RECEIVED
By APHIS BRS Document Control Officer at 11:16 am, Jul 21, 2011

MONSANTO

Petition for the Determination of Nonregulated Status for MON 87712 Soybean

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

July 19, 2011

OECD Unique Identifier: MON-87712-4

Monsanto Petition Number: 11-SY-217U

Submitted by:

Taiwo O. Koyejo, Ph.D.

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
Phone: (314) 694-4021
Fax: (314) 694-3080
E-mail: taiwo.o.koyejo@monsanto.com

Prepared by:
E. Bell, Ph.D., T.C. Best, B.S., A.H. Culler, Ph.D., E.C. Dickinson, B.S., J.C. Goley, M.S., T.O. Koyejo, Ph.D., K.E. Niemeyer, M.S., J.R. Phillips, Ph.D., G.J. Rogan, M.S., A. Skipwith, M.S., H. Su, M.S.

Contributors and/or Principal Investigators:

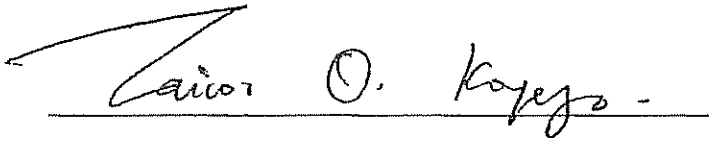
B.M. Baltazar, Ph.D., L.D., Barberis, B.S., P.L. Bommireddy, Ph.D., C.R. Brown, M.A., D.B. Carson, Ph.D., F.G. Dohleman, Ph.D., M. Elrod, M.S., A. Evans, M.P.H., A.C. Fitzgerald, M.S., C.W. Garnaat, M.S., T. Geng, Ph.D., S.-W. Hoi, M.S., K.M. Huizinga, Ph.D., N. Ivleva, Ph.D., T.A. Kaempfe, B.S., M.S. Koch, Ph.D., K.R. Lawry, M.S., K.J. Mathis, S.A. Nolte, Ph.D., S. Paul, M.S., S.L. Phillips, M.S., C. Jiang, Ph.D., A. Silvanovich, Ph.D., D. Stojsin, Ph.D., S.J. Tauchman, M.S., Q. Tian, Ph.D., H. Tu, M.S., J.L. Vicini, Ph.D., R. Wang, M.S.

RELEASE OF INFORMATION

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

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.



Taiwo O. Koyejo, Ph.D.
Regulatory Affairs Manager

Address:
Monsanto Company
800 North Lindbergh Blvd., C3SD
St. Louis, MO 63167

Tel: (314)-694-4021
Fax: (314)-694-3080

EXECUTIVE SUMMARY

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

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived soybean product, MON 87712, any progeny derived from crosses between MON 87712 and conventional soybean, and any progeny derived from crosses of MON 87712 with biotechnology-derived soybean that have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Soybean is one of the largest U.S. crops based on the acreage planted and quantity harvested each year. Global demand for soybean is expected to increase and as a major global supplier of soybean, the U.S. must increase soybean production to help meet growing demand. Increased soybean productivity in the U.S. has been accomplished by both increasing the area under cultivation and through yield increases per unit area. Based on recent trends in farm production and land area, most OECD (Organization for Economic Cooperation and Development) countries, including the U.S. and Canada, are predicted to face the challenge of expanding agricultural output by raising productivity on a stable or reduced land area. Thus, much of the projected expansion in soybean production in the future is expected to come from increased yield rather than increased area under production.

Improvement in soybean yield remains one of the major objectives for plant breeders. Gains in major crop yields over the years can, in-part, be attributed to genetic improvement through traditional breeding. Breeders have crossed plants with different genetic backgrounds and selected traits resulting in higher yields, compositional improvements, and desirable production traits. Biotechnological approaches complement traditional soybean breeding efforts by targeting some of these same major characteristics as traditional breeding.

Crop yield results from a sequential growth and development process – first the plant grows vegetatively and produces photosynthetic tissue, followed by flowering and the production of seeds, and finally seed filling and maturation. Actual yield performance is a complex parameter that is dependent on a number of genetic and environmental factors, that influence a crop's opportunity to realize its full yield potential. Improvements in crop yield have been a primary focus of conventional breeding. The genetic changes that resulted in crop domestication and yield improvement in conventional varieties have been

shown by modern molecular biology analysis to have been typically achieved through the selection and safe use of plant genes encoding transcriptional regulator proteins. Agricultural biotechnology provides the opportunity to further enhance crop yields through the introduction of new genetic elements that use or modify existing pathways in the plant.

Monsanto Company has developed the biotechnology-derived soybean line MON 87712, which will be used in traditional breeding programs to produce commercial soybean varieties with increased yield opportunity. The yield increase in MON 87712 is achieved using the *BBX32* gene from the plant *Arabidopsis thaliana* that produces a protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night processes and results in increased availability of assimilates[1] in MON 87712 compared to an appropriate comparator without this gene. Increased assimilate availability in MON 87712 is supported by the measurement of factors indicative of an extended period of photosynthetic activity in MON 87712 and evidence of changes in diurnal metabolism during the reproductive phase of the soybean plant, as well as by the significantly higher yield of MON 87712 when compared to control, as observed in multisite field studies in the U.S.

Data and Information Presented Confirms the Lack of Plant Pest Potential of MON 87712 Compared to Conventional Soybean

The data and information presented in this petition demonstrate that MON 87712 is unlikely to pose an increase in plant pest potential, including weediness or adverse environmental impacts, compared to conventional soybean. The conclusion on the food, feed, and environmental safety of MON 87712 was based on multiple, well-established lines of evidence:

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

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

3. Data demonstrated that the BBX32 protein in MON 87712 is unlikely to be a toxin or allergen based on extensive information collected.

4. A compositional assessment of harvested seed and forage confirmed that MON 87712 is compositionally equivalent to conventional soybean.

5. An extensive evaluation of phenotypic and agronomic characteristics and environmental interactions of MON 87712 demonstrated no increased plant pest potential or adverse environmental impact compared to conventional soybean.

---

[1] An Assimilate is a product of plant metabolism (Salisbury and Ross, 1992) from processes such as carbon and nitrogen fixation.

6. An assessment of the potential impact on non-target organisms (NTOs) and endangered species indicated that MON 87712 is unlikely to have adverse effects on these organisms compared to conventional soybean.

7. Evaluation of MON 87712 using typical cultivation and management practices for soybean concluded that deregulation of MON 87712 is expected to have minimal impact on soybean agronomic practices or land use, while providing the opportunity to increase the yield of soybean and help meet growing global demand for soybean.

Soybean is a Highly Domesticated Crop Lacking Weedy Characteristics

There is a longstanding history of safe use and consumption of conventional soybean and processed soybean products. Soybean is grown as a commercial crop in over 35 countries. Domestication occurred as early as 1000 B.C. and soybean is now the most widely grown oilseed crop in the world, with approximately 258.4 million metric tons of harvested seed produced in 2010, which represented 58% of world oilseed production that year.

During the period from 2004 to 2010, U.S. growers planted between 75.2 and 76.6 million acres of soybean per year. The commercial soybean species in the U.S. (*Glycine max* L. Merr.) does not exhibit weedy characteristics, does not invade established ecosystems, and does not outcross to weedy or wild relatives. Soybean is not listed as a weed in major weed references, nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Soybean does not possess attributes commonly associated with weeds, such as the ability to disperse, invade, or become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Due to a pronounced lack of dormancy it is known that soybean seed can germinate quickly under adequate temperature and moisture conditions, and can potentially grow as a volunteer plant. However, volunteer soybean plants are generally killed by frost during the autumn or winter of the year it germinated. Furthermore, if volunteer plants survive, they do not compete well with the succeeding crop, and are controlled readily via mechanical or other chemical means. Finally, since wild populations of *Glycine* species are not known to exist in the U.S., there is no opportunity for soybean, including MON 87712, to outcross to wild or weedy relatives.

Conventional Soybean A3525 is an Appropriate Comparator to MON 87712

Soybean variety A3525 is the parental line of MON 87712, which was derived from a single plant transformant of the A3525 variety. A3525 was used as the conventional soybean comparator to support the safety assessment of MON 87712. MON 87712 and the conventional control A3525 have similar genetic backgrounds with the exception of the *BBX32* expression cassette, thus, the effect of the *BBX32* expression cassette and the expressed BBX32 protein can be assessed in an unbiased manner using a comparative safety assessment.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA in MON 87712

MON 87712 was developed through *Agrobacterium*-mediated transformation of conventional soybean A3525 meristem tissue with plasmid vector PV-GMAP5779. PV-GMAP5779 contains two T-DNAs, each delineated by Left and Right Border regions to facilitate transformation. The first T-DNA, designated as T-DNA I, contains the *BBX32* coding sequence, derived from *Arabidopsis thaliana,* under regulation of the *e35S* promoter and the *E6* 3′ untranslated region. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* coding sequence under the regulation of the *FMV/EF-1α* promoter, *EF-1α* leader, *EF-1α* intron, *CTP2* targeting sequence, and the *E9* 3′ untranslated region. During transformation, both T-DNAs were inserted into the soybean genome where T-DNA II, containing the *cp4 epsps* expression cassette, functioned as a marker gene for the selection of transformed plantlets. Subsequently, conventional self-pollinated breeding methods and segregation, along with a combination of analytical techniques, were used to isolate those plants that contained the *BBX32* expression cassette (T-DNA I) but did not contain the *cp4 epsps* expression cassette (T-DNA II), resulting in the production of marker-free MON 87712.

Molecular characterization of MON 87712 by Southern blot analyses confirmed that one copy of the *BBX32* expression cassette (T-DNA I) was integrated into the soybean genome at a single locus. No T-DNA II or backbone DNA sequences from plasmid vector PV-GMAP5779 were detected in MON 87712. The complete DNA sequence of the insert and adjacent genomic DNA sequence in MON 87712 confirmed the integrity of the inserted *BBX32* expression cassette within the inserted sequences and identified the 5′ and 3′ insert-to-genomic DNA junctions. Additionally, Southern blot analysis of MON 87712 demonstrated that the inserted DNA has been maintained through five generations of breeding, thereby, confirming the stability of the insert over multiple generations.

Data Confirms BBX32 Protein Safety

MON 87712 contains the *BBX32* expression cassette that encodes the BBX32 protein. BBX32 is a transcriptional accessory protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night processes and results in increased availability of assimilates in the plant. BBX32 is a protein that has homologs in food plants with a history of safe use.

A multistep approach was used to characterize and assess the safety of BBX32 protein in MON 87712. This detailed characterization and assessment confirmed that the BBX32 protein in MON 87712 is safe for human and animal consumption. The assessment involved: 1) characterization of the physicochemical and functional properties of BBX32; 2) quantification of BBX32 levels in plant tissues; 3) comparison of the amino acid sequence of BBX32 in MON 87712 to known allergens, gliadins, glutenins, toxins, and other biologically-active proteins known to have adverse effects on mammals; 4) evaluation of the digestibility of BBX32 protein in simulated gastric and intestinal fluids; 5) documentation of the presence of related proteins in several plant species currently

consumed; and 6) investigation of the potential mammalian toxicity through an oral gavage assay.

Western blot analysis demonstrated that BBX32 was detected in leaf and root of MON 87712, and was below the limit of detection in harvested seed and forage. Bioinformatics analysis determined that BBX32 does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins. In addition, BBX32 was rapidly digested in *in vitro* assays using simulated gastric and intestinal fluids and did not show any adverse effects when administered to mice via oral gavage. The acute gavage study results, combined with undetectable levels in harvested seed and forage result in margins of exposure (MOE) exceeding one million for the most exposed population in the U.S. The protein safety assessment supports the conclusion that BBX32 poses no meaningful risk to the environment, human, or animal health.

MON 87712 is Compositionally Equivalent to Conventional Soybean

Detailed compositional analyses were conducted in accordance with soybean-specific OECD guidelines to assess levels of key nutrients and anti-nutrients in MON 87712 compared to levels present in the parental conventional soybean control A3525 as well as conventional commercial reference varieties. These compositional comparisons were made by analyzing the harvested seed and forage harvested from plants grown at each of eight field sites in the U.S. during the 2009 field season. The conventional commercial reference varieties used to establish a range of natural variability for the key nutrients and anti-nutrients in conventional commercial soybean varieties have a history of safe consumption. Nutrients assessed in this study included proximates, fiber, amino acids, fatty acids, and vitamin E in harvested seed, and proximates and fiber in forage. The anti-nutrients assessed in harvested seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitors, and isoflavones.

The combined-site analysis was conducted to identify statistically significant differences (α=0.05) between MON 87712 and the conventional control A3525. The results from the combined-site data were reviewed using considerations relevant to food and feed safety and nutritional quality. These considerations included assessments of: 1) the relative magnitude of the differences in the mean values of nutrient and anti-nutrient components of MON 87712 and the conventional control, 2) whether the MON 87712 component mean value was within the range of natural variability of that component as represented by the 99% tolerance interval of the conventional commercial reference varieties grown concurrently in the same field trial, 3) the reproducibility of the statistically significant combined-site component differences at individual sites, and 4) the differences within the context of natural variability of commercial soybean composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

Assessment of the analytical results concluded that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 87712 soybean. In addition, the levels of assessed components in MON 87712 were compositionally equivalent to the conventional control and within the

range of variability of the conventional commercial reference varieties that were grown concurrently in the same field trial. It is concluded that harvested soybean seed and soybean forage produced from MON 87712 are compositionally equivalent to harvested seed and forage of the conventional soybean and that the introduction of *BBX32* in MON 87712 does not have a meaningful impact on the composition and therefore on the food and feed safety, nutritional quality, and familiarity (as discussed below) of MON 87712 compared to conventional soybean.

MON 87712 Does Not Change Soybean Plant Pest Potential or Environmental Interactions

Plant pest potential of a biotechnology-derived crop is assessed on the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, knowledge of the introduced trait, the receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 87712 included the parental conventional control A3525 as a comparator. This evaluation used a weight of evidence approach and considered statistical differences between MON 87712 and the conventional control A3525 with respect to reproducibility, magnitude, and directionality of any detected differences. Conventional commercial reference varieties were used to establish a range representative of natural variability in soybean for each characteristic assessed, and provided a context from which to further evaluate any statistical differences. Characteristics assessed included: seed dormancy and germination, pollen morphology, and symbiont interactions in the greenhouse, plant phenotypic observations, environmental interaction evaluations, and volunteer potential and persistence outside of cultivation evaluations conducted in the field. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 87712 does not differ from the conventional control A3525 in any biologically meaningful way indicative of increased pest potential or adverse environmental impact. Thus, MON 87712 is unlikely to have increased weediness or plant pest potential compared to conventional soybean.

Seed dormancy and germination characterization demonstrated that the seed of MON 87712 had germination characteristics not fundamentally different from seed of the conventional control A3525. In particular, the lack of hard seed, a well-recognized characteristic of weediness affecting seed germination, supports a conclusion of no increased weediness of MON 87712 when compared to the conventional control A3525. For pollen characteristics and symbiont interactions, no statistically significant differences were observed between MON 87712 and the conventional control A3525 for any of the parameters measured, including pollen viability and diameter, nodule number and dry weight, shoot total nitrogen, and shoot and root dry weight. These results support the conclusion that MON 87712 is not likely to exhibit increased plant pest potential compared to conventional soybean.

The field evaluation of phenotypic, agronomic, and environmental interaction characteristics of MON 87712 also support the conclusion that MON 87712 is not likely to have an increased plant pest potential compared to conventional soybean. The evaluations were conducted at 19 replicated field sites in soybean production regions across the U.S. These assessments included plant growth and development characteristics, as well as observations for plant responses to abiotic stressors, plant-disease, and plant-arthropod interactions. The observed phenotypic characteristics were not fundamentally different between MON 87712 and the conventional control A3525.

In the combined-site analysis, no statistically significant differences were detected between MON 87712 and the conventional control A3525 for seedling vigor, days to 50% flowering, days to 50% end of flowering, plant height, lodging, pod shattering, grain moisture, or 100 seed weight. Statistically significant differences were observed between MON 87712 and the conventional control A3525 for early stand count, days to 50% senescence, days to physiological maturity, final stand count, and yield. Although significantly different from the conventional control A3525, the mean values of MON 87712 for early stand count, and final stand count were within the range of commercial reference varieties for each characteristic and thus would not be adverse in terms of pest potential. Differences in days to 50% senescence, days to physiological maturity, and yield were consistent with the mode of action of the introduced trait in MON 87712. The increase in yield is agronomically desirable and would not contribute to increased weediness potential of MON 87712 without changes in a combination of other characteristics associated with weediness.

Evaluations of volunteer potential and persistence outside of cultivation from field-grown plants provide information useful in assessing potential weediness characteristics of MON 87712 compared to the conventional control A3525. Evaluation of volunteer potential demonstrated no statistically significant differences between MON 87712 and the conventional control A3525. Evaluation of persistence outside of cultivation demonstrated a few statistically significant differences between MON 87712 and the conventional control A3525, however these differences were not seen across all sites and were small in magnitude. Taken together, these comparative assessments indicate that MON 87712 is not likely to have increased weediness or plant pest potential compared to conventional soybean.

In summary, the phenotypic, agronomic, and environmental interaction data were generated and evaluated to characterize MON 87712, and to assess whether the introduction of the trait in MON 87712 alters the plant pest potential compared to conventional soybean. The evaluation, using a weight of evidence approach, considered the reproducibility, magnitude, and direction of detected differences between MON 87712 and the conventional control, and comparison to the range of the commercial reference varieties. The results indicated that MON 87712 does not possess weediness characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant adverse environmental impact compared to conventional soybean.

