb fragment contains the *CTP2* targeting sequence, *cp4 epsps* coding sequence, and the *E9* 3′ nontranslated region. Plasmid PV-GMGOX20 DNA digested with *Not* I or a combination of *Not* I and *Nco* I was used as a positive hybridization control and size estimator. Individual Southern blot was examined with the *FMV*/*Tsf1* promoter + *Tsf1* leader probe, *Tsf1* intron probe, *CTP2* targeting sequence + *cp4 epsps* coding sequence probe, or *E9* 3′ nontranslated sequence probe (probes 4, 5, 6, and 7 respectively; Figure IV-1; Section IV). The expected DNA fragments identified by probes 4-7 are summarized in Table V-1.

C.1. *FMV/Tsf1* Promoter + *Tsf1* Leader

The A3244 control DNA digested with *Not* I (Figure V-4; lanes 1 and 7) or the combination of *Not* I and *Nco* I (lanes 3 and 9) showed no detectable bands when hybridized with the *FMV/Tsf1* promoter + *Tsf1* leader probe (probe 4, Figure IV-1; Section IV). Plasmid PV-GMGOX20 DNA digested with *Not* I and mixed with control DNA produced the expected size band at 4.1 kb (lanes 5 and 6). MON 89788 DNA digested with *Not* I (lanes 2 and 8) produced the expected band of 4.1 kb, and the DNA digested with the combination of *Not* I and *Nco* I (lanes 4 and 10) produced a single expected size band of 1.8 kb. There were no additional bands detected using the promoter and leader sequence probe. Based on the results presented in Figure V-4, it is concluded that MON 89788 contains no additional *FMV/Tsf1* promoter or *Tsf1* leader elements other than those associated with the intact *cp4 epsps* cassette.

C.2. *Tsf1* Intron

The A3244 control DNA digested with *Not* I (Figure V-5; lanes 1 and 7) or the combination of *Not* I and *Nco* I (lanes 3 and 9) was hybridized with the *Tsf1* intron probe (probe 5; Figure IV-1; Section IV). Results indicated that there were no detectable hybridization bands, as expected for the negative control. As positive control, plasmid PV-GMGOX20 DNA digested with the combination of *Not* I and *Nco* I (lanes 5 and 6) produced the expected size band of 1.8 kb. MON 89788 DNA digested with *Not* I (lanes 2 and 8) or with the combination of *Not* I and *Nco* I (lanes 4 and 10) produced the expected bands of 4.1 kb or 1.8 kb, respectively. No additional bands were detected using the *Tsf1* intron probe. These results indicate that MON 89788 contains no additional *Tsf1* intron elements other than that associated with the intact *cp4 epsps* cassette.

C.3. *CTP2* Targeting Sequence + *cp4 epsps* Coding Sequence

Hybridization of the *Not* I-digested (Figure V-6; lanes 1 and 7) or *Not* I- and *Nco* I-digested A3244 DNA (lanes 3 and 9) with the *CTP2* targeting sequence + *cp4 epsps* coding sequence probe (probe 6, Figure IV-1; Section IV) showed no detectable hybridization bands. Positive control plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size band of 4.1 kb (lanes 5 and 6). MON 89788 DNA digested with *Not* I (lanes 2 and 8) produced the expected size band of 4.1 kb, and the same source of DNA digested with a combination of *Not* I and *Nco* I (lanes 4 and 10) produced the expected size band of 2.3 kb. As there are no unexpected bands on the Southern blot, the results indicate that MON 89788 contains no additional *CTP2* targeting sequence or *cp4 epsps* coding sequence elements other than those associated with the intact *cp4 epsps* gene cassette.

C.4. *E9 3′* Nontranslated Sequence

The A3244 control DNA digested with *Not* I (Figure V-7; lanes 1 and 7) or a combination of *Not* I and *Nco* I (lanes 3 and 9) showed no detectable hybridization bands when examined with the *E9 3′* nontranslated sequence probe (probe 7, Figure IV-1; Section IV). Positive control plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size band of 4.1 kb (lanes 5 and 6). MON 89788 DNA digested with *Not* I (lanes 2 and 8) or a combination of *Not* I and *Nco* I (lanes 4 and 10) produced the expected size band of 4.1 kb or 2.3 kb, respectively. There were no additional bands detected using the *E9* 3' nontranslated sequence probe. These results indicate that MON 89788 contains no additional *E9* elements other than those associated with the intact *cp4 epsps* gene cassette.

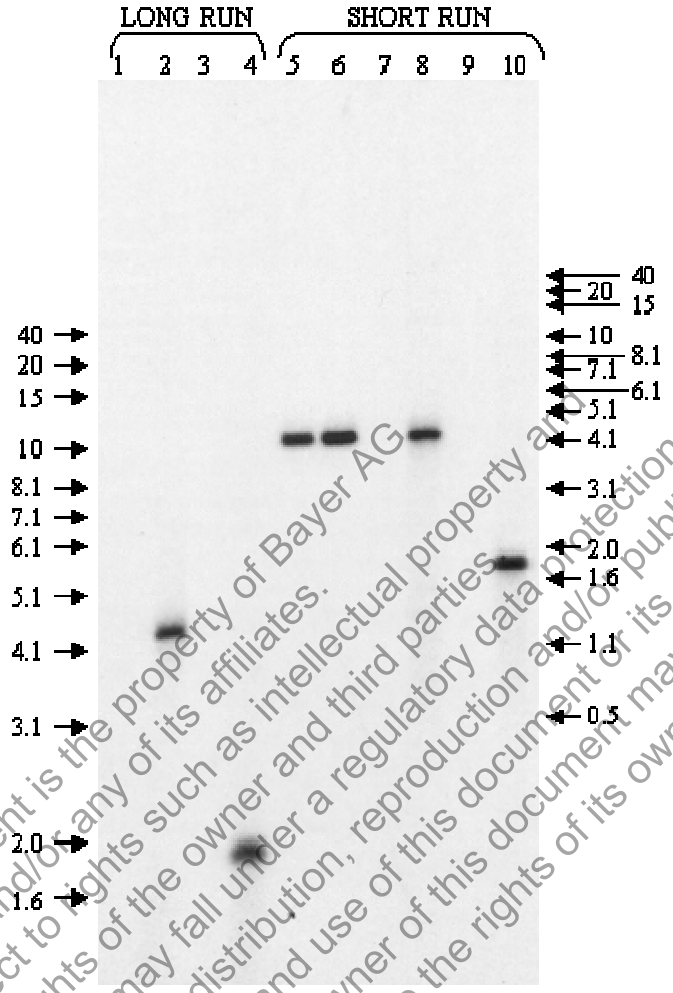


Figure V-4. Southern Blot Analysis of MON 89788: P-*FMV/Tsf1* + L-*Tsf1*
The blot was hybridized with a ^{32}P-labeled probe that spanned the *FMV/Tsf1* promoter and *Tsf1* leader (probe 4, Figure IV-1; Section IV). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

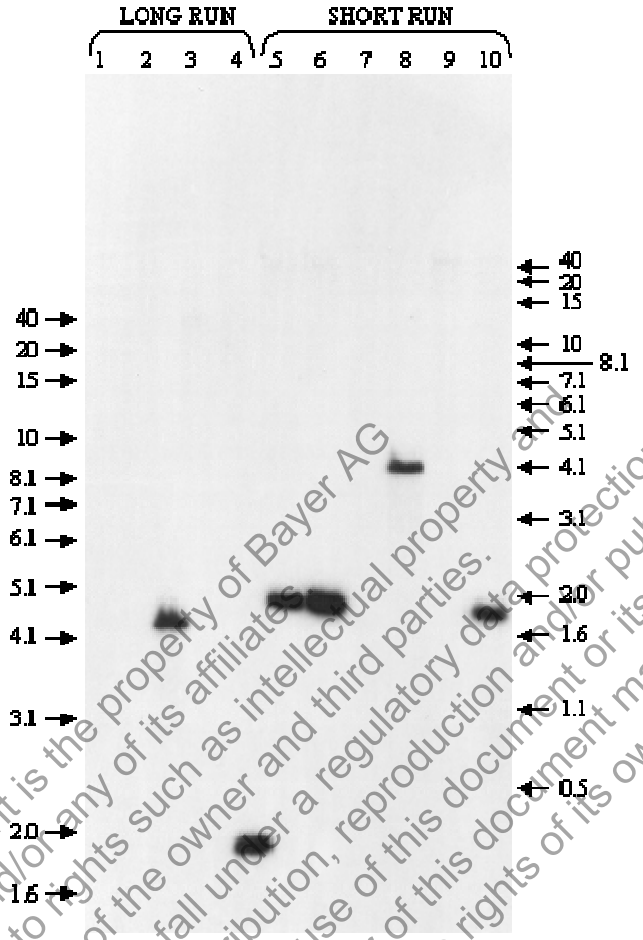


Figure V-5. Southern Blot Analysis of MON 89788: I-*Tsf1*

The blot was hybridized with a ^{32}P-labeled probe that spanned the *Tsf1* intron (probe 5, Figure IV-1; Section IV). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional mixed with PV-GMGOX20 (*Not* I/*Nco* I) [1 copy]
6: Conventional mixed with PV-GMGOX20 (*Not* I/*Nco* I) [2 copies]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

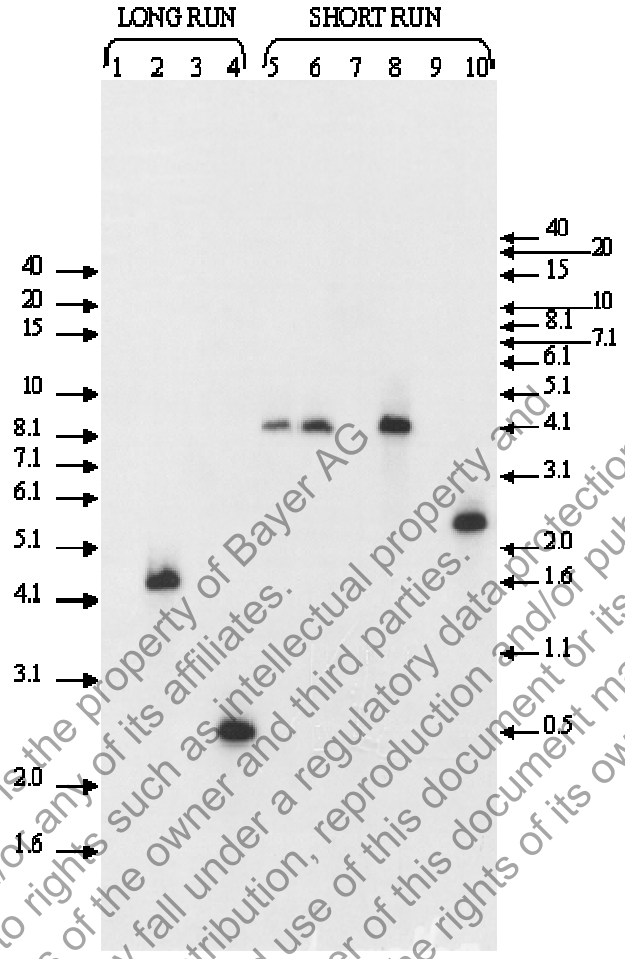


Figure V-6. Southern Blot Analysis of MON 89788: TS-*CTP2* + CS-*cp4 epsps*
The blot was hybridized with a ^{32}P-labeled probe that spanned the *CTP2* targeting sequence and *cp4 epsps* coding sequence (probe 6, Figure IV-1; Section IV). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

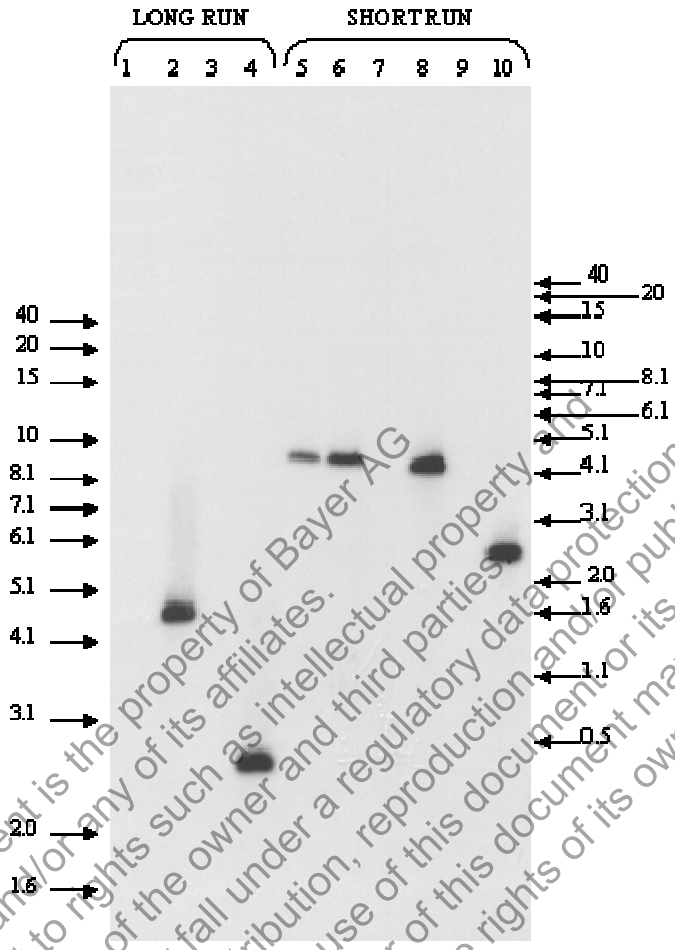


Figure V-7. Southern Blot Analysis of MON 89788: T-*E9*

The blot was hybridized with a ^{32}P-labeled probe that spanned the E9 3' nontranslated sequence (probe 7, Figure IV-1; Section IV). Each lane contains ~10 µg of digested genomic DNA isolated from leaf. Lane designations are as follows:

Lane 1: Conventional (*Not* I)
2: MON 89788 (*Not* I)
3: Conventional (*Not* I/*Nco* I)
4: MON 89788 (*Not* I/*Nco* I)
5: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [0.5 copy]
6: Conventional (*Not* I/*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
7: Conventional (*Not* I)
8: MON 89788 (*Not* I)
9: Conventional (*Not* I/*Nco* I)
10: MON 89788 (*Not* I/*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

D. Southern Blot Analyses of MON 89788 across Multiple Generations

To assess the stability of the T-DNA in MON 89788, Southern blot analysis was performed using MON 89788 DNA across four generations. For reference, the breeding history of MON 89788 is presented in Figure V-8, and the generations examined span R4 to R7. The expected Southern hybridization DNA banding pattern for these analyses is summarized in Table V-1.

D.1. Generational Stability of the Insert

DNA samples from four generations of MON 89788 were isolated and subjected to digestion with *Nco* I to determine the generational stability of the inserted T-DNA. The blot was hybridized simultaneously with three overlapping probes, which, taken together, span the entire T-DNA region of plasmid PV-GMGOX20 (probes 1, 2, and 3, Figure IV-1; Section IV).

Hybridization of A3244 control DNA digested with *Nco* I (Figure V-9; lane 1) showed no detectable hybridization bands, as expected for the negative control. Plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size bands of 4.1 and 5.6 kb (lane 2). Hybridization of MON 89788 DNA digested with *Nco* I produced two bands of 2.6 kb and ~3.5 kb (lanes 3 – 8). This is the same restriction pattern observed for the R5 generation shown in Figure V-2 (lanes 4 and 10). The results of this analysis establish the stability of the inserted DNA over four generations of MON 89788.

D.2. Confirmation of the Absence of PV-GMGOX20 Backbone Sequence

The four generations of MON 89788 material utilized to assess generational stability were also examined for the absence of backbone sequence by Southern blot. MON 89788 and control DNA samples were digested with *Nco* I and the blot was hybridized simultaneously with three overlapping probes, which taken together, span the entire backbone sequence of plasmid PV-GMGOX20 (probes 8, 9, and 10, Figure IV-1; Section IV).

Hybridization of the A3244 control DNA digested with *Nco* I did not detect any bands (Figure V-10; lane 1), as expected for the negative control. Hybridization of plasmid PV-GMGOX20 DNA digested with *Not* I produced the expected size band of 5.6 kb (lane 2). MON 89788 DNA from four generations showed no detectable hybridization signal (lanes 3-8). Consistent with the results depicted in Figure V-3, these results indicate that the generations examined do not contain any detectable backbone sequence from the transformation vector PV-GMGOX20.

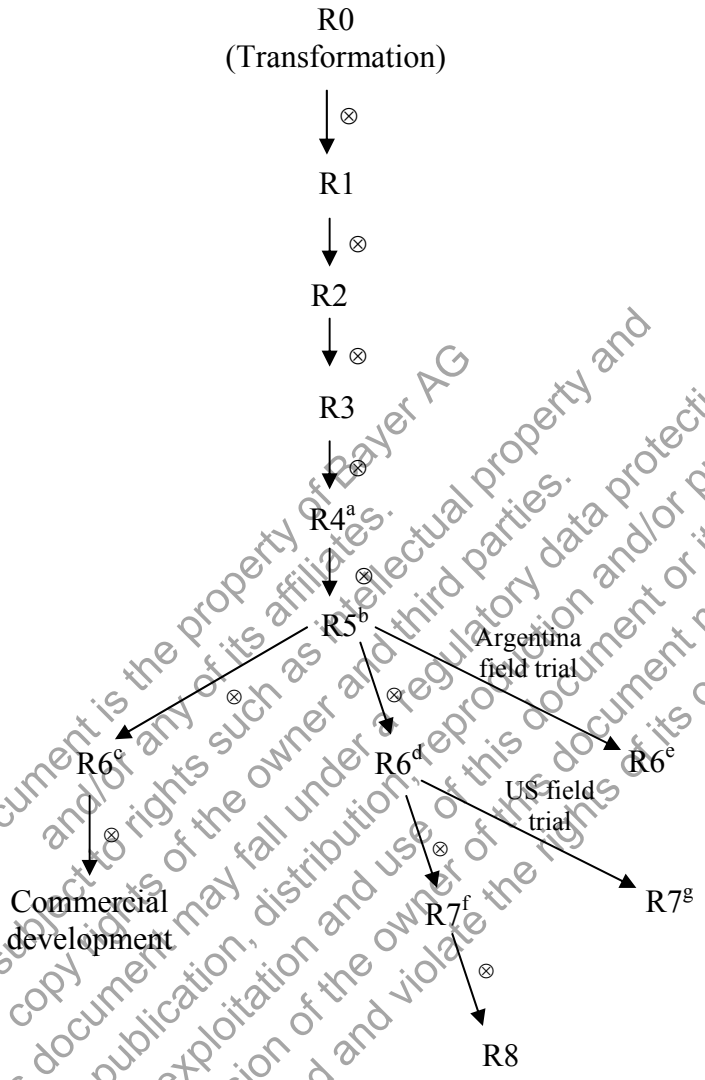


Figure V-8. MON 89788 Breeding Diagram
All generations are self-pollinated (⊗). R1 generation was used for segregation analysis and the selection of homozygous plants (Section V.F.). $R5^b$ seed material was used either for commercial development (on the left) or for regulated field trials (on the right). Generation $R5^b$ was used in the molecular analyses and was the starting seed for Argentina field trial, and the resulting seed ($R6^e$) was used in the protein characterization studies. $R6^d$ was the seed source for U.S. field trial, and the resulting seed ($R7^g$) was used in the composition and expression analyses. Seed lot $R7^f$ was the seed source for additional field trial. Generation $R6^c$ represents the materials entering commercial development. Seed lots $R4^a$, $R5^b$, $R6^c$, $R6^d$, $R6^e$, and $R7^f$ were used in molecular generation stability analyses.

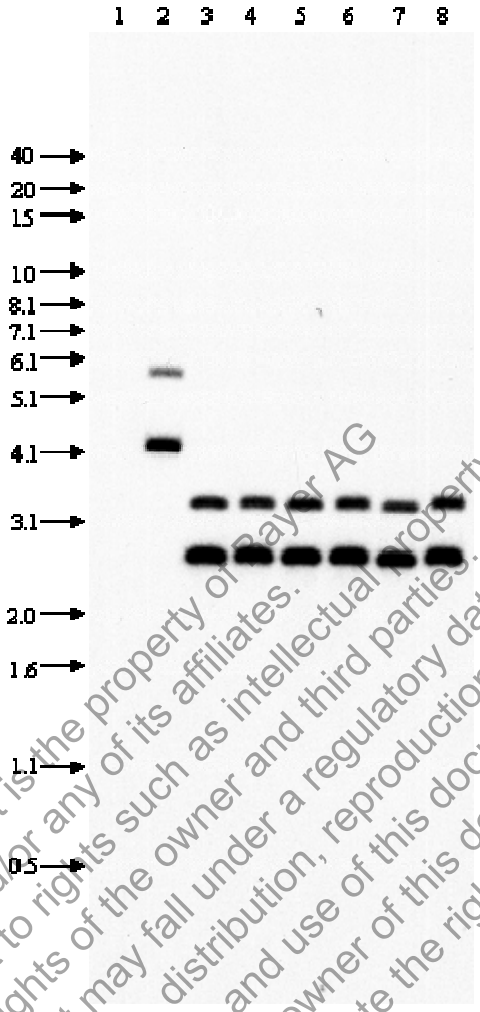


Figure V-9. Generational Stability Analyses of MON 89788 Using Insert and Copy Number Probes

The blot was hybridized simultaneously with three overlapping ^{32}P-labeled T-DNA probes (probes 1, 2, and 3, Figure IV-1, Section IV). Each lane contains ~10 µg of digested genomic DNA isolated from leaf material. The breeding history of MON 89788 is illustrated in Figure V-8. Lane designations are as follows:

Lane 1: Conventional (*Nco* I)
2: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
3: MON 89788 – R4[a] (*Nco* I)
4: MON 89788 – R5[b] (*Nco* I)
5: MON 89788 – R6[c] (*Nco* I)
6: MON 89788 – R6[d] (*Nco* I)
7: MON 89788 – R6[e] (*Nco* I)
8: MON 89788 – R7[f] (*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

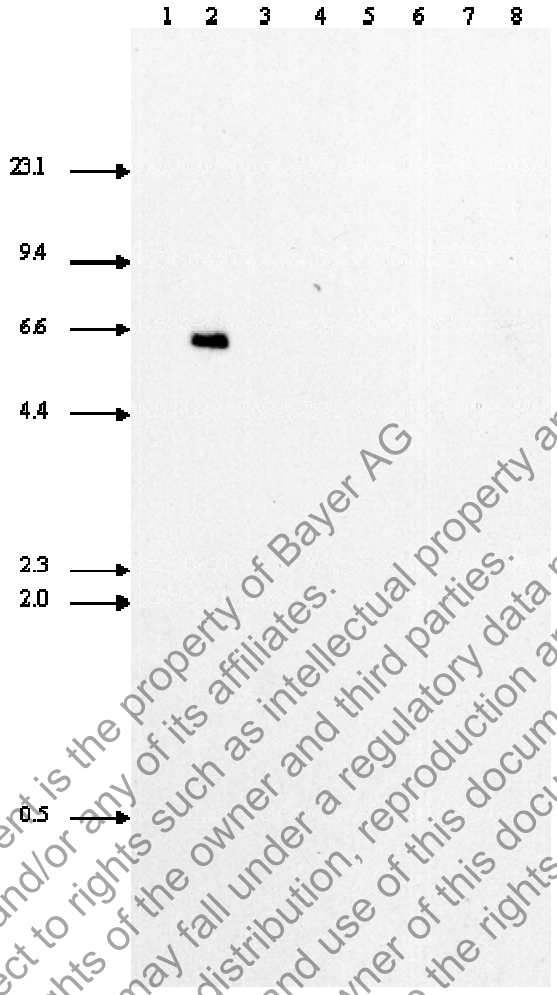


Figure V-10. Generational Stability of MON 89788 Using PV-GMGOX20 Backbone Probes

The blot was hybridized simultaneously with three ^{32}P-labeled probes that span the entire backbone sequence (probes 8, 9, and 10, Figure IV-1; Section IV) of plasmid PV-GMGOX20. Each lane contains ~10 µg of digested genomic DNA isolated from leaf material. Lane designations are as follows:

Lane 1: Conventional (*Nco* I)
2: Conventional (*Nco* I) mixed with PV-GMGOX20 (*Not* I) [1 copy]
3: MON 89788 – R4[a] (*Nco* I)
4: MON 89788 – R5[b] (*Nco* I)
5: MON 89788 – R6[c] (*Nco* I)
6: MON 89788 – R6[d] (*Nco* I)
7: MON 89788 – R6[e] (*Nco* I)
8: MON 89788 – R7[f] (*Nco* I)

→ Symbol denotes size of DNA, in kilobase pairs, obtained from MW markers on ethidium bromide-stained gel.

E. Organization of the Genetic Elements in MON 89788

The organization of the genetic elements within the insert of MON 89788 was confirmed by DNA sequence analyses. Several PCR primers were designed with the intent to amplify three overlapping DNA fragments spanning the entire length of the inert (Appendix B). The amplified DNA fragments were subjected to DNA sequencing analyses. Results confirm that the arrangement of the genetic elements is identical to that in plasmid PV-GMGOX20 and is as depicted in Figure V-1.

F. Inheritance of the Glyphosate Tolerance Trait in MON 89788

During the development of the MON 89788, phenotypic segregation data were generated and analyzed across several generations. The expected segregation ratio for each generation is summarized in Table V-2, and summaries of these analyses are presented in Table V-3. The presence and gene copy number of the *cp4 epsps* gene was determined by quantitative PCR, a method sometimes referred to as TaqMan (Schmidt and Parrott, 2001; Bubner and Baldwin, 2004). The presence of the glyphosate-tolerance trait of individual plants was determined by CP4 EPSPS ELISA and/or by treatment with glyphosate.

After self-pollination of the R0 plant, the R1 seeds were germinated, and the resulting plants were expected to segregate on a 3:1 ratio of positive:negative based on glyphosate-tolerance phenotype (Table V-2). Selected R1 plants that survived the glyphosate treatment (29 out of 43; Table V-3) were subjected to quantitative PCR analyses, and a single plant that was homozygous for *cp4 epsps* expression cassette was selected. This homozygous plant was self-pollinated to give rise to a population of R2 plants, and the segregation ratio for R2 and the subsequent generation is expected to maintain a population of 100% positive (1:0 for positive:negative plants) for the glyphosate-tolerance trait (Table V-2).

Table V-2. Selection Process and Expected Segregation Ratio during MON 89788 Development

Generation	Expected Ratio and Selection
R0	Plant was self-pollinated to produce R1 seed; no Chi-square analysis
R1	3:1 (positive:negative) based on glyphosate-tolerance phenotype
R1 Homozygous plant selection	Homozygous plant selection was conducted using TaqMan for *cp4 epsps* from the segregating R1 population.
R2	1:0 positive:negative (homozygous progeny, derived from R1 selection)
R3	1:0 positive:negative (homozygous progeny established in field plots, derived from homozygous selection)

Phenotype frequency was compared by means of a Chi-square analysis (Little and Hills, 1978), which was performed on the R1 generation to determine heritability and phenotype stability of the *cp4 epsps* expression cassette in MON 89788. The Chi-square analysis is based on testing the observed to the expected trait segregation ratio according to Mendelian principles, and the Chi-square test was computed as:

$$\chi^2 = \Sigma \left[(| o - e | - 0.5)^2 / e \right]$$

where, o = observed frequency of the genotype, e = expected frequency of the genotype, and 0.5 = Yates correction factor for analysis with one degree of freedom (df).

The χ^2 value in the R1 generation indicated no significant differences between the observed and expected phenotypic ratio for MON 89788 as the Chi-square was less than the critical value of 3.84 at $p<0.05$ (Table V-3). Following the selection of the homozygous event, the subsequent generations were no longer segregating, and the expected and the observed segregation ratios are identical. The results of this analysis are consistent with the finding of a single chromosomal insertion of the *cp4 epsps* gene cassette that segregates according to Mendel's laws of genetics. These results are also consistent with the molecular characterization data indicating a single insertion site of the *cp4 epsps* cassette.

Table V-3. Glyphosate-Tolerant Trait Segregation Patterns of MON 89788

Generation	# of Plants; (% Germ.)[1]	Expected[2]		Observed[3]		Chi-square
		Positive	Negative	Positive	Negative	
R1	43	32.25	10.75	29	14	1.31[4]
R2	58	58	0	58	0	Fixed
R3	240; (80%)	192	0	192[5]	0	Fixed
R3	240; (85%)	204	0	204[5]	0	Fixed
R3	240; (85%)	204	0	204[5]	0	Fixed

[1] Percent germination based on visual estimation (plant stand, in 5% increments).
[2] Expected number of glyphosate-tolerant plants.
[3] Observed number of glyphosate-tolerant plants by ELISA and glyphosate application.
[4] Not significant at $p \leq 0.05$ (Chi-square = 3.84 at 1df)
[5] Number of plants (observed positives) was calculated based on #seed planted × percent germination

G. Conclusions of Molecular Characterization

Molecular analyses were performed to characterize the integrated DNA insert in MON 89788. Southern blot genomic analyses were used to determine the DNA insert number (number of integration sites within the soybean genome), copy number (the number of copies within one insert), the intactness of the *cp4 epsps* gene expression

cassette, and to establish the absence of plasmid backbone sequences in the plant. The stability of the DNA insert across multiple generations was also demonstrated by Southern blot fingerprint analysis. In addition, DNA sequencing analyses were performed to confirm the organization of the elements within the DNA insert.

Data show that one intact copy of the *cp4 epsps* expression cassette was integrated at a single chromosomal locus contained within a ~5.7 kb *Xmn* I/*Bpl* I restriction fragment. No additional elements from the transformation vector PV-GMGOX20, linked or unlinked to the intact DNA insert, were detected in the genome of MON 89788. Additionally, backbone sequence from PV-GMGOX20 was not detected. Generational stability analysis demonstrated that the expected Southern blot fingerprint of MON 89788 has been maintained across four generations of breeding, thereby confirming the stability of the DNA insert over multiple generations. These generations were also shown not to contain any detectable backbone sequence from plasmid PV-GMGOX20. In addition, DNA sequence analyses confirmed the organization of the genetic elements within the *cp4 epsps* expression cassette of MON 89788, which is identical to that in plasmid PV-GMGOX20 and is as depicted in the schematic of Figure V-1. Finally, heritability and stability of the glyphosate-tolerance phenotype were as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

VI. Characterization of the Introduced CP4 EPSPS Protein

This section summarizes the evaluation of the CP4 EPSPS protein produced in MON 89788 and establishes the equivalence between the plant-produced and *E. coli*-produced CP4 EPSPS proteins. As the *E. coli*-produced CP4 EPSPS has been used previously in a number of safety assessment studies, including the simulated gastric fluid (SGF) and acute mouse gavage, demonstration of protein equivalence between *E. coli*- and MON 89788-produced CP4 EPSPS proteins allows utilization of the existing data to confirm the safety of the CP4 EPSPS protein in MON 89788. Results indicate that the MON 89788-produced CP4 EPSPS protein is equivalent to the *E. coli*-produced protein, which is also equivalent to the CP4 EPSPS proteins produced in other Roundup Ready crops including Roundup Ready soybean. Data also support a conclusion of safe consumption based on several lines of evidence, all of which have been submitted to FDA as part of the pre-market consultation.

A. EPSPS Biochemistry and Mode of Action

The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; EC2.5.1.19) family of enzymes is ubiquitous to plants and microorganisms. EPSPS proteins have been isolated from both sources, and its properties have been extensively studied (Harrison et al., 1996; Haslam, E., 1993; Klee et al., 1987; Schonbrunn et al., 2001; Steinrüchen and Amrhein, 1984). The shikimate pathway and the EPSPS protein are absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). The bacterial and plant enzymes are mono-functional with molecular weight of 44-48 kDa (Kishore et al., 1988). EPSPS proteins catalyze the transfer of the enolpyruvyl group from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai and Stallings, 2001). Due to the specificity of EPSPS for its substrates, the only known catalytic product generated is EPSP, which is the penultimate product of the shikimic acid pathway. Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules. It has been estimated that aromatic molecules, all of which are derived from shikimic acid, represent 35% or more of the dry weight of a plant (Franz et al., 1997).

MON 89788 contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp.* strain CP4 (*cp4 epsps*). The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The CP4 EPSPS protein is structurally similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS (Padgette et al., 1996). In conventional plants, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of S3P, thereby depriving plants of essential amino acids (Steinrücken and Amrhein, 1980; Haslam, 1993). In Roundup Ready plants, which are tolerant to the Roundup family of agricultural herbicides, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the presence of glyphosate (Padgette et al., 1996). The CP4 EPSPS protein expressed in

MON 89788 is identical to the CP4 EPSPSs in other Roundup Ready crops including Roundup Ready soybean, Roundup Ready canola, Roundup Ready sugar beet, and Roundup Ready Flex cotton.

B. Characterization of the CP4 EPSPS Protein Produced in MON 89788

The CP4 EPSPS protein was purified from MON 89788 grain, and the biochemical characteristic of the protein was compared to that of the *E. coli*-produced reference standard. The analyses employed for characterization or establishment of the identity of MON 89788-produced CP4 EPSPS protein included: (1) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish equivalence of the apparent molecular weight between MON 89788- and *E. coli*-produced proteins, (2) immunoblot analysis to establish immunoreactivity equivalence between MON 89788- and *E. coli*-produced proteins using anti-CP4 EPSPS antibody, (3) N-terminal sequence analysis, (4) matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry to generate tryptic peptide map, (5) CP4 EPSPS enzymatic activity analysis to demonstrate functional equivalence between MON 89788- and *E. coli*-produced proteins, and (6) glycosylation analysis to establish equivalence of the glycosylation status between MON 89788- and *E. coli*-produced proteins. The conclusions of the characterization are summarized below. The materials and methods, and detailed results of the characterization can be found in Appendix C.

The CP4 EPSPS protein isolated from MON 89788 was purified and characterized, and results confirmed the equivalence between MON 89788- and *E. coli*-produced CP4 EPSPS proteins. The apparent molecular weight was estimated by SDS-PAGE. Since the MON 89788-derived CP4 EPSPS migrated comparably to the *E. coli*-produced protein on SDS-PAGE, the apparent molecular weight of these two proteins was determined to be equivalent. This result is consistent with the deduced amino acid sequence based on the DNA sequence analysis. On the basis of western blot analysis, the electrophoretic mobility and immunoreactive properties of the MON 89788-produced CP4 EPSPS protein were demonstrated to be comparable to those of the *E. coli*-produced CP4 EPSPS reference standard. The N-terminus of the CP4 EPSPS derived from MON 89788 was consistent with the predicted amino acid sequence translated from the *cp4 epsps* coding sequence, and the MALDI-TOF mass spectrometry analysis also yielded peptide masses consistent with the expected peptide masses from the translated *cp4 epsps* coding sequence. In addition, the MON 89788- and the *E. coli*-produced CP4 EPSPS reference standard were found to be equivalent based on functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 89788 and established its equivalence to the *E. coli*-produced CP4 EPSPS protein standard. Furthermore, since all CP4 EPSPS proteins isolated from other Roundup Ready crops have established equivalence to the *E. coli*-produced protein standard previously, by inference, the MON 89788-derived CP4 EPSPS protein is likely to possess equivalent biochemical and physiological characteristics with the CP4 EPSPSs expressed in other Roundup Ready crops, all of which have been deregulated by USDA-APHIS.

C. Safety Assessment Summary of the CP4 EPSPS Protein

The EPA has previously reviewed and established a tolerance exemption for CP4 EPSPS and the genetic material necessary for the production of this protein in or on all raw agricultural commodities (40 CFR §180.1174). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, the lack of homology to toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. Similar safety assessments were conducted on MON 89788 and the CP4 EPSPS protein it produced, and similar conclusion of safety was reached. The comprehensive food and feed safety and nutritional assessment of MON 89788 was submitted to the FDA, which included the following conclusions:

1. The donor organism, *Agrobacterium* sp. strain CP4, is not a known human or animal pathogenicity and is not know to induce allergenic responses in human. Additionally, the safety of the *Agrobacterium* sp. strain CP4 as the donor organism has been reviewed previously as a part of the safety assessment for other Roundup Ready crops.

2. A history of safe use of CP4 EPSPS protein has been demonstrated, based on the similarity of the CP4 EPSPS protein in MON 89788 to EPSPS proteins naturally present in food crops (e.g., soybean and corn) and in microbial food sources such as bakers yeast (*Saccharomyces cerevisiae*). The CP4 EPSPS protein is functionally equivalent to native plant EPSPS proteins except for the lack of affinity for glyphosate. In addition, there is experience of safe use of the CP4 EPSPS protein since the introduction of Roundup Ready crops in 1996, which include Roundup Ready soybean, Roundup Ready Flex cotton, and Roundup Ready Corn 2.

3. No biologically relevant structural similarities were observed between the CP4 EPSPS protein and allergens, toxins, and pharmacologically active proteins, which suggests that CP4 EPSPS is not likely to pose a human health concern. This conclusion is also supported by the rapid degradation of CP4 EPSPS protein in simulated digestive fluids.

4. The acute oral toxicity study demonstrated that the CP4 EPSPS protein did not cause any adverse effects in mice with a No Observable Effect Level (NOEL) of equal to or greater than 572 mg/kg.

5. The margin of exposures to CP4 EPSPS derived from consumption of MON 89788 were determined to be approximately 58,000 for the overall U.S. population, and 1,500 for non-nursing infants. These large margins of exposure indicate that there is no meaningful risk to human health from dietary exposure to food products derived from MON 89788.

MON 89788 produces the same CP4 EPSPS as that in other Roundup Ready crops including Roundup Ready soybean. The above studies reconfirm the safety of the CP4 EPSPS in MON 89788 and in other Roundup Ready crops previously deregulated, which also establish the food and feed safety of the CP4 EPSPS protein produced in MON 89788.

D. Levels of the CP4 EPSPS Protein in MON 89788

CP4 EPSPS protein levels in tissues derived from MON 89788 were determined by a validated enzyme-linked immunosorbent assay (ELISA). The levels of the CP4 EPSPS protein in over-season leaf (OSL), grain, root, and forage were determined in tissues collected from MON 89788 produced in replicated field trials across five U.S. field locations during 2005. CP4 EPSPS protein levels for all tissue types were calculated on a µg/g fresh weight (FW) basis. Moisture content was determined in each tissue type, and protein levels in these tissues were converted to a dry weight (DW) basis by calculation. Materials and methods are described in detail in Appendix D.

For MON 89788, the mean CP4 EPSPS protein levels across sites for OSL1, OSL2, OSL3, OSL4, grain, root, and forage were 300, 340, 330, 290, 150, 74, and 220 µg/g DW, respectively (Table VI-1). The levels of the CP4 EPSPS protein from the conventional control (A3244) were less than the assay limits of detection (LOD) in all tissue types. The mean CP4 EPSPS expression level in grain from MON 89788 is lower than that from Roundup Ready soybean (Padgette et. al., 1995).

Table VI-1. Summary of CP4 EPSPS Protein Levels in Tissue Collected from MON 89788 Produced in the U.S. During 2005

Tissue Type	CP4 EPSPS µg/g FW (SD)[1]	Range[2] (µg/g FW)	CP4 EPSPS µg/g DW (SD)[3]	Range (µg/g DW)	LOQ / LOD (µg/g FW)
OSL1[4]	54 (7.8)	40 – 66	300 (51)	220 – 380	0.57 / 0.26
OSL2[4]	60 (10)	42 – 80	340 (55)	250 – 440	0.57 / 0.26
OSL3[4]	58 (11)	40 – 79	330 (94)	200 – 520	0.57 / 0.26
OSL4[4]	75 (17)	60 – 110	290 (48)	210 – 390	0.57 / 0.26
Grain	140 (20)	98 – 170	150 (22)	110 – 180	0.34 / 0.26
Root	22 (6.0)	13 – 38	74 (27)	41 – 150	0.57 / 0.11
Forage	59 (14)	41 – 94	220 (51)	140 – 330	0.57 / 0.10

1. Protein quantities are expressed as mean µg of CP4 EPSPS/g tissue on a fresh weight (FW) basis. The mean and standard deviation (SD) were calculated across all sites.
2. Minimum and maximum values across all sites.
3. Protein quantities are expressed as mean µg of CP4 EPSPS/g tissue on a dry weight (DW) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.
4. OSL1 to OSL4 represent over-season leaves collected at the following developmental stages: OSL1: V3-V4 growth stage; OSL2: V6-V8 growth stage; OSL3: V10-V12 growth stage; OSL4: V14-V16 growth stage.

Note: Sample number is 14 for forage, and 15 each for OSL1 to OSL4, grain, and root.

VII. Compositional and Nutritional Assessments of MON 89788

Compositional analyses were conducted to assess whether the nutrient and anti-nutrient levels in grain and forage tissues derived from MON 89788 are comparable to those in the conventional soybean variety, A3244, which has background genetics similar to MON 89788 but does not contain the *cp4 epsps* gene cassette. Additional conventional soybean varieties currently in the marketplace were also included in the analysis to establish a range of natural variability for each analyte, where the range of variability is defined by a 99% tolerance interval for that particular analyte. Results of the comparisons indicate that MON 89788 is compositionally and nutritionally equivalent to conventional soybean varieties currently in commerce.

Grain and forage tissues of MON 89788 and A3244 were harvested from soybeans grown in three replicated plots at each of five field sites across the U.S. during 2005. The field sites were located in regions that were conducive to the growth of soybean maturity group III varieties, and were representative of commercial soybean production. In addition, 12 conventional soybean varieties were also included as references where three varieties were grown at each of two sites and two varieties were grown at each of three sites for a total of 12 references. The 12 conventional soybean reference varieties were included to provide data for the development of a 99% tolerance interval for each component analyzed. For each compositional component, 99% tolerance interval was calculated. This interval is expected to contain, with 95% confidence, 99% of the values obtained from the population of commercial references. It is important to establish the 99% tolerance interval from representative conventional soybean varieties for each of the analytes, because such data illustrate the compositional variability naturally occurring in commercially grown varieties. By comparison to the 99% tolerance interval, any statistically significant differences between MON 89788 and the control (A3244) may be put into perspective, and can be assessed for biological relevance in the context of the natural variability in soybean. Additional information on the field design and reference varieties is presented in Appendix E.