MON 87712 Will Not Adversely Affect NTOs or Threatened or Endangered Species

Evaluation of the impacts of a biotechnology-derived crop on Non-Target Organisms (NTO) and threatened and endangered species is a component of the plant pest risk assessment. Since MON 87712 does not possess pesticidal activity, all organisms that interact with MON 87712 are considered to be NTOs. The environmental assessment demonstrated that the presence of BBX32 protein in MON 87712 did not meaningfully alter plant-arthropod interactions, including beneficial arthropods, or disease susceptibility compared to the conventional control A3525.

The biochemical information and experimental data for evaluation of MON 87712 included molecular characterization, BBX32 protein safety assessments, information from the environmental interaction assessment, demonstration of compositional equivalence to conventional soybean, and demonstration of agronomic and phenotypic equivalence to conventional soybean (with the exception of presence of BBX32 protein in MON 87712). Taken together, these data support the conclusion that MON 87712 has no reasonable mechanism to harm NTOs, nor does it pose an additional risk to threatened and endangered species compared to the cultivation of conventional soybean.

Outcrossing and gene introgression from MON 87712 to sexually-compatible species in the U.S. is unlikely since no known wild *Glycine* species related to cultivated soybean are known to be present in North America. Furthermore, should cross-pollination occur, MON 87712 and its progeny are not expected to exhibit a significant environmental impact because, as described above, evaluations have shown that the presence of BBX32 is not likely to enhance weediness or plant-pest potential. Therefore, the likelihood and environmental consequence of pollen transfer from MON 87712 to other *Glycine* species is considered negligible.

Deregulation of MON 87712 Will Not Significantly Impact Soybean Agronomic Practices or Land Use

Introduction of MON 87712 is expected to have minimal impact on current cultivation and management practices for soybean. MON 87712 has been shown to be no different from conventional soybean regarding pest potential and compositional characteristics, and has the same levels of tolerance or susceptibility to insects and diseases as commercial soybean. MON 87712 did not require any additional inputs to produce a crop. The intended increase in yield is not expected to significantly impact any of the agricultural practices farmers use to produce a soybean crop. Farmers understand the value of increased yield for their farm's productivity and profitability, and are accustomed to the incremental yield improvements for varieties obtained through traditional breeding. Growers are also accustomed to experiencing field-to-field and year-to-year yield variation based on environmental conditions and the genetics of the varieties they select for planting. Therefore, growers are capable of adjusting harvesting and storage equipment to handle increased yields. MON 87712 will provide another option to farmers to pursue better yielding varieties for their farm. MON 87712 offers the potential to improve productivity in the U.S. soybean production system, thereby helping to meet the growing global demand for soybean.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 87712 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87712 and any progeny derived from crosses between MON 87712 and conventional soybean or deregulated biotechnology-derived soybean be granted nonregulated status under 7 CFR Part 340.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION AND DEFINITIONS[2]

~	Approximately
AA	amino acid
AACC	American Association of Cereal Chemists
ac	Acres
ADF	acid detergent fiber
AAFCO	Association of Americal Feed Control Officers
AEBSF	4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride
AOAC	Association of Official Agricultural Chemists
AOCS	American Oils Chemists Society
AOSA	Association of Official Seed Analysts
APHIS	Animal and Plant Health Inspection Service
bp	Base pair
bu	Bushels
CEX	Cation exchange
CFR	Code of Federal Regulations
CO	CONSTANS protein
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase protein from Agrobacterium sp. strain CP4
CSFII	Continuing Survey of Food Intakes by Individuals
CTAB	Cetyltrimethylammonium bromide
CV	coefficient variation
DAP	days after pollination
dCTP	Deoxycytidine triphosphate
DDI	Daily Dietary Intake
DEEM	Dietary exposure evaluation model
dNTP	Deoxynucleotide triphosphate
DWCF	dry weight conversion factor
dwt	dry weight
ECL	Enhance chemiluminescence
EDTA	ethylenediamine tetraacetic acid
FA	fatty acid
FAARP	Food Allergy Research and Resource Program
FDA	Food and Drug Administration
ft	feet
fwt	fresh weight
g, µg, mg	gram, microgram, milligram
HCl	hydrogen chloride
HP	High performance
H.U.	hemagglutinating unit
IGEPAL	octylphenoxypolyethoxyethanol

[2] Alred, G.J., C.T. Brusaw, and W.E. Oliu. 2003. Handbook of Technical Writing, 7th edn., pp. 2-7. Bedford/St. Martin's, Boston, MA.

IgG	Immunoglobulin G
In	Inch
kb	kilobase
kDa	kiloDalton
LB	Laemmli Buffer
LOD	Limit of detection
LOQ	limit of quantitation
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
MS	mass spectrometry
MES	2-(N-morpholino)ethanesulfonic acid
mm, nm	millimeter, nanometer
MOE	Margin of exposure
MWCO	Molecular weight cut-off
NA	Not applicable
ND	Not determined
NDF	neutral detergent fiber
NFDM	Non-fat dry milk
NOAEL	No observable adverse effect level
NTO	Non-target organism
OECD	Organization for Economic Cooperation and Development
OSL	Over-season leaf
OSR	Over-season root
PRESS	predicted residual sum of squares
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
PVDF	polyvinylidene fluoride
PVP	Polyvinylpyrrolidone
RT	Room temperature
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SOP	Standard operating proceedure
SPS	Sucrose Phosphate Synthase
TBST	Tris-buffered saline containing Tween-20
TCEP	Tris(2-carboxyethyl)phosphine
T/C/R	test/control/reference
TIU	Trypsin Inhibitor Unit
Tm	melting temperature
TSSP	tissue-specific site pool
U.S.	United States
USDA	United States Department of Agriculture
UTR	Untranslated Region

I. RATIONALE FOR THE DEVELOPMENT OF MON 87712

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

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

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived soybean product, MON 87712, any progeny derived from crosses between MON 87712 and conventional soybean, and any progeny derived from crosses of MON 87712 with any biotechnology-derived soybean that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of MON 87712

Monsanto Company has developed the biotechnology-derived soybean line MON 87712, which will be used in traditional breeding programs to produce commercial varieties with increased yield opportunity. The yield increase in MON 87712 is achieved using the *BBX32* gene from the plant *Arabidopsis thaliana* that produces a protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night processes and results in increased availability of assimilates (products of plant metabolism) in MON 87712 compared to an appropriate comparator without this gene. Increased plant nutrient availability in MON 87712 is supported by the measurement of factors indicative of an extended period of photosynthetic activity in MON 87712 and evidence of changes in diurnal metabolism during the reproductive phase of the soybean plant, as well as by the significantly higher yield of MON 87712 when compared to control, as observed in multisite field studies in the U.S. Appendix B provides supplemental information on the function of the BBX32 protein in MON 87712. The data demonstrate how the plant BBX32 protein affects existing diurnal processes in soybean to increase yield in MON 87712. Higher yielding soybeans offer the opportunity for benefits to growers and the soybean food and feed chain, and help meet global demand for soybean.

Soybean is one of the largest U.S. crops based on the acreage planted and quantity harvested each year. In 2010, soybean was planted on 76.6 million acres in the U.S., where the harvested soybean seed had an average yield of 43.5 bushels per acre and total production was 3.33 billion bushels, resulting in a net value greater than $38.91 billion (USDA-NASS, 2011d; 2011a). Global demand for soybean is expected to increase over the next five years (USDA-ERS, 2011), and as a major global supplier of soybean, the

U.S. must increase soybean production to help meet growing demand. Although the U.S. soybean supply has trended upward since 1924 (USDA-NASS, 2011b), it has not outpaced soybean use. Stock levels for soybean were projected to slip to 140 million bushels at the end of 2010, which would mark the third lowest level in 10 years (USDA-ERS, 2011). Such low inventories indicate that future soybean productivity must not only be large enough to refill present stockpiles to levels that provide increased food and feed security, but must also be adequate enough to accommodate end-user demand until the next crop is harvested the following year.

Increased soybean productivity in the U.S. has been accomplished by both increasing the area under cultivation and through yield increases per unit area. From 1924 to 2010, soybean acreage increased almost 50-fold and yield rose at an average annual rate of approximately 0.35 bu/A (0.8%) in the U.S. (USDA-NASS, 2011b). Annual improvement in U.S. soybean yield is attributed to rapid producer adoption of agricultural improvements such as genetic and agronomic innovations that provide producers with means for reducing "on-farm" yield constraints (Specht et al., 1999). Agricultural production depends on continuing infusions of genetic resources for yield stability and growth (Day-Rubenstein and Heisey, 2006).

Based on recent trends in farm production and land area, most OECD countries, including the U.S. and Canada, are predicted to face the challenge of expanding agricultural output by raising productivity on a stable or reduced land area (OECD-FAO, 2008). Therefore, much of the projected expansion in soybean production in the future is expected to come from increased yield rather than increased area under production (OECD-FAO, 2008).

Improvement in soybean yield remains one of the major objectives for plant breeders. Gains in major crop yields over the years can be attributed to genetic improvement through traditional breeding. Breeders have crossed plants with different genetic backgrounds and selected traits resulting in higher yields, compositional improvements, and desirable production traits. Biotechnological approaches complement traditional soybean breeding efforts by targeting some of the major characteristics as traditional breeding.

Crop yield results from a sequential growth and development process – first the plant grows vegetatively and produces photosynthetic tissue, followed by flowering and the production of seeds, and finally seed filling and maturation. Yield is a complex trait that is dependent on a number of genetic and environmental factors, that influence a crop's opportunity to realize its full yield potential. Improvements in crop yield have been a primary focus of conventional breeding. The genetic changes that resulted in crop domestication and yield improvement in conventional varieties have been shown by modern molecular biology analysis to have been typically achieved through the selection and safe use of plant genes encoding transcriptional regulator proteins. Agricultural biotechnology provides the opportunity to further enhance crop yields through the introduction of new genetic elements that use or modify existing pathways in the plant.

In summary, MON 87712 is of benefit to growers, the food and feed chain, and the society as a whole, as it provides increased yield opportunity, will help global efforts to provide an adequate supply of soybeans, and help sustain a robust domestic and global livestock market for soybean and soybean products.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived crops falls primarily on three U.S. agencies: U.S. Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and in the case of plant incorporated protectants, the Environmental Protection Agency (EPA). Deregulation of MON 87712 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87712 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

I.C.1. Submission to FDA

MON 87712 falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). In compliance with this policy, Monsanto will initiate a consultation with the FDA on the food and feed safety and compositional assessment of MON 87712.

I.C.2. Submissions to Foreign Government Agencies

To support commercial introduction of MON 87712 in the U.S., regulatory submissions will be made to countries that import significant quantities of soybean or its processed fractions from the U.S. These will include submissions to a number of foreign government regulatory authorities, including: the Ministry of Agriculture, People's Republic of China; Japan's Ministry of Agriculture, Forestry, and Fisheries, Ministry of Environment, and the Ministry of Health, Labor, and Welfare; the Canadian Food Inspection Agency and Health Canada; the Intersectoral Commission for Biosafety of Genetically Modified Organisms, Mexico; the European Food Safety Authority, as well as to regulatory authorities in other soybean importing countries with functioning regulatory systems. As appropriate, notifications of importation will be made to importing countries that do not have a formal approval process.

II. THE BIOLOGY OF SOYBEAN

This section summarizes the taxonomy, biology, and use of soybean based on: 1) the consensus document for *Glycine max* (L.) Merr. prepared by the Organization for Economic Co-operation and Development (OECD, 2000), and 2) a summary prepared by USDA-APHIS (2006) and a biology document published by Canadian Food Inspection Agency-Plant Biosafety Office (CFIA, 1996).

II.A. Soybean as a Crop

Soybean is the most widely grown oilseed in the world, with approximately 258.4 million metric tons (MMT) of harvested seed produced in 2010, which represented 58% of world oilseed production that year (ASA, 2011). Soybean is grown as a commercial crop in over 35 countries. The major producers of soybean are the U.S., Brazil, Argentina, China, India, Paraguay and Canada, which accounted for approximately 95% of the global soybean production in 2010 (ASA, 2011); also see Table II 1. Approximately 35% of the 2010 world soybean production was produced in the U.S. (ASA, 2011). For the fifth consecutive year, U.S. whole soybean exports hit record levels in 2010, with exports exceeding 43.3 MMT (ASA, 2011). Approximately 45 MMT of soybeans were crushed in the U.S. in 2010 and used to supply the feed industry for livestock use or the food industry for edible vegetable oil and soybean protein isolates (ASA, 2011). U.S. stock levels were 3.8 MMT at the end of the year (ASA, 2011).

Table II-1. World Soybean Production in 2010

Country	Production (million metric tons)
U.S.	90.6
Brazil	70.0
Argentina	49.5
China	15.2
India	9.6
Paraguay	7.5
Canada	4.3
Other	11.6

Source: Soy Stats, World Soybean Production (ASA, 2011).

Soybean is now the second most planted field crop in the U.S. after corn. According to data from USDA-NASS (2011d), soybean was planted on approximately 76.6 million acres in the U.S. in 2010, producing 3.33 billion bushels of soybean with a value of $38.9 billion (USDA-NASS, 2011d).

Soybean is used in various food products, including tofu, soy sauce, soymilk, energy bars, and meat products. A major food use for soybean is purified oil, for use in margarines, shortenings, cooking, and salad oils. Soybean oil generally has a smaller contribution to soybean's overall value compared to soybean meal because the oil constitutes just 18 to 19% of the soybean's weight. Nonetheless, soybean oil accounted for approximately 29% of all the vegetable oils consumed globally, and was the second

largest source of vegetable oil worldwide, slightly behind palm oil at approximately 33% share (ASA, 2011).

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. Industrial edible and industrial uses of soybean range from a carbon/nitrogen source in the production of yeasts via fermentation to the manufacture of soaps, inks, paints, disinfectants, and biodiesel. Industrial uses of soybean have been summarized by the American Soybean Association (ASA, 2011).

II.B. History of Soybean

Domestication of soybean is thought to have taken place in China during the Shang dynasty (approximately 1500 to 1027 B.C.) or earlier (Hymowitz, 1970). However, historical and geographical evidence could only be traced back to the Chou 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., 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 disintegration of Chinese dynasties (Hymowitz and Newell, 1981).

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

Soybean was introduced into North America in the 18th century. In 1851, soybean was introduced in Illinois and subsequently throughout the Corn Belt. In 1853, soybean seeds were deposited at the New York State Agricultural Society, the Massachusetts Horticultural Society, and the Commissioner of Patents. The two societies and the Commissioner of Patents sent soybean seeds to dozens of growers throughout the U.S. Soybean has been cultivated extensively and improved through conventional breeding following its introduction in the U.S. and subsequently has become a key source of nutrients for food and feed use in the U.S. (Singh and Hymowitz, 1999).

II.C. Taxonomy and Phylogenetics of Soybean

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

Family: Fabaceae

Subfamily: Papilionoideae

Tribe: Phaseoleae

Genus: *Glycine* Willd.

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

Species: *Glycine max* (L.) Merr.

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

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

Genus		2n	Genome[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 soja grows wild in China, Japan, Korea, the Russian Far East, and Taiwan, and is commonly found in fields, hedgerows, roadsides, and riverbanks (Lu, 2004). The plant is an annual, slender in build with narrow trifoliolate leaves. The purple or very rarely white flowers are inserted on short, slender racemes. The pods are short and tawny with hirsute pubescence, producing oval-oblong seeds (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 ovoid to subspherical seeds are produced in the pods (Hymowitz, 2004).

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

II.D. The Genetics of Soybean

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

II.E. Pollination of Cultivated Soybean

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

receptive for 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybean is considered to be a highly self-pollinated species, with cross-pollination to adjacent plants of other soybean varieties occurring at very low frequency (0 to 6.3%) in adjacent plants (Caviness, 1966; Ray et al., 2003; Yoshimura et al., 2006). Pollination typically takes place on the day the flower opens. The pollen naturally comes in contact with the stigma during the process of anthesis. Anthesis normally occurs in late morning, depending on the environmental conditions. The pollen usually remains viable for two to four hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination can only take place during the short time when the pollen is viable.

II.F. Cultivated Soybean as a Volunteer

Cultivated soybean plants are annuals, and they reproduce solely by means of seeds. Volunteer soybean in rotational crops is typically not a concern in most environments where soybean is cultivated (CFIA, 1996; OECD, 2000). Soybean seed rarely exhibit any dormancy characteristics, and seed remaining in the field after harvest likely will readily imbibe water (Lersten and Carlson, 2004), germinate, and will be killed by frost or field cultivation. If the soybean seed did become established, volunteer plants would not compete well with the succeeding crop, and could be controlled readily by either mechanical or chemical means (OECD, 2000).

II.G. Characteristics of the Recipient Plant

The conventional soybean variety A3525, used as the recipient for the *BBX32* expression cassette insertion that produced MON 87712, was developed by Asgrow Seed Company. A3525 is a mid−maturity group III soybean variety.

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

Soybean variety A3525 is the parental line of MON 87712 and was used as the conventional soybean comparator (hereafter referred to as the conventional control) in the safety assessment of MON 87712. MON 87712 and the conventional control A3525 have similar genetic backgrounds with the exception of the *BBX32* expression cassette, thus, the effect of the *BBX32* expression cassette could therefore be assessed in an unbiased manner in the comparative safety assessment. In addition, reference materials were used to establish ranges of natural variability or responses representative of commercial soybean varieties. Conventional commercial reference varieties refer to commercial soybean varieties that were derived only through conventional methods. Commerial reference varieties refer to commercial soybean varieties that were derived through conventional methods and/or Roundup Ready soybean varieties. The commercial reference varieties used at each location were selected based on their availability and agronomic fit for the respective geographic region.

III. DESCRIPTION OF THE GENETIC MODIFICATION

MON 87712 was developed through *Agrobacterium tumefaciens*-mediated transformation of conventional soybean A3525 meristem tissue utilizing transformation plasmid vector PV-GMAP5779. This section describes the plasmid vector, the donor genes, and the regulatory elements used in the development of MON 87712 and the deduced amino acid sequence of the BBX32 protein produced in MON 87712. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences.

III.A. The Plasmid Vector PV-GMAP5779

PV-GMAP5779 was used for the transformation of conventional soybean to produce MON 87712 and is shown in Figure III-1; PV-GMAP5779 is approximately 11.4 kb and contains two T-DNAs, each delineated by Left and Right Border regions to facilitate transformation. The first T-DNA, designated as T-DNA I, contains the *BBX32* coding sequence under regulation of the *e35S* promoter and the *E6* 3′ untranslated region. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* coding sequence under the regulation of the *FMV/EF-1α* promoter, *EF-1α* leader, *EF-1α* intron, *CTP2* targeting sequence, and the *E9* 3′ untranslated region. During transformation, both T-DNAs were inserted into the soybean genome (Section III.B) where T-DNA II, containing the *cp4 epsps* expression cassette, functioned as a marker gene for the selection of transformed plantlets. Subsequently, conventional self-pollinated breeding methods and segregation, along with a combination of analytical techniques, were used to isolate those plants that contained the *BBX32* expression cassette (T-DNA I) but did not contain the *cp4 epsps* expression cassette (T-DNA II).

The backbone region of PV-GMAP5779, which is located outside both of the T-DNAs contains two origins of replication (*ori-V*, *ori-pBR322*) for maintenance of the plasmid vector in bacteria, a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer (*rop*) protein for the maintenance of the plasmid vector copy number in *E. coli*. A description of the genetic elements and their prefixes (e.g., P-, L-, I-, TS-, OR-, B-, CS-, and T-) in PV-GMAP5779 is provided in Table III-1.

III.B. Description of the Transformation System

MON 87712 was developed through *Agrobacterium*-mediated transformation of soybean, based on the method described by Martinell et al. (2002), which allows for the generation of transformed plants without the utilization of callus. Briefly, meristem tissues were excised from the embryos of germinated conventional seed. After co-culturing with the *Agrobacterium* carrying the vector, the meristems were placed on selection medium containing glyphosate, carbenicillin, cefotaxime, and ticarcillin/clavulanate acid mixture, to inhibit the growth of untransformed plant cells and excess *Agrobacterium*. The meristems were then placed in media conducive to shoot and root development. Rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

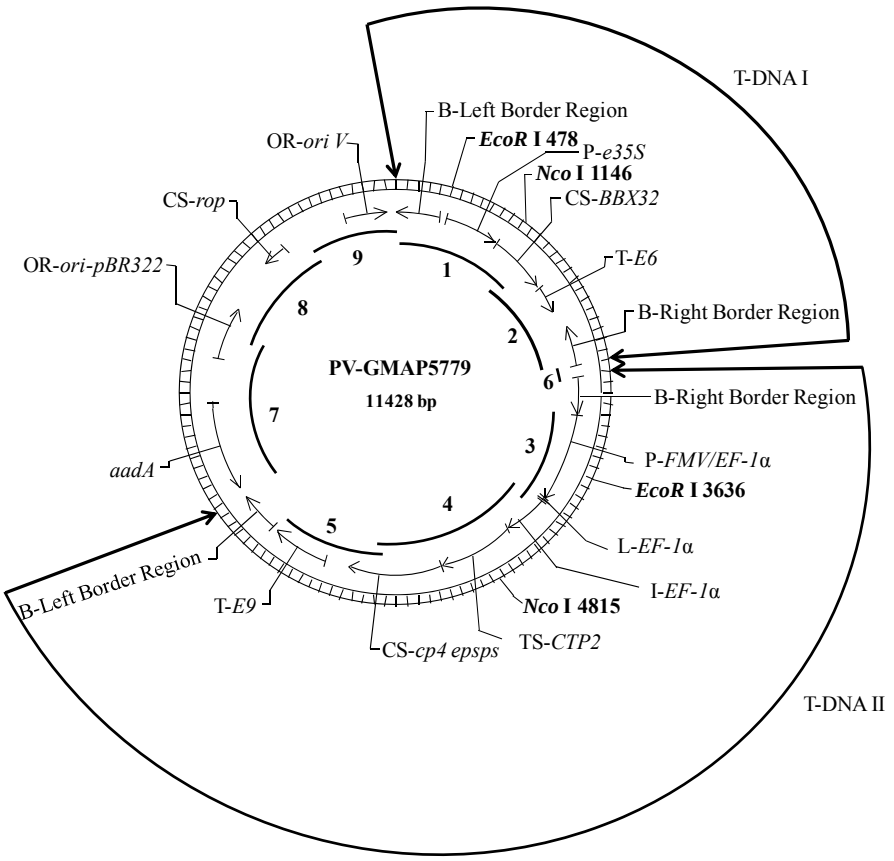
The R_0 plants generated through this transformation were self-pollinated to produce R_1 plants, and the unlinked insertions of T-DNA I and T-DNA II were segregated. A non-lethal dose of glyphosate was applied to R_1 plants and those plants with minor herbicide injury were selected for further analyses, whereas plants showing no injury, indicating that they contained the *cp4 epsps* coding sequence from T-DNA II, were eliminated from further development. Subsequently, plants that were homozygous for T-DNA I were identified by a quantitative non-polymerase chain reaction (non-PCR) based analysis. MON 87712 was selected as the lead event based on superior phenotypic characteristics and its molecular profile. The major development steps of MON 87712 are depicted in Figure III-2. The result of this process was the production of marker-free MON 87712 soybean.

III.C. The *BBX32* Coding Sequence and the BBX32 Protein (T-DNA I)

The expression cassette present in MON 87712 contains the coding region for BBX32 protein from *Arabidopsis thaliana* (Figure III-3) (Holtan et al., 2011; Khanna et al., 2009; Putterill et al., 1995). The presence of BBX32 modulates aspects of diurnal biology to increase yield by the increased capacity for growth and reproductive development (refer to Appendix B for more details).

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

The *cp4 epsps* expression cassette (T-DNA II) that is not present in MON 87712 encodes a 47.6 kDa CP4 EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996b). The *cp4 epsps* coding sequence is the codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996a). CP4 EPSPS protein confers tolerance to glyphosate and was used as a selectable marker during the transformation selection process. Plants that did not contain the *cp4 epsps* expression cassette were isolated through conventional self-pollinated breeding methods and segregation, along with a combination of analytical techniques.



Probe Number	DNA Probe Type	Start Position (bp)	End Position (bp)	Total Length (~kb)
1	T-DNA I Probe 1	1	1610	1.6
2	T-DNA I Probe 2	1556	2549	1.0
3	T-DNA II Probe 1	3078	4383	1.3
4	T-DNA II Probe 2	4304	6048	1.7
5	T-DNA II Probe 3	5954	7075	1.1
6	Backbone Probe 1	2550	2720	0.2
7	Backbone Probe 2	7487	9245	1.8
8	Backbone Probe 3	9116	10620	1.5
9	Backbone Probe 4	10543	11428	0.9

Figure III-1. Circular Map of PV-GMAP5779 Showing Probe 1 through Probe 9
A circular map of the plasmid vector PV-GMAP5779 used to develop MON 87712 is shown. PV-GMAP5779 contains two T-DNAs, designated as T-DNA I and T-DNA II. Genetic elements and restriction sites used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The probes used in the Southern analyses are shown on the interior of the map.

*The Left and Right border regions of T-DNA II share 100% identity with T-DNA I, which were covered by Probe 1 and Probe 2 and thus not included in the T-DNA II probes.

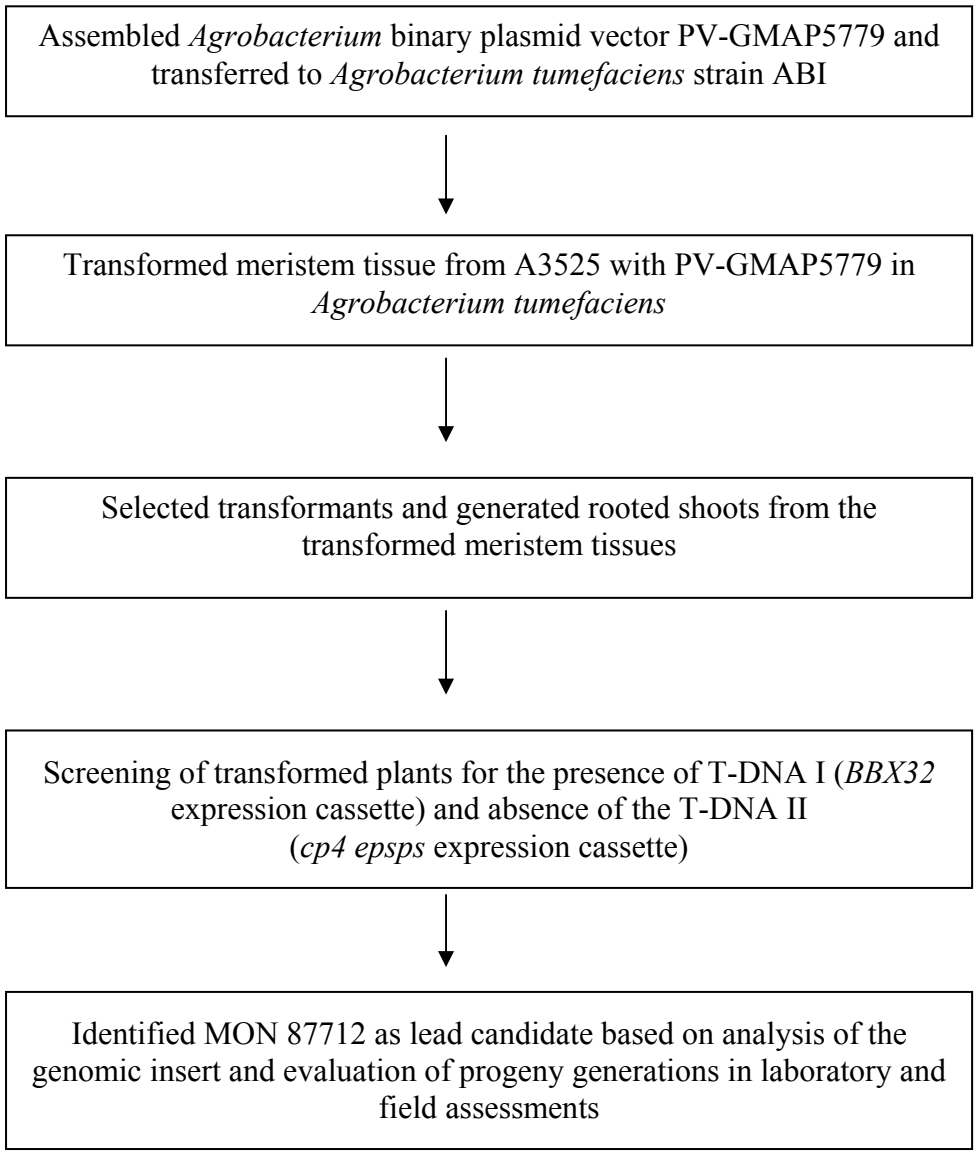


Figure III-2. Schematic of the Development of MON 87712

III.E. Regulatory Sequences

The *BBX32* coding sequence in T-DNA I is under the regulation of the *e35S* promoter, and the *E6* 3′ untranslated region. The *e35S* promoter is the promoter for 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987), which functions to direct transcription in plant cells. The *E6* 3′ untranslated region is the 3′ untranslated region of the *E6* gene from *Gossypium barbadense* (cotton) encoding a fiber protein involved in early fiber development (John, 1996), which functions to direct polyadenylation of the RNA transcripts.

T-DNA II contains the *cp4 epsps* coding sequence under the regulation of the *FMV/EF-1α* promoter, *EF-1α* leader, *EF-1α* intron, the *CTP2* targeting sequence, and the *E9* 3′ untranslated region. The *FMV/EF-1α* promoter is the chimeric promoter consisting of enhancer sequences from the 35S RNA promoter of figwort mosaic virus (FMV) (Richins et al., 1987) combined with the promoter of the *EF-1α* gene from *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989), which functions to direct transcription in plant cells. The *EF-1α* leader is the 5′ leader (exon 1) sequence of the *EF-1α* gene from *Arabidopsis thaliana* that encodes elongation factor EF-1α (Axelos et al., 1989), which is involved in regulating gene expression. The *EF-1α* intron is the intron with flanking splice sites of the *EF-1α* gene from *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989), which is involved in regulating gene expression (Curie et al., 1991). The *CTP2* targeting sequence is the targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region (Herrmann, 1995; Klee et al., 1987), which functions to direct transport of the CP4 EPSPS protein to the chloroplast. The *E9* 3′ untranslated region is the 3′ untranslated region from *Pisum sativum* (pea) *rbcS* gene family encoding the small subunit of ribulose bisphosphate carboxylase protein (Coruzzi et al., 1984), which functions to direct polyadenylation of the mRNA.

III.F. T-DNA Borders

PV-GMAP5779 contains Left and Right Border regions (Figure III-1 and Table III-1) that were derived from *Agrobacterium tumefaciens* (Barker et al., 1983; Depicker et al., 1982; Zambryski et al., 1982). The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation. Left and Right Border regions separate the T-DNA from the plasmid backbone region and are involved in the efficient transfer into the soybean genome. Because PV-GMAP5779 is a 2T-DNA vector, it contains two Left Border regions and two Right Border regions, where one border region set flanks T-DNA I and the other border region set flanks T-DNA II.

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

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-GMAP5779 in bacteria and are referred to as plasmid backbone. The origin of replication, *ori V*, is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid RK2 (Stalker et al., 1981). The origin of replication, *ori-pBR322*, is required for the maintenance of

the plasmid in *E. coli* and is derived from the plasmid vector pBR322 (Sutcliffe, 1979). Coding sequence *rop* encodes the repressor of primer (ROP) protein which is necessary for the maintenance of plasmid vector copy number in *E. coli* (Giza and Huang, 1989). The selectable marker *aadA* is a bacterial promoter and coding sequence for an enzyme from transposon Tn*7* that confers spectinomycin and streptomycin resistance (Fling et al., 1985) in *E. coli* and *Agrobacterium* during molecular cloning. Because these elements are outside the border regions, they are not expected to be transferred into the soybean genome. The absence of the backbone sequence in MON 87712 has been confirmed by Southern blot analyses (see Section IV.B).

Table III-1. Summary of Genetic Elements in the PV-GMAP5779

Genetic Element	Location in Plasmid Vector	Function (Reference)
T-DNA I		
B[1]-Left Border Region	1-442	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Intervening Sequence	443-510	Sequence used in DNA cloning
P[2]-*e35S*	511-1123	Promoter from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells
Intervening Sequence	1124-1147	Sequence used in DNA cloning
CS[3]-*BBX32*	1148-1825	Coding sequence of the *BBX32* gene from *Arabidopsis thaliana* encoding a zinc finger protein (B-box type) (Khanna et al., 2009; Putterill et al., 1995) which modulates aspects of diurnal biology (Holtan et al., 2011)
Intervening Sequence	1826-1839	Sequence used in DNA cloning
T[4]-*E6*	1840-2154	3′ UTR region of the *E6* gene from *Gossypium barbadense* (cotton) encoding a fiber protein involved in early fiber development (John, 1996) that directs polyadenylation of mRNA
Intervening Sequence	2155-2192	Sequence used in DNA cloning
B-Right Border Region	2193-2549	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Backbone		
Intervening Sequence	2550-2720	Sequence used in DNA cloning
T-DNA II		
B-Right Border Region	2721-3077	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)

Table III-1. Summary of Genetic Elements in the PV-GMAP5779 (continued)

Intervening Sequence	3078-3099	Sequence used in DNA cloning
P-*FMV/EF-1α*	3100-4139	Chimeric promoter consisting of enhancer sequences from the 35S RNA promoter of figwort mosaic virus (FMV) (Richins et al., 1987) combined with the promoter of the *EF-1α* gene from *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989) that directs transcription in plant cells
L^5- *EF-1α*	4140-4185	Leader (exon 1) sequence of the *EF-1α* gene from *Arabidopsis thaliana* that encodes elongation factor EF-1α (Axelos et al., 1989) that is involved in regulating gene expression
I^6- *EF-1α*	4186-4807	Intron with flanking splice sites of the *EF-1α* gene from *Arabidopsis thaliana* encoding elongation factor EF-1α (Axelos et al., 1989) that involved in regulating gene expression (Curie et al., 1991)
Intervening Sequence	4808-4816	Sequence used in DNA cloning
TS^7-*CTP2*	4817-5044	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al., 1987)
CS-*cp4 epsps*	5045-6412	Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein that provides herbicide tolerance (Barry et al., 2001; Padgette et al., 1996a)
Intervening Sequence	6413-6418	Sequence used in DNA cloning
T-*E9*	6419-7061	3′ UTR sequence from *Pisum sativum* (pea) rbcS gene family encoding the small subunit of ribulose bisphosphate carboxylase protein (Coruzzi et al., 1984) that directs polyadenylation of the mRNA
Intervening Sequence	7062-7075	Sequence used in DNA cloning
B-Left Border Region	7076-7486	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Backbone		
Intervening Sequence	7487-7581	Sequence used in DNA cloning

Table III-1. Summary of Genetic Elements in the PV-GMAP5779 (continued)

aadA	7582-8470	Bacterial promoter, coding sequence, and 3' UTR for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase from the transposon Tn*7* (Fling et al., 1985) that confers spectinomycin and streptomycin resistance
Intervening Sequence	8471-9000	Sequence used in DNA cloning
OR[8]-*ori-pBR322*	9001-9589	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	9590-10016	Sequence used in DNA cloning
CS-*rop*	10017-10208	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening Sequence	10209-10945	Sequence used in DNA cloning
OR-*ori V*	10946-11342	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	11343-11428	Sequence used in DNA cloning

[1]B, Border.
[2]P, Promoter.
[3]CS, Coding Sequence.
[4]T, Transcription Termination Sequence.
[5]L, Leader.
[6]I, Intron.
[7]TS, Targeting Sequence.
[8]OR, Origin of Replication.