A total of 63 components were analyzed in grain and forage samples. Components for forage samples included proximates (protein, fat, ash, and moisture), acid detergent fiber (ADF), neutral detergent fiber (NDF), and carbohydrates by calculation. Components for grain samples included proximates (protein, fat, ash, and moisture), ADF, NDF, amino acids, fatty acids (C8-C22), phytic acid, trypsin inhibitor, isoflavones, lectins, raffinose, stachyose, Vitamin E, and carbohydrates by calculation. The methods employed for these analyses are presented in Appendix E.

Statistical analyses of the compositional data were conducted using a mixed model analysis of variance with data from each of five sites, and a combination of all five field sites. Each individual analyte for MON 89788 was compared to that of the conventional control, A3244, for each of the five sites and for the combination of all five sites (i.e., the combined-site). The statistical significance is defined at the level of $p<0.05$. Of the 63 components analyzed, 14 minor fatty acids had greater than 50% of the analytical values that were below the limit of quantitation. These fatty acids are known to occur at low or

non-detectable levels in soybean oil (Codex Standard, 2005), and were not included in the statistical analyses.

Statistical analyses of the remaining 49 components (63 minus the 14) between MON 89788 and A3244 were conducted. The overall data set was examined for evidence of biologically relevant changes. Based on this evaluation and the results of statistical analyses, analytes for which the levels were not statistically different were deemed to be present at equivalent levels between MON 89788 and A3244. Analyses using data from the combination of all five sites (combined-site) indicated that there were no statistical differences in the levels of 92% of the analytes (45 of the 49). Statistical analyses for the combined-site data are presented in Appendix E, Table E-1 for forage and Table E-2 for grain. Analyses using the five single-site analyses indicated that there were no statistically significant differences in the levels of 91% of the analytes (223 of the 245) between MON 89788 and A3244.

For the combined-site analyses, statistical differences between MON 89788 and A3244 were observed for four analytes, which included forage moisture, and grain daidzein, glycitein, and Vitamin E (Table VII-1). The differences observed are generally small (1.6 – 11%), and the mean levels of MON 89788 are well within the 99% tolerance intervals for the conventional soybeans. The mean levels of MON 89788 grain daidzein, glycitein, and Vitamin E are also well within the ranges for conventional soybeans reported in the International Life Science Institute Crop Composition Database (ILSI-CCD; ILSI, 2004) as well as in the literature (Appendix E, Table E-3 for forage and E-4 for grain). The mean levels of forage moisture for both MON 89788 and A3244 are below that of the ILSI-CCD and literature ranges; however, the difference between MON 89788 and A3244 is only 1.6%. Therefore, it was concluded that MON 89788 and A3244 are compositionally and nutritionally equivalent based on analyses of the combined-site data.

The reproducibility and trends across sites were also examined, and comparisons to conventional soybean varieties using the 99% tolerance intervals were made. There were no analytes that were consistently and statistically different across sites. Statistically significant differences were observed in as many as two sites for only one analyte, raffinose. Since the differences observed were lower for MON 89788 at one site (AR) while higher at the other (IL-2), and there is no evidence of any trend across sites, it is concluded that the statistical differences are not biologically relevant.

For the remaining 16 analytes where statistically significant differences were observed in only one site, the differences between MON 89788 and A3244 were not reproducible across sites, and no consistent trends were observed. In addition, all mean levels of MON 89788 analytes were well within the 99% tolerance interval for conventional soybeans that were grown concurrently in all sites. It is concluded that these analytes where the statistical differences were observed in only site were not biologically different between MON 89788 and A3244.

Based on the data and information presented above, it was concluded that soybean grain and forage derived from MON 89788 are compositionally and nutritionally equivalent to those of the conventional soybeans. The few statistical differences between MON 89788 and A3244 are likely to reflect the natural variability of the components since the mean levels of analytes for MON 89788 are well within the 99% tolerance intervals for conventional soybeans.

Table VII-1. Summary of Statistical Differences between Component Levels of MON 89788, A3244 and Conventional Varieties

Analytical Component (Units)[1]	MON 89788 Mean	A3244 Mean	Difference (MON 89788 minus A3244) % of A3244	p-Value	MON 89788 (Range)	Conventional Tol. Int.[2]
Statistical Differences Observed in Combined-Site Analyses						
Forage Moisture (% FW)	72.07	73.21	-1.55	0.006	[67.90 - 77.60]	[60.84, 83.36]
Daidzein (ug/g DW)	993.67	1073.57	-7.44	0.021	[631.32 - 1571.41]	[0, 1925.63]
Glycitein (ug/g DW)	91.77	102.61	-10.56	0.037	[53.78 - 162.52]	[0, 287.45]
Vitamin E (mg/100g DW)	2.71	2.52	7.41	0.015	[1.88 - 3.72]	[0, 7.00]
Statistical Differences Observed in More Than One Site and Not in the Combined-Site						
Site AR Raffinose (% DW)	0.65	0.81	-20.02	0.024	[0.58 - 0.71]	[0, 1.01]
Site IL-2 Raffinose (% DW)	0.42	0.33	25.45	0.035	[0.40 - 0.43]	[0, 1.01]
Statistical Differences Observed in One Site and Not in the Combined-Site						
Site AR Phenylalanine (% DW)	2.00	2.01	-0.41	0.014	[2.00 - 2.01]	[1.70, 2.45]
Site AR Palmitic (% DW)	2.21	2.40	-7.73	0.004	[2.17 - 2.25]	[1.32, 2.64]
Site AR Stearic (% DW)	0.76	0.81	-5.43	0.024	[0.75 - 0.77]	[0.37, 1.28]
Site AR Oleic (% DW)	3.30	3.68	-10.31	0.001	[3.24 - 3.36]	[2.06, 6.43]
Site AR Linoleic (% DW)	10.27	11.02	-6.86	0.005	[10.06 - 10.42]	[7.75, 11.22]
Site AR Linolenic (% DW)	1.45	1.55	-6.16	0.029	[1.41 - 1.48]	[0.84, 1.69]

Table VII-1 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 89788 vs. A3244 and Conventional Reference Varieties

Analytical Component (Units)[1]	MON 89788 Mean	A3244 Mean	Difference (MON 89788 minus A3244) % of A3244	p-Value	MON 89788 (Range)	Conventional Tol. Int.[2]
Statistical Differences Observed in One Site and Not in the Combined-Site						
Site AR Arachidic (% DW)	0.060	0.064	-6.35	0.021	[0.058 - 0.060]	[0.031, 0.094]
Site AR Eicosenoic (% DW)	0.048	0.053	-8.60	0.032	[0.047 - 0.049]	[0.021, 0.065]
Site AR Behenic (% DW)	0.066	0.070	-5.85	0.034	[0.064 - 0.068]	[0.034, 0.091]
Site AR ADF (% DW)	21.17	16.10	31.47	0.003	[19.28 - 23.94]	[9.62, 28.57]
Site AR Carbohydrates (% DW)	38.13	36.02	5.88	0.048	[37.77 - 38.42]	[27.86, 45.79]
Site AR Fat (% DW)	18.82	20.41	-7.79	0.002	[18.42 - 19.17]	[15.38, 21.95]
Site AR Stachyose (% DW)	2.32	2.83	-18.13	0.010	[2.10 - 2.50]	[1.19, 3.31]
Site IL-2 Genistein (ug/g DW)	762.46	849.88	-10.29	0.032	[721.05 - 797.84]	[0, 1387.95]
Site IL-2 Grain Moisture (% FW)	8.53	7.48	14.04	0.045	[8.19 - 9.13]	[4.64, 9.94]
Site NE Grain NDF (% DW)	17.42	19.91	-12.51	0.023	[16.79 - 18.39]	[13.26, 26.33]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

VIII. Phenotypic and Ecological Assessments of MON 89788

This section provides an evaluation of the phenotypic and agronomic characteristics (including plant-symbiont associations), and the environmental interactions of MON 89788 compared to the control, A3244. The A3244 is a conventional soybean variety that has background genetics similar to MON 89788 but does not contain the *cp4 epsps* gene cassette. These data support a determination that MON 89788 is no more likely to pose a plant pest risk or to have an increased environmental impact compared to conventional soybean. The conclusions are based on the results of the multiple studies reported herein.

The evaluation of the phenotypic and agronomic characteristics, and ecological interactions was conducted to assess potential differences between MON 89788 and A3244 in the context of ecological risk. The results were also considered relative to the data generated on commercial reference varieties. The phenotypic, agronomic, and environmental interaction evaluations are based on replicated laboratory, greenhouse, and/or multi-site field trials and experiments. In evaluating the phenotypic and agronomic characteristics of MON 89788, data were collected that address specific ecological risks regarding pest potential based on the considerations of USDA-APHIS. The characterization encompass six general data categories: 1) germination, dormancy and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant; 5) plant-symbiont associations; and 6) plant interactions with insect, disease and abiotic stressors.

A. Interpretation of Phenotypic and Ecological Interaction Data

Familiarity is a useful approach to evaluate the potential environmental impact of a genetically-modified plant. The concept of familiarity is based on the fact that the genetically-modified 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 genetically-modified plant and its conventional counterpart. Phenotypic, agronomic and ecological interactions assessment can be used to support familiarity of the genetically-modified plant to the conventional counterpart, and a subset of the data (e.g., certain dormancy or pre-harvest seed loss characteristics) can be used for an assessment of enhanced weed potential. Based on the collection of all data, an assessment can be made whether a plant is likely to pose an increased plant pest potential or to have an increased environmental impact compared to conventional soybean.

During the processes of data collection, summarization and analysis, experienced scientists familiar with each experimental design and evaluation criteria were involved in all steps. This oversight ensured that the evaluation system was functioning appropriately, measurements were taken properly, and data were consistent with expectations based on experience with the crop. In addition, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an

unexpected plant response. These scientists did not indicate any unexpected observations or issues in the course of the studies. Data were then submitted to statistical analysis.

Characteristics for which no significant differences are detected support familiarity of MON 89788 to conventional soybean as it relates to ecological risk assessment. Detected differences are considered in the context of whether they are reproducible and whether they are adverse in terms of potential environmental impact. On the basis of these data, one can assess the probability of increased pest potential of MON 89788, and whether the phenotypic, agronomic, or environmental interactions characteristics of the plant have been adversely changed beyond the intended introduced trait.

A tiered approach is used to assess whether a detected difference is, or is not, of biological or ecological concern. When no statistically significant differences in phenotypic characteristics are detected between the genetically-modified crop and an appropriate control, a conclusion of no contribution to pest potential can be made. A detected difference would be interpreted in the context of reproducibility and pest potential (i.e., whether or not the difference increased pest potential and in particular weed potential of the genetically-modified crop) as described in Figure VIII-1. During the assessment, a "no" answer at any step indicates that there is not a biological or ecological concern for the crop in terms of pest potential and subsequent steps are not considered.

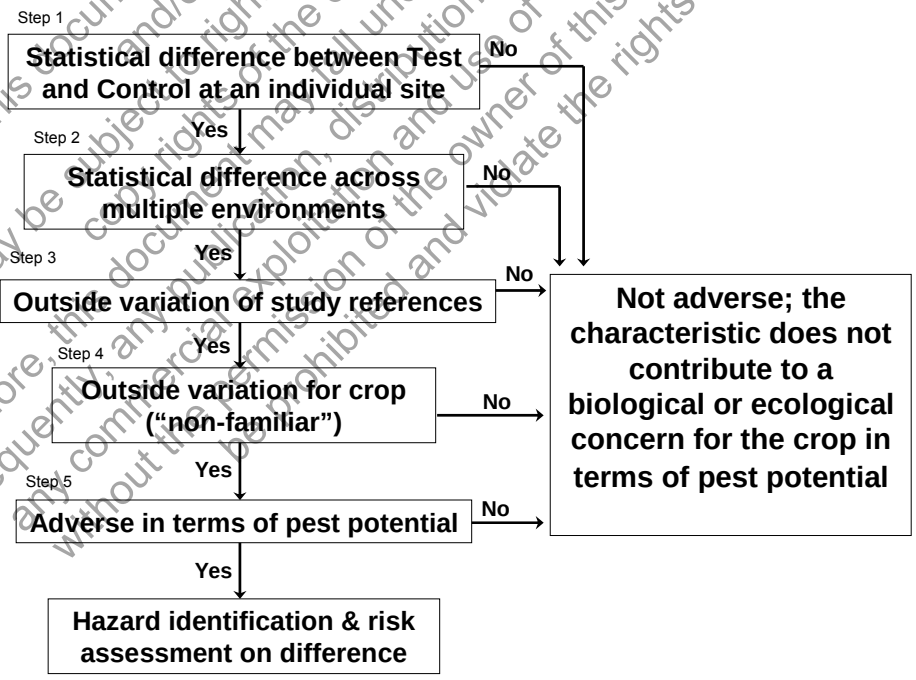


Figure VIII-1. Schematic Diagram of Data Interpretation Methods

- Steps 1-2. A significant difference between the test and control for a characteristic at an individual site is assessed in the context of whether or not a difference is detected across multiple environments.

- Step 3. If a significant difference is detected when the data are pooled across multiple environments, the test mean value is assessed relative to the range of the commercially available reference varieties.

- Step 4. If the mean of the test material is outside the range of the commercially available references, the test material value is considered in the context of known values common to the crop.

- Step 5. If the mean of the test material is outside the range of values common to the crop, the test material is considered "non-familiar" for that characteristic. The detected difference is then assessed for whether or not it is adverse in terms of pest and weed potential.

- If an adverse effect (hazard) is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced pest potential of the crop itself, the impact of significant differences detected in other measured characteristics, and potential for, and effects of, trait transfer to a sexually compatible species. Higher tier experimentation could be conducted to further elucidate any potential adverse effects identified.

B. Phenotypic, Agronomic and Ecological Interactions Characteristics

As a significant part of the evaluation of MON 89788, plant phenotypic and agronomic characteristics including seed dormancy and germination, phenotypic, agronomic and ecological interactions, pollen characteristics, and symbiont interactions were evaluated.

B.1. Seed Dormancy and Germination Characteristic

Seed dormancy (e.g. hard seed) is a survival mechanism for plants and is an important characteristic that is often associated with plants that are weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003). Dormancy mechanisms, including hard seed, vary with species and tend to involve complex processes. Standardized germination assays of the Association of Official Seed Analysts (AOSA, 2002) are used as a baseline to measure the germination potential of soybean seed, where they are evaluated for various germination parameters at the optimum temperature of growth (20/30°C) (Table VIII-1). In addition, five other temperature regimes of 10, 20, 30, 10/20, and 10/30°C were used to assess other seed germination properties. The temperature regimes and types of observation are listed in Table VIII-1. For the alternating temperature regimes of 10/20, 10/30, or 20/30°C, the lower temperature was maintained for 16 hours, and the higher temperature for eight hours.

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 89788 and A3244, where A3244 served as a comparable control with background genetics similar to MON 89788 but didn't contain the *cp4 epsps* gene cassette. In addition, 12 commercially available soybean varieties were included as references to provide baseline values common to soybeans. The seed lots for MON 89788, A3244 and references were produced during 2005 at Arkansas, Illinois, and Ohio, which represented environmentally relevant conditions for soybean production. The experimental methods and individual site data of these comparisons are presented in Appendix F.

A total of 25 comparisons were made between MON 89788 and A3244 seed germination parameters across three seed production sites (Table VIII-2). No statistically significant differences were detected between MON 89788 and A3244 for percent germinated, viable hard, dead, or viable firm swollen seed in the 10, 20, 30, 10/20, and 10/30° C temperature regimes. In addition, no statistically significant differences were detected between MON 89788 and the control for percent normal germinated, abnormal germinated, viable hard, or viable firm swollen seed in the AOSA temperature regime (20/30° C). Under the same temperature regime, one statistical difference was detected between MON 89788 and A3244, where percent dead seed was lower for MON 89788 compared to A3244 (5.7 vs. 10.1%). The mean value of percent dead seed for MON 89788 was within the reference range and 99% tolerance interval of the reference seed. In addition, this difference was not detected in any of the five additional temperature regimes. Therefore, this single difference detected in only one temperature regime is not likely to have biological relevance in terms of increased weed potential. No viable hard (dormant) seed were observed for MON 89788 or A3244 from any site in any temperature regime. The results support a conclusion that there is no increased weed potential of MON 89788 compared to the conventional soybeans based on the germination and dormancy parameters assessed.

Table VIII-1. Seed Dormancy and Germination Parameters Evaluated

Category and Characteristic	Evaluation Regime (Temperature °C†)	Evaluation Description
Normal-Germinated	20/30	Seedlings that exhibited normal developmental characteristics and possessed both a root and a shoot.
Abnormal-Germinated	20/30	Germinated, but insufficient root and shoot development, lacked a shoot, shoot with deep cracks or lesions, or exhibited mechanical damage.
Total Germinated	10, 20, 30, 10/20, 10/30	Seedlings that had germinated.
Dead	10, 20, 30, 10/20, 10/30, 20/30	Seeds that had visibly deteriorated and had become soft to the touch.
Viable Hard	10, 20, 30, 10/20, 10/30, 20/30	Seeds that did not imbibe water and remained hard to the touch.
Viable Firm Swollen	10, 20, 30, 10/20, 10/30, 20/30	Seeds that had visibly swollen (imbibed water) and were firm to the touch but lacked any evidence of growth.

†Constant temperature maintained at ~10, 20, or 30°C. In alternating temperatures of ~10/20, 10/30, or 20/30°C, the lower temperature was maintained for 16 hours and the higher temperature for eight hours.

Table VIII-2. Germination Characteristics of MON 89788 and A3244

Temperature Regime	Germination Category[1]	Mean % (SE)[2]		References			
				Range[3]		99% Tol. Interval[4]	
		MON 89788	A3244	Min	Max	LL	UL
10°C	Total Germinated	94.1 (2.2)	94.5 (2.0)	49.3	99.3	12.0	100.0
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0	0.3	0.0	0.3
	Dead	5.7 (2.1)	5.2 (1.9)	0.8	49.5	0.0	87.0
	Viable Firm Swollen	0.3 (0.2)	0.3 (0.3)	0.0	1.3	0.0	2.2
20°C	Total Germinated	92.3 (2.8)	90.6 (3.3)	44.5	99.3	6.0	100.0
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0	0.3	0.0	0.3
	Dead	7.8 (2.8)	9.3 (3.3)	0.8	55.5	0.0	94.1
	Viable Firm Swollen	0.0 (nv)	0.1 (0.1)	0.0	0.0	nv	nv
30°C	Total Germinated	94.4 (1.6)	93.9 (1.7)	57.0	98.5	22.3	100.0
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0	0.0	nv	nv
	Dead	5.6 (1.6)	6.1 (1.7)	1.5	43.0	0.0	77.7
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0	0.0	nv	nv

Table VIII-2 (continued). Germination Characteristics of MON 89788 and A3244

Temperature Regime	Germination Category[1]	Mean % (SE)[2]		References			
				Range[3]		99% Tol.	
		MON 89788	A3244	Min.	Max	LL	UL
10/20°C	Total Germinated	94.5 (2.2)	94.2 (2.0)	46.3	99.0	13.0	100.0
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0	0.0	nv	nv
	Dead	5.4 (2.2)	5.8 (2.0)	1.0	53.3	0.0	86.7
	Viable Firm Swollen	0.1 (0.1)	0.0 (nv)	0.0	0.3	0.0	0.4
10/30°C	Total Germinated	94.1 (2.2)	93.9 (2.4)	50.3	99.5	15.7	100.0
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0	0.0	nv	nv
	Dead	5.9 (2.2)	6.1 (2.4)	0.5	49.5	0.0	84.1
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0	0.3	0.0	0.3
20/30°C (AOSA)	Normal Germinated	78.4 (6.5)	73.2 (6.1)	12.5	94.5	0.0	100.0
	Abnormal Germinated	15.9 (4.3)	16.5 (3.5)	4.5	36.5	0.0	68.9
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0	0.0	nv	nv
	Dead	5.7 (2.7)*	10.1 (2.8)	0.8	55.8	0.0	100.0
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0	0.3	0.0	0.3

***** Indicates a statistically significant difference between the MON 89788 and A3244 at $p \leq 0.05$.
[1] Germinated seed in the AOSA temperature regime were categorized as either normal-germinated or abnormal-germinated seed.
[2] SE = standard error.
[3] Minimum and maximum mean values from twelve commercial soybean varieties.
[4] 99% tolerance interval with 95% confidence. LL = lower limit; UL = upper limit.
Note: nv = no variability in the data.

B.2. Field Phenotypic, Agronomic Characteristics and Ecological Interactions

Plant growth, development and yield characteristics were assessed under field condition to identify any unintended phenotypic effects or ecological interactions in MON 89788 relative to the conventional control, A3244, and commercially available soybean. The purpose of these field evaluations was to assess whether the introduction of the glyphosate-tolerant trait altered the phenotypic and agronomic characteristics or the plant-insect, plant-disease, or plant-abiotic stressor interactions of MON 89788 compared to the control. Certain growth, reproduction, and pre-harvest seed loss characteristics (such as lodging and pod shattering) can be used for an assessment of enhanced weed potential of MON 89788.

Field trials were conducted at 17 locations during 2005 to thoroughly evaluate phenotypic, agronomic and ecological interaction characteristics. These 17 locations provided a diverse range of environmental and agronomic conditions representative of the majority of commercial soybean production regions in the U.S., including regions where MON 89788 would be anticipated to be produced (Table VIII-3). A randomized complete block design with three replications was employed for the comparisons and analyses. Glyphosate herbicide was not applied to the experimental plots. The categories of phenotypic characteristics and ecological interactions evaluated are listed in Table VIII-4. Plant growth stage was assessed several times during the growing season. Observational data on the presence of stressors and any differential responses to biotic (pests and disease) and abiotic stressors were collected. In addition to the qualitative data collected at the 17 sites, insects were collected, identified, and quantified, and insect-specific damage was rated at three of the 17 sites. The methods and detailed results of these individual site data comparisons are presented and discussed in Appendix G, while the across-site analyses are summarized below.

B.2.1. Field Phenotypic and Agronomic Characteristics

A total of 11 different phenotypic characteristics were evaluated. For the across-site analyses, no significant differences were detected between MON 89788 and A3244 for early stand count, seedling vigor, days to 50% flowering, flower color, lodging, pod shattering, final stand count, seed moisture, seed test weight, or yield (Table VIII-5). The only significant difference detected in the across-site analyses was the reduced plant height for MON 89788 compared to A3244 (30.6 vs. 32.3 inches; Table VIII-5). Although plant height for MON 89788 was reduced compared to the control, the mean value observed for MON 89788 falls well within the range of values observed for the commercial soybean varieties. Furthermore, the magnitude of the difference in plant height is small (approximately 5%), and decreased plant height is unlikely to contribute to increased weed potential.

For the growth stage comparisons, there was no qualitative difference observed between MON 89788 and A3244 in 113 out of 114 observations (Appendix G, Table G-4). The single exception was during the third observation at the IA2 site, where MON 89788 was

evaluated as more mature than the control (R3 vs. R2, respectively). This single difference in growth stage was not observed in any of the other sites during the same period, and was also not observed during any other developmental stage. In addition, the growth stage of MON 89788 was within the range observed for the reference soybean varieties. This single observation of growth stage difference was not reproducible across sites and is not likely to be biologically meaningful in terms of increased pest potential of MON 89788 compared to A3244. The results of the phenotypic and agronomic analyses support familiarity and the conclusion of no increased pest potential of MON 89788 compared to A3244.

B.2.2. Ecological Interaction Analyses

Data on the susceptibility to insect pest, disease and response to abiotic stressor were also collected from each of the 17 field sites during the season. The purpose of these evaluations was to assess whether plant-disease or plant-insect interactions, or plant response to abiotic stressors of MON 89788 were altered compared to the A3244 control.

The reported severity of specific insect, disease, and abiotic stressor symptoms represents the range of ratings observed across the three replications at each site (Appendix G, Tables G-5, G-6, G-7, respectively). MON 89788 and A3244 were considered qualitatively different if the ratings across all three replications for a specific ecological stressor of MON 89788 did not overlap with that of A3244 (e.g., none vs. moderate to severe). The ratings observed among the commercial reference varieties provide qualitative assessment data common to soybeans for each parameter assessed.

Across all sites, a total of 12 insect categories (species or group), 18 disease categories (species or group), and 10 abiotic stressors were evaluated. Of the 216 disease and 224 responses to abiotic stressor observations, no qualitative differences were observed in MON 89788 compared to A3244 (Appendix G, Tables G-6 and G-7). Of the 221 insect stressor observations, a single qualitative difference was observed. The severity of symptoms caused by leafhopper was lower in MON 89788 plots than the control plots at the MO1 site at the fourth observation time (none vs. slight; Appendix G, Table G-5). This qualitative difference was not considered biologically meaningful since leafhopper symptoms were not different between MON 89788 and A3244 at other sites or at other observation periods at MO1. In addition, the symptoms on MON 89788 fell within the range observed in the reference soybean varieties. Leafhopper resistance is not a known characteristic attributed to the glyphosate-tolerant trait, nor had it been observed in Roundup Ready soybean to date.

Specific pest and beneficial insects plus spiders were collected and quantified at the IL1, MO1, and MO2 sites (Appendix G, Table G-8). The insects and spiders were collected three times during the season from each replicate plot using a beat sheet sampling method. The number of insect collected was low for most species. No statistical differences were detected in insect abundance on MON 89788 compared to the control for 63 out of 66 comparisons. Abundance on MON 89788 was higher compared to A3244 for corn earworm during the second collection at IL1 (0.7 vs. 0.0), southern corn

rootworm during the second collection at MO1 (2.0 vs. 0.0), and tarnished plant bug during the third collection at MO2 (0.3 vs. 0.0). No significant differences were detected between MON 89788 and A3244 at other sites or during other collections for southern corn rootworm and tarnished plant bug. Corn earworm was not observed at the other two sites or during the other collections at the IL1 site. Since no reproducible differences were observed across sites or across collections, it was concluded that there were no quantitative differences for pests, beneficial insects, and spiders between MON 89788 and A3244.

Quantitative assessments of plant damage caused by defoliation or fluid feeding due to specific insects at the IL1, MO1, and MO2 are presented in Appendix G, Table G-9. Overall, plant damage was low with only 6 of the 48 comparative assessments having damage ratings exceeding a mean value of "1" (represents 10% damage or defoliation). No significant differences were detected in MON 89788 compared to A3244 in any of the 48 comparisons. The results of the insect abundance and plant damage assessments indicate that plant-insect interactions of MON 89788 were not altered compared to A3244.

Table VIII-3. Field Phenotypic Evaluation Sites for MON 89788 during 2005

Location	Location Code	USDA-APHIS Notification Number
Jackson Co., Arkansas	AR	05-070-01n
Jefferson Co., Iowa	IA1	05-049-14n
Benton Co., Iowa	IA2	05-049-14n
Clinton Co., Illinois	IL1	05-049-14n
Stark Co., Illinois	IL2	05-049-14n
Warren Co., Illinois	IL3	05-049-14n
Clinton Co., Illinois	IL4	05-070-01n
Warren Co., Illinois	IL5	05-070-01n
Hendricks Co., Indiana	IN1	05-066-01n
Boone Co., Indiana	IN2	05-066-01n
Pawnee Co., Kansas	KS	05-049-14n
Shelby Co., Missouri	MO1	05-066-01n
Lincoln Co., Missouri	MO2	05-066-01n
York Co., Nebraska	NE	05-049-14n
York Co., Nebraska	NE2	05-070-01n
Fayette Co., Ohio	OH	05-067-01n
Fayette Co., Ohio	OH2	05-070-01n

Table VIII-4. Phenotypic Characteristics Evaluated in U.S. Field Trials during 2005

Category and Characteristic	Evaluation Timing	Evaluation Description
Plant Growth and Development		
Early stand count	Seedling	Number of emerged plants in rows 2 and 3 of each plot
Seedling vigor	Seedling	Rated on a 1 - 9 scale, where 1 - 3 = excellent, 4 - 6 = average, and 7 - 9 = poor vigor
Days to 50% flowering	Flowering	Days from planting until approx. 50% of the plants in each plot are flowering
Flower color	Flowering	Color of flowers: purple, white, or mixed
Plant height	Maturity	Distance from the soil surface to the uppermost node on the main stem of five representative plants per plot
Lodging	Maturity	Rated on a 0 - 9 scale, where 0 = completely up and 9 = completely down
Pod shattering	Maturity	Rated on a 0 - 9 scale, where 0 = no shattering and 9 = completely shattered
Final stand count	Maturity	Number of plants in rows 2 and 3 of each plot
Seed moisture	Harvest	Percent moisture content of harvested grain
Seed test weight	Harvest	Mass of 100 harvested seed (g / 100 seed)
Yield	Harvest	Bushels of harvested grain produced per acre, adjusted to 13% moisture
Growth stage monitoring	Recurring	Average growth stage, using guidelines outlined in Soybean Growth and Development (ISU, 2004), recorded every 2-3 weeks from approx. V2 until R8

Table VIII-4 (continued). Phenotypic Characteristics Evaluated in U.S. Field Trials during 2005

Category and Characteristic	Evaluation Timing	Evaluation Description
Ecological Interactions		
Insect, disease, abiotic stressors	Recurring	Qualitative assessment of specific stressors rated on a 0 - 9 rating scale, where 0 = no stressor symptoms and 9 = most severe stressor symptoms, recorded every 4 weeks beginning at approx. V2-V4
Insect damage	Recurring	Quantitative assessment of damage by specific insects rated on a 0 - 9 rating scale, where 0 = no damage and 9 = 90% defoliation, recorded every 4 weeks beginning at approx. V2 at the IL1, MO1, and MO2 sites
Insect abundance	Recurring	Quantitative assessment of insects collected every 4 weeks beginning at 4 weeks after plants reached approx. V2 stage at the IL1, MO1, and MO2 sites

Table VIII-5. Plant Growth and Development Data Across 17 Locations during 2005

			References			
			Range[1]		Tolerance Interval[2]	
Phenotypic Characteristics (units)	**MON 89788**	**A3244**	**Min.**	**Max.**	**LL**	**UL**
Early stand count (# plants/2 rows)	291	299	193	360	115	419
Seedling vigor rating	2.5	2.4	1.7	5.0	0.0	5.9
Days to 50% flowering	44	45	33	50	27	60
Flower color[3]	Purple	Purple	—	—	—	—
Plant height (in)	30.6*	32.3	19.2	42.6	9.1	52.9
Lodging rating	0.5	0.6	0.0	5.2	0.0	5.4
Pod shattering rating[3]	0.0	0.0	0.0	0.2	0.0	0.3
Final stand count (# plants/2 rows)	266	270	178	297	119	359
Seed moisture (%)	11.5	11.7	8.8	15.1	6.6	17.3
Seed test weight (g/100 seed)	15.0	15.2	13.5	17.4	11.2	19.2
Yield (bu/ac)	48.4	50.0	15.9	65.2	2.5	90.1

***** Indicates a statistically significant difference between MON 89788 and A3244 at $p \leq 0.05$.
[1] Reference range = minimum and maximum mean values observed among the references.
[2] 99% tolerance interval with 95% confidence.
[3] Not statistically analyzed due to lack of variation.

B.3. Pollen Characteristics

The purpose of this study was to assess whether the introduction of the glyphosate-tolerant trait altered pollen morphology or pollen viability characteristics of MON 89788 compared to the control, A3244. Soybean flower samples were collected from three replications of MON 89788, A3244, and four commercial soybean varieties grown in Missouri under randomized complete block design. The plants were not treated with glyphosate. Flowers from five plants of each plot were collected, pollen was removed and stained with Alexander's stain (Alexander, 1980). Pollen viability, grain diameter, and general pollen morphology were evaluated for MON 89788, A3244, and reference soybean varieties. The mean and weighted mean of MON 89788 were compared to that of A3244 for pollen grain diameter and percent viable pollen, respectively.

No statistically significant differences were detected at $p \leq 0.05$ between MON 89788 and A3244 for average pollen grain diameter and percent viable pollen (Table VII-6). No visual differences between MON 89788 and the control material were observed in general pollen morphology. The lack of differences between pollen collected from MON 89788 compared to the conventional control for the assessed characteristics support familiarity of MON 89788 to A3244.

Table VIII-6. Pollen Grain Diameter and Viability Analyses

Pollen Characteristic	MON 89788 Mean (SE)[1]	A3244 Mean (SE)[1]	Reference Range[2] Min.-Max. (SE)
Average Diameter (µm)	23.7 (0.3)	23.1 (0.3)	21.6 - 23.4 (0.3)
Viability (%)[3]	82.0 (2.4)	75.3 (2.4)	56.4 - 80.1 (2.4)

[1] Mean or Least Square Mean and associated standard error for each characteristic.
[2] Reference range is the minimum and maximum mean value observed among the four reference varieties.
[3] Weighted Least Square Means are reported for viability.

B.4. Symbiont Interactions

Members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae* form a highly complex and specific symbiotic relationship with leguminous plants (Gage, 2004). The *Rhizobium*-legume 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, can then be utilized by the plant. In soybean, atmospheric nitrogen is fixed into organic nitrogen through a symbiotic association with the bacterium *Bradyrhizobium japonicum.*

The purpose of this study was to assess whether the introduction of the glyphosate-tolerant trait altered the symbiotic association between *Bradyrhizobium japonicum* and MON 89788 compared to conventional soybean. MON 89788, A3244 control, and reference plants were produced from seed germinated in an environmental chamber. Germinated seedlings were then planted in pots containing nitrogen-free potting medium and grown in a greenhouse. Seedlings were inoculated with a solution containing *B. japonicum* at planting, and then re-inoculated after plants emerged from the potting medium. The pots were arranged in a randomized split-block design with eight replications. Four and six weeks after emergence, eight plants per group were excised at the surface of the potting medium, and shoot and root plus nodule material were removed from the pots. Nodules were then separated from roots prior to enumeration and determination of dry weight. MON 89788 was compared to A3244 for the following parameters at each of the two sampling periods: nodule number, nodule dry weight, shoot dry weight, root dry weight, and shoot total nitrogen.

No significant differences were detected between MON 89788 and the control for any of the parameters measured, including nodule number, shoot total nitrogen, and biomass (dry weight) of nodules, shoot material, and root material at each of the two sampling periods (Table VIII-7). Based on the assessed characteristics, the results support the conclusion that the introduction of the glyphosate-tolerant trait does not alter the symbiotic relationship between *B. japonicum* and MON 89788 compared to the conventional soybean.

Table VIII-7. Symbiont Interaction Assessment of MON 89788 and A3244

Measurement Endpoint	Sampling Period	MON 89788 (Mean)	A3244 (Mean)	Standard Error	Treatments SSD[1]
Nodule Dry Weight (mg/plant)	4 week	0.10	0.10	0.016	NS
	6 week	0.35	0.38	0.067	
Nodule Number (per plant)	4 week	41.25	51.75	6.84	NS
	6 week	147.13	153.25	17.59	
Root Dry Weight (mg/plant)	4 week	0.95	0.88	0.16	NS
	6 week	2.60	2.66	0.30	
Shoot Dry Weight (mg/plant)	4 week	1.22	1.15	0.27	NS
	6 week	4.96	5.43	0.82	
Shoot Total Nitrogen (%DW[2])	4 week	3.31	3.04	0.21	NS
	6 week	2.87	2.73	0.17	

[1] SSD, statistical significance of differences: NS, not significant at 5% level (P>0.05).

C. Overall Conclusions for Phenotypic, Agronomic and Ecological Interactions Evaluation

Phenotypic and agronomic characteristics of MON 89788 were evaluated and compared to those of A3244 to establish a comparative assessment framework in the context of familiarity, plant pest potential, and increased environmental impact. These assessments included 11 plant growth and development characteristics, five seed germination parameters, two pollen characteristics, more than 200 observations for each of plant-insect, plant-disease and plant-abiotic stressor interactions, and five plant-symbiont characteristics. In addition, forage and grain composition analyses (Section VII) are also considered as they provide analytical context for the assessment of familiarity.

Results from the phenotypic and agronomic assessments indicate that MON 89788 does not possess characteristics that would confer a plant pest risk or increased environmental impact over conventional soybean. Data on environmental interactions also indicate that MON 89788 does not confer any increased susceptibility or tolerance to specific disease, insect, or abiotic stressors. Data from composition analyses support the conclusion of equivalence between MON 89788 and A3244 based on nutritional and anti-nutritional components, which substantiate the assessment of familiarity between MON 89788 and A3244. These conclusions are consistent with our knowledge for Roundup Ready soybean where no increased weed potential or altered susceptibility to disease, insect, or abiotic stressors have been observed compared to conventional soybeans. Taken together, these data conclude that MON 89788 is not likely to pose increased plant pest risk or to have increased environmental impact compared to conventional soybean.

IX. Factors Influencing an Ecological Assessment of MON 89788

This section provides relevant information regarding the introduced trait, interactions with pest and non-pest organisms, potential to become a weed, gene flow potential, agronomic practices, occurrence of weeds and their control in conventional and herbicide-tolerant soybeans, and volunteer management practices that has been used to conduct an environmental assessment of MON 89788.

MON 89788 is being developed as a second-generation product that is expected to enhance soybean yield potential. Farmers planted Roundup Ready soybean on approximately 87% of U.S. acres in 2005 (USDA-NASS, 2005a) due to the economic, weed control, and convenience benefits it provides growers. From an ecological perspective, the transition to MON 89788 is not expected to alter either the crop rotational practices or volunteer control measures currently being utilized by U.S. soybean growers.

A. Characterization of the Trait

A.1. Safety and Nutrition

Several Roundup Ready crops that produce the CP4 EPSPS protein have been reviewed by regulatory agencies and cleared for environmental release in one or more countries around the world, including the United States. These products are Roundup Ready alfalfa, canola, corn, cotton, soybean, and sugar beet. Extensive compositional data demonstrate that these crops containing the CP4 EPSPS protein are compositionally and nutritionally equivalent to their conventional counterparts (Padgette et al., 1996; Taylor et al., 1999; Sidhu et al., 2000; McCann et al., 2005). Likewise, the safety assessment of the CP4 EPSPS protein, which is the same protein produced in MON 89788, has included a protein characterization demonstrating the lack of similarity to known allergens and toxins and the long history of safe consumption of similar proteins. In addition, data confirm the CP4 EPSPS protein digestibility *in vitro*, and the lack of acute oral toxicity in mice. Collectively, these data establish the safety of the CP4 EPSPS protein.

Similar to the Roundup Ready crops listed above, compositional analyses of field-generated MON 89788 seed and forage tissues were conducted to assess the levels of key nutrients, anti-nutrients, and other components for comparison to conventional soybean. The compositional analysis of MON 89788 (Section VII and Appendix E) demonstrated that there were few (26 out of 294 comparisons) significant differences ($p<0.05$) between MON 89788 and the control, where the mean levels for all components associated with statistically significant differences fell within the 99% tolerance interval for conventional soybean varieties. Therefore, these observed differences are unlikely to be biologically meaningful nor are they likely to contribute to an alteration in pest potential. Soybean seed and forage from MON 89788 is therefore considered to be nutritionally equivalent to the seed and forage of conventional soybean.

A.2. Interactions with Pest and Non-pest Organisms: Field Observations and Change in Toxicants

Extensive phenotypic and ecological assessments of MON 89788 have been presented in Section VIII. Included in these assessments were more than 200 observations for each of plant-insect and plant-disease stressor interactions. Data support the conclusion that MON 89788 does not confer an increased susceptibility or tolerance to the diseases and insects evaluated compared to A3244. In addition, composition analyses of soybean seed and forage (Section VII) have concluded that the levels of key nutrients and anti-nutrients in MON 89788 are comparable to those in conventional soybeans. Basked on these extensive plant-stressor and compositional assessments, MON 89788 is not expected to exert increased environmental impact compared to conventional soybean.

As discussed in Section VI, the CP4 EPSPS protein produced in MON 89788 is similar to the EPSPS proteins that exist ubiquitously in plants and microorganisms. Based on this history of occurrence, the CP4 EPSPS protein is not expected to possess biological activity towards pest and non-pest organisms through ingestion. The lack of toxicity is further supported by field experimentation conducted on Roundup Ready crops producing the CP4 EPSPS protein. There were no differences observed in the diversity and abundance of Collembola between Roundup Ready soybean and conventional soybean grown under the same management systems (Bitzer et al., 2002). Other studies conducted with commercial Roundup Ready soybean under various weed management systems also concluded that Roundup Ready trait had no apparent direct impact on arthropods, although weed management and phenotypic differences associated with plant variety influenced arthropod populations (Jasinski et al., 2003; McPherson et al., 2003; Buckelew et al., 2000). A similar lack of effect on Collembola and arthropods is expected for MON 89788.

Even though CP4 EPSPS was not known to exert adverse effects to pest and non-pest organisms, a number of studies were conducted to examine the potential effects of Roundup Ready crops to arthropods (Goldstein, 2003; Boongird et al., 2003; Jamornman et al., 2004; Harvey et al., 2003). Representative pollinators, soil organisms, beneficial arthropods and pest species were exposed to pollen, seed, and foliage tissues from Roundup Ready crops. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to Roundup Ready crops producing the CP4 EPSPS protein. These results are consistent with the data generated for MON 89788, and support the conclusion that MON 89788 is not likely to exert increased environmental impact compared to conventional soybean.

B. Ecological Characterization of MON 89788

B.1. Potential for MON 89788 to Become a Weed

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 does not possess any of the attributes commonly associated with weeds, 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. It is recognized that in some agricultural systems, soybean can volunteer in a rotational crop; however, volunteer plants are controlled through tillage or use of appropriate herbicides (see Section D.9). In addition, since the wild populations of *Glycine* species are not known to exist in the U.S., it is unlikely that MON 89788 would out-cross to weedy relatives and become a problem weed. This is also supported by the fact that there are no known reports of Roundup Ready soybeans becoming a problem weed after ten years of commercial cultivation.