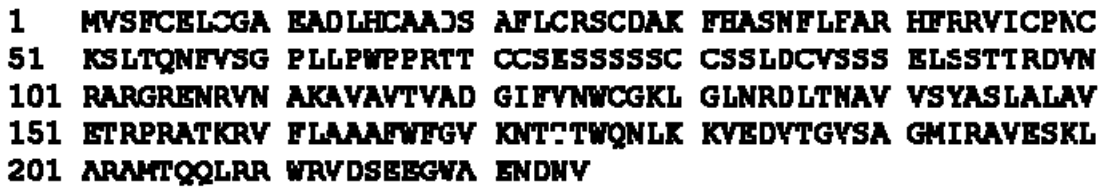


Figure III-3. Deduced Amino Acid Sequence of the BBX32 Protein in MON 87712
The amino acid sequence of the BBX32 protein was deduced from the full-length coding nucleotide sequence present in PV-GMAP5779 (see Table III-1 for more detail).

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

A multi-faceted approach was taken to characterize the genetic modification that produced MON 87712. The results confirmed that MON 87712 contains a single copy of the *BBX32* expression cassette (T-DNA I) that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations (Section IV.G.). Additionally, the results confirmed that T-DNA II and plasmid vector backbone sequences are not detected in MON 87712. These conclusions were based on several lines of evidence: 1) Southern blot analyses to assay the entire soybean genome for the presence of T-DNA I sequences and the absence of T-DNA II and plamid vector backbone sequences derived from PV-GMAP5779, and to confirm that a single copy of T-DNA I was inserted at a single site and is stably inherited; 2) DNA sequence analyses to determine the exact sequence of the inserted DNA and the DNA sequences flanking the 5' and 3' ends of the insert; 3) DNA sequence comparison of the inserted DNA sequence to the T-DNA I sequence in PV-GMAP5779 to confirm that only the expected sequences were integrated; and 4) sequence comparison of the DNA sequences flanking 5' and 3' end of the T-DNA I insert to the insertion site sequence in conventional soybean to identify any rearrangements that occurred at the insertion site during transformation. Taken together, the characterization of the genetic modification demonstrates that MON 87712 contains a single copy of T-DNA I that was inserted at a single locus of the genome.

Southern blot analyses were used to determine the number of copies and the insertion sites of T-DNA I, as well as the presence or absence of T-DNA II and plasmid vector backbone sequences. The Southern blot strategy was designed to ensure that all potential inserted segments would be identified. The entire soybean genome was assayed with probes that spanned the complete plasmid vector PV-GMAP5779 to detect the presence of T-DNA I, as well as the absence of T-DNA II and plasmid vector backbone sequences. This was accomplished by using probes that were less than 2 kb in length, ensuring a high level of sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at 0.1 copies per genome equivalent. Two sets of restriction enzymes were specifically chosen to fully characterize T-DNA I and detect any potential segments from the plasmid vector PV-GMAP5779. The restriction enzyme sets were chosen such that each enzyme set cleaves once within the inserted T-DNA and at least once within the known DNA sequence flanking the 5' or 3' end of the insert. As a result, the enzyme sets produce overlapping segments that contain the entire insert sequence and adjacent 5' and 3' flanking DNA sequence. Therefore, at least one segment containing a portion of the insert with the adjacent 5'flanking DNA generated by one set of the enzyme(s) is of a predictable size and overlaps with another predictable size segment containing a portion of the insert with the adjacent 3' flanking DNA generated by another set of the enzyme(s). This two set enzyme design ensures that the entire insert is identified in a predictable hybridization pattern. Additionally, this two enzyme set design also maximizes the possibility of detecting an insertion elsewhere in the genome that could be overlooked if that band co-migrated with an expected band.

To determine the number of copies and the insertion sites of T-DNA I, and the presence or absence of T-DNA II and the plasmid vector backbone sequences, duplicated samples that consisted of equal amounts of digested DNA were run on the agarose gel. One set of samples was run for a longer period of time (long run) than the second set (short run). The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows the detection of small molecular weight DNA. The molecular weight markers on the left of the figures were used to estimate the sizes of the bands present in the long run lanes of the Southern blots, and the molecular weight markers on the right of the figures were used to estimate the sizes of bands present in the short run lanes of the Southern blots. Southern blot results demonstrated that MON 87712 contains a single copy of T-DNA I at a single insertion site in the soybean genome, and no additional genetic elements, including backbone sequences, from PV-GMAP5779 were detected in MON 87712 (Figure IV-2 through Figure IV-8).

The PCR and DNA sequence analyses complement the Southern blot analyses. PCR and DNA sequence analyses performed on MON 87712 determined the complete DNA sequence of the insert and adjacent DNA sequences, confirmed the organization of the elements within the insert, and determined the 5′ and 3′ insert-to-plant junctions (Figure IV-9 and Figure IV-10). In addition, DNA sequencing analyses confirmed each genetic element in the insert and the sequence of the insert matches the corresponding sequence in PV-GMAP5779. Furthermore, genomic organization at the MON 87712 insertion site was determined by comparing the 5' and 3' flanking sequences of the insert to the sequence of the insertion site in conventional soybean.

The stability of T-DNA I present in MON 87712 across multiple generations (R_3, R_4, R_5, R_6, and R_7) was demonstrated by Southern blot fingerprint analysis. Genomic DNA from five generations of MON 87712 (Figure IV-11) was digested with one of the enzyme sets used for the insert and copy number analysis and was hybridized with a probe that detects restriction segments that encompass the entire T-DNA I insert (Figure IV-12). This fingerprint strategy consists of two border segments that assess not only the stability of T-DNA I, but also the stability of genomic DNA directly adjacent to T-DNA I. Generational stability analysis demonstrated that the expected Southern blot fingerprint of MON 87712 was maintained through five generations of the breeding history, thereby confirming the stability of T-DNA I in MON 87712.

Segregation analysis was employed to examine the genetic behavior of the T-DNA I insert in MON 87712. The results from this analysis showed heritability and stability of the insert occurred as expected across multiple generations (Figure IV-13, Table IV-3) which corroborates the molecular insert stability analysis and establishes that T-DNA I is inherited according to Mendelian principles of inheritance.

A circular map of PV-GMAP5779 annotated with the probes used in the Southern blot analysis is presented in Figure III-1. A linear map depicting restriction sites within the insert, as well as the DNA flanking the insert in MON 87712 is shown in Figure IV-1. Based on the plasmid map and the linear map of the insert, a table summarizing the expected DNA segments for Southern analyses is presented in Table IV-1. The genetic elements within the MON 87712 insert are summarized in Table IV-2. The results from

the Southern blot analyses are presented in Figure IV-2 through Figure IV-8. PCR amplification of the MON 87712 insert and the insertion site in conventional control for DNA sequence analysis are shown in Figure IV-9 and Figure IV-10, respectively. The generations used in the generational stability analysis are depicted in the breeding history shown in Figure IV-11 and the results from the generational stability analysis are presented in Figure IV-12. The breeding path for the segregation data is shown in Figure IV-13 and the results for the segregation analysis are presented in Table IV-3. Materials and methods used for the characterization of the insert in MON 87712 are found in Appendix C.

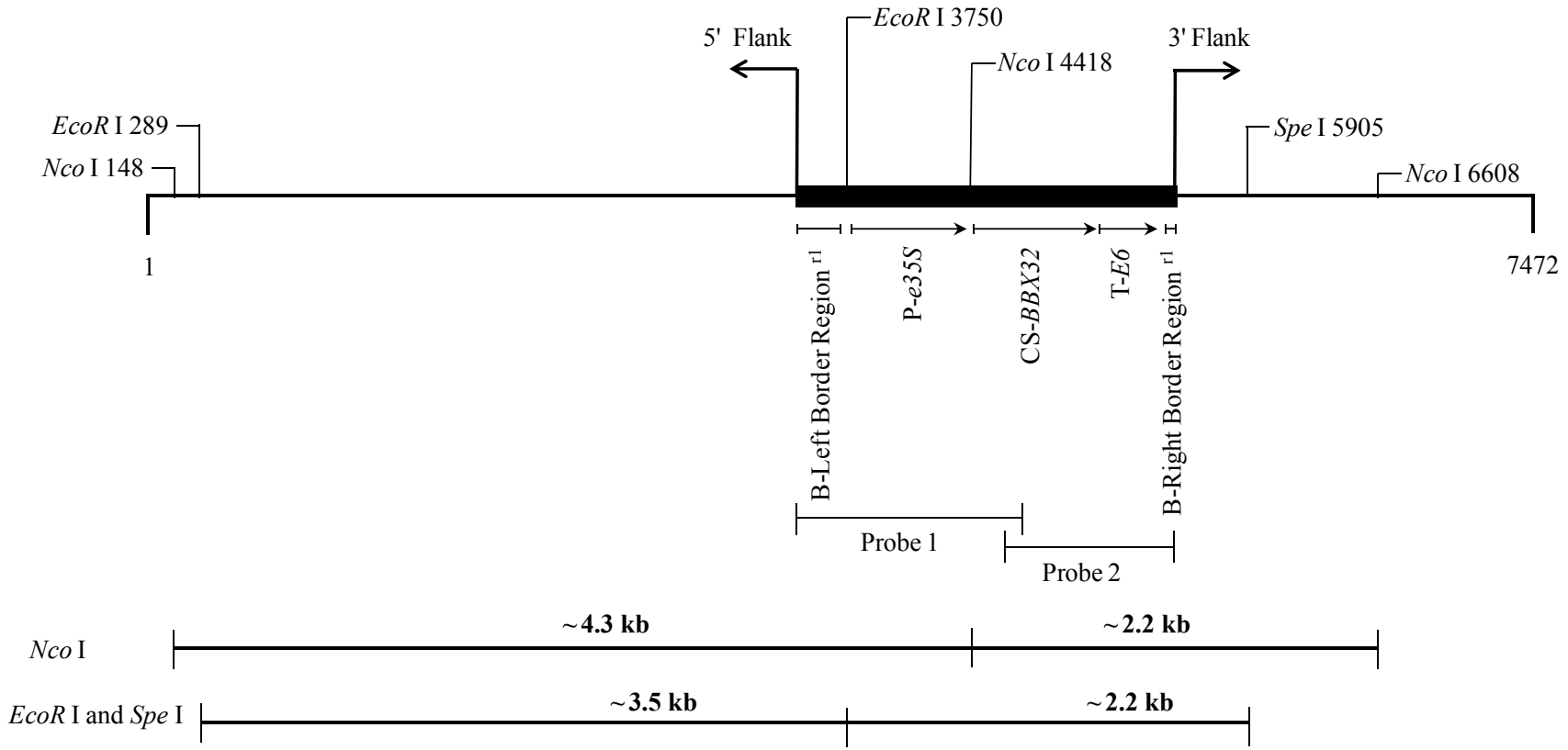


Figure IV-1. Schematic Representation of the Insert and Flanking DNA Sequences in MON 87712

DNA derived from the T-DNA I of PV-GMAP5779 integrated in MON 87712. Right-angled arrows indicate the ends of the integrated T-DNA I and the beginning of the flanking sequence. Identified on the map are the genetic elements within the insert, as well as restriction sites for enzymes with positions relative to the size of the DNA sequence (flanks and insert) used in the Southern analyses. The relative locations of the T-DNA I probes and the expected sizes of restriction segments are indicated. This schematic diagram is not drawn to scale. Locations of T-DNA I probes are approximate. Probes are described in Figure III-1.

Table IV-1. Summary Chart of the Expected DNA Segments Based on Hybridizing Probes and Restriction Enzymes Used in MON 87712 Analysis

Southern Blot Analysis		**T-DNA I**		**T-DNA II**		**Backbone**			**Insert Stability**
Southern Blot Figure		**IV-2**	**IV-3**	**IV-4**	**IV-5**	**IV-6**	**IV-7**	**IV-8**	**IV-12**
Probe Used		1	2	3, 5	4	6	7, 9	8	1
Probing Target	**Digestion enzyme**	**Expected Band Sizes on each Southern Blot**							
PV-GMAP5779	*Nco* I	~7.8 kb ~3.7 kb	~3.7 kb	~7.8 kb ~3.7 kb	~7.8 kb ~3.7 kb	~7.8 kb* ~3.7 kb	~7.8 kb ~3.7 kb*	~7.8 kb	~7.8 kb ~3.7 kb
Probe Templates[1]	N/A	~~[2]	~~[2]	~1.3 kb ~1.1 kb	~~[2]	~~[2]	~1.8 kb ~0.9 kb	~~[2]	~~[2]
MON 87712	*Nco* I	~4.3 kb ~2.2 kb	~2.2 kb	None	None	None	None	None	~4.3 kb ~2.2 kb
	EcoR I and *Spe* I	~3.5 kb ~2.2 kb	~2.2 kb	None	None	None	None	None	--[3]

*these bands were detected by the probes due to sequence homology to the other digested segment of PV-GMAP5779.

[1]probe template spikes were used as positive hybridization controls in Southern blot analyses when multiple probes were hybridized to the blot simultaneously.

[2]'~~' indicates that probe template was not used.

[3]'--' indicates that the particular restriction enzyme or the combination of the enzymes was not used in the analysis

Table IV-2. Summary of Genetic Elements in MON 87712

Genetic Element	Location in Sequence	Function (Reference)
T-DNA I		
Sequence flanking 5' end of insert	1-3493	DNA adjacent to the 5' end of the inserted DNA
B[1]-Left Border Region[r1]	3494-3714	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Intervening Sequence	3715-3782	Sequence used in DNA cloning
P[2]-*e35S*	3783-4395	Promoter from the 35S RNA of cauliflower mosaic virus (CaMV) (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells
Intervening Sequence	4396-4419	Sequence used in DNA cloning
CS[3]-*BBX32*	4420-5097	Coding sequence of the *BBX32* gene from *Arabidopsis thaliana* encoding a zinc finger protein (B-box type) (Khanna et al., 2009; Putterill et al., 1995) which modulates aspects of diurnal biology (Holtan et al., 2011)
Intervening Sequence	5098-5111	Sequence used in DNA cloning
T[4]-*E6*	5112-5426	3′ UTR region of the *E6* gene from *Gossypium barbadense* (cotton) encoding a fiber protein involved in early fiber development (John, 1996) that directs polyadenylation of mRNA
Intervening Sequence	5427-5464	Sequence used in DNA cloning
B-Right Border Region[r1]	5465-5507	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Sequence flanking 3'end of insert	5508-7472	DNA adjacent to the 3' end of the inserted DNA

[1]B, Border.
[2]P, Promoter.
[3]CS, Coding Sequence.
[4]T, Transcription Termination Sequence.
[r1]Superscripts in Left and Right Borders indicate that the border sequences in MON 87712 were truncated compared to the border sequences in PV-GMAP5779.

IV.A. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA I in MON 87712

The copy number and insertion sites of T-DNA I sequences in the soybean genome were evaluated by digesting the R_3 generation of MON 87712 and the conventional control genomic DNA samples with two sets of restriction enzymes, *Nco* I and a combination of *EcoR* I and *Spe* I and hybridized Southern blots with probes that span the T-DNA I (Figure III-1). Each restriction digest is expected to produce a specific banding pattern on the Southern blots (Table IV-1). Any additional copies and/or integration sites would be detected as additional bands.

The restriction enzyme *Nco* I cleaves once within the inserted DNA and once within the known 5' and 3' flanking sequences (Figure IV-1). Therefore, if T-DNA I sequences were present as a single copy at a single integration site in MON 87712, the digestion with *Nco* I was expected to generate two border segments with expected sizes of ~4.3 kb and ~2.2 kb (Figure IV-1 and Table IV-1). The combination of *EcoR* I and *Spe* I cleaves once within the inserted DNA and once within the known 5′ and 3′ flanking sequences in MON 87712 (Figure IV-1). Therefore, if T-DNA I sequences were present as a single copy at a single integration site in MON 87712, the digestion with *EcoR* I and *Spe* I was expected to generate two border segments with expected sizes of ~3.5 kb and ~2.2 kb (Figure IV-1 and Table IV-1).

The Southern blots were hybridized with probes spanning the entire T-DNA I sequence (Figure III-1, Probe 1 and Probe 2). Each Southern blot contained a negative control and positive controls. Conventional control genomic DNA digested with *Nco* I spiked with either digested PV-GMAP5779 DNA and/or probe template(s) served as positive hybridization controls. Conventional control genomic DNA digested with appropriate restriction enzymes was used as a negative control. The results of this analysis are shown in Figure IV-2 and Figure IV-3.

IV.A.1. Probe 1

Conventional control genomic DNA digested with *Nco* I (Figure IV-2, lane 1 and lane 7) or a combination of *EcoR* I and *Spe* I (Figure IV-2, lane 3 and lane 9) and hybridized with Probe 1 (Figure III-1) showed no detectable hybridization bands, as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 DNA previously digested with *Nco* I produced two expected size bands at ~7.8 kb and ~3.7 kb (Figure IV-2, lane 5 and lane 6). Detection of the spiked controls indicates that the probe hybridized to its target sequences.

MON 87712 DNA digested with *Nco* I and hybridized with Probe 1 (Figure III-1) produced two expected bands at ~4.3 kb and ~2.2 kb (Figure IV-2, lane 2 and lane 8). The ~4.3 kb band represents the 5′ end of the inserted DNA and the adjacent DNA flanking the 5′ end of the insert. The ~2.2 kb band represents the 3′ end of the inserted DNA and the adjacent DNA flanking the 3′ end of the insert.

MON 87712 DNA digested with a combination of *EcoR* I and *Spe* I and hybridized with Probe 1 produced the two expected bands at ~3.5 kb and ~2.2 kb (Figure IV-2, lane 4 and lane 10). The ~3.5 kb band represents the 5′ end of the inserted DNA and the adjacent DNA flanking the 5′ end of the insert. The ~2.2 kb band represents the 3′ end of the inserted DNA and the adjacent DNA flanking the 3′ end of the insert. The results presented in Figure IV-2 indicate that the sequence covered by Probe 1 resides at a single detectable locus of integration in MON 87712.

There is a faint line on the blot in lane 1 through lane 6 at ~0.7 kb, which is continuous between the lanes, indicating that it did not result from hybridization with any DNA. This conclusion is confirmed by the fact that this line at ~0.7 kb is absent in the corresponding lanes in the short run.

IV.A.2. Probe 2

Conventional control genomic DNA digested with *Nco* I (Figure IV-3, lane 1 and lane 7) or a combination of *EcoR* I and *Spe* I (Figure IV-3, lane 3 and lane 9) and hybridized with Probe 2 (Figure III-1) showed no detectable hybridization bands, as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 DNA previously digested with *Nco* I produced one expected band at ~3.7 kb (Figure IV-3, lane 5 and lane 6). Detection of the spiked controls indicates that the probe hybridized to its target sequences.

MON 87712 DNA digested with *Nco* I and hybridized with Probe 2 (Figure III-1), produced one expected band at ~2.2 kb (Figure IV-3, lane 2 and lane 8). The ~2.2 kb band represents the 3′ end of the inserted DNA and the adjacent DNA flanking the 3′ end of the insert.

MON 87712 DNA digested with a combination of *EcoR* I and *Spe* I (Figure IV-3, lane 4 and lane 10) and hybridized with Probe 2 produced one expected band at ~2.2 kb. The ~2.2 kb band represents the 3′ end of the inserted DNA and the adjacent DNA flanking the 3′ end of the insert. The results presented in Figure IV-3 indicate that the sequence covered by Probe 2 resides at a single detectable locus of integration in MON 87712.

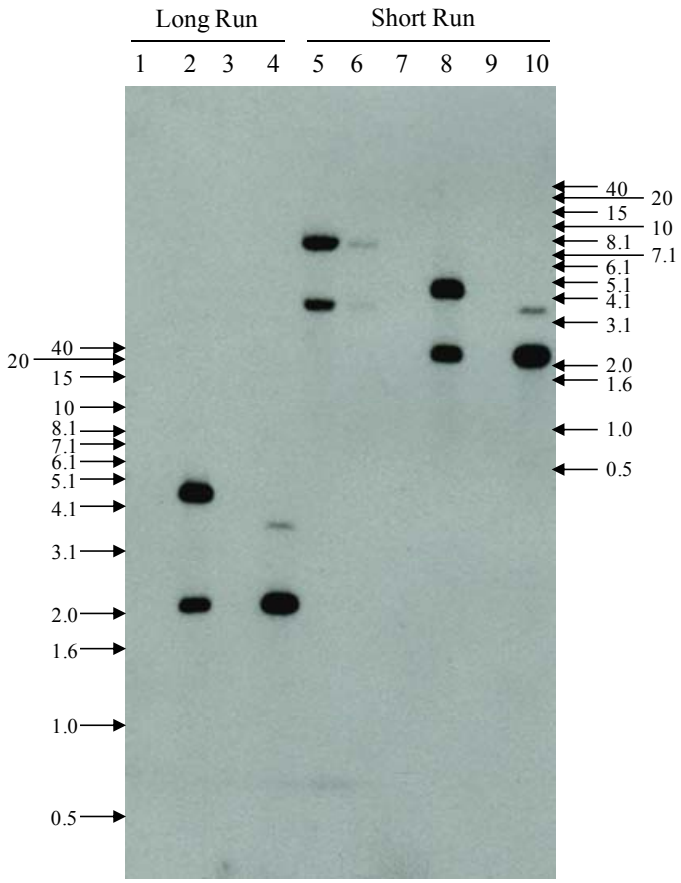


Figure IV-2. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA I in MON 87712: Probe 1

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA I sequence (Figure III-1, Probe 1). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~0.1 genome equivalent]
7	Conventional Control (*Nco* I)
8	MON 87712 (*Nco* I)
9	Conventional Control (*EcoR* I and *Spe* I)
10	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

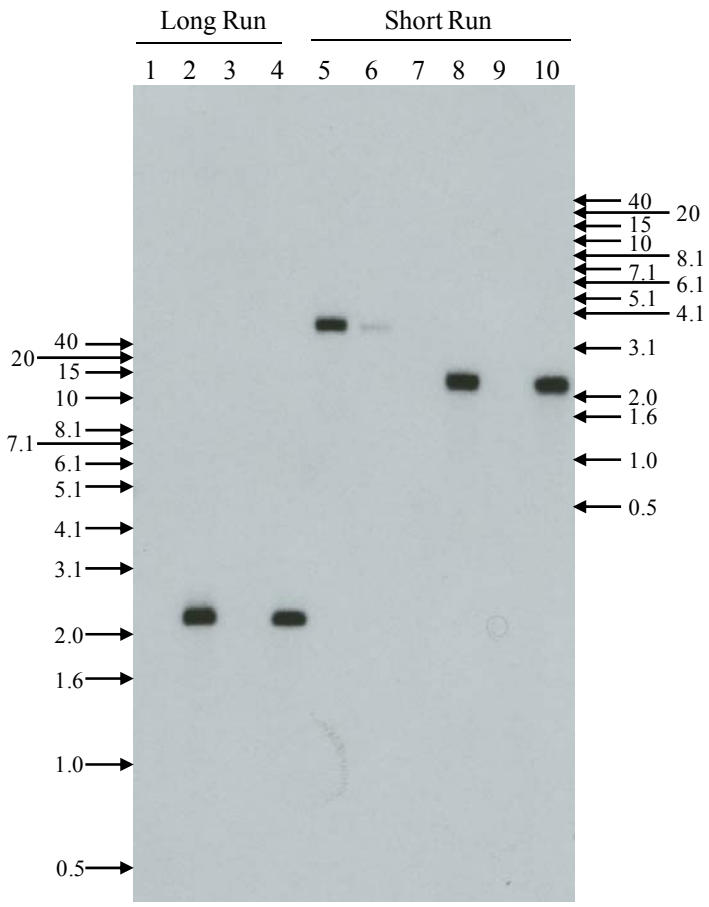


Figure IV-3. Southern Blot Analysis to Determine Insert and Copy Number of T-DNA I in MON 87712: Probe 2

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA I sequence (Figure III-1, Probe 2). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~0.1 genome equivalent]
7	Conventional Control (*Nco* I)
8	MON 87712 (*Nco* I)
9	Conventional Control (*EcoR* I and *Spe* I)
10	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

IV.B. Southern Blot Analysis to Determine the Presence or Absence of T-DNA II Sequences in MON 87712

The presence or absence of T-DNA II sequences in MON 87712 was evaluated by digesting the R_3 generation of MON 87712 and the conventional control genomic DNA samples with two sets of restriction enzymes: *Nco* I and a combination of *EcoR* I and *Spe* I. Each Southern blot was hybridized with overlapping probes spanning the T-DNA II sequence, except for the border regions (Figure III-1, Probe 3, Probe 4, and Probe 5), since the border sequences of T-DNA II share 100% homology to the border sequences of T-DNA I. Therefore, these border regions were covered by Probe 1 and Probe 2. If T-DNA II sequences are present in MON 87712, then probing with the T-DNA II sequences would result in the detection of hybridization bands. The results of these analyses are shown in Figure IV-4 and Figure IV-5.

IV.B.1. Probe 3 and Probe 5

Conventional control genomic DNA digested with *Nco* I (Figure IV-4, lane 1 and lane 8) or a combination of *EcoR* I and *Spe* I (Figure IV-4, lane 3 and lane 10) and hybridized with Probe 3 and Probe 5 (Figure III-1) showed no detectable hybridization bands, as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 DNA previously digested with *Nco* I produced two expected bands at ~7.8 kb and ~3.7 kb (Figure IV-4, lane 5). Conventional control genomic DNA digested with *Nco* I and spiked with probe templates (Figure III-1, Probe 3 and Probe 5) generated from PV-GMAP5779 produced the expected bands at ~1.3 kb and ~1.1 kb, respectively (Figure IV-4, lane 6 and lane 7). Detection of the spiked controls indicates that the probes hybridized to their target sequences.

MON 87712 DNA digested with *Nco* I (Figure IV-4, lane 2 and lane 9) or a combination of *EcoR* I and *Spe* I (Figure IV-4, lane 4 and lane 11) and hybridized with Probe 3 and Probe 5 (Figure III-1) produced no detectable bands, as expected. The results presented in Figure IV-4 indicate that MON 87712 contains no detectable T-DNA II elements from Probe 3 and Probe 5 of PV-GMAP5779.

IV.B.2. Probe 4

Conventional control genomic DNA digested with *Nco* I (Figure IV-5, lane 1 and lane 7) or a combination of *EcoR* I and *Spe* I (Figure IV-5, lane 3 and lane 9) and hybridized with Probe 4 (Figure III-1) produced endogenous hybridization signals. These signals are not visible in the reported figure (Figure IV-5). However, these endogenous signals are observed at >40 kb (*Nco* I digest, lane 1, lane 2, and lane 5 through lane 8) and ~1.0 kb (*EcoR* I and *Spe* I digest, lane 3, lane 4, lane 9, and lane 10) in a darker exposure (data not shown). These hybridization signals most likely result from Probe 4 hybridizing to homologous sequences residing in the soybean genome.

Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 DNA previously digested with *Nco* I produced two expected bands at ~7.8 kb and ~3.7 kb (Figure IV-5, lane 5 and lane 6) in addition to the endogenous hybridization

bands discussed above. Detection of the spiked controls indicates that the probe hybridized to its target sequence.

MON 87712 DNA digested with *Nco* I (Figure IV-5, lane 2 and lane 8) or a combination of *EcoR* I and *Spe* I (Figure IV-5, lane 4 and lane 10) and hybridized with Probe 4 (Figure III-1) produced no detectable bands other than the endogenous hybridization bands discussed above. The results presented in Figure IV-5 indicate that MON 87712 contains no detectable T-DNA II elements from Probe 4 of PV-GMAP5779 other than the endogenous soybean sequences.

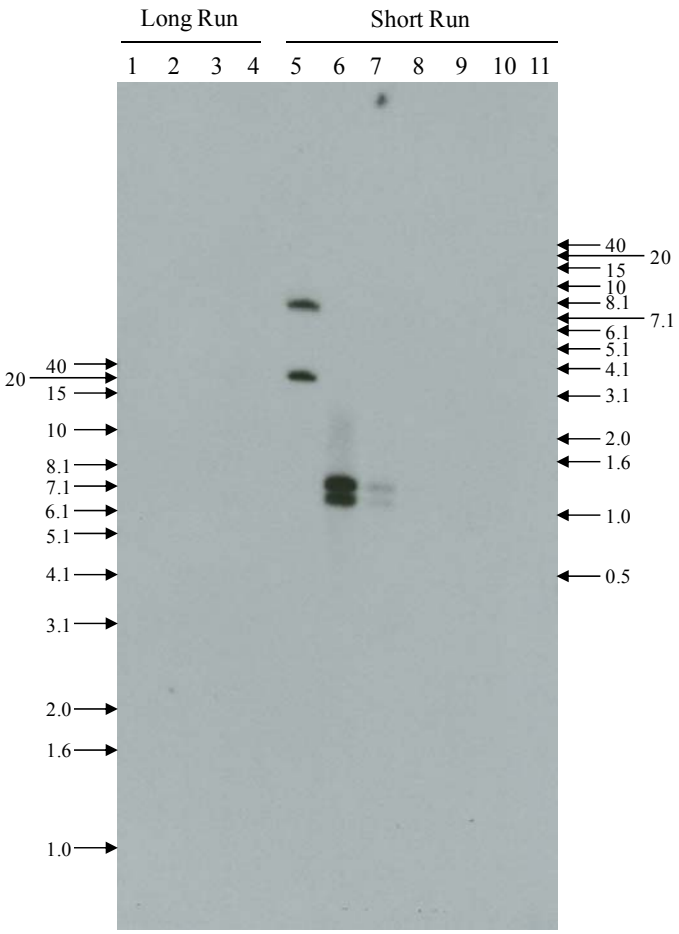


Figure IV-4. Southern Blot Analysis to Detect the Presence or Absence of T-DNA II Sequences in MON 87712: Probe 3 and Probe 5

The blot was hybridized with two ^{32}P-labeled probes that spanned portions of the T-DNA II sequence (Figure III-1, Probe 3 and Probe 5). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with Probe 3 and Probe 5 [~1.0 genome equivalent]
7	Conventional Control (*Nco* I) spiked with Probe 3 and Probe 5 [~0.1 genome equivalent]
8	Conventional Control (*Nco* I)
9	MON 87712 (*Nco* I)
10	Conventional Control (*EcoR* I and *Spe* I)
11	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

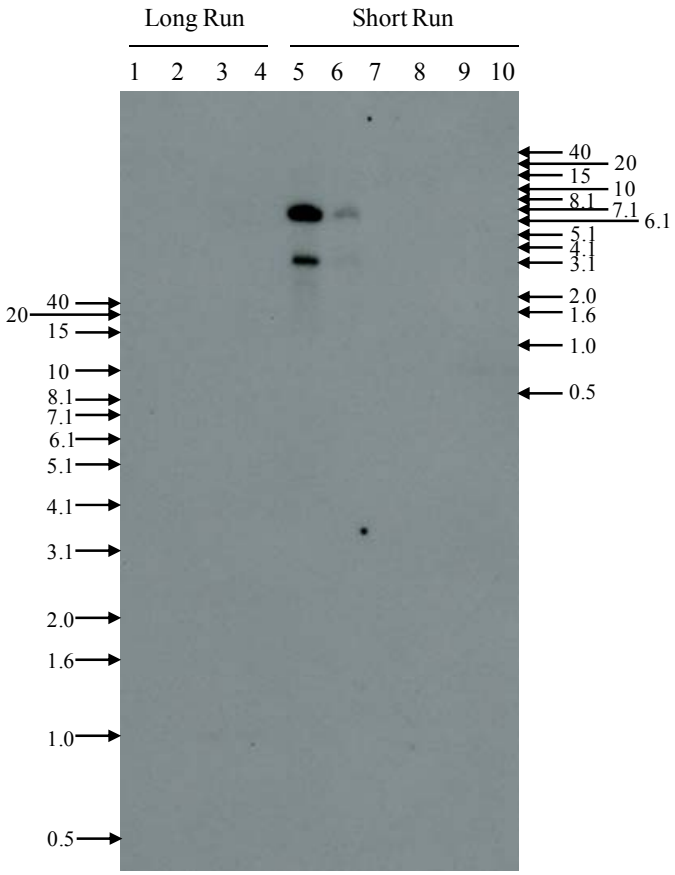


Figure IV-5. Southern Blot Analysis to Detect the Presence or Absence of T-DNA II Sequences in MON 87712: Probe 4

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA II sequence (Figure III-1, Probe 4). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~0.1 genome equivalent]
7	Conventional Control (*Nco* I)
8	MON 87712 (*Nco* I)
9	Conventional Control (*EcoR* I and *Spe* I)
10	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

IV.C. Southern Blot Analysis to Determine the Presence or Absence of PV-GMAP57779 Backbone Sequences in MON 87712

The presence or absence of PV-GMAP5779 backbone sequences in the soybean genome was evaluated by digesting the R_3 generation of MON 87712 and the conventional control genomic DNA samples with two sets of restriction enzymes: *Nco* I and a combination of *EcoR* I and *Spe* I. Digested genomic DNA was hybridized with overlapping probes spanning the main backbone sequence of PV-GMAP5779 (Figure III-1, Probe 7, Probe 8, and Probe 9) and one individual probe covering the backbone sequence between T-DNA I and T-DNA II (Figure III-1, Probe 6). If backbone DNA sequences were present in MON 87712, then hybridizing with overlapping probes corresponding to the backbone sequence should result in the detection of hybridization bands on the Southern blot. The results of this analysis are shown in Figure IV-6 through Figure IV-8.

IV.C.1. Plasmid Vector Backbone Probe 6

Conventional control genomic DNA digested with *Nco* I (Figure IV-6, lane 1 and lane 7) or a combination of *EcoR* I and *Spe* I (Figure IV-6, lane 3 and lane 9) and hybridized with Probe 6 (Figure III-1) showed no detectable hybridization bands, as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 previously digested with *Nco* I produced two expected bands at ~7.8 kb and ~3.7 kb, respectively (Figure IV-6, lane 5 and lane 6). The ~3.7 kb band was expected, because this segment contained Probe 6 sequence. The ~7.8 kb band was also detected because a small region of the intervening sequence contained in Probe 7 sequence in this ~7.8 kb segment is identical to a portion of Probe 6. Detection of the spiked controls indicates that the probe hybridized to its target sequence.