Empirical studies used to assess the weed potential of MON 89788 include evaluation of the dormancy and germination of the seed, and phenotypic characteristics of the plants (Section VIII). Based on these data, it is concluded that MON 89788 is no more likely to become a weed than conventional soybean. Furthermore, several years of qualitative assessments and post-trial monitoring of the MON 89788 fields have not revealed differences in survivability or persistence relative to other varieties of soybean (list of trials found in Appendix A). Collectively, these findings conclude that MON 89788 has no increased weed potential compared to conventional soybean.

B.2. Potential Impact of MON 89788 on Non-pest Organisms

During the phenotypic field trials at 17 locations in 2005 (Section VII; Appendix C), each field site was rated at four to five time intervals during the season for specific insects (pest and non-pests), diseases, and abiotic stressors. The purpose of these trials was to assess whether the plant-disease or plant-insect interactions of MON 89788 were altered compared to commercially available soybeans. Twelve insect categories (species or group), 18 disease categories and 10 abiotic stressors were evaluated. Out of the 221 insect observations, only one difference in insect presence between MON 89788 and A3244 was noted during one of the observation intervals at a single site. A single difference in plant-insect interaction at one site does not indicate a trend; therefore, the single difference is not considered to have biological significance. Out of the 216 disease and 224 abiotic stressor observations, no differences were detected between MON 89788 and A3244. These results support the conclusion that MON 89788 does not have altered ecological interactions relative to other soybeans.

C. Potential for Pollen-Mediated Gene Flow

C.1. Vertical Gene Flow

Assessment of cross pollination in soybean

Soybean is considered to be a self-pollinated species, although natural crossing can occur (OECD, 2000; Garber and Odland, 1926; Caviness, 1966). In studies with conventional soybean where conditions have been optimized to ensure close proximity and flowering synchrony, cross pollination has been found to be low. Cross pollination frequencies vary with growing conditions, genotypes, and physical placement of the plants. The results of published studies on cross pollination in soybean (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 0.03 to 3.62%. 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. For example, the following cross pollination rates at extended distances have been reported: 0.02% at 8.2 m (Caviness, 1966), 0.05% at 5.4 m (Ray et al., 2003), and 0% at 6.5 m (Abud et al., 2003).

Cross pollination with wild species

The genus *Glycine* is subdivided into two subgenera, the subgenus *Soja* that includes cultivated soybean and the wild annual species, and the subgenus *Glycine* that includes the wild perennial species (Hymowitz and Singh, 1987; Hymowitz, 2004). Species within both subgeneras have been evaluated for their ability to cross with cultivated soybean. Crosses with species in other genera have not been documented (Hymowitz, 2004; OECD, 2000). Therefore, the cross pollination discussion will focus on species of subgeneras *Glycine* and *Soja*.

Hybridization with wild perennial species of subgenus Glycine

There are no wild relatives of subgenus *Glycine* in North America. Therefore, the only opportunities for inter-subgeneric hybridization would occur in Australia, West Central and South Pacific Islands, China, Papua New Guinea, Philippines, and Taiwan, where those species are endemic (Hymowitz and Singh, 1987; Hymowitz, 2004). Nonetheless, there are no known reports of successful natural hybridization between cultivated soybean and these wild perennial species. All inter-subgeneric hybrids were obtained through *in vitro* seed culture (Hymowitz, 2004). The resulting F1 hybrids were generally sterile and further progeny have been obtained only in a few cases and with great difficulty. Consequently, the possibility in North America of natural gene transfer between cultivated soybean and wild species of the subgenus *Glycine* does not exist.

Hybridization with the wild annual species of subgenus Soja

The subgenus *Soja* includes the cultivated soybean *G. max* and the wild annual species *G. soja*. *G. soja* is found in China, Taiwan, Japan, Korea, and Russia and can hybridize naturally with the cultivated soybean, *G. max* (Hymowitz, 2004). Hybridization between female *G. soja* and male *G. max* was less successful then hybridization in the opposing 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 F1 hybrids of *G. max* (2n=40) and *G. soja* (2n=40) carry similar genomes and are fertile (Singh and Hymowitz, 1989).

The subgenus *Soja* also contains a form known as *G. gracilis* (Hymowitz, 2004). *G. 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*. *G. gracilis* may be an intermediate in the speciation of *G. max* from *G. soja* (OECD, 2000) or a hybrid between *G. soja* and *G. max* (Hymowitz and Singh, 1987). Inter-species fertile hybrids between *G. max* and *G. soja* and between *G. max* and *G. gracilis* have been easily obtained (Dorokhov et al., 2004; OECD, 2000).

Importantly, the frequency of crop-to-wild relative gene introgression, which is defined as the permanent incorporation of genes from one population or species to another after hybridization, in soybean is reported to be exceedingly low (Stewart et al., 2003). In conclusion, gene transfer between cultivated soybean and wild species of the subgenus *Soja* may occur, but not in North America, where wild relatives of subgenus *Soja* are not naturally present. The glyphosate-tolerant trait will not be expected to enhance the pest potential if out-crossing to a wild relative were to occur.

C.2. Transfer of Genetic Information to Species with Which Soybean Cannot Interbreed (Horizontal Gene Flow)

Monsanto is not aware of any reports regarding the unaided transfer of genetic material from soybean species to other species with which soybean cannot sexually interbreed.

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

Distance from Pollen Source	% Cross-Pollination	Comments	Reference
0.3 m	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 m	0.07 to 0.18%	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	Garber and Odland, 1926
0.1 m	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 m	0.2 to 1%	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 m 2.7 – 4.6 m 6.4 – 8.2 m 10 – 15.5 m	0.03 to 0.44 % 0.007 to 0.04% 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 honeybee.	Abrams et al., 1978
0.1 – 0.6 m	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 m	0.09 to 1.63%	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	Ahrent and Caviness, 1994
0.5 m 1.0 m 6.5 m	0.44 to 0.45% 0.04 to 1.4% none detected	Frequency by distance was investigated. Experiment conducted in a single year. Single male and female parental varieties.	Abud et al., 2003
0.9 m 5.4 m	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 m	1.8%	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties.	Ray et al., 2003

D. U.S. Agronomic Practices

D.1. Introduction

This section provides a summary of the important agronomic practices in the U.S. for producing soybean. Discussions will include soybean production, growth and development, general management practices, weed occurrence and their management, soybean rotational crops, and volunteer soybean management. Discussion will be provided on the current use of Roundup Ready soybean and the expected use of MON 89788.

Soybeans are planted in over 30 states, demonstrating its wide adaptation to soils and climate. The soil, moisture, and temperature requirements for producing soybean are generally similar to those for corn. Therefore, the majority of soybean is produced in the same states as corn. Proper seedbed preparation, good genetics, proper planting dates and plant population, and good integrated pest management practices are important to optimizing the yield potential and economic returns of soybean.

Annual and perennial weeds are perceived to be the greatest pest problem in soybean production. Soybean insects and diseases are generally considered less problematic. 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 herbicides. Numerous selective herbicides are available for preplant, preemergence, and postemergence control of annual and perennial weeds in soybean. Roundup agricultural herbicides applied in the Roundup Ready soybean system have been a widely adopted and effective weed control management program since its introduction in 1996.

As discussed in Section D.9., volunteer soybean is not considered a significant concern in rotational crops primarily because of climatic conditions and tillage practices. Additionally, mechanical and chemical control methods are available to manage the occasional volunteer soybean plant.

D.2. U.S. Soybean Production

Soybeans first entered North America in the 18th century (Hoeft et al., 2000). Sometime during the 1930s, soybeans started to be processed industrially in the U.S. for edible oil and protein meal. Since that time, it has become the most widely grown protein/oilseed crop in the world, with the U.S. producing approximately 40% of global soybean supply (Soy Stats, 2005). The U.S. exported a record 1.1 billion bushels (29.94 million metric tons) of soybean, which accounted for 48 percent of the world's soybean exports in 2004 (Soy Stats, 2005). The U.S. exported $[redacted] worth of soybean globally in 2004 (Soy Stats, 2005). China is the largest export market for U.S. soybeans with purchases totaling

$[redacted]. Japan is the second largest export market with sales of $[redacted] in the same year. Other significant markets include the European Union and Mexico.

The production of soybeans is highly dependent upon soil and climatic conditions. In the U.S., the soil and climatic requirements for growing soybeans are very similar to corn. The soils and climate in the eastern half of the U.S. provide sufficient water supplies under dry land conditions to produce soybean. The general water requirement for a high-yielding soybean crop is approximately 20 inches of water during the growing season, which is similar to corn (Hoeft et al., 2000). Soil texture and structure are key components to the level of water availability in soils, where medium-textured soils hold more available water, such that soybean roots will penetrate deeper in medium-textured soils than in clay soils. Irrigation is used on approximately 9% of the acreage to supplement the water supply during dry periods in the western and southern soybean growing regions (Soy Stats, 2005).

Most of the soybean acreage is grown as a full-season crop. Six to nine percent of the soybean acreage is planted in a double-crop system following winter wheat south of 35º North latitude (Boerma and Specht, 2004; CTIC, 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 soybean planting date, and available soil moisture. Economic factors include expected soybean price and expected economic return (Boerma and Specht, 2004).

The U.S. soybean acreage in the past ten years has varied from approximately 64.2 to 75.2 million acres, with the lowest acreage recorded in 1996 and the highest in 2004 (Table IX-2). Average soybean yields have varied from 33.9 to 43.3 bushels per acre. Soybean production ranged from 2.38 to 3.12 billion bushels over the past ten years, with 2004 being the largest production year on record. According to data from USDA-NASS (2006a), soybeans were planted on approximately 72.1 million acres in the U.S. in 2005, producing 3.09 billion bushels of soybean (Table IX-2). This was the second largest U.S. soybean crop on record. The average yield in 2005 of 43.3 bushels per acre was the highest yield on record. The value of soybean reached $[redacted] in the U.S. in 2005. In comparison, corn and wheat values in 2005 were $[redacted] and $[redacted], respectively (USDA-NASS, 2006b).

For purposes of this agronomic practices discussion, soybean production is divided into three major soybean growing regions accounting for 99.2% of the 2005 U.S. soybean acreage – Midwest region (IL, IN, IA, KS, KY, MI, MN, MO, NE, ND, OH, SD, and WI), Mid-South region (AL, AR, LA, MS, and TN) and the Eastern Coastal region (DE, GA, MD, NJ, NC, PA, SC, and VA) (Table IX-3). The vast majority of soybeans were grown in the Midwest region, which represents 84.2% of the total U.S. production. The Mid-South and Eastern Coastal regions represented 9.4% and 5.6% of the production, respectively. Among the three regions, the Midwest region produced the highest average yield at 45.2 bushels per acre in 2005, and average state yields in this region ranged from 36.0 to 53.0 bushels per acre. The average yield in the Mid-South region was 35.4

bushels per acre, with states within this region averaging from 33.0 to 38.0 bushels per acre. The Eastern Coastal region spans a large area from north to south and subsequently results in a large variation in average state yields. The average yield in this region was 29.7 bushels per acre, with individual state averages ranging from 20.5 to 42.0 bushels per acre.

Managing input costs is a major component to the economics of producing a soybean crop. Key decisions on input costs include choosing what seed or 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 2003 was $[redacted] per acre according to statistics compiled by the American Soybean Association (Soy Stats, 2005). The value of the production less operating cost was reported to be $[redacted] per acre. A summary of all potential production costs and returns from this farmer survey are presented in Table IX-4.

Table IX-2. Soybean Production in the U.S., 1996 – 2005[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
2005	72,142	71,361	43.3	3,086,432	[redacted]
2004	75,208	73,958	42.2	3,123,686	
2003	73,404	72,476	33.9	2,453,665	
2002	73,963	72,497	38.0	2,756,147	
2001	74,075	72,975	39.6	2,890,682	
2000	74,266	72,408	38.1	2,757,810	
1999	73,730	72,446	36.6	2,653,758	
1998	72,025	70,441	38.9	2,741,014	
1997	70,005	69,110	38.9	2,688,750	
1996	64,195	63,349	37.6	2,380,274	

[1]Source: USDA-NASS, 2006c.

Table IX-3. U.S. Soybean Production by Region and State in 2005

Region/State	Acres Planted[1] (thousands)	Acres Harvested[1] (thousands)	Average Yield[1] (bushels/acre)	Total Production[1] (×1000 bushels)	Value[2] (billions $)
Midwest Region					
Illinois	9,500	9,450	47.0	444,150	[redacted]
Indiana	5,400	5,380	49.0	263,620	
Iowa	10,100	10,050	53.0	532,650	
Kansas	2,900	2,850	37.0	105,450	
Kentucky	1,260	1,250	43.0	53,750	
Michigan	2000	1,990	39.0	77,610	
Minnesota	6,900	6,800	45.0	306,000	
Missouri	5,000	4,960	37.0	183,520	
Nebraska	4,700	4,660	50.5	235,330	
North Dakota	2,950	2,900	37.0	107,300	
Ohio	4,500	4,480	45.0	201,600	
South Dakota	3,900	3,850	36.0	138,600	
Wisconsin	1,610	1,580	44.0	69,520	
Region Totals	**60,720**	**60,200**	**45.2**	**2,719,100**	[redacted]
Mid-South					
Alabama	150	145	33.0	4,785	[redacted]
Arkansas	3,030	3,000	34.0	102,000	
Louisiana	880	850	34.0	28,900	
Mississippi	1,610	1,590	37.0	58,830	
Tennessee	1,130	1,100	38.0	41,800	
Region Totals	**6,800**	**6,685**	**35.4**	**236,315**	[redacted]
Eastern Coastal Region					
Delaware	185	182	26.0	4,732	[redacted]
Georgia	180	175	26.0	4,550	
Maryland	480	470	34.0	15,980	
New Jersey	95	91	28.0	2,548	
New York	190	188	42.0	7,896	
North Carolina	1,490	1,460	27.0	39,420	
Pennsylvania	430	420	41.0	17,220	
South Carolina	430	420	20.5	8,610	
Virginia	530	510	30.0	15,300	
Region Totals	**4,010**	**3,916**	**29.7**	**116,256**	[redacted]

[1] Source: USDA-NASS, 2006a.
[2] Source: USDA-NASS, 2006b.

Table IX-4. U.S. Soybean Production Costs and Returns in 2003[1]

Production Cost or Return Category	Itemized Costs	Return per Planted Acre ($ USD)
Total Gross Value of Production		[redacted]
Operating Costs:	Seed	[redacted]
	Fertilizer	
	Soil conditioners	
	Manures	
	Chemicals	
	Custom operations	
	Fuel, lube and electricity	
	Repairs	
	Purchased irrigation water	
	Interest on operating capital	
Total, operating costs		[redacted]
Allocated overhead:	Hired labor	[redacted]
	Opportunity cost of unpaid labor	
	Capital recovery of machinery and equipment	[redacted]
	Opportunity cost of land (rental rate)	[redacted]
	Taxes and insurance	[redacted]
	General farm overhead	
Total, allocated overhead		[redacted]
Total cost listed		[redacted]
Value of production less total cost listed		[redacted]
Value of production less operating costs		[redacted]

[1] Source: Soy Stats, 2005. Supporting Information: Yield = 36 bushels/acre, Price = $[redacted]/acre, Enterprise size = 268 planted acres, Irrigated = 9%, Dry land = 91%.

D.3. Production Management Considerations

Pre-Season

Crop rotation, tillage system, row spacing, planting equipment, seed or variety selection(s), and soil fertility are areas that require production decisions well in advance of planting the soybean crop. Many of the decisions in this area are made immediately after harvest of the previous crop or sooner. There are many benefits to crop rotation, with the majority of the soybean acreage planted in a two-year corn-soybean rotation (see Section D.8). 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 will usually 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 conserving soil moisture. In 2004, approximately 29.3 million acres (38.6%) of soybean were planted in a no-till system (CTIC, 2004). Slow soybean emergence and growth plus 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 emergence, but have little effect on soybean yield (Pedersen, 2006). Improved planters for establishment of good soybean populations and planting Roundup Ready soybean to effectively control weeds in no-till fields have made no-till a viable production system for soybean. Researchers still recommend some spring tillage on fine-textured and poorly drained soils for proper seedbed preparation.

Most field crops, including soybean, respond very 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 nutrients are nitrogen, phosphorus and potassium. Since soybean is a legume and fixes its own nitrogen, soybean does not respond to additional nitrogen. Therefore, nitrogen fertilizer is seldom applied prior to planting a soybean crop. Although not common, deficiencies 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. Soybean is adversely affected when the pH is below approximately 5.8 (Hoeft et al., 2000). Since soybean is grown in rotation with corn and other crops, pH should be maintained at about 6.0 to 6.5 on acidic soils.

Soil tests are the only reliable way to determine the pH, phosphorus, and potassium levels in the soil. Fertilizer requirements are subsequently determined based on these 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.

Subsequently, no additional phosphorus or potassium fertilizers are required for the soybean crop. However, soybean plants require large amounts of phosphorus and potassium; therefore, fertilizer is often needed in some of the southern growing areas due to differences in crop rotations and soil types.

Soybean varieties are developed and adapted to certain geographical zones and are separated into ten maturity groups – Group 00 to Group VIII. Groups 00 and 0 are the earliest maturity groups and are adapted best to the region 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. Groups IV, V, and VI are adapted to the southern states (Zhang et al., 2004). Groups VII and VIII, when available, are also planted in the southern states ([redacted] 2006; personal communication).

Row spacing is important to maximize soybean yield. Research in the Midwest over the past twenty 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, 2006). In the southern states, the advantage from narrow rows is less consistent or 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 (Hoeft et al., 2000).

Planting and Early Season

An understanding of the growth stages of soybean is important for the proper timing of certain management practices, such as herbicide and insecticide applications. In addition, the impact of certain weather conditions 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, 2006). 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 R8.

Adequate soil moisture and warm temperatures facilitate rapid seed germination and emergence. The ideal soil temperature for soybean germination and emergence is 77º F (Pedersen, 2006). 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 50º F 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 is in early to mid May. Yields begin to drop off 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 north and the first week of May in the south (Pedersen, 2006). 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. 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 five plants per square foot (Hoeft et al., 2000). Therefore, a full stand of soybean is approximately eight to 10 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, to account for the losses in germination, emergence, and seedling diseases. The accuracy of the planting equipment can also impact the decision on seeding rate. Soybean seed is usually sold by weight. Therefore, the farmer must know the number of seeds per pound for the particular soybean varieties being planted for accurate seeding rates.

The soybean plant 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 the bacteria are not native to most U.S. soils, inoculation of the soybean seed is recommended when soybeans have not been grown in a field for three to five years.

High quality seed is essential for controlling seedling diseases. The most important seedling diseases in soybean are *Phytophthora* and *Pythium* (Pedersen, 2006). Many soybean varieties have race-specific resistance to *Phytophthora.* Treating soybean seed with a fungicide (e.g., mefenoxam) is effective against damping-off disease (seedling blight) caused by common soil fungi, such as *Phytophthora* and *Pythium.* Additional fungicides are available for control of other seedling diseases.

Soybean cyst nematode is one of the most damaging pests of soybean throughout the Midwest (Faghihi and Ferris, 2006). This nematode can cause yield losses up 50%. 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 and sorghum. Planting resistant varieties is also an effective management strategy to prevent losses from this pest. Several public

and private soybean varieties offer sources of resistance to certain races of nematode. Nematicides are no longer recommended as a management approach due to their limited effectiveness to reduce soybean cyst nematode populations and high mammalian toxicity.

Mid to Late Season

Ideal daytime temperatures for soybean growth are between 75º F and 85º F (Hoeft et al., 2000). Warmer temperatures result in larger plants and earlier flowering. Sustained temperatures below 75º F 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 70º F. 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 northern region. 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. These are grown in the northern and central U.S. Determinate varieties increase their height very little after flowering and are generally 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 for 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 period is during the seed-filling stages to assure high rates of photosynthesis. High humidity and temperatures during seed development and maturity can result in poor seed quality since these conditions promote the development of reproductive-stage diseases.

Asian soybean rust is a foliar fungal disease that typically infests soybeans during reproductive stages of development and can cause defoliation and reduce yields significantly (University of Illinois, 2006). Soybean rust is caused by the fungus *Phakopsora pachyrhizi*. Concerns about this disease have been increasing since it was

first detected in Louisiana in 2004. Foliar application of fungicides is the standard disease management practice to limit yield losses due to soybean rust at this time.

Bean leaf beetle and soybean aphids (*Aphis glycines*) are the most common threats from insects (Pedersen, 2006). Late-season feeding by second-generation green leaf beetles can cause considerable damage and require an insecticide treatment. Thiamethoxam was recently approved as a seed treatment to protect soybean from green leaf beetle. Thiamethoxam moves systemically throughout the plant and protects it by either direct contact or stomach activity following ingestion. Soybean aphid also can reach economic threshold levels requiring an insecticide treatment.

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, soybeans 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 variety, weather, and timeliness of harvest (Scott and Aldrich, 1970). Farmers should plant shatter-resistant varieties to minimize pre-harvest losses. Timely harvest when the moisture content is 13 to 14% will also minimize losses. Proper operation and adjustment of the combine is essential to minimizing harvest losses in the field.

D.4. Occurrence of Weeds and Losses Due to Weeds in Soybean Production

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 were rated less problematic. Weed control in soybean is essential to optimizing yields. Weeds compete with soybean for light, nutrients, and soil moisture. 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 soybeans for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001). Generally, the competition increases with increasing weed density. The relationship between weed density and soybean yield loss is best described by a hyperbolic equation (Cousens, 1985). At low densities, increases in weed density result in a linear increase in yield loss. At high densities, incremental increases in weed density result in a lower yield loss until the yield loss plateaus and no additional incremental yield loss occurs. Research at the University of Wisconsin in 1998 and 1999 showed that low densities of giant foxtail and common lambsquarters resulted in soybean yield losses of 11% and 1%, respectively (Conley et al., 2003), whereas yield losses were 95% and 50% when the two weeds were at high densities. Research in North Carolina reported that soybean yield was reduced by 62% when ragweed was allowed to grow in soybean (Coble et al., 1981).

The time period that weeds compete with the soybean crop influences the level of yield loss. The critical period for weed control is defined as the maximum length of time weeds can be allowed to compete without affecting crop yields (Zimdahl, 1987). In general, the later the weeds emerge, the less impact the weeds will have on yield. The critical period of competition for weeds emerging simultaneously with soybean varies depending upon weed species, weed populations, and environmental conditions. The critical period will be shorter with high weed populations and highly competitive weed species. Soybean withstands early season weed competition longer than corn, 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.

Numerous studies have been conducted to determine the critical time period weeds can grow in a soybean crop without impacting yields. This is especially important with the high adoption rates of postemergence herbicides and herbicide-tolerant soybean in recent years, as weeds are allowed to emerge and grow with the crop for a period of time. Most research indicates that no yield reductions occur when weeds are allowed to emerge with the crop and are controlled by four weeks after soybean emergence. Research by Purdue University has shown that in a total postemergence Roundup Ready soybean system, with moderate to heavy weed infestations, an initial weed control operation must be done according to one of three criteria to minimize yield losses due to weed competition (Johnson et al., 2004). These criteria consist of either: (1) controlling weeds by four to five weeks after planting, (2) controlling weeds before they reach six to nine inches in height, or (3) controlling weeds before soybean reaches the V3 stage of growth. Environmental conditions, weed densities, and variety can slightly shift optimal management times in either direction for any of the criteria.

Wisconsin research studies with drilled soybean showed no measurable yield loss from weeds competing prior to the V4 stage (Mulugeta and Boerboom, 2000). However, yields declined rapidly if allowed to compete beyond the V4 stage. Whereas, soybean in 30-inch rows were shown to be more sensitive to early season weed competition, and weeds needed to be removed by the V2 to V4 stage to avoid yield losses.

Research in no-till, double-crop soybean indicates that glyphosate herbicide applications made when the weeds were eight or 12 inches in height were the most consistent for reducing weed biomass and maintaining soybean yield potential (Dewell et al., 2003).

Extension weed specialists survey farmers to determine the most common or troublesome weeds in various crops. Common weeds are those species which can be found abundantly infesting a significant portion of the acreage for a given crop throughout the state. These weeds are most commonly present when no weed management intervention has occurred. The most common weeds in soybean for each region are presented in Tables IX-5, IX-6, and IX-7. The number of states within each region reporting each weed as a common weed in soybean in their state is also provided. In the Midwest region (Table IX-5), the top five weed species are foxtail spp. (foxtail species group), pigweed, velvetleaf, lambsquarters, and cocklebur. The most frequently reported common weeds

in the Mid-South region (Table IX-6) are morning glory spp., prickly sida, sicklepod, palmer amaranth, and broadleaf signalgrass. Some of the weed species common in the Midwest region do not exist in the Mid-South region and visa versa. The most common weeds in the Eastern Coastal region (Table IX-7) are common ragweed, cocklebur, and morning glory spp. Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in soybean.

Some extension weed specialists also denote certain weed species as troublesome or problematic weeds. Troublesome weeds are most likely to be inadequately controlled with typical weed control measures and interfere with crop production. Some of these species may not be widely distributed but can be difficult to manage. These species may initially be controlled effectively with typical herbicide programs but can continue to emerge throughout the season, making it difficult to keep the crop weed free until harvest. Weed species that are considered troublesome or problematic may require high levels of weed management to achieve effective control.

Weed species shifts are also studied extensively by conducting weed surveys over a period of time. Extension weed specialists at Purdue University conducted mail surveys with Indiana's farmers in 1996, 2000, and 2004 (Gibson et al., 2005). Results indicated that giant ragweed, Canada thistle, lambsquarters, cocklebur, and horseweed (marestail) were among the ten most problematic weeds in all three surveys. Waterhemp, chickweed, and dandelion showed up on the top-ten list for the first time in 2004. Although some grass species, such as foxtail species, are among the most common in Indiana, no grass species were listed by the farmers as most problematic in the 2004 survey. However, johnsongrass and shattercane were included among the ten most problematic weeds in the 1996 and 2000 surveys.

Table IX-5. Common Weeds in Soybean Production: Midwest Region

Foxtail spp. (12) [1]	Ragweed, giant (3)	Dandelion (1)
Pigweed spp. (11)	Shattercane (3)	Johnsongrass (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 amaranth (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 [redacted] Extension Weed Specialist, University of Illinois - Personal Communication (2006).

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

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

KS: [redacted] Extension Weed Specialist, Kansas State - Personal communication (2006).

KY, MO: Webster et al., 2005.

MI: Davis, A., K. Renner, C. Sprague, L. Dyer and D. Mutch, 2005, Integrated Weed Management: "One Year's Seeding", Extension bulletin E-2931. Michigan State University. List is not ranked in order of importance or frequency.

NE: [redacted] Extension Weed Specialist, University of Nebraska – Personal communication (2006).

ND: Zollinger, 2000.

SD: [redacted] Extension Weed Specialist, South Dakota State University – Personal communication (2006).

Table IX-6. Common Weeds in Soybean Production: Mid-South Region

Morningglory spp. (5) [1]	Pigweed spp. (3)	Ragweed, common (1)
Prickly sida (5)	Crabgrass spp. (2)	Ragweed, giant (1)
Johnsongrass (4)	Palmer amaranth (2)	Red rice (1)
Sicklepod (4)	Cocklebur (1)	Smartweed (1)
Signalgrass, broadleaf (4)	Copperleaf, hophorn (1)	Spurge, nodding/hyssop (1)
Barnyardgrass (3)	Florida pusely (1)	Spurge, Prostrate (1)
Hemp sesbania (3)	Horseweed (marestail) (1)	
Nutsedge spp. (3)	Poinsettia, wild (1)	

[1] Number provided in parenthesis is the number of states out of the five total states in the Mid-South region reporting each weed as a common weed.

Sources:

AL, LA, MS, TN: Webster et al., 2005.

AR: [redacted] Extension Weed Specialist, University of Arkansas - Personal communication (2006).

Table IX-7. Common Weeds in Soybean Production: Eastern Coastal Region

Ragweed, common (8)[1]	Jimsonweed (4)	Dandelion (1)
Cocklebur (7)	Sicklepod (3)	Goosegrass (1)
Morningglory spp. (7)	Florida pusely (2)	Nightshade, Eastern black (1)
Crabgrass spp. (6)	Johnsongrass (2)	Panicum, Texas (1)
Foxtail spp. (6)	Palmer amaranth (2)	Prickly sida (1)
Lambsquarters (6)	Quackgrass (2)	Shattercane (1)
Pigweed spp. (6)	Arrowleaf sida (1)	Signalgrass, broadleaf (1)
Velvetleaf (6)	Beggarweed, Florida (1)	Smartweed spp. (1)
Nutsedge spp. (5)	Burcucumber (1)	
Panicum, fall (5)	Canada thistle (1)	

[1] Number provided in parenthesis is the number of states out of the eight 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.

GA, NC, SC: Webster et al., 2005.

NY: [redacted] Extension Weed Specialist, Cornell University – Personal Communication (2006).

VA: [redacted] Extension Weed Specialist, Virginia Tech – Personal Communication (2006).

D.5. Methods of Weed Control in Conventional 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 the root systems, or causing sufficient physical injury to kill the plants. 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 herbicide (2,4-D) for weed control in corn in 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), allidochlor (1956), amiben (1958), trifluralin (1959), linuron (1960), and alachlor (1969) led the way for numerous selective herbicides in soybean (Agranova, 2006). Bentazon (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 IX-8 provides a summary of herbicide use in soybeans in the U.S. from 1995 through 2001. Weed control programs in conventional soybean consist of preemergence herbicides used alone or in a tank mixture with other preemergence herbicides. Applications are made as preplant incorporated or preemergence surface applications prior to or at planting. Tank mixtures of two preemergence herbicides are 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 seldomly were used in conventional soybean prior to 1995. Soybeans planted in a no-till system would receive a preplant burndown herbicide application for broad-spectrum control of existing weeds at time of planting. Therefore, multiple herbicides and/or multiple applications are generally made in conventional soybean. 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 post applications or two post applications (Gianessi et al., 2002).

It is important to observe the herbicide use in 1995, as this is prior to the introduction of Roundup Ready soybean. 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 IX-8). 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 the 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 soybeans.

Herbicide programs in conventional soybean have not changed significantly since 1995. Several new active ingredients have been introduced, including carfentrazone, sulfentrazone, flufenacet, 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 troublesome weeds species.

Tables IX-9 and 10 provide a summary of the crop tolerance of herbicides applied in soybean production and the efficacy of these herbicides on 26 common weed species identified in Section D.4. These tables list only the most commonly used herbicides in soybean production. Glyphosate applied postemergence 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 26 weed species, but they generally have a mixture of grass and broadleaf weed species. These ratings are intended to facilitate the selection of a herbicide program for a soybean crop, which offers the best overall control of the weed species. Glyphosate is considered to have better control (80%+) on more grass and broadleaf weed species than any of the other herbicides. 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 IX-8. Herbicide Use in Soybeans in the U.S. from 1995 through 2001[1]

	% 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: Gianessi et al., 2002.

Table IX-9: Crop Tolerance and Grass Weeds 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**	**QG**	**NSy**
Preplant Incorporated													
Trifluralin	1	9	9	9	9	9	9	7	7	3	9	0	0
Preplant or Preemergence													
Chlorimuron/tribenuron	2	3	6	3	3	3	5	0	0	0	4	0	3
Cloransulam	0	5	NA	5	5	5	NA	0	0	0	NA	0	0
Flumioxazin	2	3	NA	3	3	5	NA	0	0	0	NA	0	0
Flumioxazin/cloransulam	2	5	NA	5	5	5	NA	5	0	0	NA	0	0
Imazaquin	1	5	7	5	5	5	5	5	5	2	5	0	6
Imazethapyr	1	6	NA	7	7	7	NA	7	7	3	NA	0	4
Metribuzin	2	6	6	5	6	6	7	2	2	0	4	0	2
Pendimethalin	2	8	9	9	9	8	9	7	7	3	4	0	0
s-Metolachlor	1	8	8	9	8+	8+	9	5	4	0	8	0	8+
Postemergence													
Bentazon/acifluorfen	2	3	4	5	3	5	3	0	0	0	0	0	7
Chlorimuron	2	0	0	0	0	0	0	0	0	0	0	0	8
Clethodim	0	9	9	8+	9	9	9	9	9	9	8	9	0
Cloransulam	1	0	0	0	0	0	0	0	0	0	0	0	6
Clorimuron/thifensulfuron	0	0	NA	0	0	0	NA	0	0	0	NA	0	8
Fluazifop/fenoxaprop	0	9	8	8+	9	9	9	9	9	9	7	9	0
Flumiclorac	2	0	NA	0	0	0	NA	0	0	0	NA	0	0
Fomesafen	2	2	3	5	2	5	3	0	0	0	0	0	0
Glyphosate	0*	8+	9	8+	8+	9	8	9	9	9	8	9	7
Glyphosate/imazethapyr	1	9	NA	8+	9	9	NA	9	9	8+	NA	8+	7
Imazamox	2	6	NA	7	7	7-8+	NA	NA	NA	NA	NA	0	4
Imazethapyr	1	6	7	7	7	7-8	5	8	8	5	4	0	5
Lactofen	3	2	4	3	2	3	4	0	0	0	0	0	0
Thifensulfuron	2+	0	NA	0	0	0	NA	0	0	0	NA	0	0

[1] All weed control ratings except for BS, GG, and RR are from the 2006 Weed Control Guide for Ohio and Indiana, Ohio State University and Purdue University. Ratings for BS, GG and RR are from Weed Control Guidelines for Mississippi, Mississippi State University. 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%, where ratings of five or less are rarely of commercial significance. 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, QG = quackgrass, and NSy = yellow nutsedge.

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

NA denotes not available. *Rating based on glyphosate applied to Roundup Ready soybeans.

Table IX-10: Broadleaf Weeds 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	0	2	0	0	8+	2	7	9	0	4	4	3	8
Preplant or Preemergence														
Chlorimuron/tribenuron	0	8	9	8	5	9	9	9	9	4	6	9	8+	5
Cloransulam	0	8	9	8	NA	9	8	NA	9	NA	NA	8	8+	5
Flumioxazin	9	4	7	3	NA	9	NA	NA	9	NA	NA	7	7	8
Flumioxazin/cloransulam	9	8	9	8	NA	9	8	NA	9	NA	NA	9	8+	8
Imazaquin	9	8	8	8	0	9	6-8	9	9	9	5	9	7	5
Imazethapyr	9	7	6	6	NA	9	7	NA	9	NA	NA	9	8	5
Metribuzin	4	5	7	5	9	9	2-8	9	9	9	8	9	7	7
Pendimethalin	0	0	2	0	0	8+	2	7	9	4	2	4	4	7
s-Metolachlor	8	0	5	2	0	6	0	8	8	4	3	4	0	7
Postemergence														
Bentazon/acifluorfen	7	8+	9	7+	9	6	8-9	7	9	7	2	9	8	8+
Chlorimuron	3	9	8	8	8	2	8-9	6	9	2	7	8	8	5
Clethodim	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cloransulam	0	9	9	9	3	0	8-9	2	5	2	7	8	9	5
Clorimuron/thifensulfuron	4	9	8	8	NA	8	8	NA	9	NA	NA	9	9	5
Fluazifop/fenoxaprop	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Flumiclorac	5	7	7	5	NA	7	6	NA	7	NA	NA	5	9	7
Fomesafen	8	7	8+	8	9	5	8-9	8	9	2	3	7	6	9
Glyphosate	8	9	8+	8+	7	8	6-9	9	9	7	8	8	8	8+
Glyphosate/imazethapyr	9	9	8+	8+	NA	8+	8	NA	9	NA	NA	9	9	8
Imazamox	9	8	7	8	NA	8	7	NA	9	NA	NA	8	9	5
Imazethapyr	9	9	6	7	0	6	7-9	6	9	6	0	9	9	5
Lactofen	8+	8	9	8	9	4	8-9	8	9	8	5	6	7	9
Thifensulfuron	4	6	4	3	NA	8	NA	NA	9	NA	NA	8	8	8+

[1] All weed control ratings except for HS, MG, PA, PS, and SP are from the 2006 Weed Control Guide for Ohio and Indiana, Ohio State University and Purdue University. Ratings for HS, MG, PA, PS, and SP are from Weed Control Guidelines in Soybeans for Mississippi, Mississippi State University. 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%, where ratings of five or less are rarely of commercial significance. 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, LQ = lambsquarters, MG = morninglory spp., HS = hemp sesbania, PA = palmer and spiny amaranth, PW = pigweed, PS= prickly sida, SP = sicklepod, SW = smartweed, VL = velvetleaf, and WH = waterhemp.

NA denotes not available.

D.6. Method of Weed Control in Herbicide-Tolerant Soybean

Herbicide-tolerant soybeans were introduced to provide farmers with additional options to improve crop safety and/or improve weed control. Herbicide-tolerant soybeans enable the use of certain herbicides in soybeans that previously would not provide satisfactory crop safety when applied postemergence to conventional soybeans. The first introduced herbicide-tolerant soybean was sulfonylurea-tolerant soybean (STS), which was developed through mutational (conventional) breeding. STS was introduced in 1993 to increase tolerance to the sulfonylurea class of herbicides, such as chlorimuron and thifensulfuron. Roundup Ready soybean was subsequently introduced in 1996. Currently, Roundup Ready soybean is planted on 87% of the U.S. soybean acreage (USDA-NASS, 2005a).

Roundup Ready soybean contains in-plant tolerance to postemergence applications of Roundup agricultural herbicides providing a total weed control program in soybean production. The Roundup Ready soybean system – that is, planting Roundup Ready soybean and applying Roundup agricultural herbicides in crop – has become the standard weed control program in soybean production. Farmers incorporate other herbicides and cultural practices as part of the Roundup Ready cropping system where appropriate. Glyphosate, the active ingredient in Roundup agricultural herbicides, provides broad-spectrum control of annual and perennial grass and broadleaf weeds. The current Roundup herbicide label allows postemergence applications to Roundup Ready soybean from emergence (cracking) throughout the flowering stage of soybean development. A single application of glyphosate up to 1.5 pounds acid equivalent per acre or multiple applications up to 2.25 pounds acid equivalent per acre can be made during this application window. A preharvest application of glyphosate up to 0.75 pounds acid equivalent per acre can be made up to 14 days prior to harvest.

Starting with a weed-free field and making timely postemergence in-crop applications are critical to obtaining excellent weed control and maximum yield potential with the Roundup Ready soybean system. In no-till systems, a preplant burndown herbicide application is recommended to control any emerged weeds present at planting. In-crop applications of Roundup agricultural herbicides are recommended when the weeds are two to eight inches tall. A sequential application may be required to control new flushes of weeds, depending on the weed species present and soybean row spacing.

Table IX-11 shows the herbicide usage in soybean in the 11 primary soybean production states in 2004. Glyphosate-containing herbicides were used on 91% of the treated area. Although some of this usage was as a preplant burndown application, the predominant use was a postemergence application in Roundup Ready soybean. In contrast to herbicide use in 1995 (Table IX-8), postemergence glyphosate applications on Roundup Ready soybean largely replaced the previous widespread use of the ALS-inhibiting herbicides (imazethapyr, imazaquin) and the dinitroanaline herbicides (pendimethalin, trifluralin), which were used in combination. The ineffectiveness of ALS-inhibitors on waterhemp and the excellent control of glyphosate on this weed is one of the contributors to the rapid adoption of Roundup Ready soybean (Gianessi et al., 2002).

The adoption of the Roundup Ready soybean system has been rapid and widespread since its introduction. There are several reasons for the rapid adoption of the Roundup Ready soybean system over conventional weed control systems in soybean production. The primary reasons are: (1) more effective weed control, (2) excellent crop tolerance, (3) no carryover issues to subsequent crops, (4) longer herbicide application window, (5) only one herbicide required, (6) lower cost, (7) convenience and ease of use, and (8) it allows adoption of reduced and no-tillage systems (Marra et al., 2004; Gianessi, 2005).

Tables IX-9 and 10 provide the efficacy of postemergence use of glyphosate on the common weed species in soybean. The weed control ratings for glyphosate herbicide support the superior effectiveness of glyphosate compared to other herbicides. As previously mentioned, glyphosate is rated 8 or above (80%+ control) on more weed species than any of the other herbicides. Other herbicides will control only certain types of weeds, either grasses and/or broadleaf weeds, while glyphosate is effective on a broad spectrum of annual and perennial grasses and broadleaf weeds. Additionally, glyphosate is generally more effective on taller weeds. One of the key reasons for the rapid adoption of the Roundup Ready soybean system is the ability of glyphosate to control weed species that were difficult and expensive to control using other herbicides (Gianessi, 2005).

Crop safety is also an important consideration in selection of a soybean herbicide. When used in the Roundup Ready soybean system, Roundup agricultural herbicides provide excellent crop tolerance compared to other herbicides. Glyphosate is rated excellent for crop tolerance when used in the Roundup Ready soybean system (Table IX-9). Only four other herbicides receive this highest crop safety rating.

The Roundup Ready soybean system also provides greater flexibility in replanting and crop rotations. Glyphosate does not have recropping restrictions that limit planting options that exist with many other herbicides. Imazethapyr, an ALS-inhibitor herbicide, requires an 18-month interval between application and planting of cotton, oats, sorghum, or sweet corn. Fomesafen, a diphenyl ether, requires an 18-month interval between application and planting of alfalfa, sorghum, or sugar beets, based on product labels.

Convenience is another important feature of the Roundup Ready soybean system (Marra et al., 2004). Convenience is equated to less labor time, less management effort, or more flexibility in timing field operations. The other herbicide programs for conventional soybean usually involve two or more herbicides applied multiple times during the season, often supplemented with tillage. The longer window of application, simplicity of use with one herbicide, ease of controlling multiple weed species and less difficulty with weather disruptions were key features that incentivized growers to adopt the Roundup Ready soybean system.

Farmers also increased the use of conservation tillage with adoption of the Roundup Ready soybean system (Marra et al., 2004). Farmers value conservation tillage benefits such as reduced tillage costs and conserving soil. The proportion of farmers using

reduced tillage increases with Roundup Ready soybean adoption. Tillage trips decrease when the percent of soybean acres in no-till systems increase, and time saved from tillage activities increases as farmers shift from conventional soybean to the Roundup Ready soybean system (Marra et al., 2004). Market research in North Carolina indicates that there was 24 to 25% fewer tillage passes per season with the Roundup Ready soybean system compared to conventional soybean (Marra et al., 2004). The average total number of tillage passes per season for conventional soybean was 1.73 per acre compared to 1.39 per acre for the Roundup Ready soybean system in 2001 and 2002.