MON 87712 genomic DNA digested with *Nco* I (Figure IV-6, lane 2 and lane 8) or in combination with *EcoR* I and *Spe* I (Figure IV-6, lane 4 and lane 10) and hybridized with Probe 6 (Figure III-1) produced no detectable bands, as expected. The data indicate that MON 87712 contains no detectable backbone elements from Probe 6 of PV-GMAP5779.

IV.C.2. Plasmid Vector Backbone Probe 7 and Probe 9

Conventional control genomic DNA digested with *Nco* I (Figure IV-7, lane 1 and lane 8) or a combination of *EcoR* I and *Spe* I (Figure IV-7, lane 3 and lane 10) and hybridized with Probe 7 and Probe 9 (Figure III-1) showed no detectable hybridization bands, as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 previously digested with *Nco* I produced two expected bands at ~7.8 kb and ~3.7 kb, respectively (Figure IV-7, lane 5). The ~7.8 kb band was expected, because this segment contained Probe 7 and Probe 9 sequence. The faint ~3.7 kb band (stronger signal observed in darker exposure, data not shown) was also detected because a small region of the intervening sequence contained in Probe 6 sequence of this ~3.7 kb segment is identical to a portion of Probe 7 (refer to Section IV.C.1, Probe 6).

Conventional control genomic DNA digested with *Nco* I and spiked with probe templates (Figure III-1, Probe 7 and Probe 9) generated from PV-GMAP5779 produced the expected bands at ~1.8 kb and ~0.9 kb, respectively (Figure IV-7, lane 6 and lane 7). Detection of the spiked controls indicates that the probes hybridized to their target sequences.

MON 87712 genomic DNA digested with *Nco* I (Figure IV-7, lane 2 and lane 9) or a combination with *EcoR* I and *Spe* I (Figure IV-7, lane 4 and lane 11) and hybridized with Probe 7 and Probe 9 (Figure III-1) produced no detectable bands, as expected. The data indicate that MON 87712 contains no detectable backbone elements from Probe 7 and Probe 9 of PV-GMAP5779.

IV.C.3. Plasmid Vector Backbone Probe 8

Conventional control genomic DNA digested with *Nco* I (Figure IV-8, lane 1 and lane 7) or a combination of *EcoR* I and *Spe* I (Figure IV-8, lane 3 and lane 9) and hybridized with Probe 8 (Figure III-1) showed no detectable hybridization bands, as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 previously digested with *Nco* I produced an expected band at ~7.8 kb (Figure IV-8, lane 5 and lane 6). Detection of the spiked controls indicates that the probe hybridized to its target sequences.

MON 87712 genomic DNA digested with *Nco* I (Figure IV-8, lane 2 and lane 8) or in combination with *EcoR* I and *Spe* I (Figure IV-8, lane 4 and lane 10) and hybridized with Probe 8 (Figure III-1) produced no detectable bands, as expected. The data indicate that MON 87712 contains no detectable backbone elements from Probe 8 of PV-GMAP5779.

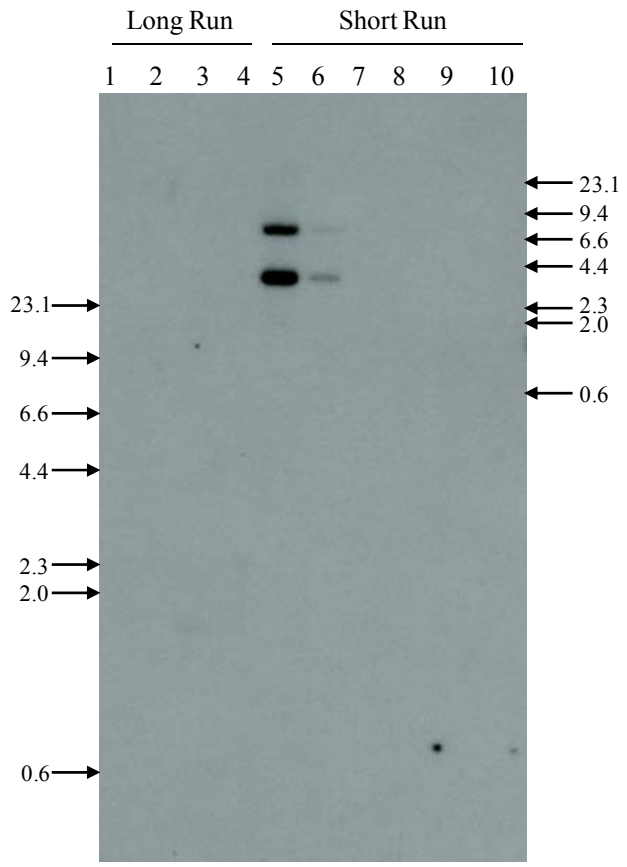


Figure IV-6. Southern Blot Analysis to Determine the Presence or Absence of PV-GMAP5779 Backbone Sequences in MON 87712: Probe 6

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the PV-GMAP5779 backbone sequence (Figure III-1, Probe 6). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~0.1 genome equivalent]
7	Conventional Control (*Nco* I)
8	MON 87712 (*Nco* I)
9	Conventional Control (*EcoR* I and *Spe* I)
10	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hin*d III Fragments (Invitrogen) on the ethidium bromide stained gel.

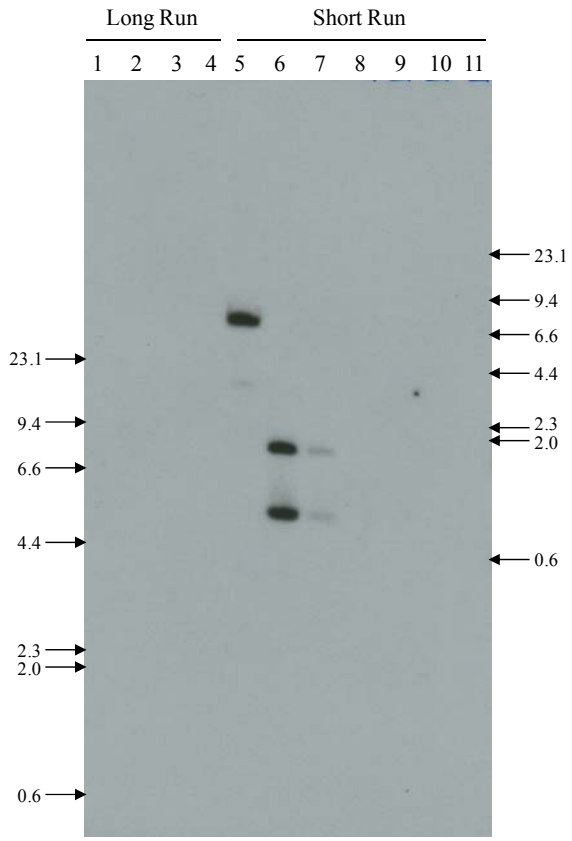


Figure IV-7. Southern Blot Analysis to Determine the Presence or Absence of PV-GMAP5779 Backbone Sequences in MON 87712: Probe 7 and Probe 9
The blot was hybridized with two ^{32}P-labeled probes that spanned portions of PV-GMAP5779 backbone sequence (Figure III-1, Probe 7 and Probe 9). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with Probe 7 and Probe 9 [~1.0 genome equivalent]
7	Conventional Control (*Nco* I) spiked with Probe 7 and Probe 9 [~0.1 genome equivalent]
8	Conventional Control (*Nco* I)
9	MON 87712 (*Nco* I)
10	Conventional Control (*EcoR* I and *Spe* I)
11	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hin*d III Fragments (Invitrogen) on the ethidium bromide stained gel.

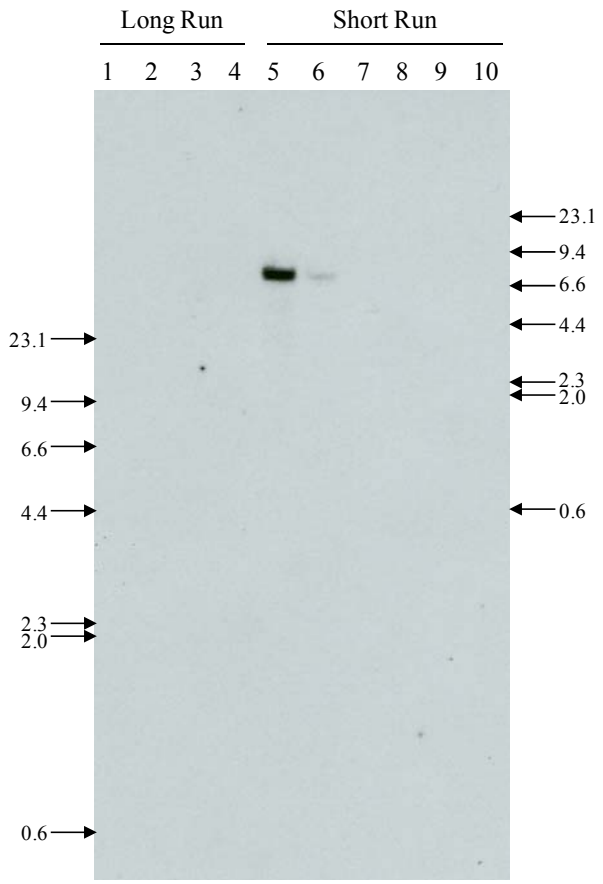


Figure IV-8. Southern Blot Analysis to Determine the Presence or Absence of PV-GMAP5779 Backbone Sequences in MON 87712: Probe 8

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the PV-GMAP5779 backbone sequence (Figure III-1, Probe 8). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	MON 87712 (*Nco* I)
3	Conventional Control (*EcoR* I and *Spe* I)
4	MON 87712 (*EcoR* I and *Spe* I)
5	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
6	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~0.1 genome equivalent]
7	Conventional Control (*Nco* I)
8	MON 87712 (*Nco* I)
9	Conventional Control (*EcoR* I and *Spe* I)
10	MON 87712 (*EcoR* I and *Spe* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hin*d III Fragments (Invitrogen) on the ethidium bromide stained gel.

IV.D. Organization and Sequence of the Insert and Adjacent DNA in MON 87712

The organization and sequence of the elements within the MON 87712 insert was confirmed by DNA sequence analyses. PCR primers were designed with the intent to amplify three overlapping DNA regions that span the entire length of the T-DNA I insert and the associated DNA flanking the 5' and 3' ends of the insert (Figure IV-9). The amplified DNA segments were subjected to DNA sequence analyses. The analyses determined that the DNA sequence of the MON 87712 insert is 2014 bp long (Table IV-2) and is identical to the corresponding T-DNA I sequence of PV-GMAP5779 as described in Table III-1. From the sequence analyses, 3493 base pairs flanking the 5' end of the MON 87712 insert and 1965 base pairs flanking the 3' end of the MON 87712 insert (Table IV-2) were also determined.

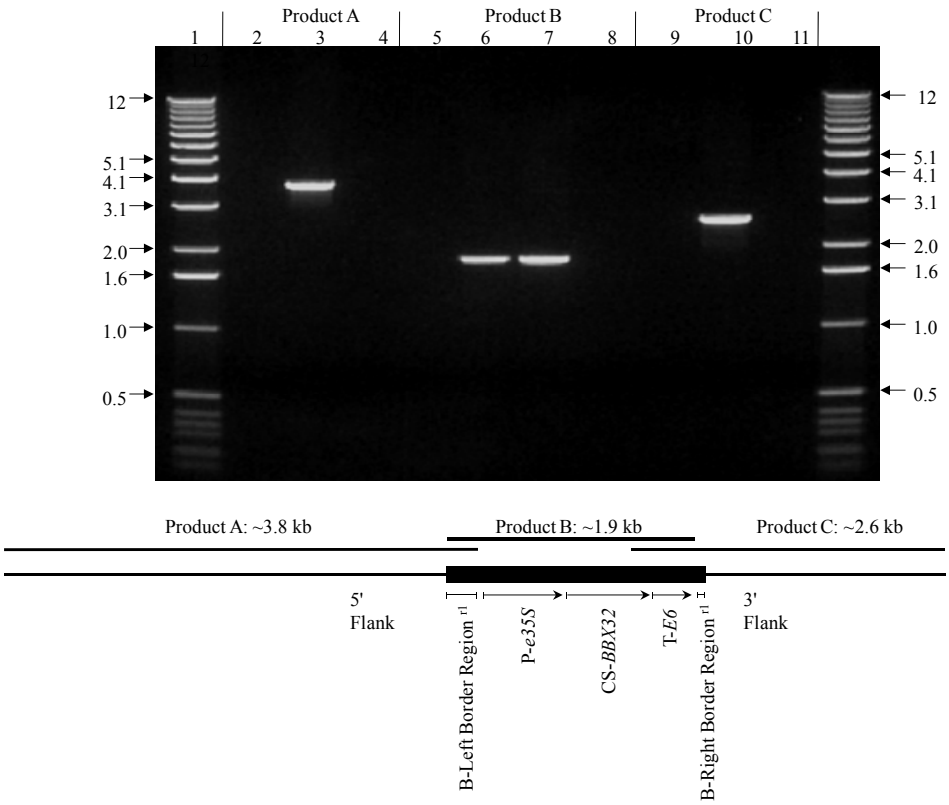


Figure IV-9. Overlapping PCR Analysis Across the Insert in MON 87712
PCR was performed on both conventional control genomic DNA and MON 87712 genomic DNA using three pairs of primers to generate overlapping PCR fragments from MON 87712 for sequencing analysis. To verify the PCR products, 4 µl of each of the PCR reactions was loaded on the gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 87712 that appears at the bottom of the figure. This figure is a representative of the data generated in the study. Lane designations are as follows:

Lane	Description
1	1 kb DNA Ladder
2	Conventional Control
3	MON 87712
4	No template DNA control
5	Conventional Control
6	MON 87712
7	PV-GMAP5779
8	No template DNA control
9	Conventional Control
10	MON 87712
11	No template DNA control
12	1 kb DNA Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1 kb DNA Ladder (Invitrogen) on the ethidium bromide stained gel.

IV.E. PCR and DNA Sequence Analyses to Examine the MON 87712 Insertion Site

PCR and sequence analyses were performed on genomic DNA extracted from MON 87712 and the conventional control to examine the integrity of the DNA insertion site in MON 87712. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure IV-10). The amplified PCR product from the conventional control was subjected to DNA sequence analysis. Sequence alignments were performed between the conventional control sequence and the sequences flanking the 5' and 3' end of the MON 87712 T-DNA I insert. The alignment analyses indicated a 42 base pair deletion from the conventional genomic DNA occurred upon T-DNA I insertion in MON 87712. This deletion presumably resulted from double stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

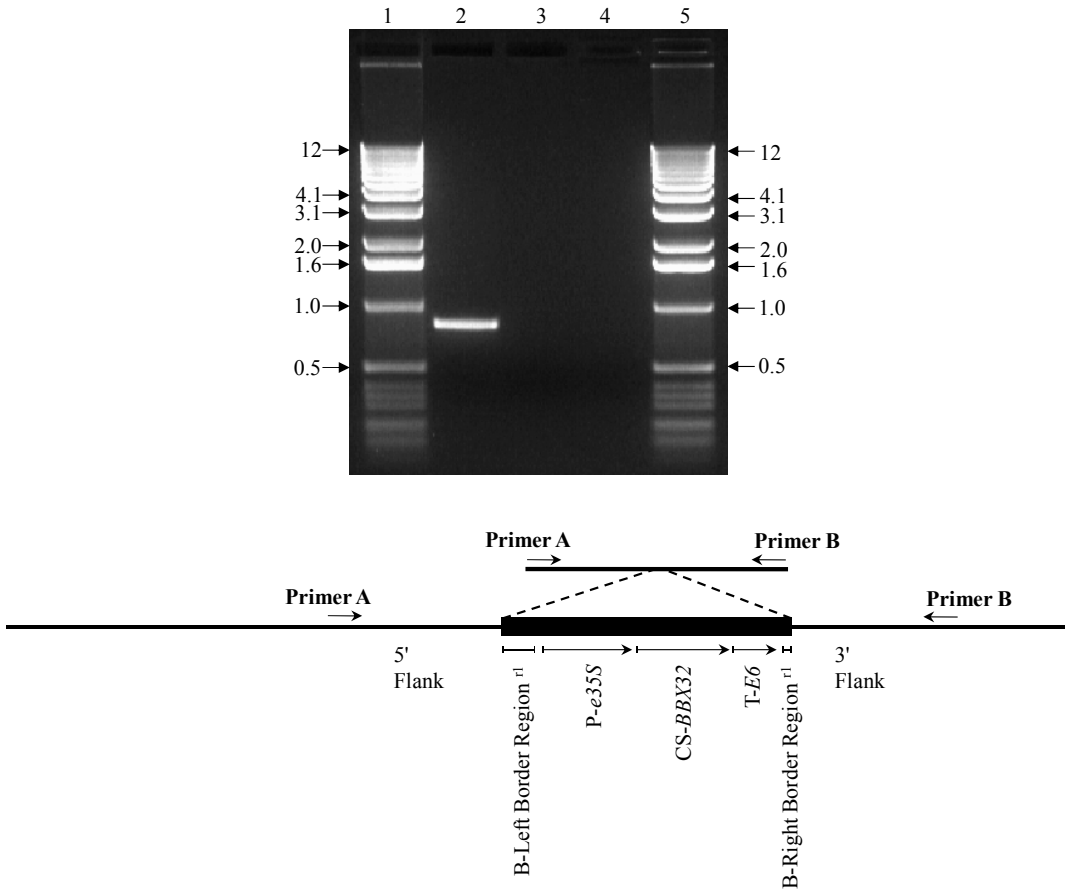


Figure IV-10. PCR Amplification of the MON 87712 Insertion Site

PCR analysis was performed to evaluate the insertion site. PCR was performed on conventional control DNA and MON 87712 DNA using Primer A, specific to the 5' flanking sequence, and Primer B, specific to the 3' flanking sequence of the insert in MON 87712. The DNA generated from the conventional control PCR was used for sequencing analysis. This illustration depicts the MON 87712 insertion site in the conventional control (upper panel) and the MON 87712 insert (lower panel). Approximately 9 µl of each of the PCR reactions was loaded on the gel. This figure is representative of the data generated in the study. Lane designations are as follows:

Lane	Description
1	1 kb DNA Ladder
2	Conventional Control
3	MON 87712
4	No template DNA control
5	1 kb DNA Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1kb DNA Ladder (Invitrogen) on the ethidium bromide stained gel.