Full adopters of the Roundup Ready soybean system perceived a net benefit on average of over $[redacted] per acre (Marra et al., 2004). While farmers estimate the seed costs to be higher with the Roundup Ready soybean system, the herbicide and application costs are lower. The average savings on herbicides alone was $[redacted] per acre for full adopters of the Roundup Ready soybean system according to the national market research study. Another study reports the average cost difference between the Roundup Ready soybean system and conventional programs it replaced was $[redacted] per acre (Gianessi, 2005).

D.7. Roundup RReady2Yield Soybean MON 89788

Developments in biotechnology and molecular-assisted breeding have enabled Monsanto to develop the second-generation glyphosate-tolerant soybean product, MON 89788. In considering MON 89788 adoption, soybean production practices will remain the same for MON 89788 as they are for the Roundup Ready soybean system, including: rotational crops, tillage systems, row spacing, and planting and harvesting machinery. In addition to compatible agronomic practices, MON 89788 will continue to provide growers flexibility and simplicity in weed control, and allow them to reap the environmental benefits associated with the use of conservation-tillage and integrated weed management practices that are facilitated by the Roundup Ready soybean system.

In addition to providing the agronomic and environmental benefits, MON 89788 and varieties containing the trait have the potential to enhance yield and thereby further benefit farmers and the soybean industry. MON 89788 was developed by introduction of the *cp4 epsps* gene cassette containing a promoter that has been used in other crops such as Roundup Ready Flex cotton (Fincher et al., 2003). In addition, the transformation was based on a new technique of *Agrobacterium*-mediated gene delivery to soybean meristem, where cells were induced directly to form shoots and give rise to transgenic plants (Martinell et al., 2002). This new technique allowed direct transformation of the gene cassette into elite soybean germplasm such as the Asgrow soybean variety A3244 (Paschal, 1997), which is known for its superior agronomic characteristics and high yielding property (Tylka and Marett, 1999). Using elite germplasm as the base genetics, the superior agronomic characteristic of A3244 can be introgressed to other soybean varieties through crosses with MON 89788 containing the *cp4 epsps* cassette. In general, MON 89788 has been found to have a 4 to 7% yield advantage compared to Roundup Ready soybeans in the same elite genetic background (A3244) while maintaining the weed control and crop safety benefits of the Roundup Ready soybean system. As a

result, MON 89788 will be an excellent agronomic base trait for future breeding improvements and multi-trait products.

Table IX-11. Agricultural Chemical Applications Registered for Soybean Use in AR, IA, IL, IN, KS, MN, MO, NE, ND, OH, and SD in 2004[1]

Herbicide	Chemical Family	Mode of Action (MOA)	Area Applied (Percent)	Total Area Applied (Percent/MOA)	Quantity Applied (1000 lbs)	Total Quantity Applied (1000 lbs/MOA)
Glyphosate	glycine	EPSPS inhibitor	87	91	57,701	60,498
Glyphosate, diam. salt	glycine	EPSPS inhibitor	2		1,184	
Sulfosate	glycine	EPSPS inhibitor	2		1,613	
Pendimethalin	dinitroanaline	tubulin inhibitor	4	9	2,082	4,771
Trifluralin	dinitroanaline	tubulin inhibitor	5		2,689	
Bentazon	benzothiadiazinone	PSII inhibitor	*	8	221	961
Metribuzin	triazinone	PSII inhibitor	2		278	
Sulfentrazone	triazolinone	PSII inhibitor	6		462	
Chlorimuron-ethyl	sulfonylurea	ALS inhibitor	7	16	77	284
Cloransulam-methyl	triazolopyrimidine	ALS inhibitor	2		36	
Flumetsulam	triazolopyrimidine	ALS inhibitor	*		9	
Imazamox	imidazolinone	ALS inhibitor	2		27	
Imazaquin	imidazolinone	ALS inhibitor	1		36	
Imazethapyr	imidazolinone	ALS inhibitor	3		97	
Thifensulfuron	sulfonylurea	ALS inhibitor	1		1	
Tribenuron-methyl	sulfonylurea	ALS inhibitor	*		1	
Alachlor	chloroacetamide	not well understood	*	1	240	1,129
Metolachlor	chloroacetamide	not well understood	*		164	
S-Metolachlor	chloroacetamide	not well understood	1		725	

Table IX-11 (continued). Agricultural Chemical Applications Registered for Soybean Use in AR, IA, IL, IN, KS, MN, MO, NE, ND, OH, and SD in 2004[1]

Herbicide	Chemical Family	Mode of Action (MOA)	Area Applied (Percent)	Total Area Applied (Percent/MOA)	Quantity Applied (1000 lbs)	Total Quantity Applied (1000 lbs/MOA)
Paraquat	bipyridilium	PSI disruption	*	<1	115	115
Clethodim	cyclohexenone	ACCase inhibitor	2	4	145	329
Fenoxaprop	aryloxyphenoxy propionate	ACCase inhibitor	1		88	
Fluazifop-P-butyl	aryloxyphenoxy propionate	ACCase inhibitor	1		25	
Quizalofop-P-ethyl	aryloxyphenoxy propionate	ACCase inhibitor	*		12	
Sethoxydim	cyclohexenone	ACCase inhibitor	*		59	
Acifluorfen	diphenyl ether	PPO inhibitor	*	5	52	517
Flumiclorac-pentyl	N-phenylphthalimide	PPO inhibitor	1		6	
Flumioxazin	N-phenylphthalimide	PPO inhibitor	1		57	
Fomesafen	diphenyl ether	PPO inhibitor	2		346	
Lactofen	diphenyl ether	PPO inhibitor	1		56	
2,4-D	phenoxy	auxin type	2	6	771	1854
2,4-DP, dimeth. salt	phenoxy	auxin type	1		200	
2,4-D (acetic acid)	phenoxy	auxin type	1		375	
2,4-D (butoxy ester)	phenoxy	auxin type	1		236	
MCPA (sodium salt)	phenoxy	auxin type	1		272	

* Area receiving application is less than 0.5 percent.

[1]Data derived from USDA-NASS, Agricultural Statistics Board. Agricultural Chemical Usage 2004 Field Crops Summary (USDA-NASS, 2005b). Planted acreage for the eleven primary soybean production states was 61.2 million acres, which represents 81.4% of total planted acres. Acetamide was not transferred from USDA-NASS table, as this is a subgroup of total chloroacetamides.

D.8. Crop Rotation Practices in Soybean

The well-established farming practice of crop rotation is still a key management tool for farmers. The purposes of growing soybean in rotation are 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 reduce runoff of nutrients, herbicides, and insecticides (Boerma and Specht, 2004; Al-Kaisi et al., 2003). According to USDA Economic Research Service (ERS) crop residue management studies, 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 have been rotated at a slightly lower level of 75% and 70%, respectively. Although the benefits of crop rotations can be substantial, the farmer must make cropping decisions by evaluating both the agronomic and economic returns on various cropping systems. Crop rotations also afford farmers 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 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 response to being grown after a number of years without soybean. The yields of both corn and soybeans are 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.

Crop rotations may change over a long period of time due to economic conditions and market opportunities. Roundup Ready soybean has provided farmers more profit opportunities than conventional soybean primarily by reducing input costs. In addition, Roundup Ready soybean has provided farmers greater flexibility to grow soybean in fields with weed infestations, which previously were considered to be too problematic or unproductive for growing soybean. However, crop rotation practices for soybean production have not changed significantly since the introduction of Roundup Ready soybean in 1996.

Unique to the southern portion of the Midwest and the Mid-South 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, farmers sometimes do not plant if soils are too dry at the time of wheat harvest. Soybean is typically grown in a corn-wheat-soybean rotation sequence when soybeans are grown in a double-cropping system. In the northern soybean growing areas, wheat will follow soybean in the rotation.

Agronomic practices such as rotations for soybean vary from state to state. However, there are similarities among states within certain growing regions. This section provides a detailed description and quantitation of the rotational cropping practices immediately following soybean production, by state. This assessment accounts for 99+% of the total soybean acreage. These data are presented in Tables IX-12 through IX-15.

The majority of the U.S. soybean acreage (71%) is rotated to corn (Table IX-12). The second largest rotational crop following soybean is soybean. Approximately 13.2% of the soybean acreage is rotated back to soybean the following year. Wheat follows soybean on approximately 10.5% of the U.S. soybean acreage, with rice, cotton, and sorghum the next largest rotational crops following soybean. However, these three crops were planted on only 4% of the soybean acreage. Other minor rotational crops that follow soybean production are listed in Tables IX-12 through IX-15.

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 U.S. (Table IX-12), this percentage is 34.8% 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 12.1% (GA) to 89.6% (NJ). In the Midwest region where 84% of the soybean is grown, 34.7% of the rotational crop area was planted with soybeans during the previous growing season.

One rotation choice available to farmers is to plant another Roundup Ready crop following the production of Roundup Ready soybeans. To determine the likelihood that the rotational crops planted after MON 89788 will be another Roundup Ready crop, an assessment has also been provided in Tables IX-12 through IX-15. This assessment is based on current agronomic practices following soybean production. Roundup Ready alfalfa, canola, corn, cotton, soybean, and sugar beets have been deregulated by the USDA and were considered as potential Roundup Ready crops following soybean production. For the purposes of this assessment, the adoption rates used for Roundup Ready corn, cotton, and soybean in 2005 were obtained from the USDA-NASS Acreage Summary report (USDA-NASS, 2005a). The percentages for Roundup Ready corn, cotton, and soybean in the following tables were assumed to be the total percentage of herbicide-tolerant crops, since the USDA-NASS report does not show the percentages of each individual herbicide-tolerant trait. Therefore, this is a slight overstatement for Roundup Ready corn, cotton, and soybean since other herbicide-tolerant traits are

planted. Considering Roundup Ready alfalfa and sugar beets were recently deregulated by the USDA, no current adoption rates were available for these crops from the USDA-NASS report. Therefore, adoption rates for Roundup Ready alfalfa and sugar beets were assumed to be 50% to represent the projected adoption rate for these products.

This assessment showed that the percentage of the total rotational crop acreage that may be rotated from Roundup Ready soybean to another Roundup Ready crop (Table IX-12 - Column K) is estimated to be 10.5% in the U.S. and ranges from 4.4% (KS) to 70.1% (MS) across the soybean growing states. The percentage is 8.7% in the Midwest region, which is the largest soybean growing region.

Table IX-12. Rotational Practices in the U.S. Following Soybean Production

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in the U.S.[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
United States	**71530**	Corn	76650	66.3	50851	71.1	24.1	12238		
		Soybean	71530	13.2	9470	13.2	88.9	8418		
		Sorghum	3805	20.6	783	1.1	NA			
		Cotton	6968	15.9	1107	1.5	74.2	821		
		Wheat	32148	23.4	7523	10.5	NA			
		Barley	1729	2.0	39	0.05	NA			
		Oats	2784	3.4	94	0.1	NA			
		Rice	2654	38.5	1023	1.4	NA			
		Alfalfa	2295	7.2	165	0.2	50	83		
		Sugar Beets	943	14.1	133	0.2	50	67		
		Potatoes	351	9.2	32	0.05	NA			
		Dry Beans	1231	2.8	35	0.05	NA			
		Dry Peas	540	5.5	30	0.04	NA			
		Millet	275	14.2	39	0.05	NA			
		Flax	928	6.4	59	0.08	NA			
		Other[9]	759	19.5	148	0.2	NA			
			Total: 205589		**Total: 71530**			**Total: 21627**	**34.8**	**10.5**

The United States summary (Table IX-12) was developed by compiling the data from all three regional summaries. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2005 planting data (USDA-NASS, 2006a); "other" crop and newly seeded alfalfa acreages are based on 2005 planting data from the Individual States data which was obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp (USDA-NASS, 2006d).

[2] Column E is obtained by dividing Column F by Column D.

[3] Column F is obtained by multiplying Column B by Column G.

[4] Column G is obtained by dividing Column F by Column B.

[5] Column H is obtained by dividing Column I by Column F.

[6] Column I is obtained by compiling the data from all three regional summaries.

[7] Column J is obtained by dividing Column B by Column D Total.

[8] Column K is obtained by dividing Column I Total by Column D Total.

[9] Various vegetables.

Table IX-13. Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	Total Soybean Acres[1]	Major Crops Following Soybean In Rotation	Total Acreage of Rotational Crop in States[1]	% Rotational Crop Following Soybean[2]	Rotational Crop Acres Following Soybean[3]	% Rotational Crop of Total Soybean[4]	% Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	% Soybean Acres Preceding Major Rotations[7]	Estimated % Roundup Ready Crops as Major Rotations[8]
Region	**60720**	Corn	69960	67.6	47269	77.8	23.5	11091		
		Soybean	60720	7.5	4582	7.5	87.1	3993		
		Sorghum	3515	18.0	634	1.0	NA			
		Cotton	514	14.6	75	0.1	74.7	56		
		Wheat	29733	24.5	7274	12.0	NA			
		Barley	1495	2.6	39	0.06	NA			
		Oats	2325	4.0	94	0.2	NA			
		Rice	216	81.0	175	0.3	NA			
		Alfalfa[9]	2017	8.2	165	0.3	50	83		
		Sugar Beets	943	14.1	133	0.2	50	67		
		Potatoes	288	11.2	32	0.1	NA			
		Dry Beans	1206	2.9	35	0.06	NA			
		Dry Peas	540	5.5	30	0.05	NA			
		Millet	275	14.2	39	0.06	NA			
		Flax	928	6.4	59	0.1	NA			
		Other[10]	463	18.4	85	0.1	NA			
			Total: 175137		**Total: 60720**			**Total: 15289**	**34.7**	**8.7**
IL	**9500**	Corn	12100	73	8835	93.0	11%	972		
		Soybean	9500	3	238	2.5	81%	192		
		Sorghum	85	89	76	0.8	NA			
		Wheat	630	56	352	3.7	NA			
			Total: 22315		**Total: 9500**			**Total: 1164**	**42.6**	**5.2**
IN	**5400**	Corn	5900	82	4860	90	15%	729		
		Soybean	5400	5	270	5	89%	240		
		Wheat	360	75	270	5	NA	NA		
			Total: 11660		**Total: 5400**			**Total: 969**	**46.3**	**8.3**

Table IX-13 (continued). Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	Total Soybean Acres[1]	Major Crops Following Soybean In Rotation	Total Acreage of Rotational Crop in States[1]	% Rotational Crop Following Soybean[2]	Rotational Crop Acres Following Soybean[3]	% Rotational Crop of Total Soybean[4]	% Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	% Soybean Acres Preceding Major Rotations[7]	Estimated % Roundup Ready Crops as Major Rotations[8]
IA	**10100**	Corn	12800	77	9797	97	25	2449		
		Soybean	10100	2	202	2	91	184		
		Alfalfa[9]	150	67	101	1	50	51		
			Total: 23050		**Total: 10100**			**Total: 2684**	**43.8**	**11.6**
KS	**2900**	Corn	3650	40	1450	50	40	580		
		Soybean	2900	10	290	10	90	261		
		Sorghum	2750	5	145	5	NA			
		Wheat	10000	10	1015	35	NA			
			Total: 19300		**Total: 2900**			**Total: 841**	**15.0**	**4.4**
KY	**1260**	Corn	1250	86	1071	85	32	343		
		Soybean	1260	10	126	10	84	106		
		Wheat	390	16	63	5	NA			
			Total: 2900		**Total: 1260**			**Total: 449**	**43.4**	**15.5**
MI	**2000**	Corn	2250	62	1400	70	25	350		
		Soybean	2000	5	100	5	76	76		
		Wheat	600	83	500	25	NA			
			Total: 4850		**Total: 2000**			**Total: 426**	**41.2**	**8.8**
MN	**6900**	Corn	7300	72	5244	76	33	1731		
		Soybean	6900	3	207	3	83	172		
		Wheat	1820	68	1242	18	NA			
		Sugarbeets	491	21	104	1.5	50	52		
		Dry Beans	145	24	35	0.5	NA			
		Other[11]	221	31	69	1	NA			
			Total: 16877		**Total: 6900**			**Total: 1954**	**40.9**	**11.6**

Table IX-13 (continued). Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	Total Soybean Acres[1]	Major Crops Following Soybean In Rotation	Total Acreage of Rotational Crop in States[1]	% Rotational Crop Following Soybean[2]	Rotational Crop Acres Following Soybean[3]	% Rotational Crop of Total Soybean[4]	% Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	% Soybean Acres Preceding Major Rotations[7]	Estimated % Roundup Ready Crops as Major Rotations[8]
MO	**5000**	Corn	3100	58	2650	53	18	477		
		Soybean	5000	30	1500	30	89	1335		
		Sorghum	135	74	100	2	NA			
		Cotton	440	17	75	1.5	74	56		
		Wheat	590	85	500	10	NA			
		Rice	216	81	175	3.5	NA			
			Total: 9481		**Total: 5000**			**Total: 1868**	**52.7**	**19.7**
NE	**4700**	Corn	8500	41	3525	75	30	1058		
		Soybean	4700	10	470	10	91	428		
		Sorghum	340	69	235	5	NA			
		Wheat	1850	25	470	10	NA			
			Total: 15390		**Total: 4700**			**Total: 1485**	**30.5**	**9.7**
ND	**2950**	Corn	1410	63	885	30	32	283		
		Soybean	2950	21	620	21	89	551		
		Wheat	9090	15	1328	45	NA			
		Sugar Beets	255	12	30	1	50	15		
		Dry Peas	540	5	30	1	NA			
		Flax	890	7	59	2	NA			
			Total: 15135		**Total: 2950**			**Total: 849**	**19.5**	**5.6**
OH	**4500**	Corn	3450	98	3375	75	9	304		
		Soybean	4500	10	450	10	77	347		
		Wheat	860	78	675	15	NA			
			Total: 8810		**Total: 4500**			**Total: 650**	**51.1**	**7.4**

Table IX-13 (continued). Rotational Practices Following Soybean Production in the Midwest Region

A	B	C	D	E	F	G	H	I	J	K
State	Total Soybean Acres[1]	Major Crops Following Soybean In Rotation	Total Acreage of Rotational Crop in States[1]	% Rotational Crop Following Soybean[2]	Rotational Crop Acres Following Soybean[3]	% Rotational Crop of Total Soybean[4]	% Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	% Soybean Acres Preceding Major Rotations[7]	Estimated % Roundup Ready Crops as Major Rotations[8]
SD	**3900**	Corn	4450	63	2808	72	53	1488		
		Soybean	3900	2	78	2	95	74		
		Sorghum	180	43	78	2	NA			
		Wheat	3315	24	780	20	NA			
		Barley	65	60	39	1	NA			
		Oats	380	21	78	2	NA			
		Millet	140	28	39	1	NA			
			Total: 12430		**Total: 3900**			**Total: 1562**	**31.4**	**12.6**
WI	**1610**	Corn	3800	36	1369	85	24	328		
		Soybean	1610	2	32	2	84	27		
		Wheat	208	39	81	5	NA			
		Oats	400	4	16	1	NA			
		Alfalfa[9]	650	10	64	4	50	32		
		Potatoes	68	47	32	2	NA			
		Other[12]	152	11	16	1	NA			
			Total: 6888		**Total: 1610**			**Total: 388**	**23.4**	**5.6**

The Midwest region summary (Table IX-13) was developed by compiling the data from all the states within the region. Unlike the individual state data, the data in Column G for this regional summary were obtained by dividing Column F by Column B and the data in Column H were obtained by dividing Column I by Column F. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2005 planting data (USDA-NASS, 2006a); "other" crop and newly seeded alfalfa acreages are based on 2005 planting data from the Individual States data which were obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp (USDA-NASS, 2006d).

[2] Column E is obtained by dividing Column F by Column D.

[3] Column F is obtained by multiplying Column B by Column G.

[4] The rotational crop percentages are based on estimates from personal communications (2006) with individual state Extension Crop Production Specialist; Extension Agronomists – Soybean, Corn and Cotton; Extension Weed Control Specialist on Soybean and Corn; and/or Monsanto Technology Development Representatives.

[5] Roundup Ready rotational crop adoption rates for corn, soybean and cotton are based on 2005 planting data (USDA-NASS, 2005a). The percentages for Roundup Ready corn, cotton and soybean represent the percentages for total herbicide-tolerant traits. Percentages of herbicide-tolerant alfalfa and sugar beets are future market adoption estimates.

[6] Column I is obtained by compiling the data from all the states within the region.
[7] Column J is obtained by dividing Column B by Column D Total.
[8] Column K is obtained by dividing Column I Total by Column D Total.
[9] Newly seeded alfalfa.
[10] Various vegetables.
[11] Sweet corn and green peas.
[12] Sweet corn, green peas, and onions.

Table IX-14. Rotational Practices Following Soybean Production in the Mid-South Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
Region	**6800**	Corn	1830	53.2	974	14.3	32.0	312		
		Soybean	6800	61.3	4170	61.3	91.7	3822		
		Sorghum	213	69.8	149	2.2	NA			
		Cotton	4060	10.7	433	6.4	73.9	321		
		Wheat	740	30.7	227	3.3	NA			
		Rice	2438	34.8	848	12.5	NA			
			Total: 16081		**Total: 6800**			**Total: 4455**	**42.3**	**27.7**
AL	**150**	Corn	220	24	53	35	32	17		
		Soybean	150	5	8	5	84	6		
		Cotton	550	14	75	50	74	56		
		Wheat	100	15	15	10	NA			
			Total: 1020		**Total: 150**			**Total: 79**	**14.7**	**7.7**
AR	**3030**	Corn	240	88	212	7	32	68		
		Soybean	3030	64	1939	64	92%	1784		
		Sorghum	66	92	61	2	NA			
		Wheat	220	96	212	7	NA			
		Rice	1643	37	606	20	NA			
			Total: 5199		**Total: 3030**			**Total: 1852**	**58.3**	**35.6**
LA	**880**	Corn	340	26	88	10	32	28		
		Soybean	880	65	572	65	84	480		
		Sorghum	90	98	88	10	NA			
		Cotton	610	22	132	15	74	98		
			Total: 1920		**Total: 880**			**Total: 606**	**45.8**	**31.6**

Table IX-14 (continued). Rotational Practices Following Soybean Production in the Mid-South Region

A	B	C	D	E	F	G	H	I	J	K
State	Total Soybean Acres[1]	Major Crops Following Soybean In Rotation	Total Acreage of Rotational Crop in States[1]	% Rotational Crop Following Soybean[2]	Rotational Crop Acres Following Soybean[3]	% Rotational Crop of Total Soybean[4]	% Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	% Soybean Acres Preceding Major Rotations[7]	Estimated % Roundup Ready Crops as Major Rotations[8]
MS	**1610**	Soybean	1610	85	1369	85	96	1314		
		Rice	265	91	242	15	NA			
			Total: 1875		**Total: 1610**			**Total: 1314**	**85.9**	**70.1**
TN	**1130**	Corn	650	96	622	55	32	199		
		Soybean	1130	25	283	25	84	237		
		Cotton	640	35	226	20	74	167		
			Total: 2420		**Total: 1130**			**Total: 603**	**46.7**	**24.9**

The Mid-South region summary (Table IX-14) was developed by compiling the data from all the states within the region. Unlike the individual state data, the data in Column G for this regional summary were obtained by dividing Column F by Column B and the data in Column H were obtained by dividing Column I by Column F. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2005 planting data (USDA-NASS, 2006a); "other" crop and newly seeded alfalfa acreages are based on 2005 planting data from the Individual States data which were obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp (USDA-NASS, 2006d).

[2] Column E is obtained by dividing Column F by Column D.

[3] Column F is obtained by multiplying Column B by Column G.

[4] The rotational crop percentages are based on estimates from personal communications (2006) with individual state Extension Crop Production Specialist; Extension Agronomists – Soybean, Corn and Cotton; Extension Weed Control Specialist on Soybean and Corn ;and/or Monsanto Technology Development Representatives.

[5] Roundup Ready rotational crop adoption rates for corn, soybean and cotton are based on 2005 planting data (USDA-NASS, 2005a). The percentages for Roundup Ready corn, cotton and soybean represent the percentages for total herbicide-tolerant traits. Percentages of herbicide-tolerant alfalfa and sugar beets are future market adoption estimates.

[6] Column I is obtained by compiling the data from all the states within the region.

[7] Column J is obtained by dividing Column B by Column D Total.

[8] Column K is obtained by dividing Column I Total by Column D Total.

Table IX-15. Rotational Practices Following Soybean Production in the Eastern Coastal Region

A	B	C	D	E	F	G	H	I	J	K
State	**Total Soybean Acres[1]**	**Major Crops Following Soybean In Rotation**	**Total Acreage of Rotational Crop in States[1]**	**% Rotational Crop Following Soybean[2]**	**Rotational Crop Acres Following Soybean[3]**	**% Rotational Crop of Total Soybean[4]**	**% Roundup Ready Rotational Crop Option[5]**	**Acreage of Roundup Ready Rotational Crop Option[6]**	**% Soybean Acres Preceding Major Rotations[7]**	**Estimated % Roundup Ready Crops as Major Rotations[8]**
Region	**4010**	Corn	4860	53.7	2608	65.0	32.0	835		
		Soybean	4010	17.9	718	17.9	84.0	603		
		Cotton	2394	25.0	599	14.9	50	446		
		Wheat	1675	1.3	22	0.5	NA			
		Other[9]	277	22.8	63	1.6	NA			
			Total: 13216		**Total: 4010**			**Total: 1883**	**30.3**	**14.2**
DE	**185**	Corn	160	98	157	85	32	50		
		Soybean	185	15	28	15	84	23		
			Total: 345		**Total: 185**			**Total: 73**	**53.6**	**21.3**
GA	**180**	Corn	270	7	18	10	32	6		
		Cotton	1220	13	162	90	66	107		
			Total: 1490		**Total: 180**			**Total: 113**	**12.1**	**7.6**
MD	**480**	Corn	470	92	432	90	32	138		
		Soybean	480	10	48	10	84	40		
			Total: 950		**Total: 480**			**Total: 179**	**50.5**	**18.8**
NJ	**95**	Corn	80	89	71	75	32	23		
		Other[10]	26	91	24	25	NA			
			Total: 106		**Total: 95**			**Total: 23**	**89.6**	**21.5**
NY	**190**	Corn	990	18	181	95	32	58		
		Other[11]	75	13	10	5	NA			
			Total: 1065		**Total: 190**			**Total: 58**	**17.8**	**5.4**
NC	**1490**	Corn	750	95	715	48	32	229		
		Soybean	1490	25	373	25	84	313		
		Cotton	815	46	373	25	78	291		
		Other[12]	67	44	30	2	NA			
			Total: 3122		**Total: 1490**			**Total: 832**	**47.7**	**26.7**

Table IX-15 (continued). Rotational Practices Following Soybean Production in the Eastern Coastal Region

A	B	C	D	E	F	G	H	I	J	K
State	Total Soybean Acres[1]	Major Crops Following Soybean In Rotation	Total Acreage of Rotational Crop in States[1]	% Rotational Crop Following Soybean[2]	Rotational Crop Acres Following Soybean[3]	% Rotational Crop of Total Soybean[4]	% Roundup Ready Rotational Crop Option[5]	Acreage of Roundup Ready Rotational Crop Option[6]	% Soybean Acres Preceding Major Rotations[7]	Estimated % Roundup Ready Crops as Major Rotations[8]
PA	**430**	Corn	1350	31	421	98	32	135		
		Soybean	430	2	9	2	84	7		
			Total: 1780		**Total: 430**			**Total: 142**	**24.2**	**8.0**
SC	**430**	Corn	300	79	237	55	32	76		
		Soybean	430	30	129	30	84	108		
		Cotton	266	16	43	10	74	32		
		Wheat	170	13	22	5	NA			
			Total: 1166		**Total: 430**			**Total: 216**	**36.9**	**18.5**
VA	**530**	Corn	490	77	376	71	32	120		
		Soybean	530	25	133	25	84	111		
		Cotton	93	23	21	4	74	16		
			Total: 1113		**Total: 530**			**Total: 247**	**47.6**	**22.2**

The Eastern Coastal region summary (Table IX-15) was developed by compiling the data from all the states within the region. Unlike the individual state data, the data in Column G for this regional summary were obtained by dividing Column F by Column B and the data in Column H were obtained by dividing Column I by Column F. NA denotes not applicable. All acreages are expressed as 1000s of acres.

[1] Acreage planted of the specific crops is based on 2005 planting data (USDA-NASS, 2006a); "other" crop and newly seeded alfalfa acreages are based on 2005 planting data from the Individual States data which were obtained from Quick Stat searches on http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp (USDA-NASS, 2006d).

[2] Column E is obtained by dividing Column F by Column D.

[3] Column F is obtained by multiplying Column B by Column G.

[4] The rotational crop percentages are based on estimates from personal communications (2006) with individual state Extension Crop Production Specialist; Extension Agronomists – Soybean, Corn and Cotton; Extension Weed Control Specialist on Soybean and Corn; and/or Monsanto Technology Development Representatives.

[5] Roundup Ready rotational crop adoption rates for corn, soybean and cotton are based on 2005 planting data (USDA-NASS, 2005a). The percentages for Roundup Ready corn, cotton and soybean represent the percentages for total herbicide-tolerant traits. Percentages of herbicide-tolerant alfalfa and sugar beets are future market adoption estimates.

[6] Column I is obtained by compiling the data from all the states within the region.

[7] Column J is obtained by dividing Column B by Column D Total.

[8] Column K is obtained by dividing Column I Total by Column D Total.

[9] Sweet corn and other vegetables.

[10] Sweet corn, onions, and other vegetables.

[11] Sweet corn and onions.

[12] Cucumbers, sweet potatoes, and Irish potatoes.

VIII.D.9 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 the combine is improperly adjusted for efficient harvesting. Volunteer soybean in rotational crops is typically not a concern in the Midwest region because the soybean seed is typically not viable after the winter period. 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 is normally not a concern in rotational crops such as corn, cotton, rice, and wheat that are the significant rotational crops following soybean due to control measures that are available for volunteer soybean when they arise. 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 Roundup Ready soybean) in each of the major rotational crops. Table IX-16 provides control ratings on volunteer Roundup Ready soybean for several herbicides used in the major rotational crops.

To provide control of volunteer soybean in corn, postemergence applications of AAtrex (atrazine), Clarity (dicamba), Distinct (diflufenzopyr + dicamba), Hornet (flumetsulam + clopyralid), and Widematch (clopyralid + fluroxypyr) provide excellent control (Zollinger, 2005). In wheat, Bronate Advanced (bromoxynil), Clarity (dicamba) and Widematch postemergence provide excellent control of volunteer soybean (Zollinger, 2005).

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, where the unharvested soybean seed can produce volunteer plants. Preplant applications of paraquat or herbicide mixtures containing paraquat will effectively control volunteer Roundup Ready soybean (Montgomery et al., 2002; Murdock et al., 2002). Recent research in North Carolina indicates Envoke (trifloxysulfuron) will provide excellent postemergence control of soybean with 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 utilized in producing rice ([redacted] 2006; personal communication). If volunteer plants should emerge in rice, the postemergence applications of Grasp (penoxsulam), Permit (halosulfuron) and Regiment (bispyribac) typically used for weed control in rice will effectively alleviate competition from volunteer soybean (Dillon et al., 2006).

Table IX-16. Ratings for Control of Volunteer Roundup Ready Soybean in Labeled Rotational Crops[1]

Product	Rate (Product/Acre)	Soybean V2 – V3	Soybean V4- V6
Corn[2]			
AAtrex	0.38 qts	E	P
	0.50 qts	E	F
Clarity	4 fl oz	E	E
	5 fl oz	E	E
Distinct	1 oz	E	G
	2 oz	E	E
Hornet	1 oz	E	F
	2 oz	E	F-G
Widematch	0.25 pt	E	G
Wheat[2]			
Bronate Advanced	0.8 pt	E	E
Clarity	4 fl oz	E	E
	5 fl oz	E	E
Widematch	0.25 pt	E	G
Cotton[3]			
Envoke	0.1 oz	E	E
Rice[4]			
Grasp	2 oz	E	NA
Permit	1 oz	E	NA
Regiment	0.4 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] Zollinger, 2005.

[3] York et al., 2005.

[4] Dillon et al., 2006.

E. Weed Resistance to Glyphosate

The risk of weeds developing resistance and the potential impact of resistance on the usefulness of a herbicide vary greatly across different 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 the glyphosate molecule. This research includes an evaluation of some of the factors that can contribute to the development of weed resistance. Detailed information regarding glyphosate stewardship is presented in Appendix J.

X. Summary of Environmental Assessment

The phenotypic evaluations of MON 89788 included an assessment of seed germination and dormancy characteristics, plant growth and development characteristics, pollen characteristics, ecological interaction characteristics, plant-symbiont characteristics, and compositional components. These studies were conducted across a broad range of environmental conditions and agronomic practices to represent the conditions that MON 89788 would likely to encounter in commercial production. These detailed characterizations and comparisons demonstrate that MON 89788 is not likely to pose an increased pest potential compared to conventional soybean currently grown in the U.S. In addition, the glyphosate-tolerance trait and the CP4 EPSPS protein produced in MON 89788 are identical to those present in Roundup Ready soybean 40-3-2 that was previously granted a determination of nonregulated status by APHIS, and has been widely planted in the U.S. and global soybean areas.

The environmental consequences of pollen transfer from MON 89788 to other soybeans are considered negligible due to limited movement of soybean pollen and the safety of the introduced trait. Additionally, the potential for outcrossing to sexually compatible species is also unlikely because of the lack of sexually compatible species in the U.S. The agronomic consequences of volunteer MON 89788 soybean plants are expected to be minimal as these plants are easily controlled by mechanical means or by one of a number of herbicides currently registered for the control of soybean plants. There is no indication that MON 89788 would have an adverse impact on beneficial or non-pest organisms, including threatened or endangered organisms.

From an ecological perspective, MON 89788 is similar to the commercial Roundup Ready soybean products used in the U.S. since 1996. Farmers familiar with the Roundup Ready soybean system would continue to employ the same crop rotational practices and/or volunteer control measures currently in place for Roundup Ready soybean system.

XI. Adverse Consequences of Introduction

Monsanto knows of no study data or observations associated with MON 89788 that will result in adverse environmental consequences from its introduction. MON 89788 is a second-generation glyphosate-tolerant soybean product that expresses the same CP4 EPSPS protein as in Roundup Ready soybean 40-3-2. As demonstrated by field and laboratory studies, the only biologically relevant phenotypic difference between MON 89788 and conventional soybean is the expression of CP4 EPSPS protein in MON 89788, which provides tolerance to application of Roundup agricultural herbicide. Successful adoption of MON 89788 is expected to increase the economic benefits to the growers, and maintain the environmental and weed control benefits afforded by the current Roundup Ready soybean products that are grown on the majority of U.S. soybean acres.

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Appendices

Appendix A. USDA Notifications for MON 89788 Field Trials

Field trials of MON 89788 were conducted under notification in the U.S. since 2001. In the submitted notifications, MON 89788 was designated as GM_A19788 or as PV-GMGOX20. MON 89788 and GM_A19788 refer to the same transformation event.

The protocols for these trials include field performance, agronomics, and generation of field materials and data necessary for this petition. In addition to the phenotypic assessments made on MON 89788, 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 that are not yet due, mainly from the 2005-2006 seasons, are still in preparation. A list of trials conducted with MON 89788 under USDA notification and the status of the final reports for these trials are provided in Table A-1.

The observations made during these trials and provided as part of the final field reports provide confirmatory information regarding the following characteristics: insect susceptibility, weediness, disease susceptibility, plant growth and plant stand. The qualitative assessments contained in the final reports indicate that MON 89788 performed similarly to the control, A3244, and other control materials in the trials. Over numerous years and geographies of field testing there were no reports in any of the trials of notable or unexpected plant phenotypes or interactions with plant pests with MON 89788 relative to controls. These results are as expected, and are consistent with the more detailed and quantitative phenotypic and agronomic assessment of MON 89788 described in Section VIII of the petition. The combination of these qualitative reports and more quantitative assessments support a conclusion of no enhanced survivability for MON 89788 relative to conventional soybeans.

Table A-1. USDA Notifications for MON 89788 Field Trials[1]

USDA Reference Number	Effective Date	Release Sites (by State) Covered by Notification
	2001 Field Trials	
01-206-02n	8/24/2001	PR
	2002 Field Trials	
02-074-05n	4/14/2002	IL
02-263-12n	10/20/2002	PR
	2003 Field Trials	
03-070-10n	4/10/2003	IA, IL, KS
03-181-05n	7/30/2003	PR
03-258-10n	10/27/2003	PR
	2004 Field Trials	
04-068-21n	5/14/2004	IA, IL, IN, MD, OH
04-068-24n	5/14/2004	IL, IN
04-072-02n	5/14/2004	IA, IL, KS
04-072-03n	5/14/2004	IA
04-098-03n	5/12/2004	IA
04-218-02n	10/5/2004	PR
04-230-02n	9/16/2004	PR
04-278-06n	11/1/2004	PR
04-321-01n	12/15/2004	PR
	2005 Field Trials	
05-049-14n*	3/24/2005	IA, IL, KS, NE
05-056-08n	4/11/2005	AR
05-056-09n*	4/1/2005	IL
05-060-15n	4/1/2005	IA, IL
05-060-16n	4/1/2005	IA, IL, IN, KS
05-060-17n	4/1/2005	IL
05-066-01n*	4/1/2005	IN, MO
05-067-01n*	3/22/2005	OH
05-070-01n	4/13/2005	AR, IL, NE, OH
05-073-09n*	4/11/2005	IL, IN
05-073-10n	4/26/2005	AR, IL, MD, OH, WI
05-073-11n	4/12/2005	IA
05-073-12n	4/12/2005	IL
05-080-06n	5/4/2005	IA, IL, IN, MN, PA

Table A-1 (continued). USDA Notifications for MON 89788 Field Trials

2005 Field Trials (continued)		
05-122-01n*	6/9/2005	PR
05-122-02n*	7/22/2005	HI
05-151-07n*	6/28/2005	PR
05-217-02n*	9/28/2005	PR
05-271-07n*	10/12/2005	IL, MO, NE
05-291-01n*	11/7/2005	IN
05-325-03n*	12/12/2005	PR
2006 Field Trials		
06-031-05n*	3/8/2006	AR, IA, IL, IN, MI, MN, NE, OH
06-033-01n*	3/14/2006	IL
06-033-02n*	3/23/2006	IA, IL, IN
06-038-06n*	4/6/2006	IA, MN, WI
06-038-08n*	4/6/2006	IA, IL, IN, MN, ND,WI
06-040-01n*	3/15/2006	PR
06-045-16n*	5/18/2006	PR
06-045-18n*	5/18/2006	PR
06-045-19n*	5/18/2006	PR
06-045-20n*	5/10/2006	AR, IL, IN, MD, NC
06-045-29n*	4/17/2006	IA, IL, IN, OH
06-058-01n*	5/11/2006	AR, IA, IL, MD, MN
06-058-02n*	5/11/2006	AL, GA
06-058-05n*	4/3/2006	IL, IN, MI
06-058-06n*	4/24/2006	IA
06-058-08n*	4/24/2006	IA, PR
06-058-10n*	4/11/2006	MN
06-061-07n*	5/11/2006	IA, IN, MI, NE, WI
06-069-11n*	4/24/2006	PR
06-072-07n*	5/19/2006	IL, WI
06-072-08n*	5/19/2006	IL
06-076-10n*	5/5/2006	AL, GA, LA
06-076-11n*	5/25/2006	IA, IL, KS, KY, MN, NE, OH, SD
06-086-07n*	5/15/2006	WI
06-087-07n*	5/25/2006	IA, IL, IN, MO

Table A-1 (continued). USDA Notifications for MON 89788 Field Trials

2006 Field Trials (continued)		
06-087-08n*	5/15/2006	IL
06-093-02n*	5/5/2006	IN
06-094-04n*	6/8/2006	PR
06-118-03n*	5/9/2006	IA

* Final Test Reports in preparation and not due prior to the submission of this petition.
1. This list contains notifications granted through June 15, 2006 and MON 89788 was planted. In these notifications MON 89788 is also designated as GM_A19788, or as PV-GMGOX20.

Appendix B. Materials and Methods Used for Molecular Analyses of MON 89788

Materials

The DNA used in molecular analyses was isolated from leaf tissue of MON 89788 collected in 2005 from seed lot GLP-0405-15118-S. Additional DNA extracted from various generations of leaf tissues were used in generation stability analyses. The control DNA was isolated from the leaf tissue of a conventional soybean variety, A3244. The reference substances included the PV-GMGOX20 plasmid and the size estimation molecular weight standards. As a positive control on Southern blots, PV-GMGOX20 plasmid DNA was digested with a restriction enzyme or combination of enzymes to produce the banding patterns that were most relevant to the assessment of the test substance digested with appropriate enzyme(s). The plasmid DNA was either added to undigested A3244 soybean genomic DNA and digested, or was digested first and then added to pre-digested A3244 soybean genomic DNA. The molecular weight standards include the 1 kb DNA Extension Ladder (Invitrogen) and λ DNA/*Hin*d III fragments (Invitrogen) for size estimations on Southern blots. The 500 bp DNA ladder (Invitrogen) was used for size estimations for the PCR analyses.

Characterization of the Materials

The quality of the source materials from MON 89788 and A3244 were verified by PCR analysis to confirm the presence or absence of MON 89788 except the materials used in the generational stability analyses where the identity of the materials was confirmed by the generation stability Southern blots themselves. The stability of the genomic DNA was confirmed in each Southern analysis by observation of the digested DNA sample on an ethidium bromide-stained agarose gel.

DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA samples from MON 89788 and A3244 used in the insert and copy number, insert integrity, backbone analysis, and PCR analyses were isolated from soybean leaf tissues that were ground to a fine powder in liquid nitrogen using a mortar and pestle. DNA was extracted from the processed leaf tissue using the Sarkosyl DNA isolation method by Fulton et al. (1995) with the following exceptions. Instead of recovering DNA by centrifugation, the DNA was spooled using a glass hook and placed in a microcentrifuge tube containing 70% ethanol. Also, during one of the isolations, RNAse A was added to the extraction buffer to minimize the co-purification of RNA.

Genomic DNA used in the generational stability analysis was isolated using the following method. Leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Approximately 2 ml equivalents of fresh leaf tissue powder were transferred to 13 ml conical tubes, and ~10 ml of CTAB extraction buffer [1.5% CTAB, 75 mM Tris pH 8.0, 100 mM EDTA, 1.05 M NaCl, 0.75% PVP (40K)] were added to the tissue. The samples were incubated at 68°C for 45-50 minutes and were mixed halfway through the incubation. Samples were split into 13 ml conical tubes (2/sample) containing 5 ml of chloroform. The suspensions were mixed by inversion for 2 minutes. The two phases were separated by centrifugation at ~10,300 x *g* for 8 minutes at room temperature. The aqueous (upper) layer was transferred to a clean 13 ml tube and the chloroform extraction

was repeated as above with 5 ml of chloroform. The aqueous layer was transferred to a clean 13 ml tube containing 5 ml of 100% ethanol to precipitate the genomic DNA. The genomic DNA of like samples was spooled into a 13 ml tube containing 10 ml of 70% ethanol. Samples were centrifuged at ~5,100 x *g* for 5 minutes at room temperature to pellet the DNA. The pellet was transferred with an inoculating loop to a microcentrifuge tube containing 1 ml of 70% ethanol. The DNA was spun for 1 minute at maximum speed in a microcentrifuge. Ethanol was removed with a pipette tip and the samples were allowed to air dry for 1-2 hours. The DNA was resuspended in TE buffer and stored in a 4°C refrigerator until use.

Quantification of Genomic DNA

Quantification of DNA samples was performed using a Hoefer DyNA Quant 200 Fluorometer with Roche molecular size marker IX as a DNA calibration standard.

Restriction Enzyme Digestion of Genomic DNA

Approximately 10 µg of genomic DNA were used for restriction enzyme digestions. When digesting genomic DNA with *Not* I (Roche), *Nco* I (Roche), or the combination of *Not* I and *Nco* I (Roche), 10X buffer H (Roche) was used. When digesting genomic DNA with the restriction enzyme combination of *Bpl* I (Fermentas) and *Xmn* I (New England Biolabs), buffers 10X Tango buffer and 2.5 mM SAM (Fermentas) were used. Finally, 100X BSA (New England Biolabs) was added to all digests to a final concentration of 1X. Overnight digests were performed at 37°C in a total volume of 500 µl using 100 units of the appropriate restriction enzyme(s).

DNA Probe Preparation for Southern Blot Analyses

Probes were prepared by PCR amplification of the PV-GMGOX20 template using a standard procedure based on Sambrook and Russell (2001). Approximately 25 ng of each template was used to generate the probe labeled with ^{32}P-dCTP (~6000 Ci/mmol) by random priming method (RadPrime DNA Labeling System, Invitrogen) or by PCR. Probe positions relative to the genetic elements in plasmid PV-GMGOX20 are depicted in Figure IV-1.

Southern Blot Analyses of Genomic DNA

Digested DNA was separated using 0.8% (w/v) agarose gel electrophoresis. Except for generational stability analyses, DNA samples were loaded on the gels for a long run and a short run in an effort to provide better resolution of larger DNA fragments while retaining smaller DNA fragments on the gel. After transferring the DNA to the membrane, Southern blots were hybridized at 65^{o}C except when probing with the *Tsf1* intron sequence and the *E9* 3' nontranslated sequence. These elements contain A-T rich sequences; therefore, it is necessary to lower the hybridization temperature to 60^{o}C. Multiple exposures of each blot were then generated using Kodak Biomax MS film in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

DNA Sequence Analyses of the Insert

The organization of the elements within the T-DNA of MON 89788 was confirmed using DNA sequencing analyses. Several PCR primers were designed with the intent to

amplify three overlapping DNA fragments (Products A, B and C) spanning the entire length of the insert. The PCR for Products A and B were conducted using 50 ng of genomic DNA or 6 ng of plasmid DNA as templates in a 50 µl reaction volume containing a final concentration of 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM each dNTP, and 2.5 µl of Platinum Taq DNA polymerase (Invitrogen). The PCR for Product C was conducted using 50 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM each dNTP, and 1 unit of Accuprime *Taq* (Invitrogen) DNA polymerase mix. The amplification of Product A was performed under the following cycling conditions: 94°C for 3 minutes, 35 cycles at 94°C for 30 seconds, 59°C for 30 seconds, 72°C for 3 minutes, and 1 cycle at 72°C for 10 minutes. The amplification of Product B was performed under the following cycling conditions: 94°C for 3 minutes, 35 cycles at 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 3 minutes, and 1 cycle at 72°C for 10 minutes. The amplification of Product C was performed under the following cycling conditions: 94°C for 3 minutes, 35 cycles at 94°C for 15 seconds, 55°C for 30 seconds, 68°C for 3 minutes, and 1 cycle at 68°C for 5 minutes. Aliquots of each PCR product were separated on 1.0 % (w/v) agarose gels and visualized by ethidium bromide staining to verify that the products were of the expected size prior to sequencing. The PCR products were sequenced with primers used for PCR amplification as well as multiple primers designed internal to the amplified sequences. All sequencing was performed by the Monsanto Genomics Sequencing Center using dye-terminator chemistry.

Appendix C. Materials, Methods and Results for Characterization of the CP4 EPSPS Protein Produced in MON 89788

Materials

The MON 89788-produced CP4 EPSPS protein was isolated from grain of MON 89788. The grain used for the isolation of CP4 EPSPS protein was produced in Argentina field production during the 2004-2005 season. The identity of the grain sample containing MON 89788 was confirmed by event-specific PCR. The isolated MON 89788-produced CP4 EPSPS protein was stored in a –80°C freezer in a buffer solution containing 50 mM Tris-HCl pH 7.5, 50 mM KCl, 2 mM DTT, 0.5 mM PMSF, 1 mM benzamidine-HCl, and 25% (v/v) glycerol. Data supporting the extraction and isolation of the CP4 EPSPS protein from the grain of MON 89788 conducted prior to the initiation of this plan are archived under APS lot 60-100085.

The *E. coli*-produced CP4 EPSPS protein (APS lot 20-100015) was used as a reference standard to establish equivalence in select analyses. These analyses included molecular weight determination by SDS-PAGE, immunoblot analysis, glycosylation analysis, and the functional enzymatic assay. The CP4 EPSPS protein was stored in a -80°C freezer in a buffer solution [50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl, and 25% (v/v) glycerol] at a total protein concentration of 3.8 mg/mL.

Description of Assay Controls

Protein molecular weight markers were used to calibrate SDS-PAGE gels and verify protein transfer to PVDF membranes. The *E. coli*-produced CP4 EPSPS reference standard protein was used in the generation of the standard curve to estimate the total protein concentration using the Bio-Rad protein assay. It was also used as the positive control in the immunoblot analysis. Beta-lactoglobulin protein and PTH-amino acid standards were used to verify the performance of the amino acid sequencer. A peptide mixture was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. Transferrin and *E. coli*-produced CP4 EPSPS proteins were used as the positive control and the negative control, respectively, in glycosylation analysis.

Protein Purification

The CP4 EPSPS protein was purified from an extract of ground grain of MON 89788, using a combination of isoelectric precipitation, ammonium sulfate fractionation, hydrophobic interaction chromatography, anion exchange chromatography, and cellulose phosphate affinity chromatography.

Approximately one kilogram of pre-chilled MON 89788 grain material was ground and defatted in hexane, air-dried, and stored in a −80°C freezer prior to protein extraction. The ground and defatted material (100 g) was mixed in Buffer A [1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 10% (v/v) glycerol, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine-HCl, 0.5 mM PMSF, and 1% (w/v) PVPP, pH 7.4] at 1:50 sample weight to buffer volume ratio. The sample-buffer suspension was homogenized and the crude homogenate was clarified by centrifugation and filtration. The 11S globulin protein in the extract was removed by lowering the pH of the supernatant to 5.5 by addition of ~50.5 ml of 1 N HCl (Liu, 1999). The protein precipitate was removed by

centrifugation followed by filtration. The resultant 4.55 L supernatant was subjected to 40% ammonium sulfate protein fractionation. The solution was stirred and centrifuged, and the remaining supernatant was subject to a 70% ammonium sulfate fractionation. The pellet was collected by centrifugation and was re-suspended in 500 ml of Buffer B [50 mM Tris-HCl, 10% (v/v) glycerol, 0.5 mM PMSF, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine-HCl, 1.25 M ammonium sulfate, pH 7.56]. The supernatant was filtered and the volume was brought to 740 ml with Buffer B.

The sample was loaded onto a 206 ml (5 cm × 10.5 cm column) Phenyl Sepharose 6 Fast Flow (high sub) hydrophobic resin column, which was equilibrated with 5 column volume (CVol) of Buffer B. The unbound proteins were removed with 2 CVol of Buffer B. The bound CP4 EPSPS protein was eluted with a linear salt gradient of 100-40% of Buffer B in 1 CVol followed by a 40-0% gradient of Buffer B in 8 CVol. Fractions containing the CP4 EPSPS protein, identified based on phosphate release activity assay and immunoblot analysis, were pooled to a final volume of ~500 ml. The pooled sample was concentrated and desalted by diafiltration against Buffer C [50 mM Tris-HCl, 10% (v/v) glycerol, 0.5 mM PMSF, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine-HCl, pH 7.5]. The final volume of the concentrated sample was brought to 125 ml, and it was clarified by centrifugation.

The protein solution of ~125 ml was loaded onto an anion exchange column (Source 15Q resin; 45 ml; 2 cm × 14.2 cm column), which was equilibrated with Buffer C prior to sample loading. The resin was washed with 3 CVol of Buffer C and the bound CP4 EPSPS protein was eluted with a linear salt gradient of 0-25% of 1 M NaCl in 4 CVol of Buffer C followed by 25-100% in 3 CVol. Fractions containing CP4 EPSPS protein were identified using SDS-PAGE, immunoblot analysis, and phosphate release activity assays.

Fractions containing the highest amount of CP4 EPSPS protein were buffer exchanged into Buffer D [50 mM MES, 1 mM DTT, 1 mM benzamidine-HCl, 15% (v/v) glycerol, pH 5.8] and applied to a 7 ml pre-cycled cellulose phosphate cation exchange resin (1.6 cm × 3.5 cm column). Prior to sample loading, the cellulose phosphate column was equilibrated with at least 200 ml of Buffer D and the bound protein was eluted with Buffer D, pH 5.8, containing 0.5 mM phosphoenolpyruvate (PEP) and 0.5 mM shikimate-3-phosphate (S3P). The MON 89788-produced CP4 EPSPS protein found in the flow-through fractions and the column wash were pooled and prepared for anion exchange column chromatography. One of the major contaminant proteins was removed by cellulose phosphate affinity column chromatography.

A pooled sample of ~13 ml containing the CP4 EPSPS protein was buffer exchanged against Buffer E (50 mM bis-tris propane, 0.5 mM PMSF, 2 mM DTT, 1 mM EDTA, and 1 mM benzamidine-HCl, pH 8.5) and concentrated to a final volume of 5 ml prior to loading onto a Mono Q column (Amersham, 5/50 GL; 0.5 cm × 5 cm). Unbound proteins were removed with 5 CVol of Buffer E and the bound CP4 EPSPS protein was eluted with Buffer E containing 1 M NaCl with a linear salt gradient of 0-50% in 12 CVol followed by 50-100% in 8 CVol. Fractions containing CP4 EPSPS protein, identified by SDS-PAGE, were pooled and buffer exchanged against Storage Buffer [50 mM Tris-HCl,

50 mM KCl, 25% (v/v) glycerol, 0.5 mM PMSF, 2 mM DTT, 1 mM benzamidine-HCl, pH 7.5]. The volume of the concentrated protein sample was brought to 2.4 ml in Storage Buffer. Prior to the protein characterization, the protein sample was assigned to the APS program as lot 60-100085.

Molecular Weight and Purity Estimation – SDS-PAGE

Aliquots of stock solutions of the MON 89788-produced CP4 EPSPS and reference standard protein were each diluted with 5× loading buffer [312 mM Tris-HCl, 20% (v/v) 2-mercaptoethanol, 10% (w/v) SDS, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8)] and water to a final concentration of 0.2 µg/µL. Molecular weight markers (Bio-Rad broad-range) were diluted to a final total protein concentration of 0.9 µg/µL. The MON 89788-produced CP4 EPSPS protein was analyzed in duplicate at 1, 2, and 3 µg total protein per lane. The *E. coli*-produced CP4 EPSPS reference standard (APS lot 20-100015) was analyzed at 1 µg total protein. All samples were heated at 98-99°C for 5 min and loaded onto a pre-cast tris-glycine 4→20% polyacrylamide gradient 10-well mini-gel (Invitrogen, Carlsbad, CA). Electrophoresis was performed at a constant voltage of 150 V for 78 or 90 min. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) glacial acetic acid for 30 min, stained 16 h with Brilliant Blue G-Colloidal stain (Sigma, St. Louis, MO), destained with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol followed by 25% (v/v) methanol.

Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA). Molecular weight markers were used to estimate the apparent molecular weight of each observed band. All visible bands within each lane were quantified using Quantity One software. For the MON 89788-produced CP4 EPSPS protein, purity was estimated as the percent optical density of the 44 kDa band relative to all bands detected in the lane. Apparent molecular weight and purity were reported as an average of all six loadings containing the MON 89788-produced CP4 EPSPS protein.

Immunoblot Analysis – Immunoreactivity

Aliquots of the stock solutions of the MON 89788-produced CP4 EPSPS protein and reference standard were diluted to a final purity-corrected protein concentration of 0.2 ng/µL in water and in 5× loading buffer. Samples were then heated to ~100°C for 5 min and loaded onto a pre-cast tris-glycine 4→20% polyacrylamide gradient 10-well gel. The MON 89788-produced CP4 EPSPS protein and reference standard protein were loaded at three different loadings of 1, 2, and 3 ng per lane. Electrophoresis was performed at a constant voltage of 140 V for 20 min followed by a constant voltage of 200 V for 47 min. Pre-stained molecular weight markers included during electrophoresis (Bio-Rad Precision Plus Dual Color, Hercules, CA) were used to verify electro-transfer of protein to the membrane and to estimate the molecular weight of the immunoreactive bands. Samples were electrotransferred to a 0.45 micron PVDF membrane (Invitrogen, Carlsbad, CA) for one h at a constant current of 300 mA.

The membrane was blocked for one h with 5% (w/v) NFDM in PBST. The membrane was probed with a 1:4000 dilution of goat anti-CP4 EPSPS antibody (lot 6844572) in 2% (w/v) NFDM in PBST for one hour. Excess antibody was removed by three washes with PBST. The membrane was probed with peroxidase-conjugated rabbit anti-goat IgG (Sigma, St. Louis, MO) at a dilution of 1:10,000 in 2% (w/v) NFDM in PBST for one hour. Excess peroxidase-conjugated IgG was removed by three washes with PBST. Immunoreactive bands were visualized using the ECL detection system (Amersham Biosciences, Piscataway, NJ) and exposed (5 s, 10 s, and 3 min) to Hyperfilm ECL film (Amersham Biosciences, Piscataway, NJ). Films were developed using a Konica SRX-101A automated film processor.

Image analysis of immunoreactive bands on blot films was conducted using a Bio-Rad model GS-800 calibrated imaging densitometer (Hercules, CA) equipped with Quantity One software Version 4.4.0. The intensity of signal detected in each lane was measured as band adjusted intensity (average band OD × band area in mm^2). The percent difference between the MON 89788- and *E. coli*-produced CP4 EPSPS proteins was calculated as shown below:

$$\left|\frac{\left(E.coli\text{ Produced CP4 EPSPS}\right)-\left(\text{Plant Produced CP4 EPSPS}\right)}{\left(E.coli\text{ Produced CP4 EPSPS}\right)}\right|\times 100$$

N-terminal Sequence Analysis

An aliquot of the MON 89788-produced CP4 EPSPS protein was diluted with 5 × loading buffer to a final purity corrected protein concentration of 272 ng/µL. Pre-stained molecular weight markers included during electrophoresis (Bio-Rad Precision Plus Dual Color, Hercules, CA) were used to verify electro-transfer of protein to the membrane and to estimate MW. The MON 89788-produced CP4 EPSPS protein was loaded in five lanes at 5.4 µg (purity corrected) per lane. The CP4 EPSPS containing samples were heated to ~99°C for 4 min prior to electrophoresis on a pre-cast tris-glycine 4→20% SDS polyacrylamide gel at 125V for 90 min. The gel was then electro-blotted to a 0.45 micron PVDF membrane for 90 min at a constant current of 125 mA in a solution containing 10 mM CAPS, 10% (v/v) methanol, pH 11. Protein bands on the membrane were visualized with Ponceau S stain (Sigma).

The protein band that migrated at 44 kDa in each of three lanes was excised individually from the membrane and pooled prior to sequence analysis. N-terminal sequence analysis was performed using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785 Programmable Absorbance Detector and Procise™ Control Software (version 2.1) was used. Chromatographic data were collected using Atlas^{99} software (version 2003R1.1). A PTH-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as peak resolution, peak area and relative amino acid chromatographic retention times. A control protein (β-lactoglobulin, Applied

Biosystems) was analyzed before and after the analysis of the CP4 EPSPS protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity.

MALDI-TOF Analysis

MALDI-TOF mass spectrometry was used to confirm the identity of the MON 89788-produced CP4 EPSPS protein. With sufficient mass accuracy, four tryptic peptides were found to be sufficient to identify a protein (Jiménez et al., 1998).

SDS-PAGE Separation of Proteins: Approximately 5.4 µg of the MON 89788-produced CP4 EPSPS protein along with broad Range molecular weight markers (Bio-Rad, Hercules, CA) were heated to 99°C for 4 min prior to electrophoresis on a pre-cast tris-glycine 4→20% polyacrylamide gel. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) glacial acetic acid for 50 min, stained with Brilliant Blue G-Colloidal stain (Sigma, St. Louis, MO), destained with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, followed by 25% (v/v) methanol.

In-gel Protein Digestion: The stained protein band that migrated at 44 kDa was excised from the gel, destained, reduced, alkylated, and subjected to an in-gel trypsin (Promega, Madison, WI) digestion (Williams et al., 1997). Briefly, each gel band was individually destained by incubation in 100 µL of 40% (v/v) methanol and 10% (v/v) glacial acetic acid. Following destaining, the gel bands were incubated in 100 µL of 100 mM ammonium bicarbonate buffer for 30 min at room temperature. Proteins were reduced in 100 µL of 10 mM dithiothreitol solution for two h at 37°C. Proteins were then alkylated by the addition of 100 µL of buffer containing 200 mM iodoacetic acid. The alkylation reaction was allowed to proceed at room temperature for 20 min in the dark. The gel bands were incubated in 100 µL of 100 mM ammonium bicarbonate buffer for 30 min at room temperature, at which time 100 µL of acetonitrile was added and the incubation was continued for an additional 30 min. The ammonium bicarbonate/acetonitrile incubations were repeated two additional times to remove the reducing and alkylating agents and salts from the gel. The gel was dried in a SpeedVac concentrator (Savant, Holbrook, NY), rehydrated with 40 µL 25 mM ammonium bicarbonate containing 33 µg/ml trypsin, and digested for 16 h at 37°C. Digested peptides were extracted with 50 µL 70% (v/v) acetonitrile containing 0.1% (v/v) TFA. Supernatant from each extraction was combined and dried in a SpeedVac concentrator. This process was repeated two more times, and the dried material was reconstituted in 10 µL of 0.1% (v/v) TFA.

Sample Preparation: A portion (5 µL) of the digested sample was desalted (Bagshaw et al., 2000) using Millipore (Bedford, MA) ZipTip® C18 pipette tips. The mixture of tryptic peptides was applied to a ZipTip C18 and eluted with 5 µL of Wash 1 [0.1% (v/v) TFA], followed by 5 µL of Wash 2 [20% (v/v) acetonitrile containing 0.1% (v/v) TFA], 5 µL of Wash 3 [50% (v/v) acetonitrile containing 0.1% (v/v) TFA], and 5 µL of Wash 4 [90% (v/v) acetonitrile containing 0.1% (v/v) TFA].

MALDI-TOF Instrumentation and Mass Analysis: Mass spectral analyses were performed as follows: mass calibration of the instrument was performed using a peptide

mixture from a Sequazyme™ Peptide Mass Standards kit (Applied Biosystems). Samples (0.3 μL) from each of the desalting steps, as well as a sample of solution taken prior to desalting, were co-crystallized with 0.75 μL α-cyano-4-hydroxy cinnamic acid (Waters, Milford, MA) on the analysis plate. All samples were analyzed in the 500 to 5000 dalton range using 100 shots at a laser intensity setting of 2603-2960 (a unit-less MALDI-TOF instrument specific value). Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). GPMAW32 software (Applied Biosystems, version 4.23) was used to generate a theoretical trypsin digest of the expected CP4 EPSPS protein sequence deduced from the nucleotide sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Experimental masses (MH+) were assigned to peaks in the 500-1000 Da range if there were two or more isotopically resolved peaks, and in the 1000-5000 Da range if there were three or more isotopically resolved peaks in the spectra. Peaks were not assessed if the peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal of ± 2 daltons from the mass analyzed. Known trypsin autocatalytic fragments were also identified in the raw data. The identity of the CP4 EPSPS protein is confirmed if ≥ 40 % of the protein sequence can be identified by matching experimental masses to the expected masses for the fragments.

Functional Activity Assay

This end-point type colorimetric assay measures the release of inorganic phosphate from one of the substrates, PEP, which is released by the action of the EPSPS enzyme. Briefly, reaction mixtures containing the isolated CP4 EPSPS enzyme with S3P were initiated by the addition of PEP. The final reagent concentrations in the assay were 50 mM HEPES (pH 7.0), 0.1 mM ammonium molybdate, 2 mM S3P, 1 mM PEP and 5 mM potassium fluoride. Reactions were incubated for two min at 25°C to allow for product formation. The reactions were quenched with malachite green (phosphate assay reagent) and fixed after two min with 33% (w/v) sodium citrate. The EPSPS-catalyzed release of inorganic phosphate from PEP was determined at a wavelength of 660 nm using a PowerWave X; (Bio-Tek) microplate reader, relative to a standard curve of inorganic phosphate treated with the malachite green (phosphate assay) reagent and 33% (w/v) sodium citrate. For CP4 EPSPS, one unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 μmole of inorganic phosphate from PEP per min at 25°C. Calculations of the specific activities were performed using Microsoft Excel 2000 version 9.0.4402 SR-1. Specific activity values were calculated based on the purity-corrected concentration of the CP4 EPSPS protein. As specified in Monsanto characterization plan, the MON 89788-produced CP4 EPSPS protein was considered equivalent to the *E. coli*-produced CP4 EPSPS protein if the average specific activity was within two-folds of the average specific activity of the *E. coli*-produced protein.

Glycosylation Analysis

Glycosylation analysis was used to determine whether the MON 89788-produced CP4 EPSPS protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 89788-produced CP4 EPSPS and the *E. coli*-produced CP4 EPSPS reference standard (in this instance, a negative control) were diluted in 5 ×

loading buffer and water to a final purity corrected concentration of ~55 ng/µL and 50 ng/µL, respectively. An aliquot of the transferrin protein (positive control) was diluted in 5 × loading buffer and water to a total protein concentration of 50 ng/µL. These samples were heated to ~100.3 °C for five min, and loaded along with Precision Plus Dual Color pre-stained protein molecular weight markers (Bio-Rad, Hercules, CA) and a No Protein Control (loading buffer only) and electrophoresed on a pre-cast tris-glycine 4→20% polyacrylamide gradient 10-well mini-gel. The transferrin and *E. coli*-produced CP4 EPSPS protein were loaded at 0.5 and 1 µg protein per lane, while the MON 89788-produced protein was loaded at 0.6 µg and 1.1 µg protein per lane. Electrophoresis was performed at a constant voltage of 140 V for 20 min followed by a constant voltage of 200 V for 47 min. After electrophoresis, proteins were electrotransferred to a 0.45 micron PVDF membrane for 75 min at a constant current of 300 mA.

Carbohydrate detection was performed directly on the PVDF membrane using the ECL detection system (Amersham Biosciences, Piscataway, NJ). The PVDF membrane was incubated in PBS for 10 min, and transferred to a solution of 100 mM acetate buffer, pH 5.5, containing the oxidation reagent, 10 mM sodium metaperiodate. The membrane was incubated in the dark for 20 min. The oxidation solution was removed from the membrane by two brief rinses followed by three sequential 10 min washes in PBS. The membrane was transferred to a solution of 100 mM acetate buffer, pH 5.5, containing 25 nM biotin hydrazide and incubated for 60 min. Biotin hydrazide solution was removed by washing in PBS as previously described. The membrane was blocked with 5% blocking agent (provided with the ECL detection system) in PBS for 60 min. The blocking solution was removed by washing in PBS as previously described. The membrane was incubated with streptavidin-HRP conjugate (diluted 1:6000) in PBS for 30 min to detect carbohydrate moieties bound to biotin. Excess streptavidin-HRP was removed by washing in PBS as previously described. Bands were visualized using the ECL detection system (Amersham Biosciences). Films were exposed (10 s, 30 s, 1 min, and 3 min) to Hyperfilm ECL film (Amersham Biosciences). Films were developed using a Konica SRX-101A automated film processor.

Results of CP4 EPSPS Molecular Weight Equivalence

The equivalence in apparent molecular weight of the purified MON 89788- and the *E. coli*-produced CP4 EPSPS proteins was demonstrated using SDS-PAGE and stained with Brilliant Blue G-Colloidal stain (Figure C-1). The MON 89788-produced CP4 EPSPS protein migrated with a molecular weight indistinguishable to that of the *E. coli*-produced protein standard analyzed concurrently (Figure C-1, lane 2 vs. lanes 3-8). Based on the comparable electrophoretic mobility, the MON 89788- and *E. coli*-produced CP4 EPSPS proteins were determined to have equivalent apparent molecular weight. The estimated molecular weight is consistent with the calculated molecular weight of 47.6 kDa based on translation of the coding sequence of *cp4 epsps*.

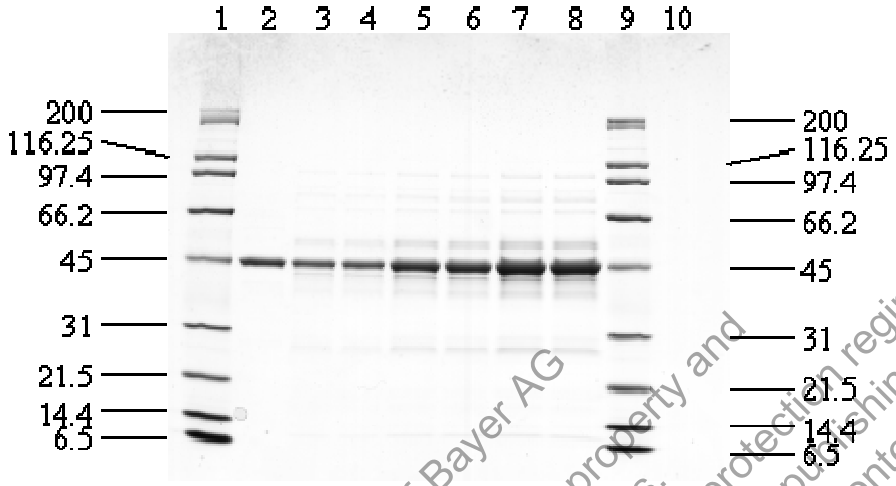

Figure C-1. SDS-PAGE Molecular Weight Analysis of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Aliquots of the purified MON 89788-produced CP4 EPSPS protein, and the *E. coli*-produced CP4 EPSPS reference standard were separated by denaturing tris-glycine 4→20% PAGE and stained with Brilliant Blue G-Colloidal stain. Amounts correspond to total protein loaded per lane. Approximate molecular weights (kDa) correspond to the markers loaded in Lanes 1 and 9.

Lane	Sample	Amount (µg)
1	MW Markers	4.5
2	*E. coli*-produced CP4 EPSPS reference standard	1
3	MON 89788-produced CP4 EPSPS protein	1
4	MON 89788-produced CP4 EPSPS protein	1
5	MON 89788-produced CP4 EPSPS protein	2
6	MON 89788-produced CP4 EPSPS protein	2
7	MON 89788-produced CP4 EPSPS protein	3
8	MON 89788-produced CP4 EPSPS protein	3
9	MW Markers	4.5
10	Empty lane	N/A

Results of CP4 EPSPS Immunoreactivity Equivalence

A western blot analysis using goat anti-CP4 EPSPS serum was conducted to determine the relative immunoreactivity of the MON 89788-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard. Results indicated that the anti-CP4 EPSPS antibody recognized the mature MON 89788-produced CP4 EPSPS protein that migrated identically to the *E. coli*-produced reference standard protein (Figure C-2). Moreover, the immunoreactive signal increased with increasing levels of the CP4 EPSPS protein. The observed immunoreactivities between the MON 89788- and *E. coli*-produced proteins were similar based on densitometric analysis of the western blot. Based on the above analysis, the MON 89788- and *E. coli*-produced CP4 EPSPS demonstrated equivalent immunoreactive properties, which confirmed the identity and equivalence of the two proteins.

Results of N-terminal Sequence Analysis

The N-terminus of the purified MON 89788-produced CP4 EPSPS protein was determined. The resulting sequence matched the predicted CP4 EPSPS N-terminal sequence translated from the *cp4 epsps* coding region (Table C-1, Observed Sequence-1 and 2). The removal of the N-terminal methionine was observed in a fraction of the purified MON 89788-produced protein. This is likely due to cellular enzyme processing in plant (Schmidt et al., 1992). This result is not unexpected as the initial methionine is frequently removed from proteins in eukaryotic organisms by an endogenous methionine aminopeptidase (Arfin and Bradshaw, 1988). Similar findings have been observed in a number of products that have been deregulated by USDA, which include Roundup Ready Flex cotton and Roundup Ready soybean (Harrison, et. al., 1996). This information, therefore, confirms the N-terminal sequence identity of the CP4 EPSPS protein isolated from MON 89788, and that this sequence is consistent with the coding region of the gene.

Results of MALDI-TOF Mass Spectrometry Analysis

The identity of the CP4 EPSPS protein was established using matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry. With appropriate mass accuracy, four tryptic peptides were found to be sufficient to identify a protein (Jiménez et al., 1998). Observed tryptic peptides were considered a match to the expected tryptic mass when differences in molecular weight of less than one Dalton were found between the observed and predicted fragment masses. Such matches were made without consideration for potential natural amino acid modifications such as glycosylation.

Using the aforementioned criteria, the identity of the MON 89788-produced CP4 EPSPS protein was assessed by MALDI-TOF mass spectrometry of chemically reduced and alkylated tryptic fragments prepared from the MON 89788-produced CP4 EPSPS protein. A total of 23 masses matched the expected tryptic digest mass fragments from the deduced amino acid sequence of the CP4 EPSPS protein. The identified masses were used to assemble a coverage map indicating the matched peptide sequences for the entire CP4 EPSPS protein (Figure C-3). This analysis confirmed the identity of the MON 89788-produced CP4 EPSPS protein.

Results of CP4 EPSPS Functional Activity Equivalence

The specific activity of the MON 89788-produced CP4 EPSPS protein was estimated using a phosphate release assay, where one unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 μmole of inorganic phosphate from PEP per minute at 25°C. The *E. coli*- and MON 89788-produced CP4 EPSPS were considered functional equivalent if the specific activity of one protein was within two-fold of the other. Results showed that the estimated specific activity was 3.7 U/mg protein for the MON 89788-produced CP4 EPSPS, and 4.4 U/mg protein for the *E. coli*-produced reference standard. The enzymatic activity assay demonstrated that the MON 89788-produced CP4 EPSPS protein was as active as the *E. coli*-produced reference standard. These results confirmed that these two proteins are functionally equivalent.

Results of CP4 EPSPS Glycosylation Equivalence

As many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher et al., 1988), glycosylation analysis was conducted to further demonstrate the equivalence between *E. coli*- and MON 89788-produced CP4 EPSPS proteins. Since non-virulent *E. coli* strains used for cloning and expression purposes lack the ability to glycosylate endogenous proteins, the *E. coli*-produced CP4 EPSPS was used as the negative control for glycosylation analysis. The positive control was represented by transferrin protein that was known to have multiple covalently linked carbohydrate modifications on each molecule. The transferrin protein, as well as the purified CP4 EPSPS proteins isolated from MON 89788 and *E. coli* were separated on SDS-PAGE, and western blot analysis was performed to detect oxidized carbohydrate moieties on the proteins.

Results of this analysis are presented in Figure C-4. No carbohydrate moieties were detected for CP4 EPSPS protein isolated from either *E. coli* or MON 89788 (lanes 5-6 and lanes 7-8, respectively). As expected, carbohydrate moieties covalently lined to transferrin were detected at the expected transferrin molecular weight of ~75 kDa (lanes 3 and 4). The additional lower molecular weight fragments in lanes 3 and 4 are likely to be the proteolytic fragments of the full-length protein. In addition, a faint band migrating at approximately 44 kDa was observed in lane 5 through lane 8. Since it was established that the *E. coli* strains used in the expression system were non-virulent, and lack the ability to glycosylate recombinant proteins (Letourneur et al., 1995), this faint band observed across *E. coli*- and MON 89788-CP4 EPSPS samples was deemed nonspecific. Taken together, the results demonstrated that, similar to the *E. coli*-produced CP4 EPSPS, the MON 89788-produced CP4 EPSPS protein is not glycosylated. This analysis also confirms the equivalence between the MON 89788- and the *E. coli*-produced CP4 EPSPS reference standard with respect to the status of glycosylation.

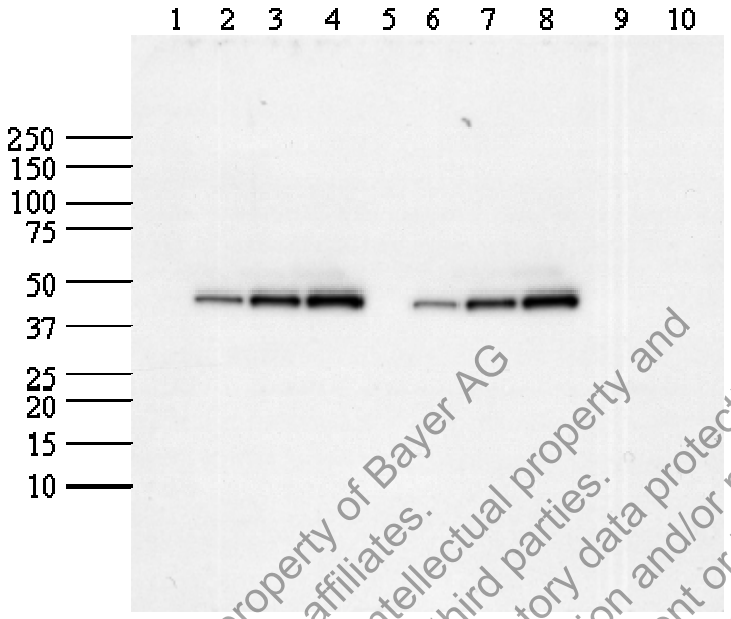

Figure C-2. Immunoblot Analysis of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Aliquots of the purified MON 89788-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were separated by denaturing tris-glycine 4→20% PAGE, electrotransferred to a PVDF membrane and detected using CP4 EPSPS polyclonal antiserum followed by development using the ECL system (10-second exposure shown). Approximate molecular weights (kDa) correspond to the markers loaded in lane 1.

Lane	Sample	Amount of CP4 EPSPS (ng)
1	MW Markers	N/A
2	*E. coli*-produced CP4 EPSPS reference standard	1
3	*E. coli*-produced CP4 EPSPS reference standard	2
4	*E. coli*-produced CP4 EPSPS reference standard	3
5	Empty Lane	N/A
6	MON 89788-produced CP4 EPSPS protein	1
7	MON 89788-produced CP4 EPSPS protein	2
8	MON 89788-produced CP4 EPSPS protein	3
9	Empty lane	N/A
10	Empty lane	N/A

Table C-1. N-terminal Amino Acid Sequence Analysis of the CP4 EPSPS Protein Purified from Grain Tissue of MON 89788

Amino acid residue # from the N-terminus	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Predicted CP4 EPSPS Sequence[1, 2]	M	L	H	G	A	S	S	R	P	A	T
	|	|	|	|	|	|	|	|	|	|	|
Observed Sequence-1[3,4]	M	X	H	G	A	X	S	(R)	(P)	(A)	(T)
Observed Sequence-2[3,4]		L	H	G	A	S	S	(R)	(P)	X	X

1 The predicted amino acid sequence of the CP4 EPSPS protein was deduced from the coding region of the full length *cp4 epsps* gene present in MON 89788.

2 The single letter IUPAC-IUB amino acid code is **A**, alanine; **G**, glycine; **H**, histidine; **L**, leucine; **M**, methionine; **P**, proline; **R**, arginine; **S**, serine, and **T,** threonine.

3 The amino acids in parentheses ( ) were tentatively designated due to high background noises. The undesignated amino acids are shown as "X" due to interferences from other amino acids.

4 Observed sequence-1 and -2 were identified after comparison to the predicted CP4 EPSPS protein sequence.

```
  1  MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL
 51  LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA
101  TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD
151  RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR
201  DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF
251  PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED
301  VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL
351  RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT
401  HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS
451  DTKAA
```

Figure C-3. MALDI-TOF Coverage Map of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Tryptic masses identified by MALDI-TOF are boxed. These identified masses yielded a coverage map equal to 50.3% (229 of 455 amino acids) of the full-length CP4 EPSPS protein, which is considered sufficient to confirm the identity of the MON 89788-produced CP4 EPSPS protein.

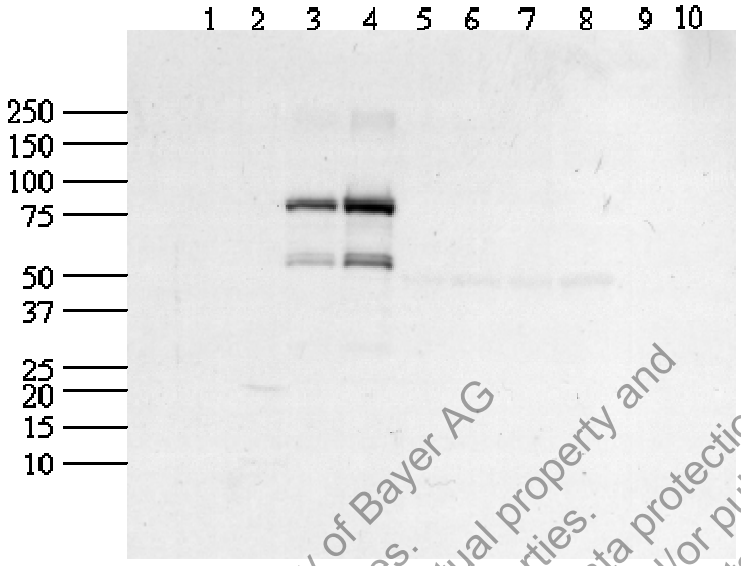

Figure C-4. Glycosylation Analysis of the CP4 EPSPS Protein Isolated from MON 89788 Grain

Aliquots of the MON 89788-produced CP4 EPSPS protein, *E. coli*-produced CP4 EPSPS reference standard (negative control), and transferrin (positive control) were separated by denaturing tris-glycine 4→20% PAGE and electrotransferred to PVDF membrane. Approximate molecular weights (kDa) in the figure correspond to the markers loaded in lane 2. Amount below refers to total protein loaded per lane for transferrin, and purity-corrected protein values for the *E. coli*- and the MON 89788-produced proteins.

Lane	Sample	Amount (µg)
1	No Protein Control	N/A
2	MW Markers (Precision Plus Dual Color)	N/A
3	Transferrin (positive control)	0.5
4	Transferrin (positive control)	1
5	*E. coli*-produced CP4 EPSPS protein (negative control)	0.5
6	*E. coli*-produced CP4 EPSPS protein (negative control)	1
7	MON 89788-produced CP4 EPSPS protein	0.6
8	MON 89788-produced CP4 EPSPS protein	1.1
9	Empty Lane	N/A
10	Empty Lane	N/A

Appendix D. Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 89788

Materials

Tissue samples analyzed in this study were produced from five field sites in the U.S. during 2005 season from seed lot GLP-0504-16045-S for MON 89788 and GLP-0504-16046-S for control. The control line was A3244, which is a conventional variety and does not contain the *cp4 epsps* coding region. Samples were stored in a -80°C freezer throughout the study. An *E. coli*-produced CP4 EPSPS protein (Monsanto APS lot # 20-100015) was used as a reference standard for the assay.

Characterization of the Materials

All samples were verified by either the chain-of-custody documentation or an event-specific PCR method. Three MON 89788 grain samples (one each from the IL-1, IL-2, and NE sites) contained less than or equal to 3.05% of the Roundup Ready soybean, and the samples were included for analyses as the low level of impurity would not impact the integrity of the study. However, two control grain samples from the IL-1 site also contained the Roundup Ready soybean, and these two samples along with their associated tissues were not analyzed.

Field Design and Tissue Collection

Field trial was initiated during the 2005 growing season at five locations in the U.S. to generate the MON 89788 and control substances. The field locations were: York County, Nebraska (NE), Clinton County, Illinois (IL-1), Warren County, Illinois (IL-2), Jackson County, Arkansas (AR), and Fayette County, Ohio (OH). The production sites were located within major soybean growing regions, and they provided a range of environmental and agronomic conditions representative of eventual MON 89788 commercial production. At each location, three replicated plots of MON 89788 and control were planted using a randomized complete block field design. Over-season leaf (OSL1, OSL2, OSL3, and OSL4), grain, root, and forage tissues were collected from each replicated plot at all field locations. Samples were tracked throughout the field production using unique sample identifiers and proper chain-of-custody documentation. Upon collection, all samples were placed in uniquely labeled bags or containers. Over-season leaf, root, and forage tissue samples were stored on dry ice and shipped frozen on dry ice to Monsanto's processing facility in Creve Coeur, MO. Grain samples were stored and shipped at ambient temperature.