IV.F. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87712

In order to demonstrate the stability of the insert in MON 87712, Southern blot analysis was performed using genomic DNA obtained from five generations of MON 87712. For reference, the breeding history diagram of MON 87712 and the generations that were tested are indicated in Figure IV-11. The MON 87712 R_3 generation was used for the molecular characterization analyses as shown in Figure IV-2 through Figure IV-8. To analyze insert stability, four additional generations, R_4, R_5, R_6, and R_7, were evaluated by Southern blot analysis and compared to the R3 generation. Genomic DNA, isolated from each of the selected generations of MON 87712, was digested with *Nco* I (Figure IV-1) and hybridized with Probe 1 (Figure III-1). Probe 1 was designed to detect both segments generated by the *Nco* I digest. Any instability associated with the insert would be detected as novel bands within the fingerprint on the Southern blot. This Southern blot contains the same controls as described in Section IV.A.1.

IV.F.1. Probe 1

Conventional control genomic DNA digested with *Nco* I and hybridized with Probe 1 showed no detectable hybridization bands (Figure IV-12, lane 1), as expected. Conventional control genomic DNA digested with *Nco* I and spiked with PV-GMAP5779 DNA previously digested with *Nco* I produced two expected bands at ~7.8 kb and ~3.7 kb (Figure IV-12, lane 2 and lane 3). Detection of the spiked controls indicates that the probe hybridized to its target sequences.

MON 87712 DNA extracted from generations R_3, R_4, R_5, R_6, and R_7 and digested with *Nco* I and hybridized with Probe 1 (Figure III-1) produced two expected bands at ~4.3 kb and ~2.2 kb (Figure IV-12, lane 4 through lane 8). These bands are consistent with the ~4.3 kb and ~2.2 kb bands detected in the MON 87712 R_3 generation (Figure IV-2, lane 2 and lane 8). The ~4.3 kb band represents the 5′ end of the inserted DNA and the adjacent DNA flanking the 5′ end of the insert. The ~2.2 kb band represents the 3′ end of the inserted DNA and the adjacent DNA flanking the 3′ end of the insert. Therefore, this result indicates that the single copy of T-DNA I in MON 87712 is stably maintained across the five generations.

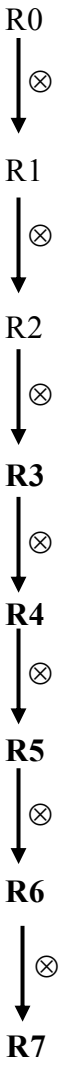


Figure IV-11. Breeding History of MON 87712
The R_3 generation was used for the molecular characterization and commercial development of MON 87712. R0 corresponds to the transformed plant. All generations were self pollinated (⊗). Generations used for insert stability analysis (R_3, R_4, R_5, R_6, and R_7) are indicated in bold text.

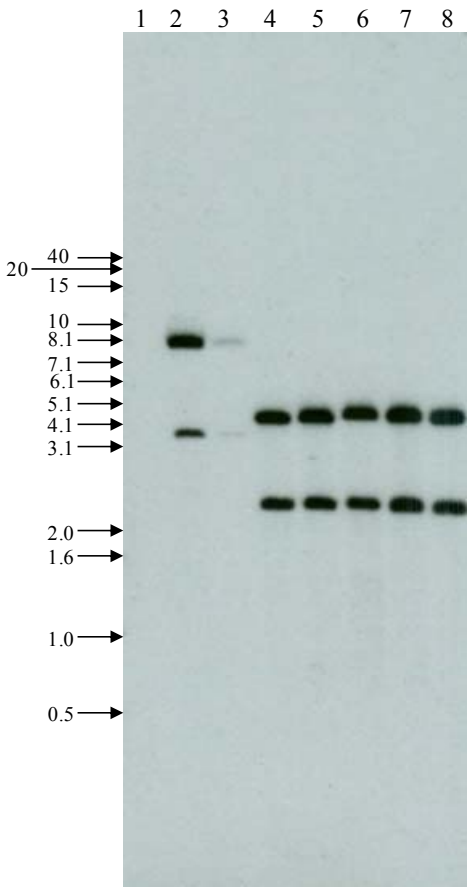


Figure IV-12. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87712: Probe 1

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA I sequence (Figure III-1, Probe 1). Each lane contains ~10 µg of digested genomic DNA isolated from leaf tissue. Lane designations are as follows:

Lane	Description
1	Conventional Control (*Nco* I)
2	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~1.0 genome equivalent]
3	Conventional Control (*Nco* I) spiked with PV-GMAP5779 (*Nco* I) [~0.1 genome equivalent]
4	MON 87712 (R_3) (*Nco* I)
5	MON 87712 (R_4) (*Nco* I)
6	MON 87712 (R_5) (*Nco* I)
7	MON 87712 (R_6) (*Nco* I)
8	MON 87712 (R_7) (*Nco* I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1 kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

IV.G. Inheritance of the Genetic Insert in MON 87712

During development of MON 87712, segregation data were generated to assess the heritability and stability of the T-DNA I present in MON 87712. Chi square (χ^2) analysis was performed over several generations to confirm the segregation and stability of T-DNA I in MON 87712. The Chi square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 87712 breeding path for generating segregation data is described in Figure IV-13. The transformed R_0 plant was self-pollinated to produce R_1 seed. From the R_1 segregating population, which consisted of 88 total plants, an individual plant (subsequently designated as MON 87712) homozygous for the *BBX32* gene was identified via Invader® analysis. Invader is a non-PCR based assay that can be used to accurately quantify DNA copy number in plant genomes (Gupta et al., 2008).

The selected R_1 MON 87712 plant was self-pollinated to give rise to a population of R_2 plants, which were in turn self-pollinated to obtain the R_3 generation. At each generation, the fixed homozygous plants were tested for the expected segregation pattern of 1:0 (positive:negative) for the *BBX32* gene using the Invader analysis.

Homozygous R3 MON 87712 plants were crossed to a Monsanto proprietary soybean line (EXP0224AAC) that did not contain the *BBX32* gene to produce F_1 hemizygous seed. The resulting F_1 plants were tested for the copy number of the *BBX32* gene by Endpoint Taqman zygosity analysis. A hemizygous F_1 plant was selected and then self-pollinated to produce F_2 seed. This process of self-pollination and Endpoint Taqman zygosity analysis was repeated for the F_2, F_3, and F_4 plants. Subsequently, assessment at each of these generations was based on zygosity, and the *BBX32* gene was predicted to segregate at a 1:2:1 (homozygous positive:hemizygous positive:homozygous negative) ratio for progeny derived from a hemizygous parental plant according to Mendelian inheritance principles.

A Chi square (χ^2) analysis was used to compare the observed segregation ratios to the expected ratios according to Mendelian inheritance principles. The $\chi 2$ was calculated as:

$$\chi^2 = \sum [(\mid o - e \mid)2 / e]$$

where o = observed frequency of the phenotype and e = expected frequency of the phenotype. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$).

The results of the χ^2 analysis of the segregating progeny of MON 87712 are presented in Table IV-3. The χ^2 value in the F2, F3, and F4 generations indicated no statistically significant difference between the observed and expected 1:2:1 segregation ratio. These results support the conclusion that the *BBX32* coding sequence in MON 87712 resides at a single locus within the soybean genome and is inherited according to Mendelian inheritance principles. These results are also consistent with the molecular characterization data that indicate MON 87712 contains a single, intact copy of the *BBX32* expression cassette that was inserted into the soybean genome at a single locus.

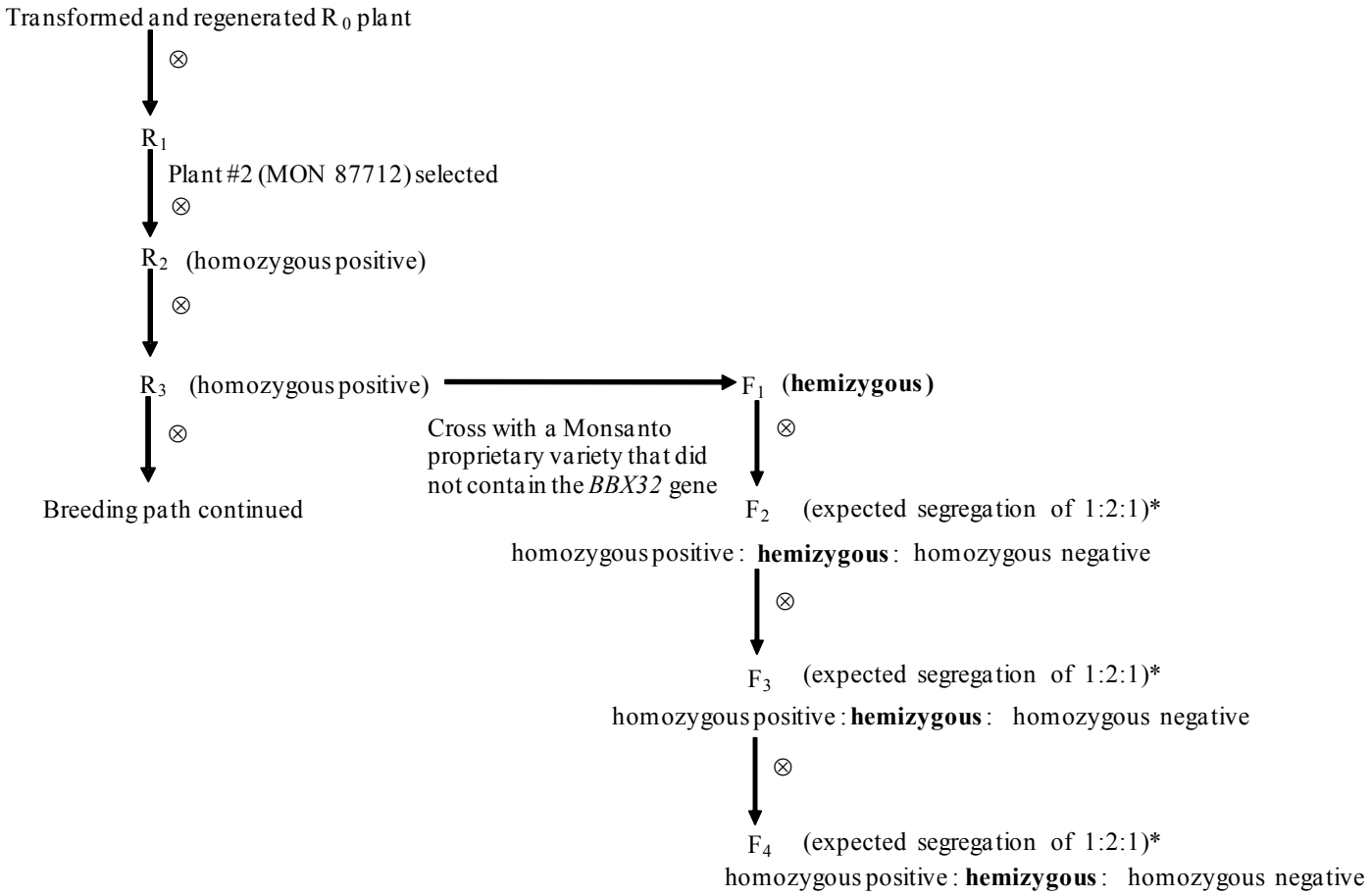


Figure IV-13. Breeding Path for Generating Segregation Data for MON 87712

⊗Self pollinated.
* Chi-square analysis conducted on segregation data from the F_2, F_3, and F_4 generations.
Note: An individual hemizygous plant from each of the F_1, F_2, and F_3 populations was self-pollinated to produce the population of the next generation.

Table IV-3. Segregation of the Expression Cassette During the Development of MON 87712

					1:2:1 Segregation				
Generation	Total Plants[1]	Observed # Plants Homozygous Positive	Observed # Plants Hemizygous	Observed # Plants Homozygous Negative	Expected # Plants Homozygous Positive	Expected # Plants Hemizygous	Expected # Plants Homozygous Negative	χ^2	Probability
F_2	218	59	98	61	54.50	109.00	54.50	2.26	0.3235
F_3	184	48	94	42	46.00	92.00	46.00	0.48	0.7873
F_4	182	44	87	51	45.50	91.00	45.50	0.89	0.6408

[1]Plants were evaluated for the presence of the *BBX32* gene by Endpoint Taqman zygosity analysis. "Total plants" refers to the total number of plants in which zygosity could be determined using the assay.

IV.H. Genetic Modification Characterization Conclusion

Molecular characterization of MON 87712 by Southern blot analyses confirmed that one copy of the *BBX32* expression cassette was integrated into the soybean genome at a single locus. No T-DNA II or backbone DNA sequences from plasmid vector PV-GMAP5779 were detected in MON 87712.

PCR and DNA sequence analyses performed on MON 87712 and conventional control determined the following: the complete DNA sequence of the insert and adjacent DNA sequences in MON 87712; the organization of the genetic elements within the insert; the expected sequence of each element in the inserted DNA; and the 5′ and 3′ insert to genomic DNA junctions. The PCR and DNA sequence analysis identified a 42 base pair deletion that occurred at the insertion site in MON 87712.

Southern blot analysis of MON 87712 demonstrated that the inserted DNA has been maintained through five generations of breeding, thereby, confirming the stability of the insert. Results from segregation analyses show heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA I in MON 87712 at a single chromosomal locus.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE BBX32 PROTEIN IN MON 87712

Characterization of the introduced protein in a biotechnology-derived crop product is important to establishing its food, feed, and environmental safety. As described in Section IV, MON 87712 contains a *BBX32* expression cassette that upon translation results in the BBX32 protein. This section summarizes: 1) the functionality of BBX32; 2) the characterization of MON 87712 BBX32; 3) the levels of MON 87712 BBX32 in plant tissues; 4) assessment of the potential allergenicity of MON 87712 BBX32 and 5) the food, feed, and environmental safety assessment of MON 87712 BBX32. The data support a conclusion that MON 87712 is safe for the environment and human or animal consumption based on several lines of evidence, all of which are summarized below.

V.A. Identity and Function of the BBX32 Protein from MON 87712

MON 87712 has been demonstrated to provide increased yield (See Appendix B), due to the insertion of the *Arabidopsis thaliana BBX32* gene. The BBX32 protein is a member of the of the B-box zinc finger family from *Arabidopsis thaliana*. This family represents a subgroup of zinc finger proteins that contain one or more B-box domains with specialized tertiary structures that are stabilized by binding zinc ions. The B-box domain is predicted to be involved in protein-protein interactions (Khanna et al., 2009). BBX32 contains a single annotated protein domain, the B-Box B1 domain (Khanna et al., 2009). The B-box zinc finger family is found in many plant species; for example, the soybean B-box family contains 61 genes (Preuss et al., submitted for publication). Homologs of BBX32 are found in many agronomically-important species, suggesting that the function of BBX32 in its source plant, *Arabidopsis thaliana*, is conserved in other plant species.

BBX32 acts as a plant transcriptional accessory protein. As described in Appendix B, BBX32 in MON 87712 plays a role in the transcriptional regulation of the plant's day/night processes, and results in increased availability of assimilates in the plant. Plant nutrient assimilation and utilization are critical processes to drive yield improvement.

V.B. Characterization and Equivalence of BBX32 Protein from MON 87712

The safety assessment of crops derived through biotechnology includes characterization of the functional and physicochemical properties of, and confirmation of the safety of, the introduced protein. The expression level of BBX32 protein in MON 87712 is low, and insufficient for use in the subsequent safety evaluations. Therefore, recombinant BBX32 protein was produced in *Escherichia coli*, using an expression vector with a *BBX32* coding sequence that matched that of the *BBX32* coding sequence in MON 87712. In order to establish the suitability of the *E. coli*-produced BBX32 for use in safety evaluations, the *E. coli*-produced and the MON 87712-produced BBX32 preparations were characterized and assessed for equivalence.

As reported in section V.C, the level of expression of BBX32 in MON 87712 is very low. Due to its low level of expression, the MON 87712-produced BBX32 protein was not isolated to a high level of purity, but instead was enriched in leaf extract to a purity of approximately 0.0001%. This low purity limited the physicochemical characterization of MON 87712-produced BBX32 and the overall equivalence analysis to characteristics that could be evaluated by western blot. *E. coli*-produced BBX32 was characterized using a panel of analytic techniques including western blot analysis, N-terminal sequence analysis, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to confirm identity, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish the apparent molecular weight of BBX32, and analysis of binding activity to a specific partner protein to demonstrate protein-protein binding functionality.