Over-season leaf tissue samples were collected from the youngest set of fully expanded trifoliate leaves at the following growth stages: OSL1 at the V3-V4 growth stage; OSL2 at the V6-V8 growth stage; OSL3 at the V10-V12 growth stage; and OSL4 at the V14-V16 growth stage. The root and forage tissues were collected at approximately the R6 growth stage, and the above-ground portion of the plant was labeled as the forage, and the below ground portion was washed and labeled as root tissue. Grain samples were collected at the R8 growth stage.

Tissue Processing and Protein Extraction

All samples produced at the field sites were shipped to Monsanto's processing facility in Creve Coeur, MO. During the processing step, dry ice was combined with the individual samples, and vertical cutters or mixers were used to thoroughly grind and mix the tissues. Processed samples were transferred into capped 15 ml tubes and stored in a -80°C freezer until use.

The CP4 EPSPS protein was extracted from all tissues using a Harbil mixer and the appropriate amount of Tris-borate buffer with L-ascorbic acid (TBA) [0.1 M Tris, 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v/v) Tween-20 at pH 7.8, and 0.2% (w/v) L-ascorbic acid]. Insoluble material was removed from the extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The clarified extracts were aliquot, and stored frozen in a -80°C freezer until ELISA analysis.

Anti-CP4 EPSPS Antibodies

The capture antibody was mouse monoclonal antibody clone 39B6 (IgG2a isotype, kappa light chain; lot 6199732) specific for CP4 EPSPS protein, and was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography. The production of the 39B6 IgG2a monoclonal antibody was performed by TSD Bioservices, Inc. (Newark, DE), and the concentration of the purified IgG2a was 3.2 mg/ml. The purified antibody was stored in a buffer containing 0.02 M $Na_2HPO_4 \cdot 7H_2O$, 0.15 M NaCl, and 15 ppm ProClin 300, pH 7.2. The detection reagent was goat anti-CP4 EPSPS polyclonal antibodies (Sigma, St. Louis, MO) conjugated to horseradish peroxidase (HRP).

CP4 EPSPS ELISA Method

The CP4 EPSPS ELISA was performed using an automated robotic workstation (Tecan, Research Triangle Park, NC). Mouse anti-CP4 EPSPS antibody was diluted in coating buffer [0.015 M Na_2CO_3, 0.035 M $NaHCO_3$, and 0.15 M NaCl, pH 9.6] at 1.0 µg/ml and immobilized onto 96-well microtiter plates, followed by incubation in a 4°C refrigerator for $\geq$ 12 h. Plates were washed with phosphate buffered saline (PBS) with 0.05% (v/v) Tween-20 (PBST), followed by the addition of CP4 EPSPS protein standard or sample extract at 100 µl per well, and incubated at 37°C for 1 h. Plates were washed with PBST, followed by the addition of goat anti-CP4 EPSPS peroxidase conjugate at 100 µl per well, and incubated at 37°C for 1 h. Plates were washed with PBST, and developed by adding TMB substrate (3,3',5,5'- tetramethyl-benzidine, Kirkegaard & Perry, Gaithersburg, MD) at 100 µl per well. The enzymatic reaction was terminated by the addition of 100 µl of 6 M H_3PO_4 per well. Quantitation of CP4 EPSPS protein levels was accomplished by interpolation from a CP4 EPSPS protein standard curve that spanned 0.456 - 14.6 ng/ml.

Moisture Analysis

A homogeneous, tissue-specific site pool (TSSP) was prepared by mixing comparable amounts (on a volumetric basis) of at least four test and control samples from each field location. Pools were prepared for all tissue types analyzed in this study. All tissues were analyzed for moisture content using an IR 200 Infrared Moisture Analyzer (Denver Instrument Company, Arvada, CO). The mean percent moisture for each TSSP was

calculated from three analyses of a given pool and used to convert the fresh weight values for the test and control substances at each site to dry weight values. A tissue-specific Dry Weight Conversion Factor (DWCF) was calculated as follows:

DWCF = 1 - [Mean Percent TSSP Moisture / 100]

The DWCF was only applied to samples with protein quantities greater than the assay limits of quantitation (LOQ). All protein values calculated on a fresh weight basis were converted into protein values reported on a dry weight basis using the following calculation.

$$Protein\ Level\ in\ Dry\ Weight = \frac{(Protein\ Level\ in\ Fresh\ Weight)}{(DWCF)}$$

Data Analyses

All ELISA plates were analyzed on a SPECTRAFluor Plus microplate reader (Tecan, Research Triangle Park, NC) using dual wavelengths. The CP4 EPSPS protein absorbance readings were determined at a wavelength of 450 nm with a simultaneous reference reading of 620 nm that was subtracted from the 450 nm reading. Data analysis was performed using Molecular Devices SOFTmax PRO version 2.4.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a µg/g FW basis. This conversion utilized the sample dilution factor and tissue-to-buffer ratio. The protein quantities in µg/g FW were also converted to µg/g DW by applying the DWCF. The arithmetic mean, standard deviation (SD), and range (FW and DW) were calculated for each tissue type across locations. Microsoft Excel 2002 (Version 10.6730.6735 SP3, Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS protein quantities in all tissues from MON 89788.

Appendix E. Materials and Methods Used for Compositional Analysis of MON 89788 Soybean Grain and Forage from Five Replicated Field Sites

Materials

MON 89788, A3244 and conventional reference soybeans were grown at five U.S. locations in 2005. MON 89788 and A3244 were grown from seed lots GLP-0504-16045-S and GLP-0504-16046-S, respectively. The control material, A3244, has background genetics representative of MON 89788 but does not contain the *cp4 epsps* coding sequence or produce the CP4 EPSPS protein. In addition, twelve conventional soybean varieties produced alongside of MON 89788 were included for the generation of 99% tolerance interval. The varieties, locations, and seed lot numbers are listed below:

Variety	Starting Seed Lot Number	Field Site
Stine/ST3600	REF-0409-15515-S	AR
Stine/ST3870	REF-0409-15516-S	AR
Asgrow/A3525	REF-0409-15502-S	IL-1
Asgrow/A3559	REF-0504-16051-S	IL-1
Asgrow/A2553	REF-0504-16052-S	IL-2
Asgrow/A3204	REF-0409-15509-S	IL-2
Stine/ST2788	REF-0409-15512-S	IL-2
Asgrow/A2804	REF-0504-16048-S	NE
Stine/ST3300	REF-0409-15514-S	NE
Asgrow/A2704	REF-0504-16053-S	OH
Stine/ST2800	REF-0409-15513-S	OH
Asgrow/A2833	REF-0504-16056-S	OH

Characterization of the Materials

The identities of the MON 89788, A3244, and reference soybean varieties were verified prior to use by examination of the chain-of-custody documentation. Additionally, the identities of the MON 89788 and A3244 grain samples were confirmed by event-specific PCR analysis to determine the presence or absence of MON 89788.

Field Production of the Samples

The field design and tissue collection process have been described previously in Appendix C with the addition of reference varieties as described above. A total of twelve different conventional soybean varieties were planted at five field locations with two to three different varieties grown at each site. Fields were managed with normal agronomic practices for soybean, and plots containing MON 89788 were treated with a commercial rate of Roundup agricultural herbicide.

Summary of Analytical Methods

Soybean grain and forage samples from MON 89788, A3244, and conventional reference materials were shipped overnight on dry ice to Covance Laboratories Inc., Madison, Wisconsin, for compositional analyses. Analyses were performed using methods that are currently used to evaluate the nutritional quality of food and feed.

The following analyses were performed on **forage** samples:

Analyte	Method Mnemonic[1]
Proximates	
Moisture	M100
Protein	PGEN
Fat	FAAH
Ash	ASHM
Acid detergent fiber	ADF
Neutral detergent fiber	NDFE

[1]analytical methods were kept on file at Covance Laboratories Inc.

The following analyses were performed on the **grain** samples:

Analyte	Method Mnemonic[1]
Proximates	
Moisture	M100
Protein	PGEN
Fat	FSOX
Ash	ASHM
Acid Detergent Fiber	ADF
Neutral Detergent Fiber	NDFE
Amino Acid composition	TAAP
Fatty Acid profile (C8-C22)	FAPM
Trypsin Inhibitor	TRIP
Lectin	LECT
Isoflavones	ISOF
Phytic acid	PHYT
Stachyose/Raffinose	SUGT
Vitamin E (alpha-tocopherol)	LCAT

[1]analytical methods were kept on file at Covance Laboratories Inc.

In addition, carbohydrate (CHO) values were estimated by calculation. The methods are described below:

Acid Detergent Fiber (ADF) The method was based on a USDA Agriculture Handbook No. 379 (1970) method. The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. Lignocellulose fraction was collected on

the frit and determined gravimetrically. The limit of quantitation for this study was 0.100%.

Amino Acid Composition (TAAP) The method used was based on AOAC International (2000a) method 982.30 that estimates the levels of 18 amino acids in the sample: alanine, arginine, aspartic acid (including asparagine), cystine (including cysteine), glutamic acid (including glutamine), glycine, histidine, isoleucine, leucine, lysine, methoinine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer. The limit of quantitation for this study was 0.1 mg/g FW. The reference standards were Beckman, K18, 2.5 µmol/mL per constituent (except cystine 1.25 µmol/mL), Lot Number S504255; Sigma, L-Tryptophan, >99% (used as 100%), Lot Number 063K0382; Fluka, L-Cysteic Acid Monohydrate, 99.9% (used as 100%), Lot Number 1157629; Sigma, L-Methionine Sulfone, >99% (used as 100%), Lot Number 012H3349

Ash (ASHM) The method used was based on AOAC International (2000b) method 923.03. The sample was placed in an electric furnace at 550 °C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of quantitation for this study was 0.1% FW.

Carbohydrates (CHO) The method used was based on an USDA Agriculture Handbook No. 74 (1973) method. The limit of quantitation for this study was 0.1% FW. The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

% carbohydrates = 100% - (% protein + % fat + % moisture + % ash)

Fat by Acid Hydrolysis (FAAH) The method used was based on AOAC International (2000c) method 922.06 and 954.02. The sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted using ether and hexane. The extract was washed with a dilute alkali solution, then evaporated under nitrogen, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again under nitrogen, dried, and weighed. The limit of quantitation for this study was 0.100%.

Fat by Soxhlet Extraction (FSOX) The method used was based on AOAC International (2000d) method 960.39. The sample was weighed into a cellulose thimble containing sand or sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. The limit of quantitation for this study was 0.1% FW.

Fatty Acids (FAPM) The method used was based on AOCS (1997a) method Ce 1-62 that estimates the levels of C8-C22 fatty acids in the samples. The lipid was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride:methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The limit of quantitation for this study was 0.00300%.

Reference Standards:
Nu Chek Prep GLC Reference Standard Hazelton No. 1, used as 100%, Lot AU22-P
Nu Chek Prep GLC Reference Standard Hazelton No. 2, used as 100%, Lot M13-0
Nu Chek Prep GLC Reference Standard Hazelton No. 3, used as 100%, Lot MA13-0
Nu Chek Prep GLC Reference Standard Hazelton No. 4, used as 100%, Lot JA13-P
Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot U-63M-J1-P
Sigma Methyl Tridecanoate, used as 100%, Lot 035K1392

Isoflavones Analysis (ISOF) The method is based on Seo and Morr (1984) and Pettersson and Kiessling (1984). The sample was extracted using a solution of hydrochloric acid and reagent alcohol heated on steam baths or hot plates. The extract was brought to volume, diluted, and centrifuged. An aliquot of the supernatant was placed onto a C18 solid-phase extraction column. Unwanted components of the matrix were rinsed off with 20% methanol and then the isoflavones were eluted with 80% methanol. The sample was analyzed on a high-performance liquid chromatography system with ultraviolet spectrophotometric quantitation and was compared against an external standard curve of known standards. The limit of quantitation for each component was 10.0 mcg/g.

Reference Standards:
Indofine, daidzein, 99+%[1], lot number 020508146
Indofine, genistein, 99+%[1], lot number 0103070
Indofine, Glycitein, 99%[1], Lot Number 0310189
Note: [1]Used as 100% in calculations

Lectin (LECT) The method used was based on Klurfeld and Kritchevsky (1987) and Liener (1955). The sample was suspended in phosphate buffered saline (PBS), shaken, and filtered. An aliquot of the resulting extract was serially diluted in 10 cuvettes containing PBS. A 10% hematocrit of lyophilized rabbit blood in PBS was added to each dilution. After 2.5 hours, the absorbance of each dilution of the sample and lectin control was read by a spectrophotometer at 620 nm, using PBS to zero the instrument. One hemagglutinating unit (H.U.) was defined as the level that caused 50% of the standard cell suspension to sediment in 2.5 hours. The limit of quantitation for this study was 0.10 H.U./mg based on a 2 g equivalent sample.

Moisture (M100) The method used was based on AOAC International (2000e) methods 926.08 and 925.09. The sample was dried in a vacuum oven at 100 °C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of quantitation for this study was 0.1% FW.

Neutral Detergent Fiber, Enzyme Method (NDFE) The method used was based on AACC (1998) methods 32.20 and a USDA Agriculture Handbook No. 379 (1970) method. Samples were placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically. The limit of quantitation for this study was 0.1% FW.

Phytic Acid (PHYT) The method used was based on Lehrfeld (1989 and 1994). The sample was extracted using 0.5M HCl with ultrasonication. Purification and concentration was done on a silica based anion exchange (SAX) column. Sample analysis was done on a macroporous polymer HPLC column PRP-1, 5µm (150 x 4.1mm) and a refractive index detector. The limit of quantitation for this study was approximately 0.100%. Reference Standard was: Aldrich, Phytic Acid Dodecasodium Salt Hydrate, 95%, Lot Number 01913EC

Protein (PGEN) The method used was based on AOAC International (2000f) methods 955.04 and 979.09 and two literature methods (Bradstreet, 1965; Kalthoff and Sandell, 1948). Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a standard acid. The percent nitrogen was calculated and converted to protein using the factor 6.25. The limit of quantitation for this study was 0.100%.

Raffinose and Stachyose (SUGT) The method is based on Mason and Slover (1971) and Brobst (1972). After extraction from the sample with deionized water, the sugars were treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl- β -D-glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane (HMDS) and trifluoracetic acid (TFA) and analyzed by gas chromatography using a flame ionization detector. The limit of quantitation for this study was calculated out to be a range of 0.179-3.571% for a 4/5 dilution. Reference Standards: Sigma, Raffinose Pentahydrate, 99%/84.0% after correction for degree of hydration, Lot Number 073K0938; Sigma, Stachyose, 99%/95.4% after correction for degree of hydration, Lot Number 103K3776

Trypsin Inhibitor (TRIP) The method is based on AOCS (1997b). The sample was ground and/or defatted with petroleum ether, if necessary. A sample of matrix was extracted for 3 hours with 0.1N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoy1-DL-arginine~p~nitroana1ide hydrochloride. The sample was allowed to react for 10 minutes at 37°C. After 10 minutes, the reaction was halted by the addition of acetic acid. The solution was filtered or centrifuged, then the absorbance was determined at 410 nm. Trypsin inhibitor unit (TIU) was determined by photometrically measuring the inhibition of trypsin's reaction with benzoyl-DL-arginine-p-nitroanalide hydrochloride. The limit of quantitation for this study was 1.00 Trypsin Inhibitor Unit/mg.

Vitamin E (LCAT) The method used was based on three literature methods (Cort et al., 1983; Speek et al., 1985; McMurray et al., 1980). The sample was saponified to break down any fat and release any vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated directly by high-performance liquid chromatography on a silica column. The limit of quantitation for this study was approximately 0.005 mg/100g. Reference Standard: USP, Alpha Tocopherol, 100%, Lot Number M.

Data Processing and Statistical Analysis

After compositional analyses were performed at Covance Laboratories Inc., data spreadsheets containing individual values for each analysis were sent to Monsanto Company for review. Data were then transferred to Certus International where they were converted into the appropriate units and statistically analyzed. The following formulas were used for re-expression of composition data for statistical analysis:

Component	From (X)	To	Formula
Proximates (excluding Moisture), Fiber, Phytic Acid, Raffinose, Stachyose	% FW	% DW	X/d
Isoflavones	µg/g FW	µg/g DW	X/d
Trypsin Inhibitor	TIU/mg FW	TIU/mg DW	X/d
Vitamin E	mg/100g FW	mg/100g DW	X/d
Amino Acids (AA)	mg/g FW	% DW	X/(10*d)
Fatty Acids (FA)	% FW	% DW	X/d

[1]d is the fraction of the sample that is dry matter.

Across samples, analytes with greater than fifty percent of observations below the assay's limit of quantitation (LOQ) were excluded from summaries and analysis. Otherwise, results below the quantitation limit were assigned a value equal to half the quantitation limit. No analytes were assigned values in this study. The following 14 analytes with >50% of observations below the LOQ of the assay were excluded from statistical analysis: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. Studentized PRESS residuals revealed the absence of outliers. No data was excluded from the statistical analyses. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point.

Statistical analyses were conducted on the converted values for each component in the soybean grain and forage using a mixed model analysis of variance for the six sets of comparisons: analysis for each of the five replicated trial sites (AR, IL-1, IL-2, NE, OH), and one for the combination of all five sites. There were a total of 49 components statistically evaluated (the initial 63 analytes minus the 14 for which >50% of the observations were below the LOQ). A total of 294 comparisons were made: 49 components with six statistical analyses each.

At the field sites, the MON 89788, A3244 and references substances were grown in single plots randomly assigned within each of three replication blocks. The compositional components for the test and control substances were statistically analyzed using a mixed model analysis of variance. The five replicated sites were analyzed both separately and combined across sites. Individual replicated site analyses used the model:

$$Y_{ij} = U + T_i + B_j + e_{ij} ,$$

where Y_{ij} = unique individual observation, U = overall mean, T_i = substance effect, B_j = random block effect, and e_{ij} = residual error.

Combined-site analyses used the model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk} ,$$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = substance effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by substance interaction effect, and e_{ijk} = residual error. For each compositional component, the values obtained for the forage and grain from the test substance were compared to the conventional control.

A range of observed values from the reference substances was determined for each analytical component. Additionally, the reference substances data were used to develop population tolerance intervals. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial references. Each tolerance interval estimate was based upon one observation per unique reference substance. Individual references with multiple observations were averaged within sites to obtain a single estimate for inclusion in tolerance interval calculations. Because negative quantities are not possible, calculated negative lower tolerance bounds were set to zero. SAS® software was used to generate all summary statistics and perform all analyses (SAS Software Release 9.1, 2002-2003). Report tables present p-values from SAS® as either <0.001 or the actual value truncated to three decimal places.

Table E-1. Statistical Summary of Combined-Site Soybean Forage Fiber and Proximate Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fiber						
Acid Detergent Fiber (% DW)	36.82 (2.35) [30.95 - 45.99]	38.23 (2.37) [31.18 - 50.89]	-1.41 (1.88) [-11.96 - 4.12]	-6.63, 3.81	0.494	(29.64 - 50.69) [19.03, 54.55]
Neutral Detergent Fiber (% DW)	36.37 (0.80) [32.77 - 41.12]	38.25 (0.86) [32.69 - 43.14]	-1.88 (1.17) [-9.45 - 6.95]	-4.29, 0.53	0.121	(31.43 - 43.70) [26.89, 46.89]
Proximate						
Ash (% DW)	6.76 (0.38) [5.20 - 8.45]	6.65 (0.39) [5.28 - 7.95]	0.11 (0.36) [-1.40 - 2.10]	-0.90, 1.12	0.775	(5.36 - 8.36) [3.50, 9.58]
Carbohydrates (% DW)	67.28 (1.06) [61.61 - 71.00]	67.40 (1.08) [64.55 - 72.30]	-0.12 (0.55) [-3.34 - 4.46]	-1.30, 1.07	0.837	(62.57 - 72.28) [55.96, 77.90]
Fat (% DW)	5.87 (0.70) [4.20 - 9.49]	6.11 (0.70) [3.96 - 8.60]	-0.24 (0.17) [-0.93 - 0.88]	-0.60, 0.12	0.176	(3.51 - 9.87) [0, 14.70]
Moisture (% FW)	72.07 (1.25) [67.90 - 77.60]	73.21 (1.25) [69.90 - 77.60]	-1.14 (0.21) [-2.60 - 0]	-1.72, -0.55	0.006	(68.50 - 78.40) [60.84, 83.36]
Protein (% DW)	20.08 (0.51) [18.41 - 23.50]	19.79 (0.52) [17.47 - 22.18]	0.29 (0.47) [-3.75 - 2.34]	-1.00, 1.58	0.572	(16.48 - 22.78) [13.55, 25.95]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table E-2. Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Alanine (% DW)	1.77 (0.017) [1.56 - 1.87]	1.77 (0.018) [1.71 - 1.83]	-0.0035 (0.018) [-0.19 - 0.069]	-0.042, 0.035	0.845	(1.62 - 1.89) [1.51, 2.00]
Arginine (% DW)	3.06 (0.082) [2.73 - 3.31]	3.07 (0.083) [2.76 - 3.34]	-0.0095 (0.037) [-0.26 - 0.33]	-0.090, 0.071	0.801	(2.61 - 3.27) [2.27, 3.60]
Aspartic Acid (% DW)	4.73 (0.068) [4.20 - 5.08]	4.72 (0.070) [4.42 - 4.98]	0.0072 (0.045) [-0.41 - 0.33]	-0.090, 0.10	0.875	(4.21 - 5.02) [3.85, 5.44]
Cystine (% DW)	0.62 (0.0084) [0.58 - 0.67]	0.62 (0.0085) [0.59 - 0.65]	-0.00028 (0.0050) [-0.044 - 0.026]	-0.011, 0.010	0.955	(0.57 - 0.65) [0.55, 0.67]
Glutamic Acid (% DW)	7.53 (0.12) [6.69 - 8.20]	7.49 (0.13) [6.97 - 7.90]	0.035 (0.075) [-0.63 - 0.53]	-0.13, 0.20	0.647	(6.62 - 8.19) [5.86, 8.96]
Glycine (% DW)	1.78 (0.020) [1.58 - 1.88]	1.78 (0.021) [1.71 - 1.86]	0.0012 (0.018) [-0.18 - 0.11]	-0.037, 0.040	0.949	(1.62 - 1.90) [1.46, 2.05]
Histidine (% DW)	1.07 (0.014) [0.95 - 1.13]	1.07 (0.015) [1.02 - 1.13]	-0.0035 (0.0099) [-0.10 - 0.057]	-0.025, 0.018	0.729	(0.96 - 1.13) [0.90, 1.21]
Isoleucine (% DW)	1.83 (0.029) [1.65 - 1.97]	1.83 (0.031) [1.70 - 1.99]	-0.0092 (0.030) [-0.22 - 0.26]	-0.071, 0.053	0.760	(1.64 - 2.00) [1.44, 2.16]

Table E-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Leucine (% DW)	3.18 (0.040) [2.81 - 3.39]	3.18 (0.042) [3.04 - 3.33]	-0.0024 (0.031) [-0.32 - 0.20]	-0.070, 0.065	0.940	(2.89 - 3.42) [2.62, 3.66]
Lysine (% DW)	2.62 (0.025) [2.33 - 2.76]	2.62 (0.026) [2.51 - 2.73]	-0.00003 (0.023) [-0.25 - 0.13]	-0.051, 0.050	0.998	(2.40 - 2.77) [2.22, 2.95]
Methionine (% DW)	0.52 (0.0059) [0.47 - 0.56]	0.53 (0.0062) [0.50 - 0.55]	-0.0081 (0.0060) [-0.040 - 0.032]	-0.021, 0.0049	0.200	(0.45 - 0.56) [0.42, 0.60]
Phenylalanine (% DW)	2.10 (0.030) [1.84 - 2.24]	2.10 (0.031) [2.00 - 2.19]	-0.0011 (0.021) [-0.21 - 0.14]	-0.047, 0.045	0.959	(1.90 - 2.29) [1.70, 2.45]
Proline (% DW)	2.05 (0.029) [1.81 - 2.21]	2.05 (0.029) [1.95 - 2.16]	0.0047 (0.020) [-0.18 - 0.12]	-0.039, 0.048	0.819	(1.86 - 2.23) [1.66, 2.38]
Serine (% DW)	2.23 (0.029) [1.93 - 2.42]	2.21 (0.030) [2.08 - 2.28]	0.019 (0.023) [-0.16 - 0.17]	-0.031, 0.069	0.432	(1.99 - 2.42) [1.84, 2.54]
Threonine (% DW)	1.58 (0.014) [1.42 - 1.68]	1.59 (0.015) [1.51 - 1.66]	-0.0073 (0.013) [-0.13 - 0.062]	-0.035, 0.020	0.573	(1.44 - 1.67) [1.38, 1.76]
Tryptophan (% DW)	0.39 (0.015) [0.34 - 0.44]	0.39 (0.015) [0.33 - 0.46]	-0.0025 (0.015) [-0.10 - 0.064]	-0.044, 0.039	0.875	(0.30 - 0.47) [0.25, 0.54]

Table E-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.41 (0.019) [1.25 - 1.48]	1.42 (0.020) [1.33 - 1.47]	-0.0091 (0.015) [-0.12 - 0.070]	-0.051, 0.033	0.582	(1.28 - 1.51) [1.18, 1.64]
Valine (% DW)	1.91 (0.035) [1.73 - 2.05]	1.93 (0.036) [1.77 - 2.11]	-0.017 (0.032) [-0.24 - 0.28]	-0.084, 0.051	0.615	(1.71 - 2.09) [1.51, 2.27]
Fatty Acid (% DW)						
16:0 Palmitic (% DW)	2.07 (0.094) [1.84 - 2.40]	2.07 (0.094) [1.71 - 2.46]	-0.0027 (0.052) [-0.21 - 0.24]	-0.14, 0.14	0.961	(1.66 - 2.35) [1.32, 2.64]
18:0 Stearic (% DW)	0.78 (0.027) [0.65 - 0.89]	0.77 (0.027) [0.61 - 0.86]	0.012 (0.018) [-0.053 - 0.14]	-0.036, 0.060	0.531	(0.63 - 1.07) [0.37, 1.28]
18:1 Oleic (% DW)	3.53 (0.14) [3.05 - 4.24]	3.54 (0.14) [2.92 - 4.09]	-0.015 (0.10) [-0.40 - 0.51]	-0.29, 0.26	0.890	(2.99 - 5.29) [2.06, 6.43]
18:2 Linoleic (% DW)	9.17 (0.47) [8.00 - 10.42]	9.25 (0.47) [7.42 - 11.29]	-0.079 (0.21) [-0.86 - 0.99]	-0.64, 0.48	0.720	(8.41 - 10.69) [7.75, 11.22]
18:3 Linolenic (% DW)	1.29 (0.063) [1.09 - 1.48]	1.30 (0.063) [1.09 - 1.60]	-0.0059 (0.028) [-0.13 - 0.15]	-0.082, 0.070	0.843	(1.02 - 1.55) [0.84, 1.69]
20:0 Arachidic (% DW)	0.061 (0.0026) [0.049 - 0.071]	0.060 (0.0026) [0.046 - 0.068]	0.0012 (0.0016) [-0.0048 - 0.012]	-0.0031, 0.0055	0.482	(0.046 - 0.076) [0.031, 0.094]

Table E-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Fatty Acid (% DW)						
20:1 Eicosenoic (% DW)	0.042 (0.0031) [0.032 - 0.050]	0.042 (0.0031) [0.029 - 0.053]	0.00036 (0.0013) [-0.0062 - 0.0073]	-0.0032, 0.0039	0.796	(0.030 - 0.057) [0.021, 0.065]
22:0 Behenic (% DW)	0.063 (0.0030) [0.050 - 0.072]	0.062 (0.0031) [0.046 - 0.071]	0.00094 (0.0014) [-0.0056 - 0.0096]	-0.0029, 0.0048	0.539	(0.046 - 0.073) [0.034, 0.091]
Fiber						
Acid Detergent Fiber (% DW)	18.01 (0.94) [14.64 - 23.94]	17.46 (0.95) [14.39 - 22.44]	0.54 (1.21) [-3.22 - 5.67]	-2.79, 3.88	0.676	(13.30 - 26.26) [9.62, 28.57]
Neutral Detergent Fiber (% DW)	18.18 (0.46) [16.38 - 20.49]	19.11 (0.48) [15.60 - 20.73]	-0.93 (0.60) [-3.35 - 2.77]	-2.34, 0.49	0.165	(14.41 - 23.90) [13.26, 26.33]
Isoflavones						
Daidzein (ug/g DW)	993.67 (114.34) [631.32 - 1571.41]	1073.57 (114.79) [747.53 - 1526.23]	-79.90 (30.47) [-272.18 - 106.63]	-146.14, -13.66	0.021	(274.88 - 1485.52) [0, 1925.63]
Genistein (ug/g DW)	797.90 (49.93) [565.26 - 996.66]	824.83 (50.35) [651.01 - 1003.02]	-26.93 (19.52) [-151.16 - 74.36]	-69.66, 15.81	0.193	(354.09 - 984.29) [0, 1387.95]
Glycitein (ug/g DW)	91.77 (9.88) [53.78 - 162.52]	102.61 (10.01) [72.93 - 148.31]	-10.84 (4.69) [-32.97 - 30.19]	-20.98, -0.70	0.037	(52.72 - 298.57) [0, 287.45]
Proximate						
Ash (% DW)	5.04 (0.12) [4.66 - 5.60]	5.03 (0.12) [4.75 - 5.46]	0.0099 (0.073) [-0.81 - 0.42]	-0.14, 0.16	0.892	(4.61 - 5.57) [4.00, 6.08]

Table E-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Proximate						
Carbohydrates (% DW)	37.07 (0.54) [35.01 - 40.24]	36.88 (0.56) [35.17 - 40.74]	0.20 (0.55) [-2.38 - 2.95]	-1.30, 1.69	0.738	(32.75 - 40.98) [27.86, 45.79]
Fat (% DW)	17.57 (0.74) [15.35 - 19.98]	17.72 (0.74) [14.40 - 20.91]	-0.15 (0.42) [-1.74 - 1.73]	-1.28, 0.99	0.745	(15.97 - 20.68) [15.38, 21.95]
Moisture (% FW)	7.76 (0.47) [6.41 - 9.35]	7.51 (0.47) [6.51 - 9.63]	0.25 (0.27) [-0.44 - 1.31]	-0.51, 1.01	0.417	(6.24 - 9.11) [4.64, 9.94]
Protein (% DW)	40.32 (0.72) [37.31 - 42.54]	40.38 (0.73) [36.96 - 42.44]	-0.069 (0.31) [-1.72 - 2.44]	-0.74, 0.60	0.828	(36.48 - 43.35) [31.50, 47.45]
Vitamin						
Vitamin E (mg/100g DW)	2.71 (0.22) [1.88 - 3.72]	2.52 (0.22) [1.58 - 3.07]	0.19 (0.065) [-0.23 - 0.66]	0.043, 0.33	0.015	(1.29 - 4.80) [0, 7.00]
Antinutrient						
Lectin (H.U./mg FW)	4.29 (0.97) [0.70 - 9.77]	4.55 (1.01) [1.44 - 10.87]	-0.26 (1.02) [-8.11 - 5.75]	-2.38, 1.86	0.800	(0.45 - 9.95) [0, 9.72]
Phytic Acid (% DW)	0.76 (0.035) [0.58 - 0.93]	0.75 (0.037) [0.51 - 1.07]	0.011 (0.044) [-0.24 - 0.30]	-0.084, 0.11	0.811	(0.41 - 0.96) [0.39, 1.07]
Raffinose (% DW)	0.52 (0.063) [0.40 - 0.71]	0.54 (0.063) [0.31 - 0.83]	-0.014 (0.041) [-0.20 - 0.11]	-0.13, 0.099	0.751	(0.26 - 0.84) [0, 1.01]

Table E-2 (continued). Statistical Summary of Combined-Site Soybean Grain Amino Acid, Fatty Acid, Fiber, Isoflavone, Proximate, Vitamin E, and Antinutrient Content for MON 89788 vs. A3244

Analytical Component (Units)[1]	MON 89788 Mean (S.E.) [Range]	A3244 Mean (S.E.) [Range]	Difference (MON 89788 minus A3244) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Conventional (Range) [99% Tol. Int.[2]]
Antinutrient						
Stachyose (% DW)	2.36 (0.070)	2.50 (0.073)	-0.15 (0.10)	-0.38, 0.085	0.183	(1.53 - 2.98)
	[2.02 - 2.85]	[2.12 - 3.04]	[-0.59 - 0.53]			[1.19, 3.31]
Trypsin Inhibitor (TIU/mg DW)	33.69 (2.84)	31.44 (2.88)	2.25 (1.56)	-2.32, 6.81	0.231	(20.79 - 55.51)
	[24.59 - 53.85]	[23.43 - 41.91]	[-4.81 - 13.99]			[5.15, 59.34]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2]With 95% confidence, interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table E-3. Literature and Historical Ranges for Components in Soybean Forage

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% DW)		
Ash	8.8-10.5[a]	6.718-10.782
Carbohydrates	not available	59.8-74.7
Fat, total	3.1-5.1[a]	1.302-5.132
Moisture (% FW)	74-79[f]	73.5-81.6
Protein	11.2-17.3[a]	14.38-24.71
Fiber (% DW)		
Acid detergent fiber (ADF)	32-38[a]	not available
Neutral detergent fiber (NDF)	34-40[a]	not available
Crude fiber	not available	13.58-31.73

[1] FW=fresh weight; DW=dry weight;
[2] Literature range references: [a]OECD, 2001.
[3] ILSI Soybean Database, 2004.
Conversions: % DW × 10^4 = µg/g DW; mg/g DW × 10^3 = mg/kg DW; mg/100g DW × 10 = mg/kg DW; g/100g DW × 10 = mg/g DW

Table E-4. Literature and Historical Ranges for Components in Soybean Grain

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% DW)		
Ash	4.61-5.94[b]; 4.29-5.88[a]	3.885-6.542
Carbohydrates	29.3-41.3[a]	29.6-50.2
Fat, total	198-277[c] g/kg DW; 160-231[d] g/kg DW	8.104-23.562
Moisture (% FW)	5.3-8.73[a] , 5.18-14.3[b]	5.1-14.9
Protein	329-436[c] g/kg DW; 360-484[d] g/kg DW	33.19-45.48
Fiber (% DW)		
Acid detergent fiber (ADF)	not available	7.81-18.61
Neutral detergent fiber (NDF)	not available	8.53-21.25
Crude fiber	5.74-7.89[a]	4.12-10.93
Amino Acids (mg/g DW = % DW × 10)		
Alanine	16.0-18.6[a,h]	15.13-18.51
Arginine	25.6-34.6[a,h]	22.85-33.58
Aspartic acid	41.8-49.9[a,h]	38.08-51.22
Cystine/Cysteine	5.4-6.6[a,h]	3.70-8.08
Glutamic acid	66.4-81.6[a,h]	58.43-80.93
Glycine	16.0-18.7[a,h]	14.58-18.65
Histidine	9.8-11.6[a,h]	8.78-11.75
Isoleucine	16.5-19.5[a,h]	15.63-20.43
Leucine	28.1-33.7[a,h]	25.90-33.87
Lysine	24.7-28.4[a,h]	22.85-28.39
Methionine	5.1-5.9[a,h]	4.31-6.81
Phenylalanine	17.8-21.9[a,h]	16.32-22.36
Proline	18.6-22.3[a,h]	16.87-22.84
Serine	19.6-22.8[a,h]	16.32-24.84
Threonine	15.1-17.3[a,h]	12.51-16.18
Tryptophan	5.6-6.3[a,h]	3.563-5.016
Tyrosine	13.5-15.9[a,h]	10.16-15.59
Valine	17.1-20.2[a,h]	16.27-22.04

Table E-4 (continued). Literature and Historical Ranges for Components in Soybean Grain

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Fatty Acids (% DW)		
12:0 Lauric	not available	not available
14:0 Myristic	not available	not available
16:0 Palmitic	1.44-2.31[f]	not available
16:1 Palmitoleic	not available	not available
17:0 Heptadecanoic	not available	not available
17:1 Heptadecenoic	not available	not available
18:0 Stearic	0.54-0.91[f]	not available
18:1 Oleic	3.15-8.82[f]	not available
18:2 Linoleic	6.48-11.6[f]	not available
18:3 Linolenic	0.72-2.16[f]	not available
20:0 Arachidic	0.04-0.7[f]	not available
20:1 Eicosenoic	not available	not available
20:2 Eicosadienoic	not available	not available
22:0 Behenic	not available	not available
Vitamins (mg/100g)	**FW[i]**	**DW**
Vitamin E	0.85[g]	0.47-6.17
Anti-Nutrients		
Lectin (H.U./mg FW)	0.8-2.4[a]	0.105-9.038
Trypsin Inhibitor (TIU/mg DW)	33.2-54.5[a]	19.59-118.68
Raffinose	not available	0.212-0.661
Stachyose	not available	1.21-3.50
Isoflavones	**mg/100g FW**	**(mg/kg DW)**
Daidzein	9.88-124.2[e]	60.0-2453.5
Genistein	13-150.1[e]	144.3-2837.2
Glycitein	4.22-20.4[e]	15.3-310.4

[1] FW=fresh weight; DW=dry weight;
[2] Literature range references: [a]Padgette et al., 1996. [b]Taylor et al., 1999. [c]Maestri et al., 1998. [d]Hartwig and Kilen, 1991. [e]USDA-ISU Isoflavone Database, 2002. [f]OECD, 2001. [g]USDA-NND, 2005. [h]Data converted from g/100g DW to mg/g DW. [i]Moisture value = 8.54g/100g.
[3] ILSI Soybean Database, 2004.
Conversions: % DW × 10^4 = µg/g DW; mg/g DW × 10^3 = mg/kg DW; mg/100g DW × 10 = mg/kg DW; g/100g DW × 10 = mg/g DW

Appendix F. Materials, Methods, and Individual Site Results for Seed Dormancy and Germination Analyses of MON 89788

Materials

The MON 89788, control, and reference starting seed were produced in Jackson County, AR; Clinton County, IL; and Fayette County, OH in 2005.

Material Type	Material Name from Each Site		
	AR	IL	OH
Test	MON 89788	MON 89788	MON 89788
Control	A3244	A3244	A3244
Reference	ST3600	A3525	A2704
Reference	ST3870	A3559	ST2800
Reference	DKB37-51	AG3401	A2833
Reference	DKB38-52	DKB31-51	AG3201

Characterization of the Materials

The presence or absence of MON 89788 was verified by event-specific polymerase chain reaction for the MON 89788 and control starting seed. The results of these verifications were as expected with two exceptions. The MON 89788 seed sample from the IL site contained ≤1.84% of Roundup Ready® soybean 40-3-2. In addition, the control seed sample from the AR site contained ≤3.05% of MON 89788. In both cases, these results were not detected in the seed samples from the other sites. Furthermore, it was determined that the level of Roundup Ready® 40-3-2 in the MON 89788 seed sample and MON 89788 in the control seed sample from the single sites was low and did not negatively affect the quality of the study or interpretation of the results**.**

Performing Facility and Experimental Methods

Dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was certified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 2000; AOSA, 2002).

Six germination chambers were used in the study and each chamber was maintained dark under one of the following six temperature regimes: constant temperature of approximately 10, 20 or 30° C or alternating temperatures of approximately 10/20, 10/30, or 20/30° C. The alternating temperature regimes were maintained at the lower temperature for 16 hours and the higher temperature for eight hours. The temperature inside each germination chamber was monitored and recorded every 15 minutes throughout the duration of the study.

Germination towels for MON 89788, control, and reference materials were prepared per facility SOPs. Each germination towel represented one replication. The types of data collected depended on the temperature regime. Each rolled germination towel in the

AOSA-recommended temperature regime (i.e., 20/30° C) was assessed periodically during the study for normal germinated, abnormal germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed as defined by AOSA guidelines (AOSA, 2002). Each rolled germination towel in the additional temperature regimes (i.e., 10, 20, 30, 10/20 and 10/30° C) was assessed periodically during the study for germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed.

Statistical Analysis

Statistical analyses were performed by the Monsanto Statistics Technology Center. Analysis of variance was conducted according to a split plot design using the Statistical Analysis System (SAS®) to compare the MON 89788 to the control material for each temperature regime. The whole plot treatment was the site effect arranged in a randomized complete block design. The sub-plot was the seed material arranged in a completely randomized design. The data were pooled across sites and MON 89788 was compared to the control for the following germination characteristics: percent germinated (categorized as percent normal germinated and percent abnormal germinated for the AOSA temperature regime), percent dead, percent viable firm swollen, and percent viable hard seeds. Seed from the three sites were tested within the same germination chamber for each temperature regime; thus, an analysis of the data pooled across sites is more appropriate than an analysis within each site. However, if an interaction between site and seed material (i.e., MON 89788 and control materials) had been detected, the MON 89788 would have been compared to the control material within sites. The means of the MON 89788 and control materials (across-sites and within-sites) and the results of the analysis of variance are reported. MON 89788 was not statistically compared to the reference materials. The reference materials provided seed germination characteristic values common to commercially available soybean. The minimum and maximum values among the individual means (reference range) and a 99% tolerance interval with 95% confidence were determined from the twelve reference materials. Data transformation was performed before the analysis of variance to validate the F-test and t-test, which requires the assumption of normality.

Individual Site Seed Dormancy and Germination Results and Discussion

MON 89788, A3244, and reference seed materials were produced at three sites to assess germination characteristics of seed grown under various environmental conditions. The individual site data presented in Table F-1 indicate that overall seed germination across all seed materials and temperature regimes was lower for seeds produced at the OH site relative to the AR and IL sites. The results were not unexpected because droughty growing conditions at the OH site may have affected the quality of the MON 89788, A3244, and reference starting seed. Although percent germination at OH was poor by seed production standards, it is representative of areas where MON 89788 will be grown for grain. In the analysis of the data, no site × seed material interactions were detected for any characteristic in any temperature regime. Therefore, MON 89788 was compared to the A3244 material across sites (Table VIII-2 in Section VIII).

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Table F-1. Germination Characteristic By-Site Analyses of MON 89788 and A3244

Temperature Regime	Germination Category[1]	AR Mean % (SE)[2]		IL Mean % (SE)		OH Mean % (SE)	
		MON 89788	A3244	MON 89788	A3244	MON 89788	A3244
10°C	Total Germinated	98.5 (1.0)	99.5 (0.3)	98.8 (0.5)	98.3 (0.9)	85.0 (3.5)	85.8 (2.5)
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
	Dead	1.5 (1.0)	0.5 (0.3)	1.0 (0.4)	1.8 (0.9)	14.5 (3.2)	13.3 (2.6)
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.3 (0.3)	0.0 (nv)	0.5 (0.5)	1.0 (1.0)
20°C	Total Germinated	98.3 (0.8)	98.5 (0.5)	99.0 (0.7)	97.8 (0.9)	79.5 (1.3)	75.5 (2.1)
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
	Dead	1.8 (0.8)	1.5 (0.5)	1.0 (0.7)	2.3 (0.9)	20.5 (1.3)	24.3 (2.3)
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.3 (0.3)
30°C	Total Germinated	95.3 (0.5)	96.3 (1.3)	99.8 (0.3)	98.8 (0.5)	88.3 (2.2)	86.8 (1.9)
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
	Dead	4.8 (0.5)	3.8 (1.3)	0.3 (0.3)	1.3 (0.5)	11.8 (2.2)	13.3 (1.9)
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)

Table F-1 (continued). Germination Characteristic By-Site Analyses of MON 89788 and A3244

Temperature Regime	Germination Category[1]	AR Mean % (SE)[2]		IL Mean % (SE)		OH Mean % (SE)	
		MON 89788	A3244	MON 89788	A3244	MON 89788	A3244
10/20°C	Total Germinated	100.0 (nv)	98.5 (0.9)	99.3 (0.5)	99.0 (0.6)	84.3 (0.5)	86.3 (1.4)
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
	Dead	0.0 (nv)	1.5 (0.9)	0.8 (0.5)	1.0 (0.6)	15.5 (0.3)	13.8 (1.4)
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.3 (0.3)	0.0 (nv)
10/30°C	Total Germinated	98.8 (0.5)	98.5 (0.3)	99.5 (0.5)	99.8 (0.3)	84.0 (1.2)	83.5 (2.5)
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
	Dead	1.3 (0.5)	1.5 (0.3)	0.5 (0.5)	0.3 (0.3)	16.0 (1.2)	16.5 (2.5)
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
20/30°C (AOSA)	Normal Germinated	92.3 (1.9)	89.5 (0.3)	89.3 (1.0)	84.3 (2.4)	45.3 (0.7)	48.5 (3.6)
	Abnormal Germinated	6.8 (1.5)	7.0 (0.4)	9.5 (1.0)	11.3 (3.7)	36.7 (6.1)	30.0 (2.3)
	Viable Hard	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)
	Dead	1.0 (0.4)	3.0 (1.1)	1.3 (0.3)	4.3 (1.3)	18.0 (5.5)	21.5 (1.7)
	Viable Firm Swollen	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)	0.0 (nv)

[1] Germinated seed in the AOSA temperature regime were categorized as either normal germinated or abnormal germinated seed.
[2] SE = standard error; nv = no variability in the data.

Appendix G. Material, Methods and Individual Site Results from Phenotypic, Agronomic and Ecological Interactions Analyses of MON 89788

Materials

The materials for phenotypic assessments include: MON 89788, A3244, and 23 commercially available soybean varieties as references. The references contain both the conventional soybeans 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 89788 and A3244 seed were confirmed by PCR analysis prior to use.

Field Sites and Plot Design

Field trials were established at 17 locations (site code in parenthesis): Jackson Co., Arkansas (AR), Jefferson Co., Iowa (IA1), Benton Co., Iowa (IA2), Clinton Co., Illinois (IL1), Stark Co., Illinois (IL2), Warren Co., Illinois (IL3), Clinton Co., Illinois (IL4), Warren Co., Illinois (IL5), Hendricks Co., Indiana (IN1), Boone Co., Indiana (IN2), Pawnee Co., Kansas (KS), Shelby Co., Missouri (MO1), Lincoln Co., Missouri (MO2), York Co., Nebraska (NE), York Co., Nebraska (NE2), Pickaway Co., Ohio (OH), and Fayette Co., Ohio (OH2). These 17 locations provided a range of environmental and agronomic conditions representative of major U. S. soybean-growing regions where the majority of commercial production of MON 89788 is expected to occur. The field cooperators at each site were familiar with the growth, production, and evaluation of the soybean characteristics.

The experiment was established at each of the 17 sites in a randomized complete block design with three replications. At the IA1, IA2, IL2, IL3, IN1, IN2, KS, NE, and OH sites, each plot consisted of four rows spaced approximately 30 inches apart and approximately 20 feet in length. The plots were planted adjacent to each other and surrounded by a border of commercially available soybean approximately 10 feet (four row) in width. At the IL1, MO1, and MO2 sites, where additional insect abundance and insect damage data were collected, each plot consisted of eight rows spaced approximately 30 inches apart and approximately 30 feet in length. At these sites, 10 feet (four rows) of commercially available soybeans were planted surrounding each plot. At the AR, IL4, IL5, NE2, and OH2 sites, each plot consisted of six rows spaced approximately 30 inches apart and approximately 20 feet in length. The plots within each replicate were separated by approximately five feet (two rows) of commercially available soybeans, and all plots were surrounded by a border of commercially available soybeans approximately 10 feet (four rows) in width.

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 in this study to avoid injury to the conventional control or reference plants and to ensure all plants were managed uniformly.

Table G-1. Starting Seed for Phenotypic Assessments

Variety	Material Type	Genotype	Sites[1]
MON 89788	Test	Glyphosate-tolerant	All
A3244	Control	Conventional	All
A3525	Reference	Conventional	IA1, IA2, IL1, IL2, IL3, IN1, IN2, KS, MO1, MO2, NE, OH, IL4
A2553	Reference	Conventional	IL5
A2704	Reference	Conventional	OH2
A2804	Reference	Conventional	NE2
A2833	Reference	Conventional	OH2
A2869	Reference	Conventional	IL2, IL3
A3204	Reference	Conventional	IN2, MO1, NE
A3204	Reference	Conventional	IL5
A3469	Reference	Conventional	IL1, KS
A3559	Reference	Conventional	IL4
ST2788	Reference	Conventional	IL5
ST2800	Reference	Conventional	IA1, IA2, OH2
ST3300	Reference	Conventional	IN1, MO2, OH, NE2
ST3600	Reference	Conventional	MO2, AR
ST3870	Reference	Conventional	AR
AG3005	Reference	Glyphosate-tolerant[2]	IA1, IA2, IL2, IL3, NE2
AG3201	Reference	Glyphosate-tolerant[2]	IN1, IN2, MO1, NE, OH2
AG3302	Reference	Glyphosate-tolerant[2]	IN1, IN2, MO1, NE, NE2
AG3401	Reference	Glyphosate-tolerant[2]	IL4
AG3905	Reference	Glyphosate-tolerant[2]	IL1, KS, OH
DKB31-51	Reference	Glyphosate-tolerant[2]	IA1, IA2, IL2, IL3, IL4, IL5
DKB37-51	Reference	Glyphosate-tolerant[2]	AR
DKB38-52	Reference	Glyphosate-tolerant[2]	IL1, KS, MO2, OH, AR

[1] The MON 89788 and A3244 materials were planted at all sites; the reference materials were site-specific.
[2] Commercially available Roundup Ready soybean (40-3-2) varieties.

Table G-2. Field and Planting Information

Site	Planting Date	Planting Rate[1]	Planting Depth	Plot Size (ft)	Soil Series, Organic Matter, pH	2004 Crop	2003 Crop
AR	06/20/2005	9.2	1 in	15 x 20	Bosket loam, 1.3%, 5.5	Rice	—
IA1	05/31/2005	9.0	1 in	10 x 20	Taintor silty clay loam, 3.5%, 6.9	Corn	Soybean
IA2	06/01/2005	9.0	2 in	10 x 20	Tama silty clay loam, 3.8%, 6.6	Corn	Soybean
IL1	05/24/2005	9.0	1 in	20 x 30	Cisne silt loam, 2.1%, 7.0	Corn	Soybean
IL2	06/01/2005	9.0	1.75 in	10 x 20	Flanagan silt loam, 3.8%, 6.4	Corn	Corn
IL3	06/03/2005	9.0	1 in	10 x 20	Sable silty clay loam, 4.3%, 6.5	Corn	Soybean
IL4	05/24/2005	7.5	1 in.	15 x 21.3	Cisne Huey complex silt loam, 1.8%, 6.8	Corn	Wheat/beans
IL5	06/03/2005	9.3	1 in.	15 x 20	Muscatine silty clay loam, 4.5%, 6.5	Corn	—
IN1	05/26/2005	9.0	1.1 in	10 x 20	Crosby silt loam, 1.1%, 5.6	Corn	Soybean
IN2	05/24/2005	9.0	1 in	10 x 20	Crosby silt loam, 2.6%, 6.2	Corn	Soybean
KS	06/06/2005	7.2	1.5 in	10 x 25	Farnum loam, 2.1%, 7.5	Corn	Alfalfa
MO1	05/28/2005	9.0	1 in	20 x 30	Putnam silt loam, 1.8%, 6.9	Corn	Soybean
MO2	06/03/2005	9.0	1.5 in	20 x 30	Keswick silt loam, 2.3%, 6.5	Corn	Soybean
NE	05/23/2005	9.0	1 in	10 x 20	Hastings silt loam, 3%, 6.2	Soybean	Soybean
NE2	05/20/2005	9.3	1 in.	15 x 20	Hastings silt loam, 3%, 6.5	Soybean	—
OH	05/24/2005	9.0	1 in	10 x 20	Brookston loam, 2.5%, 6.1	Wheat	Soybean
OH2	05/24/2005	8.0	1 in.	15 x 20	Crosby loam, 1.8%, 7	Soybean	—

— = Not provided.
[1] Seeds per foot.

Phenotypic Observations

The description of the characteristics measured and the designated developmental stages where observations occurred are listed in Section VIII, Table VIII-4.

Ecological Observations

The plots at all sites were qualitatively evaluated at least four times (except at IL3) for differential response to naturally occurring ecological stressors during the growing season. During each observation, each plot was evaluated for the severity of symptoms caused by three insect, three disease, and three abiotic stressors that commonly occur at the study sites. With a few exceptions, these stressors were predetermined by the individual site Principal Investigators (PIs) based on their experience. The ecological stressors evaluated were not artificially induced and could vary between sites. Plots were rated on the 0 – 9 scale described below but the results were reported as categorical (none, slight, moderate, or severe).

0	=	none (no symptoms observed)
1 – 3	=	slight (symptoms observed, not detrimental to plant growth and development)
4 – 6	=	moderate (intermediate between slight and severe)
7 – 9	=	severe (symptoms observed, detrimental to plant growth and development)

At the IL1, MO1, and MO2 sites, insect (and spider) abundance was quantitatively evaluated three times during the growing season using a beat sheet sampling method. The beat sheet consisted of an approximately 40 × 30 inch white sheet with a central opening to which a container lid had been glued. The attached lid had a hole in the middle to allow insects to pass through. Prior to insect collection, an empty container was attached to the lid. The beat sheet was placed flat on the ground between two sampling rows, and plants from both rows adjacent to the beat sheet were shaken vigorously. Dislodged insects that fell onto the beat sheet were brushed toward the center into the container. The container was removed from the beat sheet, filled with enough alcohol to cover the insects and plant debris, and sealed with a solid lid. Two insect sub-samples were collected from non-systematically selected plants in each plot, one from rows five and six and the other from rows six and seven. The two sub-samples from each plot were combined into a single container.

To focus the insect evaluation on the most abundant pest and beneficial species, the following predetermined selection criteria were employed. A list of important Midwestern pest and beneficial species was developed. Four randomly selected samples from each collection time point at each site were examined to determine the five most abundant pest species and the three most abundant beneficial species from the list. These eight species were then counted in each sample from each plot. Because the species

counted were site- and collection-specific, they varied from site to site and from collection to collection.

Plant damage caused by defoliation or by pre-selected fluid feeding insect species was also evaluated four times during the growing season at the IL1, MO1, and MO2 sites. Damage caused by a minimum of four specific insect species or groups commonly found at each field site were rated by the PIs using a 0 – 9 scale, where 0 = no damage or defoliation observed, 5 = 50 % damage or defoliation, and 9 = 90% damage or defoliation.

Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS® (SAS Version 9.1.3, SAS Institute, Inc. 2002-2003). The level of significance was $p \leq 0.05$. For each analyzed characteristic, MON 89788 was compared to A3244 at each site (by-site analysis) and pooled across all sites (across-site analysis). Characteristics analyzed include: Early stand count, seedling vigor, days to 50% flowering, plant height, lodging, final stand count, seed moisture, seed test weight, yield, insect damage, insect abundance.

No statistical analyses were conducted on flower color and pod shattering due to low categorical variability in the data. Growth stage monitoring and ecological stressor observations were qualitative and were not statistically analyzed. No statistical comparisons were made between MON 89788 and reference materials. For the 23 reference varieties, the minimum and maximum mean values observed across the three replications at a given site and a 99% tolerance interval with 95% confidence were calculated for each characteristic.

Individual Field Site Plant Growth and Development Results and Discussion

For the by-site analyses, no differences between MON 89788 and A3244 were detected for early stand count, flower color, pod shattering, or yield (Table G-3). A total of 14 out of 181 site × characteristic comparisons were significantly different between MON 89788 and A3244. The significant differences were distributed among seven of the 11 phenotypic characteristics. Except for plant height, all of the significant differences detected in the by-site analysis were not detected in the across-site analyses. Therefore, the differences detected in the by-site analysis were not indicative of a consistent trend, and are not likely to be biologically meaningful in terms of increased weed potential of MON 89788 compared to A3244.

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Table G-3. Phenotypic Comparison of MON 89788 and A3244 at Each Site

Site	Phenotypic Characteristics (units)											
	Early stand count (# plants/2 rows)		Seedling vigor		Days to 50% flowering		Flower color[1]		Plant height (in)		Lodging	
	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244
AR	248	257	3.0	2.7	31*	32	Purple	Purple	22.9	24.6	1.7	1.0
IA1	273	280	1.7	1.0	40	40	Purple	Purple	31.0	33.2	0.7	1.0
IA2	323	336	2.0	2.0	43	47	Purple	Purple	37.9	39.8	1.0	1.3
IL1	453	455	3.7	3.3	44	45	Purple	Purple	37.9	40.0	0.7	0.7
IL2	308	293	2.0	2.0	41	41	Purple	Purple	36.5	37.3	0.0	0.0
IL3	332	334	1.3	1.7	45	45	Purple	Purple	39.5	40.9	0.7	2.0
IL4	253	260	4.0	3.7	47	48	Purple	Purple	34.5	36.1	0.7	0.3
IL5	327	339	2.0	2.0	—	—	—	—	32.7	35.3	0.0	0.0
IN1	307	319	4.0	4.0	46	46	Purple	Purple	27.1	29.0	0.7	0.7
IN2	206	218	2.0	1.0	51	51	Purple	Purple	33.7*	36.1	0.3	1.0
KS	302	298	3.0	3.3	43	43	Purple	Purple	26.7	27.5	1.3*	0.3
MO1	194	221	3.7	4.7	54	54	Purple	Purple	19.9*	21.9	0.3	0.0
MO2	259	302	2.7	2.3	42	42	Purple	Purple	17.7*	20.6	0.0	0.0
NE	293	273	2.3*	1.3	45	46	Purple	Purple	36.7*	39.1	0.3	0.7
NE2	277	279	1.3	1.3	46	46	Purple	Purple	37.6	38.3	0.7	0.7
OH	305	325	2.0	2.0	44	45	Purple	Purple	24.8	26.7	0.0	0.0
OH2	286	287	2.0	2.3	42	43	Purple	Purple	22.6	22.0	0.0	0.0

Table G-3 (continued). Phenotypic Comparison of MON 89788 and A3244 at Each Site

Site	Phenotypic Characteristics (units)									
	Pod shattering[1]		Final stand count (# plants/2 rows)		Seed moisture (%)		Seed test weight (g/100 seed)		Yield (bu/ac)	
	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244	MON 89788	A3244
AR	0.0	0.0	235	247	13.1*	14.1	13.6*	14.6	47.1	47.8
IA1	0.0	0.0	258	276	13.3	13.2	15.7	16.3	55.5	59.1
IA2	0.0	0.0	307	309	10.4	10.4	14.8	15.0	75.6	76.8
IL1	0.0	0.0	380	362	11.9	12.0	14.1	14.1	39.4	37.3
IL2	0.0	0.0	305	292	8.2	8.4	—	—	49.4	47.8
IL3	0.0	0.0	302	294	12.1	12.4	—	—	57.5	56.7
IL4	0.0	0.0	238	230	11.4*	12.0	13.3	14.0	39.6	45.5
IL5	0.0	0.0	287	300	11.7	11.4	—	—	57.5	55.2
IN1	0.0	0.0	262	270	11.0	11.3	14.6	14.7	42.3	47.1
IN2	0.0	0.0	135	142	11.4	11.5	16.6	16.6	39.1	44.3
KS	0.0	0.0	281	272	12.4	12.9	17.0	18.0	65.5	68.6
MO1	0.0	0.0	193	216	12.4	12.5	—	—	39.0	42.0
MO2	0.0	0.0	254	287	13.6	14.2	15.9*	15.3	22.0	21.0
NE	0.0	0.0	270	260	8.8	8.7	14.7*	15.3	74.9	75.8
NE2	0.0	0.0	261	266	9.0	9.0	15.0	15.0	68.3	71.4
OH	0.0	0.0	283	301	12.4	12.3	14.0	14.8	28.3	32.7
OH2	0.0	0.0	274*	267	12.4*	12.9	15.3	14.1	21.9	20.6

* Indicates a statistically significant difference between MON 89788 and A3244 at $p \leq 0.05$.
— Dashes indicate data that are missing.
[1] Not statistically analyzed due to lack of variation.

Table G-4. Growth Stage Monitoring of MON 89788, A3244, and the Reference Soybean Varieties

		Assessment Date and Range of Growth Stages Observed									
Site	**Material**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
AR		07/11	08/01	08/23	09/13	10/03	—	—	—	—	—
	MON 89788	V2	V8-R3	V13-R5	V14-R6	V14-R8	—	—		—	—
	A3244	V2	V8-R3	V13-R5	V14-R6	V14-R8	—	—	—	—	—
	References	V2	V7-R3	V13-R5	V14-R6	V14-R8	—	—	—	—	—
IA1		06/20	07/06	07/18	08/02	08/16	08/30	09/09	10/10	—	—
	MON 89788	V2	V6	V8-R2	R4	R5	R5-R6	R6	R8	—	—
	A3244	V2	V6	V8-R2	R4	R5	R5-R6	R6	R8	—	—
	References	V2	V5-V7	V8-R2	R3-R4	R5	R5-R6	R6-R7	R8	—	—
IA2		06/28	07/12	07/26	08/09	08/23	09/06	09/27	10/18	—	—
	MON 89788	V2	V6	R3	R4	R5	R6	R7	R8	—	—
	A3244	V2	V6	R2	R4	R5	R6	R7	R8	—	—
	References	V2	V6-R1	R2-R3	R4	R5	R6	R7-R8	R8	—	—
IL1		06/16	07/08	07/24	08/13	08/31	09/17	10/03	—	—	—
	MON 89788	V2	R1	R3	R4	R5	R7	R8	—	—	—
	A3244	V2	V8-R1	R3	R4	R5	R7	R8	—	—	—
	References	V2	V7-R1	R3	R4	R4-R5	R6-R7	R8	—	—	—
IL2		06/27	07/18	08/08	08/30	09/15	09/27	10/17	—	—	—
	MON 89788	V2	R1	R2-R3	R4-R5	R5-R6	R6	R8	—	—	—
	A3244	V2	R1	R2-R3	R4-R5	R5-R6	R6	R8	—	—	—
	References	V2-V3	R1	R2-R3	R4-R5	R5-R6	R6	R8	—	—	—

Table G-4 (continued). Growth Stage Monitoring of MON 89788, A3244, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed									
Site	**Material**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
IL3		06/24	07/15	08/04	08/26	09/08	10/03	—	—	—	—
	MON 89788	V2	V6	R4	R6	R6	R8	—	—	—	—
	A3244	V2	V6	R4	R6	R6	R8	—	—	—	—
	References	V2	V6	R4	R6	R6	R8	—	—	—	—
IL4		06/17	06/21	07/06	07/21	08/11	09/08	09/29	—	—	—
	MON 89788	V2	V3	V6	V10	V16	R6	R8	—	—	—
	A3244	V2	V3	V6	V10	V16	R6	R8	—	—	—
	References	V2	V3	V6	V10	V16	R6	R8	—	—	—
IL5		06/24	07/15	08/05	08/25	09/15	09/28	—	—	—	—
	MON 89788	V2	V5	V12	R5	R7-R8	R8	—	—	—	—
	A3244	V2	V5	V12	R5	R7-R8	R8	—	—	—	—
	References	V2	V5	V12	R5	R7-R8	R8	—	—	—	—
IN1		06/24	07/06	07/19	08/03	08/10	08/15	08/29	09/12	09/21	09/21-10/04[1]
	MON 89788	V2	V5	R2	R2-R3	R4	R5	R6	R7	R7-R8	R8
	A3244	V2	V5	R2	R2	R4	R5	R6	R7	R7	R8
	References	V2	V5	R2	R2-R3	R4	R5	R6	R7	R7	R8
IN2		06/10	06/27	07/22	08/17	09/15	—	—	—	—	—
	MON 89788	V1	V3-V4	R1-R2	R4	R6-R7	—	—	—	—	—
	A3244	V1	V3-V4	R1-R2	R4	R6-R7	—	—	—	—	—
	References	VC-V1	V3-V4	R1-R2	R4	R6-R8	—	—	—	—	—

Table G-4 (continued). Growth Stage Monitoring of MON 89788, A3244, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed									
Site	**Material**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
KS		07/05	07/26	08/15	09/15	09/21	09/27	—	—	—	—
	MON 89788	V2-V3	R2-R3	R5	R6	R7	R8	—	—	—	—
	A3244	V2-V3	R3	R4-R5	R6-R7	R7-R8	R8	—	—	—	—
	References	V2-V3	R2-R3	R3-R5	R6-R7	R6-R7	R7-R8	R8[2]	—	—	—
MO1		06/20	07/08	07/27	08/15	09/14	10/09	—	—	—	—
	MON 89788	V2	V6	R2	R4	R7	R8	—	—	—	—
	A3244	V2	V6	R2	R4	R6-R7	R8	—	—	—	—
	References	V2	V6	R2	R4	R6-R7	R8	—	—	—	—
MO2		06/21	—	07/05	07/22	08/11	08/22	09/06	10/06	—	—
	MON 89788	V2	—	V5	R2	R4	R5	R6	R8	—	—
	A3244	V2	—	V5	R2	R4	R5	R6	R8	—	—
	References	V2	—	V5	R2	R4	R5	R6	R8	—	—
NE		06/17	07/05	07/26	08/15	09/06	09/26	—	—	—	—
	MON 89788	V2	V7-R1	R4	R5	R6	R8	—	—	—	—
	A3244	V2	V7-R1	R3-R4	R5	R6	R8	—	—	—	—
	References	V2	V7-R1	R3-R4	R5	R6	R8	—	—	—	—
NE2		06/13	07/05	07/26	08/15	09/06	09/26	—	—	—	—
	MON 89788	V2	V7-R1	R3	R5	R6	R8	—	—	—	—
	A3244	V2	V7-R1	R3	R5	R6	R8	—	—	—	—
	References	V2	V7-R1	R3	R5	R6	R8	—	—	—	—

Table G-4 (continued). Growth Stage Monitoring of MON 89788, A3244, and the Reference Soybean Varieties

		Date and Range of Growth Stages Observed									
Site	**Material**	**Obs. 1**	**Obs. 2**	**Obs. 3**	**Obs. 4**	**Obs. 5**	**Obs. 6**	**Obs. 7**	**Obs. 8**	**Obs. 9**	**Obs. 10**
OH		06/18	07/05	07/18	07/30	08/20	09/11	10/03	—	—	—
	MON 89788	V2	V7-V8	V10-R3	R3-R4	R5	R7	R7-R8	—	—	—
	A3244	V2	V7-V8	V10-R2	R3-R4	R5	R7	R7-R8	—	—	—
	References	V2	V7-V8	V10-R3	R3-R4	R5	R7	R7-R8	—	—	—
OH2		06/18	07/05	07/18	07/26	08/20	09/11	10/02	—	—	—
	MON 89788	V2	V7-V8	V10-V11	R3	V13-R5	R7	R8	—	—	—
	A3244	V2	V7-V8	V10-V11	R3	V13-R5	R7	R8	—	—	—
	References	V2	V7-V8	V10-V11	R3	V13-R5	R7	R8	—	—	—

Note: Obs. = Observation number; all data were collected during 2005.
[1] The date where each plot reached the R8 growth stage was recorded for this observation.
[2] Only the references that had not reached R8 in the previous observation were rated in this observation.

Table G-5. Insect Stressor Symptom Severity of MON 89788, A3244, and the Reference Soybean Varieties

Insect	Site(s)	Observation 1			Observation 2			Observation 3			Observation 4			Observation 5		
		MON 89788	A3244	Ref.	MON 89788	A3244	Ref.	MON 89788	A3244	Ref.	MON 89788	A3244	Ref.	MON 89788	A3244	Ref.
Aphid[1]	IA1	no	no	no	no	no	no	no	no-sl	no-sl	no-sl	no-sl	no-sl	no-sl	sl	no-sl
	IA2	no	no	no	no	no	no	sl	sl	sl	no	no	no	—	—	—
	IL1	no	no	no	sl	no-sl	no-sl	sl	sl	sl	no	no	no	—	—	—
	IL2, IN1, OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IL4, IN2, KS, MO1, NE, NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Bean leaf beetle	IA1	no	no	no	sl	sl	sl	sl	sl	no-sl	sl	sl	sl	sl	sl	sl
	IA2	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	—	—	—
	IL1	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	IL2, OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IL3	no	no	no	no	no	no	sl	sl	sl	—	—	—	—	—	—
	IL5	no	no	no	sl	sl	sl	sl	sl	sl	sl	sl	sl	—	—	—
	IN1	sl	sl	sl	no	no	no	no	no	no	sl	sl	sl	no	no	no
	MO1	no	no	no	sl	sl	sl	sl	sl	sl	mo	mo	mo	—	—	—
	MO2	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl-mo	—	—	—
	NE	no	no	no	no	no	no	no	no	no	sl	sl	sl	—	—	—
	NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Beet armyworm	AR	sl	sl	sl	—	—	—	—	—	—	—	—	—	—	—	—
Grasshopper	IL1	no	no	no	no	no	no	no	no	no	sl	sl	sl	—	—	—
	IL4, NE, NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IN1	—	—	—	—	—	—	—	—	—	sl	sl	sl	—	—	—
Green cloverworm	AR	—	—	—	sl	sl	sl	sl-mo	sl	sl-mo	—	—	—	—	—	—
	KS	no	no	no	sl	sl	no-sl	sl	sl-mo	sl-mo	no	no	no	—	—	—
	MO2	—	—	—	—	—	—	—	—	—	sl	sl	sl	—	—	—

Table G-5 (continued). Insect Stressor Symptom Severity of MON 89788, A3244, and the Reference Soybean Varieties

		Observation 1			Observation 2			Observation 3			Observation 4			Observation 5		
Insect	**Site(s)**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**
Japanese	IL4	sl	sl	sl	—	—	—	—	—	—	—	—	—	—	—	—
beetle	IN1	no	no	no	sl	sl	sl	sl	sl	sl	no	no	no	no	no	no
	IN2	no	no	no	sl	sl	sl	sl	sl	sl	no	no	no	—	—	—
	MO2	no	no	no	no	no	no	no	no	no	no-sl	no	no-sl	—	—	—
	OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Leafhopper[2]	IL3	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	IL5	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	MO1	sl	sl	sl	no-sl	no-sl	no-sl	sl	sl	sl	no	sl	no-sl	—	—	—
Soybean looper	AR	no	no	no	sl	sl	sl	no	no	no	no	no	no	—	—	—
Spider mite	IA1	no	no	no	no	no	no	no	no	no-sl	no-sl	no-sl	no-sl	no	no	no
	IA2, IL4, IL5, IN2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IL3	no	no	no	no	no	no	sl	sl	sl	—	—	—	—	—	—
Soybean stem borer	KS	—	—	—	no	no	no	no	no	no	no	no	no	—	—	—
Stink bug[3]	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL1	—	—	—	—	—	—	—	—	—	sl	sl	sl-mo	—	—	—
	MO2	no	no	no	no	no	no	sl	sl	sl	sl	sl	sl	—	—	—
Velvetbean caterpillar	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—

Ref. = References; NO = None; SL = Slight; MO = Moderate; SE = Severe.

[1] Including soybean aphid

[2] Including potato leafhopper

[3] Including green stink bug

— Insects not evaluated at this observation and site.

Table G-6. Disease Stressor Symptom Severity of MON 89788, A3244, and the Reference Soybean Varieties

		Observation 1			Observation 2			Observation 3			Observation 4			Observation 5		
Disease	**Sites**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**
Alternaria leaf spot																
	KS	no	no	no	no	no	no	no	no	no	no	no	no-sl	—	—	—
Anthracnose	MO2	no	no	no	no	no	no	—	—	—	—	—	—	—	—	—
Asian rust	KS, NE, NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Bacterial blight	AR	—	—	—	—	—	—	sl	sl	sl	—	—	—	—	—	—
	IN1	no	no	no	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl
Brown spot[1]	IA2	no	no	no	no	no	no	sl	sl	sl	sl	sl	sl	—	—	—
	IL1	—	—	—	—	—	—	sl	sl	sl-mo	sl	sl	sl-mo	—	—	—
	IL2, OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IL4	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	KS	no	no	no	no	no	no	sl	sl	sl	no-sl	no-sl	no-sl	—	—	—
	MO1	sl	sl	sl	no	no-sl	no-sl	no-sl	no-sl	no-sl	sl	sl	sl	—	—	—
	NE	—	—	—	—	—	—	—	—	—	sl	sl	sl	—	—	—
Brown stem rot	IA1, IL2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IL5	—	—	—	no	no	no	—	—	—	—	—	—	—	—	—
Cercospora leaf blight[2]	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	MO1	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Charcoal rot	IN1	no	no	no	no	no	no	no	no	no	no	no	no	sl	sl	sl
Frogeye leaf spot	AR	no	no	no	no	no	no	sl	sl	sl	no	no	no	—	—	—
	MO1	no	no	no	no	no	no	no	no	no	sl	sl	sl	—	—	—
	MO2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—

Table G-6 (continued). Disease Stressor Symptom Severity of MON 89788, A3244, and the Reference Soybean Varieties

		Observation 1			Observation 2			Observation 3			Observation 4			Observation 5		
Disease	**Sites**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref**
Phytophthora[3]																
	IA2, IL1, IL4, IN2, NE, NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IN1, OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Powdery mildew	IN2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	KS	—	—	—	—	—	—	—	—	—	no-sl	no-sl	no-sl	—	—	—
Pythium	IL1	no	no	no	no	no	no	—	—	—	—	—	—	—	—	—
	IL3	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	IL5	no	no	no	no	no	no	—	—	—	no	no	no	—	—	—
	NE	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Rhizoctonia	IL1	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL3	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	IL5	no	no	no	no	no	no	—	—	—	no	no	no	—	—	—
Soybean mosaic virus	MO2	—	—	—	—	—	—	sl	sl	sl	sl	sl	sl	—	—	—
Soybean rust	IN2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Stem canker	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Sudden death	IA1	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IL4, MO2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
White mold																
	IA1, IL2, OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IA2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL3	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	IL5	no	no	no	no	no	no	—	—	—	no	no	no	—	—	—

Ref. = References; NO = None; SL = Slight; MO = Moderate; SE = Severe.

[1] Including septoria brown spot and septoria leaf spot

[2] Including cercospora leaf disease

[3] Including phytophthora root rot

— Diseases not evaluated at this observation and site.

Table G-7. Abiotic Stressor Symptom Severity of MON 89788, A3244, and the Reference Soybean Varieties

		Observation 1			Observation 2			Observation 3			Observation 4			Observation 5		
Abiotic	**Sites**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref**
Chloride toxicity																
	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Cold stress[1]	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
Compaction[2]	IL1	no-sl	no-sl	no-sl	no	no	no	no	no	no	no	no	no	—	—	—
	IL3	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	IL5	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IN1	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl
	MO1	sl-mo	sl-mo	sl-se	no	no	no	no	no	no	no	no	no	—	—	—
Drought[3]																
	AR, IA2, KS, NE, NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IA1	no	no	no	sl	sl	sl	no	no	no	sl	sl	sl	no	no	no
	IL1	no	no	no	no	no	no	sl	sl	sl	no	no	no	—	—	—
	IL2	sl	sl	sl	sl	sl	sl	sl	sl	sl	no	no	no	no	no	no
	IL3	no	no	no	mo	mo	mo	mo	mo	mo	—	—	—	—	—	—
	IL4	no	no	no	sl	sl	sl	no	no	no	no	no	no	—	—	—
	IL5	no	no	no	se	se	se	se	se	se	mo	mo	mo	—	—	—
	IN1	sl	sl	sl	mo	mo	mo	no	no	no	no	no	no	no	no	no
	IN2	sl	sl	sl	no	no	no	no	no	no	no	no	no	—	—	—
	MO1	no	no	no	sl	sl	sl	no	no	no	sl	no-sl	no	—	—	—
	MO2	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl-mo	sl-mo	—	—	—
	OH, OH2	no	no	no	sl	sl	sl	sl	sl	sl	no	no	no	no	no	no
Flood[4]	AR, IL5, IN2, NE	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL3	no	no	no	no	no	no	no	no	no	—	—	—	—	—	—
	OH, OH2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Frost	NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—

Table G-7 (continued). Abiotic Stressor Symptom Severity of MON 89788, A3244, and the Reference Soybean Varieties

		Observation 1			Observation 2			Observation 3			Observation 4			Observation 5		
Abiotic	**Sites**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**	**MON 89788**	**A3244**	**Ref.**
Hail	IA1	no-sl	no-sl	no-sl	no	no	no	no	no	no	no	no	no	no-sl	no	no-sl
	IA2, IL4, MO2, NE, NE2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	KS	sl	sl	sl-mo	no	no	no	no	no	no	no	no	no	—	—	—
Heat[5]	AR	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IA1	sl	sl	sl	sl	sl	sl	no	no	no	no	no	no	no	no	no
	IL1	no	no	no	no	no	no	sl	sl	sl	—	—	—	—	—	—
	IL2	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	IN1	sl	sl	sl	sl	sl	sl	sl	sl	sl	no	no	no	no	no	no
	MO2	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	sl	—	—	—
	OH, OH2	no	no	no	sl	sl	sl	no	no	no	no	no	no	no	no	no
Herbicide injury[6]	KS	sl	sl	sl	no	no	no	no	no	no	no	no	no	—	—	—
	MO1	sl	sl	sl	no	no	no-sl	no	no	no	no	no	no	—	—	—
Wind	IA2, IL4, IN2	no	no	no	no	no	no	no	no	no	no	no	no	—	—	—
	IL1	—	—	—	—	—	—	—	—	—	sl	no-sl	no-mo	—	—	—

Ref. = References; NO = None; SL = Slight; MO = Moderate; SE = Severe.

[1] Including temperature extremes

[2] Including wet soil/ compaction due to torrential rain fall

[3] Including soil moisture extremes

[4] Including excess moisture, excess water, flooding, and soil moisture extremes

[5] Including heat stress and temperature extremes

[6] Herbicides other than glyphosate

— Abiotic stressors not evaluated at this observation and site.

Table G-8. Insect Abundance Data from Beat Sheet Samples of MON 89788, A3244, and the Reference Soybean Varieties

			Insect Abundance[1]								
			Collection 1			Collection 2			Collection 3		
Insect	Sites	Category	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range
Aphid	IL1	Pest	0.0	0.0	0.0 – 0.3	0.3	0.0	0.0 – 0.3	1.3	3.0	0.3 – 3.0
	MO1		0.3	0.3	0.0 – 0.3	0.0	0.3	0.0 – 0.7	0.0	0.0	0.0 – 0.3
	MO2		6.0	12.3	4.7 – 13.0	—	—	—	1.0	1.3	0.3 – 1.7
Bean leaf beetle	IL1	Pest	2.0	2.7	0.3 – 3.3	—	—	—	3.0	1.7	2.7 – 5.0
	MO1		0.7	0.0	0.0 – 0.3	3.3	5.3	4.7 – 5.3	0.0	0.0	0.3 – 2.3
	MO2		0.7	2.0	2.0 – 4.3	5.3	9.0	5.7 – 6.7	2.3	2.0	0.3 – 3.3
Corn earworm	IL1	Pest	—	—	—	0.7*	0.0	0.0 – 0.0	—	—	—
Flea beetle	MO1	Pest	0.0	0.0	0.0 – 0.7	2.3	1.7	0.3 – 1.7	3.3	2.3	0.7 – 2.7
	MO2		—	—	—	—	—	—	0.3	0.0	0.0 – 0.3
Garden fleahopper	MO2	Pest	5.3	4.0	0.3 – 9.3	—	—	—	—	—	—
Grape colaspis	IL1	Pest	0.3	0.3	0.0 – 1.3	—	—	—	—	—	—
	MO2		0.0	0.0	0.0 – 0.3	—	—	—	—	—	—
Green cloverworm	IL1	Pest	—	—	—	3.0	4.0	5.0 – 11.0	4.3	6.3	7.0 – 8.3
	MO1		0.7	0.0	0.0 – 0.7	1.7	1.3	0.7 – 3.7	0.0	0.7	0.3 – 1.3
	MO2		2.0	1.7	1.0 – 3.3	29.7	27.3	26.7 – 41.0	—	—	—
Japanese beetle	IL1	Pest	1.7	2.3	1.0 – 1.7	—	—	—	—	—	—
Southern corn rootworm	MO1	Pest	1.0	1.0	0.7 – 0.7	2.0*	0.0	0.0 – 0.3	—	—	—
Stink bug	IL1	Pest	0.0	0.0	0.0 – 0.3	—	—	—	1.0	2.0	0.7 – 4.0
	MO1		—	—	—	—	—	—	0.0	0.3	0.0 – 1.0
	MO2		—	—	—	0.7	1.0	1.0 – 2.0	28.7	14.7	19.3 – 29.7

Table G-8 (continued). Insect Abundance Data from Beat Sheet Samples of MON 89788, A3244, and the Reference Soybean Varieties

			Insect Damage Ratings								
			Collection 1			Collection 2			Collection 3		
Insect	Sites	Category	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range
Tarnished plant bug	IL1	Pest	—	—	—	0.3	0.3	0.3 – 2.3	—	—	—
	MO2		—	—	—	—	—	—	0.3*	0.0	0.0 – 0.0
Weevil	IL1	Pest	—	—	—	0.0	0.0	0.0 – 0.7	0.0	0.0	0.0 – 0.7
Asian ladybeetle	MO2	Beneficial	0.0	0.3	0.0 – 0.7	—	—	—	—	—	—
Lacewing	MO1	Beneficial	—	—	—	—	—	—	0.3	0.3	0.0 – 0.0
Nabis	IL1	Beneficial	0.0	0.0	0.0 – 0.7	0.7	0.3	0.3 – 1.0	1.7	3.0	0.7 – 7.7
	MO1		—	—	—	0.0	0.0	0.0 – 0.3	—	—	—
	MO2		2.0	2.7	0.3 – 2.7	0.7	1.3	1.0 – 3.0	2.0	1.0	0.3 – 1.3
Orius	IL1	Beneficial	2.3	3.7	2.0 – 4.7	4.3	5.7	2.0 – 6.7	5.0	5.3	3.0 – 10.3
	MO1		2.0	2.3	1.0 – 4.7	0.7	0.3	1.0 – 2.7	—	—	—
	MO2		1.3	3.3	1.3 – 3.0	2.3	2.0	0.3 – 1.7	1.0	0.7	1.0 – 2.0
Pink spotted ladybeetle	MO1	Beneficial	—	—	—	—	—	—	0.0	0.3	0.0 – 0.3
7 spotted ladybeetle	MO1	Beneficial	0.0	0.0	0.0 – 0.3	—	—	—	—	—	—
Spider	IL1	Beneficial	0.7	1.7	0.0 – 0.7	2.0	1.0	1.3 – 2.3	4.7	8.7	2.7 – 8.7
	MO1		0.7	0.7	0.3 – 1.0	—	—	—	—	—	—
	MO2		—	—	—	6.7	7.0	5.7 – 11.3	2.0	1.7	1.0 – 2.7

* Indicates a statistically significant difference between the MON 89788 and A3244 at $p \leq 0.05$.

— Insects not evaluated at this observation and site.

[1] MON 89788 and A3244 values represent mean number of insects/spiders collected across three replications

Table G-9. In-Field Plant Damage of MON 89788, A3244, and the Reference Soybean Varieties

		Damage[1]											
		Evaluation 1			Evaluation 2			Evaluation 3			Evaluation 4		
Insect	Sites	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range	MON 89788	A3244	Reference Range
Aphid	MO1	0.0	0.0	0.0 – 0.0	0.3	0.2	0.2 – 0.2	0.2	0.1	0.0 – 0.1	0.0	0.0	0.0 – 0.0
Bean leaf beetle	IL1	0.2	0.1	0.0 – 0.1	0.0	0.0	0.0 – 0.0	0.0	0.1	0.0 – 0.1	0.4	0.4	0.3 – 0.4
	MO1	0.0	0.0	0.0 – 0.0	0.8	1.0	0.8 – 0.9	2.2	2.3	1.9 – 2.3	4.0	4.3	4.3 – 5.0
	MO2	0.6	0.7	0.6 – 0.8	0.9	0.8	0.8 – 1.0	1.0	1.2	1.0 – 1.1	1.2	1.4	1.2 – 1.4
Grasshopper	IL1	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.3	0.2	0.2 – 0.3
	MO1	0.5	0.4	0.5 – 0.7	0.4	0.4	0.4 – 0.5	0.6	0.7	0.6 – 0.7	0.3	0.4	0.3 – 0.4
Japanese beetle	IL1	0.4	0.4	0.5 – 0.9	0.1	0.1	0.1 – 0.5	1.0	0.7	0.8 – 1.0	1.0	1.0	1.0 – 1.0
	MO2	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.1	0.1	0.1 – 0.1	0.2	0.4	0.2 – 0.5
Spider mite	MO2	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0
Stink bug	IL1	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0
	MO1	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0	0.0	0.0	0.0 – 0.0
	MO2	0.0	0.0	0.0 – 0.0	0.1	0.1	0.0 – 0.1	1.2	1.2	1.2 – 1.3	1.7	1.4	1.5 – 1.7

* Indicates a statistically significant difference between the MON 89788 and A3244 at $p \leq 0.05$ (none detected).

[1] Based on a 0 – 9 Plant Damage Scale (Where 0 = No Damage Observed, 5 = 50 % Defoliation, and 9 = 90% Defoliation). MON 89788 and A3244 values represent mean number of insects/spiders collected across three replications.

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

Plant Production

Plants of MON 89788, A3244, and four commercially available reference varieties were grown in Lincoln County, MO, in a randomized complete block design with three replications. Each plot consisted of eight rows approximately 30 ft in length with inter-row spacing of approximately 30 in.

Flower Collection

While plants were flowering, whole flowers were collected from five non-systematically selected plants from the fourth 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. Three flowers were collected from each of the five plants per plot: one from the bottom, one from the middle, and one from the top of each plant. Flowers from each selected plant were transferred to an appropriately labeled microcentrifuge tube (three flowers per tube). Flowers from different plants within a given plot were not mixed. All tubes containing flowers were maintained on wet ice from immediately after collection until the pollen was extracted and fixed.

Pollen Sample Preparation

Pollen was collected using tweezers and a dissecting needle to open the flower and brush the pollen out. Pollen from each of the three flowers per plant was removed and placed into a new, appropriately labeled microcentrifuge tube. Approximately 0.1 ml of Alexander's stain (Alexander, 1980) was added to the microcentrifuge tube containing the pollen. The tube was closed and tapped against the bench top to dislodge pollen from the tube wall. The pollen and stain solution was thoroughly mixed via vortex. Samples were heated in a water bath at approximately 55°C for approximately ten minutes and placed in cold storage (approximately 4°C). Microscope slides were prepared by labeling them with identifying sample information and by drawing a water-repellant circle in the center of the slide with a pap hydrophobic barrier pen. Approximately 0.05 ml of the pollen and stain solution was transferred to the circle on the microscope slide and a cover slip was placed over the sample.

A minimum of 100 pollen grains per sample was desired for data collection. For samples containing less than 100 pollen grains on the microscope slide, a second slide was prepared from the remaining pollen/stain solution, and evaluated along with the first slide to obtain the 100 pollen grains per sample target. Thirty-eight out of 90 samples contain less than 100 pollen grains but the variable sample size was accounted for in the statistical analysis by using weighted means.

Data Collection

All pollen samples were viewed under an Olympus Provis AX70 light/fluorescence microscope with an Olympus DP70 digital color camera. Microscope and camera software [DP Controller v1.2.1.108 and DP Manager v1.2.1.107, respectively (© 2001-

2003, Olympus Optical Co., Ltd.)] were installed on a connected computer [running Microsoft Windows 2000 Professional (© 1981-1999, Microsoft Corp.)].

Pollen viability was evaluated by counting viable and dead pollen grains. When exposed to the staining solution, viable pollen grains stained red to purple (due to the presence of vital cytoplasmic content). Dead pollen grains stained blue to green and may have appeared round to collapsed, depending on the degree of hydration. Pollen grains were counted one field of view at a time until at least 100 pollen grains had been classified or until all pollen grains present in the sample had been counted. Dense clusters of pollen or pollen grains adhering to flower parts were not counted because they did not absorb the staining solution uniformly.

Pollen grain diameter was evaluated for ten representative viable pollen grains collected from one (randomly selected) of the five plants per plot (i.e. three plants per MON 89788, A3244, and reference). Micrographs (200X) of the 10 selected pollen grains were imported into Image-Pro Plus v4.5.1.27 (© 1993-2002, Media Cybernetics, Inc.) software for diameter measurement. Pollen grain diameter was measured along the x-axis and the y-axis (perpendicular to the x-axis). Pollen general morphology was observed for one (randomly selected) of the three micrographs per MON 89788, A3244, and reference materials evaluated for pollen grain diameter.

Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS® (SAS Version 9.1, SAS Institute, Inc. 2002-2003). Weighted means of the percentage viable pollen and mean pollen grain diameter for MON 89788 were compared to A3244 at the $p \leq 0.05$ significance level. No statistical comparisons were made between MON 89788 and reference materials. Least square means (LSMean), standard error (SE), and minimum/maximum mean values were calculated for each MON 89788, A3244, and reference material.

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Appendix I. Materials and Methods for Symbiont Study

Materials

The MON 89788 and A3244 were produced in Argentina in 2005. The reference starting seed was acquired commercially (see table below). Nodules, root, and shoot tissue collected from MON 89788, A3244, and reference plants were evaluated in the study.

Materials	Material Type	Phenotype
MON 89788	Test	Glyphosate-tolerant
A3244	Control	Conventional
A2553	Reference	Conventional
A2824	Reference	Conventional
ST3600	Reference	Conventional

The presence or absence of MON 89788 in the starting seed was verified by event-specific polymerase chain reaction (PCR) analyses. Results of PCR analyses were as expected.

Greenhouse Phase and Experimental Design

Soybean seeds were germinated by incubating for four days in a tray containing moist paper towels placed in an environmental chamber set to maintain a temperature of 22 ± 3°C. Germinated seedlings were planted in 10-inch pots containing nitrogen-free potting medium (LB2 from Sun Gro Horticulture, Inc., Garland, TX) composed of peat, vermiculite, and perlite. Plants were grown in a climate-controlled greenhouse with a 14-hour photoperiod and with a target day-time temperature of 27°C and a target night-time temperature of 22°C. Actual temperatures ranged from approximately 17°C to approximately 32°C. A total of 20 pots were planted with two germinated seedlings per pot for each of the MON 89788, A3244 and reference materials. At planting, each seedling was inoculated with approximately 1×10^8 cells of *B. japonicum* (Becker Underwood, Ames, IA) delivered in a phosphate-buffered saline solution (pH 7.0). Pots were arranged in ten replicated blocks for each 4- and 6-week sampling period using a randomized split-block design. Two additional blocks (for a total of 10 blocks per sampling period) were planted to assure 8 replicate plants were sampled for each MON 89788, A3244, and reference material for the 4- and 6-week sampling periods.

Approximately one week after emergence, plants were thinned to one seedling per pot and re-inoculated with approximately 1×10^8 cells of *B. japonicum* delivered in a phosphate-buffered saline solution. Nitrogen-free nutrient solution (~250 mL) was added weekly after plants emerged from the potting medium.

Plant Harvesting/Data Collection

Four and 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 nodules were then excised from the roots of each plant, enumerated, and the fresh weight 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.

Statistical Analysis

The data consisted of five measurement endpoints from each of the two sampling periods (4- and 6-week): nodule number, nodule dry weight (mg), shoot dry weight (mg), root dry weight (mg), and shoot total nitrogen (%). Data obtained from MON 89788, A3244, and A2553, A2824, ST3600 references were analyzed.

An analysis of variance was conducted using a randomized split-block design with eight replications for each MON 89788, A3244 and reference materials at each sampling period. Data were analyzed using SAS (Version 9.1, SAS Institute, Inc. 2002-2003) with the level of statistical significance predetermined to be 5% ($p \leq 0.05$). The means of the MON 89788 and A3244 were compared. Minimum and maximum values (reference range) were determined for the three reference materials. No statistical comparisons were made between MON 89788 and the reference materials.

Appendix J. Appearance of Glyphosate Resistant Weeds

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. The issue of glyphosate resistance is important to Monsanto because it can adversely impact the utility and life cycle of our products if it is not managed properly. The risk of weeds developing resistance and the potential impact of resistance on the usefulness of a herbicide vary greatly across different modes of action and are dependent on a combination of different factors. As leaders in the development and stewardship of glyphosate products for over 30 years, Monsanto invests considerably in research to understand the proper uses and stewardship of the glyphosate molecule. This research includes an evaluation of factors that can contribute to the development of weed resistance.

A. The Herbicide Glyphosate

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #: 1071-83-6), the active ingredient in the Roundup family of nonselective, foliar-applied, postemergent agricultural herbicides, is among the world's most widely used herbicidal active ingredients. Glyphosate is highly effective against the majority of economically significant annual and perennial grasses and broadleaf weeds. Glyphosate kills plant cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Franz et al., 1997). This aromatic amino acid pathway is not present in mammalian metabolic systems (Cole, 1985). This mode of action contributes to the selective toxicity of glyphosate toward plants and to the low risk to human health from the use of glyphosate according to label directions. A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals (Williams et al., 2000). Glyphosate has favorable environmental characteristics, including a low potential to move through the soil to reach ground water and is degraded over time by soil microbes. Because it binds tightly to soil, glyphosate's bioavailability is reduced immediately after use, which is why glyphosate has no residual soil activity. An ecotoxicological risk assessment concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to non-target species, such as birds and fish, when used according to label directions (Giesy et al., 2000).

B. Characteristics Related to Resistance

Today, some 182 herbicide-resistant species and 305 biotypes within those species have been identified (Heap, 2006). A significant portion of the biotypes are resistant to the acetolactate synthase inhibitor (ALS) family of herbicides. Resistance usually has developed because of the long residual activity of these herbicides with the capacity to control weeds all year long and the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action. Glyphosate has no soil residual activity (WSSA, 2002), a unique mode of action and apparently low resistance frequency (Weersink et al., 2005). Nonetheless, the question has been raised as to whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide.

It is important to recognize that weed resistance is a herbicide-related issue, not a crop-related issue. The use of a specific herbicide with a herbicide-tolerant crop is no different than the use of a selective herbicide over a conventional crop from a weed resistance standpoint. While the incidence of weed resistance often is associated with repeated applications of a herbicide product, its development depends very much on the specific herbicide chemistry in question as well as the plant's ability to inactivate them. Some herbicide products are much more prone to develop herbicide resistance than others. Glyphosate has been used extensively for over three decades with very few cases of resistance development, particularly in relation to many other herbicides. A summary of some of those factors is described below.

B.1. Target Site Specificity

Target site alteration is a common resistance mechanism among many herbicide classes, such as ALS inhibitors and triazines, but is less likely for glyphosate.

A herbicide's mode of action is classified by the interference of a critical metabolic process in the plant by binding to a target protein and disrupting the required function. The specificity of this interaction is critical for the opportunity to develop target site-mediated resistance. Because the herbicide contacts discreet amino acids during protein binding, changing one of these contact point amino acids can interrupt this binding. Specificity of inhibitor binding is dependent on the number and type of the amino acids serving as contact points and can be measured indirectly by counting the number of unique compounds that can bind in the same site. On one extreme, glyphosate is the only herbicide compound that can bind to EPSPS. Single amino acid substitutions near the active site have been observed for EPSPS, and while glyphosate binding is slightly weaker, these enzymes are also less fit. Similarly, high specificity also is observed for glutamine synthetase, binding three compounds including phosphinothricin in the active site (Crespo et al., 1999). Paraquat and diquat are the only two herbicides inhibiting photosystem I. No target site mutations have been reported to be responsible for resistance in these systems (Powles and Holtum, 1994).

On the other extreme are target enzymes that are efficiently inhibited by a wide array of compounds, e.g., ALS and acetyl CoA carboxylase (ACCase) are inhibited by over 50 and 20 separate herbicide compounds, respectively, that bind both within and outside the active site (HRAC, 2002; Trannel and Wright, 2002), respectively. These cases demonstrate that numerous noncritical amino acids are involved outside of the active site, offering a relatively large range of permissible mutations. In these two cases, a single amino acid change can result in virtual immunity to the class of herbicides and has led directly to the preponderance of resistant weed species for these mode-of-actions, with 93 and 35 species, respectively, identified to date for ALS and ACCase herbicides.

Glyphosate competes for the binding site of the second substrate, phosphoenolpyruvate in the active site of EPSPS and is a transition state inhibitor of the reaction (Steinrücken and Amrhein, 1984). This was verified by x-ray crystal structure (Schonbrunn et al., 2001). As a transition state inhibitor, glyphosate binds only to the key catalytic residues in the

active site. Catalytic residues are critical for function and cannot be changed without a lethal or serious fitness penalty. Furthermore, very few selective changes can occur near the active site of the enzyme to alter the competitiveness of glyphosate without interfering with normal catalytic function. Therefore, target site resistance is highly unlikely for glyphosate. This was further illustrated in that laboratory selection for glyphosate resistance using whole plant or cell/tissue culture techniques were unsuccessful (Jander et al., 2003; Widholm et al., 2001; OECD, 1999).

B.2. Limited Metabolism in Plants

Metabolism of the herbicide active moiety is often a principle mechanism for the development of herbicide resistance. The lack of glyphosate metabolism or significantly slow glyphosate metabolism has been reported in several species and reviewed in various publications (Duke, 1988; Coupland, 1985). Therefore, this mechanism is unlikely to confer resistance to glyphosate in plants.

B.3. Lack of Soil Residual Activity

Herbicides with soil residual activity dissipate over time in the soil, resulting in a sublethal exposure and, in effect, resulting in low dose selection pressure. Glyphosate adsorption to soils occurs rapidly, usually within one hour (Franz et al., 1997). Soil-bound glyphosate is unavailable to plant roots, so the impact of sublethal doses over time is eliminated. Subsequently, the postemergence only activity of glyphosate allows for the use of a high dose weed management strategy.

The graph in Figure J-1 illustrates the instances of weed resistance to various herbicide families. The different slopes observed are largely due to the factors described above, which relate to chemistry and function, in addition to levels of exposure in the field. Glyphosate is a member of the glycine family of herbicides, which has experienced a limited number of resistance cases despite almost three decades of use. The ALS inhibitors and triazine families, on the other hand, have experienced extensive cases of resistance even after they were available for only a relatively short period of time.

It also is important to recognize that each herbicide targets a large number of weeds, so the development of resistance in certain species does not mean the herbicide is no longer useful to the grower. For example, resistance of certain weeds to imidazolinone and sulfonyurea chemistries developed within three to five years of their introduction into cropping systems. Nevertheless, Pursuit (imidazolinone) herbicide had a 60% share of the U.S. soybean herbicide market despite the presence of a large number of resistant weeds because it was used in combination with other herbicides that controlled the resistant species. How weed resistance impacts the use of a particular herbicide varies greatly depending on the herbicide chemistry, the biology of the weed, availability of other control practices and the diligence with which it is managed.

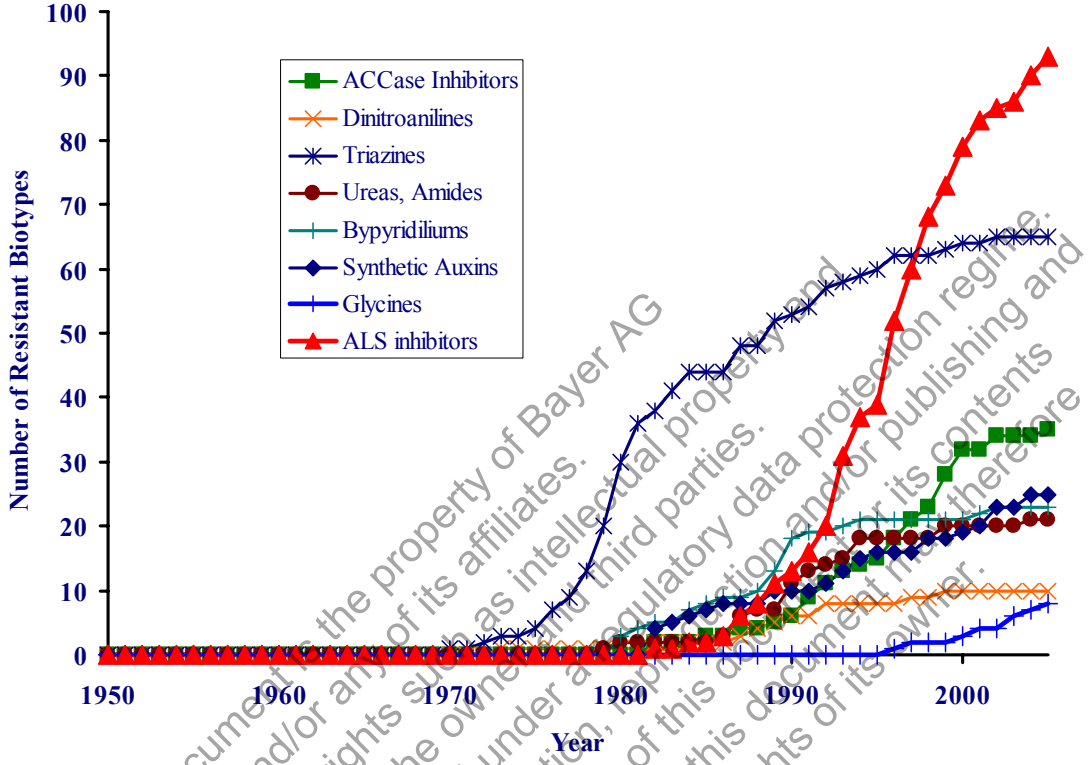

Figure J-1. Number of Herbicide Resistant Weed Species Found Over Time

Source: Heap, 2006.

C. Weeds Resistant to Glyphosate

Weed resistance generally is defined as the naturally occurring heritable ability of some weed biotypes within a given weed population to survive a herbicide treatment that should, under normal use conditions, effectively control that weed population. Thus, a resistant weed must demonstrate two criteria: (1) the ability to survive application rates of a herbicide product that once were effective in controlling it, and (2) the ability to pass the resistance trait to its seeds. Procedures to confirm resistance generally require both field and greenhouse analyses, particularly if the level of resistance is relatively low. This correlation has been particularly important for the accurate detection of glyphosate resistance, for which the levels of resistance observed have been as low as 2X the susceptible biotypes.

Herbicide tolerance differs from resistance in that the species is not controlled but has the inherent ability to survive applications of the herbicide from the beginning. In other words, the species does not develop tolerance through selection but is innately tolerant.

As part of our product stewardship and customer service policy, Monsanto investigates cases of unsatisfactory weed control to determine the cause, as described in the performance evaluation program outlined in section E of this appendix. Weed control failures following application of Roundup agricultural herbicides are most often the result of management and/or environmental issues and are very rarely the result of herbicide resistance. However, the procedures included in Monsanto's performance evaluation program provide early detection of potential resistance, with field and greenhouse protocols to investigate suspected cases and mitigation procedures established to respond to confirmed cases of glyphosate resistance.

To date, biotypes of eight weed species resistant to glyphosate have been identified and confirmed. Monsanto has worked with local scientists to identify alternative control options that have been effective in managing the resistant biotypes.

Lolium rigidum

In 1996 in Australia, it was reported that a biotype of annual ryegrass (*Lolium rigidum*) was surviving application of label recommended rates of glyphosate (Pratley et al., 1996). A collaboration was established with Charles Sturt University to develop an agronomic understanding of the biotype and investigate the mechanism of resistance. Where the biotype has been found, it has occurred as isolated patches within a field and does not appear to be widespread. The resistant biotype is effectively controlled with conservation and conventional tillage systems with other herbicides, tillage or seed removal.

A large body of biochemical and molecular biology experiments between Australian ryegrass biotypes resistant and susceptible to glyphosate indicate that the observed resistance is due to a combination of factors. The mechanism of resistance appears to be multigenic and caused by a complex inheritance pattern, which is unlikely to occur across a wide range of other species. The mechanism is yet to be fully defined despite significant research effort (Owen and Zelaya, 2005). Research by several groups has identified a difference in the translocation of glyphosate in the plant between the susceptible and resistant biotypes (Lorraine-Colwill et al., 2003; Wakelin et al., 2004).

Glyphosate resistant rigid ryegrass biotypes have been observed in orchard systems of South Africa. Similar to the Australian locations, these fields are small and isolated. Glyphosate resistant rigid ryegrass also has been found in California. Monsanto established collaborations with local scientists to identify alternative control mechanisms. The use of other herbicides, tillage, mowing, and seed removal have been effective in controlling this resistant ryegrass.

Lolium multiflorum

A population of Italian ryegrass (*Lolium multiflorum*) was reported to survive labelled rates of glyphosate by a scientist conducting greenhouse and field trials in Chile in 2001. Monsanto conducted field and greenhouse trials to confirm the resistance and worked with the researcher to identify alternative control options. Populations were also identified in Brazil in 2003 and in Oregon, U.S., in 2004.

The mechanism responsible for herbicide resistance in these biotypes is still unclear. Recently published research studies with the resistant biotype from Oregon have indicated that the resistance is not due to an altered target site (Perez-Jones et al., 2005).

The resistant biotypes have been found on only a few farms and are easily controlled through tank mixes with other herbicides and cultural agronomic practices. Italian ryegrass is not a common weed in soybean fields in the U.S.

Eleusine indica

Goosegrass is a warm season annual grass that can be found in U.S. soybean fields. A population of *Eleusine indica* (goosegrass) was reported to survive labeled rates of glyphosate in some orchard systems in Malaysia. Monsanto entered into collaborations with the University of Malaysia and identified alternative control options to effectively manage the resistant biotype. Extensive molecular investigations determined that some of the resistant goosegrass plants have a modified EPSPS that is two to four times less sensitive to glyphosate than in more sensitive biotypes (Baerson et al., 2002). However, some resistant individuals did not exhibit the enzyme modification, suggesting that different mechanisms may be at play or resistance may be due to a combination of factors.

The resistant biotypes are easily controlled through application timing (applying glyphosate during the early growth stages), other herbicides (SWCG, 2006), tillage and other cultural control practices.

Conyza canadensis

Laboratory and field investigations confirmed the presence of a glyphosate-resistant biotype of marestail (*Conyza canadensis*) in certain states of the eastern and southern U.S. (VanGessel, 2001). The mechanism of resistance in the marestail biotype is likely due to altered cellular distribution that impaired phloem loading and plastid import of glyphosate that reduced overall translocation (Feng et al., 2004).

Investigations thus far indicate that this biotype has a heritable resistance ranging up to approximately six to eight times field herbicide application rates. Current data indicates that the heritance is dominant and transmitted by a single nuclear gene. Additional studies found that resistance was not due to overexpression of EPSPS, glyphosate metabolism or reduction in glyphosate retention or uptake. Resistance also was not due to target site mutation, as the three isozymes of EPSPS identified in marestail were identical in sensitive and resistance lines (Heck et al., 2002). Tissues from both sensitive and resistant biotypes showed elevated levels of shikimate, suggesting that EPSPS remained sensitive to glyphosate. Analysis of tissue shikimate levels relative to those of glyphosate demonstrated a reduced efficiency of EPSPS inhibition in the resistant biotypes.

The resistant marestail biotype has been observed in conventional and Roundup Ready cotton and soybean fields. As in other cases, Monsanto responded to weed control

inquiries and alternative weed control options were provided. The primary recommendation is for growers to use a tank mix of glyphosate with either a dicamba or 2,4-D based herbicide in their burndown treatment. If marestail is present in-crop in Roundup Ready soybeans, then growers are advised to use a tank mix of glyphosate and cloransulam-methyl. An alternative control option is to plant a cover crop that can compete with marestail and limit its fall and winter germination.

In addition, as part of Monsanto's stewardship program, we have obtained a supplemental label, approved by EPA, which provides specific instructions on proper use of glyphosate herbicides where the resistant biotype has been confirmed. Growers are instructed to use the alternative control options, regardless of whether they had trouble controlling marestail on their farm the previous season, as a means to minimize spread of the resistant biotype. It has been recommended to growers in surrounding areas where the resistant biotype has not been confirmed that they use the alternative control options if marestail has been a difficult weed for them to control.

Ambrosia artemisiifolia

Field and greenhouse investigations have confirmed the presence of glyphosate-resistant biotypes of common ragweed in Missouri (Pollard et al., 2004a,b). A biotype of this species from Arkansas also is listed as glyphosate resistant at the website for the International Survey of Herbicide Resistant weeds (Heap, 2006). Results from greenhouse experiments with the common ragweed biotype from Missouri have demonstrated that the resistant biotype exhibited an I_{50} value that was approximately 10-fold higher than the susceptible biotype on a dry weight basis.

The mechanism of resistance in these common ragweed biotypes has not been fully elucidated. Current results from enzyme assays with the Missouri biotype indicate an elevated level of shikimate in both sensitive and resistant biotypes, suggesting that EPSPS remains sensitive to glyphosate. Over time, shikimate levels in the resistant biotype return to normal, indicating that glyphosate no longer would be present at the site of action ([redacted] 2006; personal communication). These results are consistent with a reduced translocation or exclusion mechanism for glyphosate resistance. The exclusion mechanism for glyphosate resistance has been seen previously with glyphosate-resistant horseweed and some biotypes of glyphosate-resistant rigid ryegrass and Italian ryegrass.

Field study in Roundup Ready soybeans have demonstrated effective control of the resistant common ragweed biotype from Missouri by using tank mixes of glyphosate at the labeled rate and lactofen (Pollard et al., 2004b). Monsanto also is collaborating with an academic researcher at the University of Missouri to further study this biotype. Additional field studies in corn at the site indicated that the use of atrazine preemergence or early postemergence, and the use of dicamba or 2,4-D postemergence provided excellent control of the glyphosate-resistant ragweed.

Amaranthus palmeri

Field and greenhouse investigations have been completed to confirm the presence of a glyphosate-resistant biotype of Palmer amaranth in Georgia (Heap, 2006). The resistant

biotype was controlled at approximately 27 times the herbicide rate needed to control the susceptible biotype (Haider et al., 2006).

The mechanism of resistance in this Palmer amaranth biotype has not been fully elucidated. Current results from laboratory studies indicate no differences in foliar uptake between the resistant and susceptible biotypes, and no significant differences in glyphosate translocation out of the treated leaf between the resistant and susceptible biotypes. Differences in shikimate accumulation were observed between the resistant and susceptible biotypes, with no shikimate accumulation observed in the resistant biotype until treated with the highest glyphosate concentration tested (Haider et al., 2006). The observation of no shikimate accumulation found in the resistant biotype at the lower glyphosate rates tested appears to indicate that the resistance may be due to a difference at the site of action.

Field study results in Roundup Ready cotton have demonstrated control of the glyphosate resistant Palmer amaranth biotype by using preemergence residual herbicides and tank mix of glyphosate and another effective herbicide, such as flumioxazin (Culpepper et al., 2006). Several of the herbicides used in these treatments are also approved for use in soybeans. Monsanto continues to collaborate with an academic researcher at the University of Georgia to further study this biotype.

Other Species

Outside of the U.S., populations of two additional weed species, hairy fleabane (*Conyza bonariensis*) and buckhorn plantain (*Plantago lanceolata*), have been reported to be resistant to glyphosate (Heap, 2006). Where possible, Monsanto has collaborated in the investigation of these biotypes, and supports the academic research. Various herbicides are available for control of these species, but these species commonly do not occur in U.S. soybean production.

Plant species tolerant to glyphosate, such as *Equisetum arvensis* (field horseweed), are occasionally described as resistant. This characterization is technically inappropriate because glyphosate is not commercially effective on those weeds and they generally are not listed as controlled on Roundup agricultural herbicide product labels. Other species, such as *Convolvulus arvensis* (field bindweed) that are listed on the label may be partially tolerant or difficult to control with glyphosate alone. In these cases, additional herbicides are usually recommended to be tank mixed with glyphosate. Still other species, such as *Abutilon theophrasti* (velvetleaf), are listed as controlled by glyphosate on the label and are controlled by glyphosate alone under most conditions, but a tank mix recommendation for an additional herbicide may be used in the field due to sensitive environmental conditions at the time of the herbicide application.

In summary, Monsanto has effective product stewardship and customer service practices established to directly work with the grower communities and provide appropriate control measures for the occurrence of glyphosate-resistant weeds. Monsanto has collaborated with academic institutions to study these glyphosate-resistant biotypes and findings have

been communicated to the scientific community through publications in peer-reviewed scientific journals and in presentations at scientific meetings.

D. Weed management strategies for glyphosate

A key element of good weed management is using the correct rate of glyphosate at the appropriate window of application for the weed size and species present. Higher herbicide doses result in higher weed mortality and less diversity of resistance genes in the surviving population (Matthews, 1994). Low herbicide rates also may allow both heterozygous and homozygous resistant individuals to survive (Maxwell and Mortimer, 1994), further contributing to the buildup of resistant alleles in a population. As resistance is dependent upon the accumulation of relatively weak genes, which appears may be the case for one or more of the eight weed species in which resistance to glyphosate has been found, using a lethal dose of herbicide is critical. The glyphosate rates recommended in Roundup agricultural product labeling have been evaluated for the effective control of the target weed populations, and are consistent with the high dose strategy.

Results that support these strategies have emerged from recent field research studies at several universities. Various weed management programs have been evaluated since 1998 to determine how they impact weed population dynamics. Studies were initiated in Colorado, Nebraska, Wyoming (Wilson et al., 2006), and Wisconsin (Jeschke and Stoltenburg, 2006) to evaluate continuous use of a Roundup Ready system with exclusive use of glyphosate at label and below label rates, rotation of glyphosate with herbicides with other modes-of-action, and only non-glyphosate herbicides. These treatment regimes were compared to a conventional herbicide program for each crop evaluated. General observations after eight years are:

1. Use of a continuous Roundup Ready cropping system with either glyphosate alone at labeled rates or incorporation of herbicides with other modes-of-action resulted in excellent weed control and generally lower numbers of seed in the weed seed bank,

2. Use of glyphosate at below labeled rates resulted in a weed shift to common lambsquarters at two locations (NE, WY), and

3. Weed species diversity tended to be higher in the recommended rate and application timing of glyphosate-based weed management treatments.

By using glyphosate at the recommended lethal dose, the build up of weeds with greater inherent tolerance or potential resistance alleles has been avoided over the duration of these studies. These results indicate that continuous Roundup Ready systems used over several years did not create weed shifts or resistant weeds when the correct rate of glyphosate was applied and good weed management was practiced.

E. Glyphosate stewardship program

Commercial experience, field trials and laboratory research demonstrate that one of the most important stewardship practices is achieving maximum control of weeds. This can

be accomplished by using the correct rate of glyphosate at the appropriate window of application for the weed size and species present, and using other tools or practices as necessary.

As the recognized leader on the development and commercialization of glyphosate, Monsanto is committed to the proper use and long-term effectiveness of glyphosate through a four-part stewardship program: developing appropriate weed control recommendations, continuing research to refine and update recommendations, educating growers on the importance of good weed management practices, and responding to repeated weed control inquiries through a performance evaluation program.

E.1. Develop Local Weed Management Recommendations to Ensure Maximum Practical Control is Achieved

Weed control recommendations in product labels and informational materials are based on local needs to promote the use of the management tool(s) that are most appropriate technically and economically for each region. Furthermore, growers are instructed to apply the same principles when making weed control decisions for their own farm operation. Multiple agronomic factors, including weed spectrum and population size, application rate and timing, herbicide resistance status (where applicable), and an assessment of past and current farming practices used in the region or on the specific operation are considered to ensure appropriate recommendations for the use of glyphosate to provide effective weed control. Carefully developing and regularly updating the use recommendations for glyphosate are fundamental to Monsanto's stewardship program.

Weed Spectrum

Weed spectrum refers to all the weed species present in a grower's field and the surrounding areas that may impact those fields. The spectrum may vary across regions, farm operations, and even among fields within a farm operation depending on environmental conditions and other factors. Weed control programs should be tailored on a case-by-case basis by identifying the target weeds present, considering the efficacy of glyphosate and other weed management tools against those particular weeds, and assessing if any are unlikely to be controlled sufficiently with glyphosate alone (not included on the Roundup brand agricultural herbicide label: difficult to control based on the agronomic and/or environmental conditions, or having documented resistance to glyphosate). A formulation, rate, application parameters, and additional control tools are recommended, as necessary, to optimize control of all weeds in that system.

Application Rate

Application rate is integral to the correct use of glyphosate and critical to obtaining effective weed control. Significant research is conducted to identify the appropriate rate of glyphosate that should be applied for a particular weed at various growth stages in various agronomic and environmental conditions. These rates are included in rate tables provided in product labels and other materials. In addition, Monsanto recommends that growers use the rate necessary to target the most difficult to control weed in their system to minimize weed escapes. When using tank mixes, growers should consider the

potential impacts on glyphosate efficacy through antagonism or below-recommended rates and make adjustments accordingly.

Application Timing

Application timing is based on the growth stage of weeds, the size/biomass of weeds and the agronomic and environmental conditions at the time of application. Delaying the application of glyphosate and allowing weeds to grow too large before applying the initial recommended rate of glyphosate will result in poor efficacy. Applying glyphosate at a time while weeds are under agronomic stress (e.g., insect and/or disease) or environmental stress (e.g., moisture and/or cold) can also result in poor weed control.

Compensating for a delayed application through subsequent applications may not be effective, as the first application may inhibit weed growth and impair efficacy of the second application because the weeds may not be in an active growth process.

Correct application timing is dependent on management of the weed spectrum, the size and layout of the farm operation and the feasibility to make timely applications of all weeds in each field with labor and equipment available. Monsanto recommends an application timeline that targets small weeds, and where applicable includes recommendations for inclusion of additional control tools as necessary to optimize control of all weeds on that farm.

Finally, it is important to assess current agronomic practices used in a region or on a farm operation to integrate the glyphosate recommendations into the grower's preferred management system. Variables such as tillage methods, crop rotations, other herbicide programs, other agronomic practices, and the resistance status of weeds to herbicides other than glyphosate can impact the spectrum of weeds present and the tools available to the grower.

Weed management recommendations communicated to the grower also incorporate other components of the glyphosate stewardship program, including the use of certified seed, employing sanitary practices such as cleaning equipment between fields, and scouting fields and reporting instances of unsatisfactory weed control for follow up investigation.

E.2. Continuing Research

A fundamental component of Monsanto's leadership in glyphosate stewardship is continuing research on the recommended use of glyphosate and factors impacting its effectiveness. In addition to extensive analyses conducted to determine the labeled rate of glyphosate prior to product registration, ongoing agronomic evaluations are conducted at the local level to refine weed management recommendations for specific weed species in specific locations.

Weed efficacy trials are part of ongoing efforts by Monsanto to tailor recommendations to fit local conditions and grower needs. Application rate and timing, additional control tools and other factors are included in these analyses. As a result of weed efficacy trials, changes are made to specific weed control recommendations where and when applicable,

and modifications to local recommendations are highlighted to growers through informational sheets and other methods.

E.3. Education and Communication Efforts

Another key element of effective product stewardship and appropriate product use is education to ensure that growers understand and can implement effective weed management plans and recommendations. Monsanto communicates weed management recommendations through multiple channels and materials to multiple audiences.

All internal technical and field sales representatives are required to take a weed management training course to understand the glyphosate stewardship program and the importance of proper product use. The training program is supported by ongoing weed management updates that highlight seasonal conditions and recommendations.

Monsanto weed management recommendations and the importance of sound agronomic practices are communicated to growers, dealers and retailers, academic extension, and crop consultants through multiple tools:

a. Technology training programs: Highlighting weed management principles, weed management plans, and practical management guidelines.
b. Technology Use Guide: Includes tables outlining appropriate rate and timing for different weed species and sizes. Crop specific weed resistance management guidelines are also included.
c. Grower meetings: Conducted prior to planting to emphasize the importance of following local application recommendations.
d. Marketing programs: Designed to reinforce and encourage the continued adoption and use of weed management recommendations by the grower (e.g., recommended rate and timing of application, additional weed control tools when applicable).
e. Informational Sheets: Issued to growers and dealers/retailers to highlight local recommendations for specific weeds.
f. Weedresistancemanagement.com website.

As with most stewardship efforts, education is key in helping growers and other stakeholders understand the importance of proper product use and encouraging those practices in the field.

E.4. Performance Inquiry Evaluation and Weed Resistance Management Plan

To support and enhance Monsanto's weed management principles and recommendations, Monsanto implements a performance evaluation program based on grower performance inquiries and field trial observations. The goal of the program is to continue to adapt, modify, and improve Monsanto's weed control recommendations, with a focus on:

a. Identifying particular weeds and growing conditions,
b. Providing product support to customers who are not satisfied with their level of weed control, and

c. Identifying and investigating potential cases of glyphosate resistance early so that mitigation strategies can be implemented.

The grower generally reports instances of unsatisfactory weed control following glyphosate application to Monsanto or the retailer. It is important to Monsanto, as part of its customer service and stewardship commitment, that these product performance inquiries are acted upon immediately, resolved to the satisfaction of the customer, and not repeated.

The vast majority of inquiries is due to application error or environmental conditions and resolved through a phone conversation with the grower. However, a system is in place to investigate a repeated performance inquiry for a specific weed in a specific field within the same year. The investigation considers the various factors that could account for ineffective weed control such as:

a. Application rate and timing,
b. Weed size and growth stage,
c. Environmental and agronomic conditions at time of application, and
d. Calibration of herbicide application equipment.

In all cases, the first priority is to provide control options to the grower so that satisfactory weed control is achieved for that growing season. The majority of repeated product performance inquiries is due to improper application or environmental/agronomic conditions that are not repeated. However, if the problem occurs again in that field and does not appear to be due to application or growing condition factors, then steps are taken to determine if resistance is the cause as outlined in the Monsanto Weed Resistance Management Plan.

The Monsanto Weed Resistance Management Plan consists of three elements:

a. Identification process for potential cases of glyphosate resistance,
b. Initiation of steps to respond to cases of suspected resistance, and
c. Development and communication of guidelines to incorporate resistance mitigation into weed management recommendations.

Identification of potential cases of glyphosate resistance is accomplished through evaluation of product performance inquiries and local field trials. These efforts provide an early indication of ineffective weed control that may indicate potential resistance.

If the follow up investigation clearly indicates that the observation is due to application error or agronomic/environmental conditions, then appropriate control options are recommended to the grower for that season and the grower receives increased education on the importance of proper product use. The vast majority of weed control inquiries fall into this category.

If repeated lack of control is observed and does not appear to be due to application error or environmental conditions, then a field investigation is conducted by Monsanto to more thoroughly analyze control of the weed.

The vast majority of field investigations do not repeat the insufficient control reported by the grower, largely due to characteristics of the mode of action of glyphosate that make subsequent applications by the grower ineffective. The weed usually must be in an active growth phase in order for glyphosate to be effective. Application error or environmental conditions that result in insufficient glyphosate to kill the weed often stunt its growth such that subsequent applications by the grower are ineffective. Monsanto's field investigations at this stage remove that artifact by ensuring that the weeds tested are in an active growth phase. If the field investigation confirms that agronomic factors account for the observation, then the grower receives increased education on proper application recommendations.

In addition, the internal network of Monsanto technical managers and sales representatives in the surrounding area are notified to highlight any problematic environmental conditions or application practices that may be common in that area. Critical information regarding location, weed species, weed size, rate used and the potential reason for lack of control are captured, and the results are reviewed annually by the appropriate technical manager to identify any trends or learnings that need to be incorporated into the weed management recommendations.

If the reported observation is repeated in the field investigation, then a detailed performance inquiry is conducted and greenhouse trials are initiated. If greenhouse trials do not repeat the observation and the weed is clearly controlled at label rates, then a thorough follow up visit is conducted with the grower to review the application recommendations and conditions of the operation that may be impacting weed control. The internal network of agronomic managers is notified of the results to raise awareness of performance inquiries on that weed the following season. If the greenhouse efficacy trials do indicate insufficient control at label rates, then detailed studies are conducted to determine if the weed is resistant.

Resistance is considered to be confirmed if the two criteria outlined in the Weed Science Society of America definition of resistance are deemed to be fulfilled either through greenhouse data or experience with similar cases:

1. The suspect plant is demonstrated to tolerate labeled rates of glyphosate that previously were effective in controlling it, and
2. The suspect plant is capable of passing that ability to offspring (the trait is heritable).

Additional field trials will be initiated simultaneously as these investigations are conducted to identify the most effective and efficient alternative control options for that weed in various growing conditions. The research may be conducted internally as well as through collaboration with external researchers.

If resistance is confirmed, then the scientific and grower communities are notified as appropriate and a weed resistance mitigation plan is implemented. The mitigation plan is designed to manage the resistant biotype through effective and economical weed management recommendations implemented by the grower. The scope and level of intensity of the mitigation plan may vary depending on a combination of the following factors:

a. Biology and field characteristics of the weed (seed shed, seed dormancy, etc.),
b. Importance of the weed in the agricultural system,
c. Resistance status of the weed to other herbicides with alternate modes of action, and
d. Availability of alternative control options.

These factors are analyzed in combination with economic and practical management considerations to develop a tailored mitigation strategy that is technically appropriate for the particular weed and incorporates practical management strategies that can be implemented by the grower.

Once developed, the mitigation plan is communicated to the grower community through the use of supplemental labeling, informational fact sheets, retailer training programs, agriculture media or other means, as appropriate.

The final step of the Weed Resistance Management Plan may include extensive genetic, biochemical or physiological analyses of confirmed cases of glyphosate resistant weeds in order to elucidate the mechanism of resistance. Findings of this research are communicated to the scientific community through scientific meetings and publications, and information pertinent to field applications is incorporated into weed management recommendations.

F. Summary

Development of weed resistance is a complex process that is very difficult to accurately predict. No single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance needs to be managed on a case-by-case basis and tailored for the particular herbicide and grower needs. Using good weed management principles, built upon achieving high levels of control through proper application rate, choice of cultural practices, and appropriate companion weed control tools, will allow Roundup agricultural herbicides, with their glyphosate active moiety, to continue to be used effectively.

The key principles for effective stewardship of glyphosate use, including Roundup Ready crops, include: (1) basing recommendations on local needs and using the tools necessary to optimize weed control, (2) using proper rate and timing of application, and (3) responding rapidly to instances of unsatisfactory weed control.