MON 87712-produced BBX32 protein was characterized by western blot analysis to establish identity via immunoreactivity with an anti-BBX32 antibody. Equivalence between MON 87712-produced and *E. coli*-produced BBX32 was established through comparison of the migration of the two proteins on SDS-PAGE, as detected by western blot analysis, and through bioinformatic comparison of the BBX32 coding sequence from the *E. coli* expression vector and MON 87712. The details of the materials, methods, and results are described in Appendix D, while the conclusions are summarized as below.

Western blot analysis with a monoclonal antibody developed against BBX32 showed that the MON 87712-produced BBX32 had equivalent mobility to the *E. coli*-produced BBX32 protein on the blot, indicating the proteins from both sources were immunoreactive and equivalent in apparent molecular weight. These data provide a characterization of the BBX32 protein from MON 87712, and were used to establish its equivalence to the *E. coli*-produced BBX32 protein.

The BBX32 coding sequence expressed in *E. coli* was designed to precisely match the amino acid coding sequence of the *BBX32* gene expressed in MON 87712. Sequence alignment showed that translation of the BBX32 coding sequence from the vector used to transform conventional soybean to produce MON 87712, PV-GMAP5779, and from the *E. coli* BBX32 expression vector, pMON102114, resulted in the same amino acid sequence (see Appendix D). As reported in Section IV.D, the DNA sequence of the MON 87712 insert is identical to the corresponding T-DNA I sequence of PV-GMAP5779. Thus, the pMON102114 BBX32 coding sequence is identical to the BBX32 coding sequence in MON 87712.

Direct assessments of BBX32 partner protein binding activity, glycosylation status, and N-terminal sequence , were not conducted for MON 87712-produced BBX32 due to the low purity of the plant-produced protein. Instead, these characteristics of BBX32 in MON 87712 were assessed indirectly. With respect to functional activity, the phenotypes observed for MON 87712 compared to conventional soybean, including discrete changes in gene expression (for example, Figure B-6) and increased crop yield, indicate that BBX32 is active in MON 87712. The *E. coli*-produced BBX32 was active in its partner protein binding assay. The N-terminal sequence of *E. coli*-produced BBX32 was evaluated and shown to match the N-terminus predicted by the BBX32 coding sequence

in MON 87712. Thus, the *E. coli*-produced BBX32 represents the full length BBX32 protein in MON 87712. There is no predicted N-terminal targeting or signal sequence in the BBX32 coding sequence. The BBX32 protein sequence contains a consensus potential N-glycosylation site (NTT, starting at position 172), but lacks the N-terminal signal sequence required for transport to the endoplasmic reticulum which is the gateway for both N- and O-glycosylation (Pattison and Amtmann, 2009; Vitale and Denecke, 1999). This is similar to the CP4-EPSPS, which contains potential N-glycosylation sites but has been shown to not be glycosylated (Harrison et al., 1996). In total, this suggests that MON 87712-produced BBX32 is not glycosylated.

V.C. Expression Levels of BBX32 Protein in MON 87712

The levels of BBX32 in various tissues of MON 87712 that are relevant to the risk assessment were determined by western blot analysis. For western blots, protein extracts are separated by gel electrophoresis and then transferred to a membrane that is probed with an antibody. A sensitive western blot technique was developed to separate and clearly differentiate the BBX32 protein present in MON 87712 tissue extracts from other proteins present in the tissue matrix, which allowed for the detection of this low expressing protein in the presence of other matrix proteins. The levels of the BBX32 protein in various tissues of MON 87712 were estimated by densitometric analysis of X-ray films exposed to immunoblots probed with BBX32 antibodies and visualized using chemiluminescent detection reagents (refer to Appendix E).

Tissue samples of MON 87712 and the parental conventional control A3525 were collected during the 2009 growing season from eight geographically diverse field sites in the U.S.: Jackson County, Arkansas; Parke County, Indiana; Clinton County, Illinois; Madison County, Illinois; Stark County, Illinois; York County, Nebraska; Boone County, Indiana; and Pawnee County, Kansas. These field sites were representative of soybean producing regions suitable for commercial production. At each site, four replicated plots of MON 87712 and the conventional control A3525 were planted using a randomized complete block field design. Over-season leaf (OSL-1 to OSL-4), root (OSR-3), forage (forage-1), and harvested seed tissues were collected from each replicated plot at all field sites.

Analyses of BBX32 protein levels were carried out in all seven tissue types collected as described in Table V-1. For leaf and root samples, densitometric analysis of the BBX32-specific immunoblots yielded the reported quantitative values of BBX32 protein by interpolation from standard curves prepared using purified BBX32 protein standard. The results obtained from western blot analysis are summarized in Table V-1and the details of the materials, methods, and sample collection are described in Appendix E.

The mean BBX32 protein levels were determined across eight sites and seven tissue types. BBX32 protein levels in MON 87712 across tissue types ranged from <LOD to 110 ng/g dwt. The western blot method developed was highly sensitive as indicated by the low LODs established for each tissue (Table V-1). In spite of the high sensitivity of the western technique developed, the BBX32 protein was not detected in several samples including all harvested seed, forage and OSL-4 samples. The mean protein level in root

was 3.9 ng/g dwt, though most samples were <LOD. Expression was highest in leaf tissue, specifically OSL-3 (mean protein level of 35 ng/g dwt) where BBX32 was determined in all samples. The levels in OSL-1 and OSL-2 were slightly below those of OSL-3. These results indicate that the expression of BBX32 is highest in leaf.

For seed and forage tissues the LOD of the BBX32 protein was determined by spiking serially diluted BBX32 protein reference standard into the conventional control soybean, A3525, of forage and seed tissue extracts. The LOD of BBX32 on the western blots was defined by the lowest amount of BBX32 protein that resulted in a visible band on the western blots. The LOD was determined to be 0.5 pg in forage extracts and 2.5 pg in seed extracts.

Other B-box proteins, such as the *Arabidopsis thaliana* B-box protein, CONSTANS (CO), accumulate at levels too low to be detected by western blot although functional activity is present (Suarez-Lopez et al., 2001). Similarly to BBX32 in MON 87712, Valverde et al. (2004) reported that CO over-expression in *Arabidopsis thaliana* results in growth condition-dependent detection of the protein, indicating that an over-expressed protein can still be subject to endogenous mechanisms that regulate protein abundance.

Table V-1. Summary of BBX32 Protein Levels in Leaf, Root, Forage, and Seed from MON 87712 Grown in 2009 U.S. Field Trials

Tissue[1]	Development Stage[2]	Days After Planting (DAP)	BBX32 Mean (SD) Range (ng/g fwt)[3]	BBX32 Mean (SD) Range (ng/g dwt)[4]	LOQ/LOD[5] (pg)
OSL-1	V3-V4	24-33	3.9 (4.0) 0.84-16	21 (21) 4.4-80	0.25-1.0/0.25
OSL-2	V6-V8	35-49	3.7 (1.9) 0.43-8.0	21 (11) 2.7-47	0.25-1.0/0.25
OSL-3	R2	39-63	5.0 (3.7) 1.0-14	35 (28) 5.5-110	0.25-1.0/0.25
OSL-4	R6	71-106	<LOD	NA[6]	0.25-0.5/0.25
OSR-3	R6	71-106	1.2 (0.15) 1.1-1.5	3.9 (0.54) 3.3-5.0	0.25-1.0/0.25
Forage-1	R6	71-106	ND[7]	NA	NA/0.5
Seed	R8	112-155	ND	NA	NA/2.5

[1]OSL = over-season leaf, OSR = over-season root.
[2]The development stage each tissue was collected. Soybean plant growth stages described in Soybean Growth and Development (Pedersen, 2004).
[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as nanogram (ng) of protein per gram (g) of tissue on a fresh weight basis (fwt). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites. The numbers of samples (n) used in the calculations were as follows: OSL-1 n=29, OSL-2 n= 22, OSL-3 n=31, OSR-3 n= 12). Sample collections are detailed in Appendix E.
[4]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as nanogram (ng) of protein per gram (g) of tissue on a dry weight basis (dwt). The dry weight values were calculated by dividing the ng/g fwt by the dry weight conversion factor obtained from moisture analysis data.
[5]LOQ=limit of quantitation; LOD=limit of detection. For leaf and root tissues; LOQ is the range of all lowest detectable band across all blots for the specific tissues and LOD is the lowest detectable band across the entire tissue type. For forage-1 and seed the LOD was the lowest detectable band when spiked into the corresponding tissue extracts.
[6]NA = not applicable
[7]ND = not detected

V.D. Assessment of Potential Allergenicity of the BBX32 Protein

The allergenic potential of an introduced protein is assessed by comparison of the biochemical characteristics of the introduced protein to the biochemical characteristics of known allergens (Codex Alimentarius, 2003). A protein is not likely to be associated with allergenicity if: 1) the protein is from a non-allergenic source, 2) the protein represents a very small portion of the total plant protein, 3) the protein does not share structural similarities to known allergens based on the amino acid sequence, and 4) the protein is rapidly digested in mammalian gastrointestinal systems. BBX32 has been assessed for its potential allergenicity according to these safety assessment guidelines.

1) BBX32 originates from *Arabidopsis thaliana*, an organism that has not been reported to be a source of known allergens.

2) BBX32 protein represents a minor component (less than 0.001%) of the total protein in the harvested seed of MON 87712.

3) Bioinformatics analyses demonstrated that BBX32 does not share amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes.

4) *In vitro* digestive fate experiments conducted with BBX32 demonstrate that the protein is rapidly digested in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF).

Taken together, these data support the conclusion that BBX32 does not pose a significant allergenic risk to humans or animals.

V.E. Safety Assessment Summary of BBX32 Protein in MON 87712

V.E.1. The Donor Organism is Safe

The gene encoding BBX32 is from *Arabidopsis thaliana*, a plant that has served as a model organism for plant biology (Meinke et al., 1998). *Arabidopsis thaliana* is generally not considered an allergenic or toxic source organism. Although *Arabidopsis thaliana* contains homologs of proteins previously described as allergens in other plant species (e.g., germins, lipid transfer protein, profilins, and small molecular weight calcium binding proteins), no Arabidopsis proteins have been reported in a peer-reviewed database of known allergens (FARRP, 2011). One case of occupational asthma has been reported in a laboratory worker due to exposure to Arabidopsis pollen (Yates et al., 2008).

Arabidopsis thaliana is not purposely consumed as a food source by humans, and there is no documented consumption by animals. However, certain populations of a close relative, *Arabidopsis lyrata*, have been reported to be subject to sheep grazing (Sandring et al., 2007). Arabidopsis is in the *Brassicaceae* family (Meinke et al., 1998), which contains well-known food and oilseed crops such as broccoli, cauliflower, cabbage, and canola/rapeseed. *Camelina sativa*, an emerging oilseed crop, is reported to be the cultivated species most closely related to Arabidopsis (Pilgeram et al., 2007). *Camelina*

sativa leaves are consumed as fresh greens by humans in the country of Georgia (Facciola, 1998), the meal can be used as a component of livestock feed (AAFCO, 2011), and the plants in a crop setting are grazed by roaming wildlife (Pilgeram et al., 2007). The safe consumption of near relatives of *Arabidopsis thaliana* by humans and animals supports the safety of this organism.

V.E.2. BBX32 Protein Belongs to a Common Class of Plant Proteins

BBX32 is a member of a family of B-box-containing proteins from *Arabidopsis thaliana* (Khanna et al., 2009). The B-box zinc finger family is found in many plant species including soybean, where the B-box family contains 61 genes (Preuss et al., submitted for publication). Bioinformatic searches using the BBX32 amino acid sequence as the query identify homologous sequences from several different plant species, including the food crops: citrus, grape, apple, soybean, rice, lettuce, and corn. Overall the protein sequence identity of BBX32 to homologs in these species range from ~31-43%. The overall protein sequence identity of BBX32 to its homolog in canola is 66%, indicating that *Brassicaceae* species, likely including commonly consumed species such as broccoli, contain proteins very similar to BBX32. The amino acid sequence alignment between BBX32 and its crop homologs spans the length of the proteins, with the highest identity found in the B-box domain. Thus BBX32 shares sequence identity and structural similarities with proteins present in plants currently consumed, establishing that humans and animals are exposed to this class of proteins and that no adverse effects have been attributed to this class of proteins.

V.E.3. BBX32 Protein in MON 87712 is Not Homologous to a Known Allergen or Toxin

Bioinformatics analyses were performed to assess the allergenic potential, toxicity, or biological activity of BBX32. The analysis demonstrated that BBX32 does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which could have adverse effects to human or animal health.

V.E.4. BBX32 Protein in MON 87712 is Labile in *in vitro* Digestion Assays

BBX32 was readily digestible in SGF and SIF. Rapid degradation of BBX32 in SGF and SIF suggests that it is highly unlikely that the BBX32 in MON 87712 would be absorbed in the small intestine and have any adverse effects on human or animal health.

V.E.5. BBX32 Protein in MON 87712 is Not Acutely Toxic

An acute oral toxicology study was conducted with BBX32. Results indicate that BBX32 did not cause any adverse effects in mice, with a No Observable Adverse Effect Level (NOAEL) of 29 mg/kg body weight (BW), the highest dose level tested.

Potential human health risks from consumption of foods derived from MON 87712 were evaluated using a Margin of Exposure (MOE) approach. BBX32 was not detected in harvested seed when assayed using a sensitive immunoblot method with a LOD of 2.5 pg (Table V-1) so an

expression level corresponding to the LOD of BBX32 in seed was calculated by converting the observed LOD to a ng/g fresh weight value using the following formula:

$$\text{ng/g fwt} = \frac{\text{the lowest spiked BBX32 standard with a visible band (pg)}}{\text{volume of the extract loaded per lane } (\mu\text{l}) \div (\text{buffer to tissue ratio})} \quad [3]$$

The corresponding expression level calculated for BBX32 in seed was10 ng/g fwt. To generate estimates of exposure and MOE, an expression level of BBX32 in seed tissue of 5 ng/g fwt was used. This value, which corresponds to an expression level based on one-half the LOD of the assay used to quantify BBX32 expression in MON 87712 seed, is in keeping with U.S. Environmental Protection Agency guidance for calculations of acute human exposure to small molecules in blended commodities when the substance is not detectable (U.S. EPA, 2000). A MOE was calculated between the acute mouse NOAEL (29 mg/kg BW) for BBX32 and 95th percentile "eater-only" estimates of acute dietary exposure determined using the Dietary Exposure Evaluation Model (DEEM-FCID version 2.16, Exponent Inc.). DEEM food consumption data are obtained from the 1994-1996 and 1998 USDA Continuing Survey of Food Intakes by Individuals (CSFII), and assume that 100% of soybean products consumed, excluding oil, are derived from MON 87712. The MOEs for acute dietary intake of BBX32 were estimated to be 29,000,000 for the general population and 1,070,000 for non-nursing infants, the sub-population with the highest estimated exposure. These very large MOEs, in addition to the above mentioned protein safety data for BBX32, support the conclusion that there is no meaningful risk to human health from dietary exposure to BBX32 in MON 87712.

Potential health risks to animals from the presence of MON 87712 BBX32 in feed were evaluated by calculating an estimate of daily dietary intake (DDI). BBX32 was not detected in harvested seed or in forage (see section V.C), so expression levels corresponding to the LODs of BBX32 in seed and forage were calculated as described above. These expression levels, 10 ng/g fwt in seed and 1 ng/g fwt in forage, were used to calculate the DDI. In the worst case scenario, poultry, swine, and lactating dairy cattle would be consuming no more than 0.000003% of their total protein intake as BBX32 protein from MON 87712. This very low level of exposure of animals to BBX32 in their feed, in addition to the above mentioned safety data for BBX32, supports the conclusion that there is no meaningful risk to animal health when MON 87712 is present in their diets.

Using the guidance provided by the FDA in its 1992 Policy Statement regarding the evaluation of New Plant Varieties, a conclusion of "no concern" is reached for the donor organism and BBX32 (U.S. FDA, 1992). The food and feed products containing MON 87712 or derived from MON 87712 are as safe as soybean currently on the market for human and animal consumption.

V.F. BBX32 Protein Characterization and Safety Conclusion

[3] Buffer to tissue ratio includes the dilutions factor. For forage and seed the buffer to tissue ratio was 20 and 40, with dilution factors of 2 and 4, respectively.

BBX32 is a transcriptional accessory protein that influences the expression of some diurnally regulated genes. BBX32 is derived from *Arabidopsis thaliana*, a member of the *Brassicaceae* family. BBX32 is a B-box-containing protein that has homologs in food plants with a history of safe use. BBX32 was produced in and isolated from *E. coli* and was subsequently used for the described safety studies. Expression studies using western blot analysis demonstrated that BBX32 was detected in leaf and root of MON 87712, and was expressed at levels below the limit of detection in harvested seed and forage, the other tissues assayed. The maximum expression level detected in leaf was 16 ng/g fwt, and in root the maximum expression level was 1.5 ng/g fwt; the limits of detection for forage and harvested seed gave expression values of 1 ng/g fwt and 10 ng/g fwt respectively, representing a low percentage of the total protein in soybean. Bioinformatics analysis determined that BBX32 does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins. BBX32 was rapidly digested in *in vitro* assays using simulated gastric and intestinal fluids and did not show any adverse effects when administered to mice via oral gavage at levels that resulted in large margins of exposure (MOE).

The protein safety data presented herein support the conclusion that food and feed products containing MON 87712 or derived from MON 87712 are as safe as soybean currently on the market for human and animal consumption.

VI. COMPOSITIONAL ASSESSMENT OF MON 87712

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process (Codex Alimentarius, 2009) in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop is compared to the appropriate conventional counterpart that has a history of safe use. Compositional assessments are performed using the principles and analytes outlined in the OECD consensus documents for soybean composition (OECD, 2001).

A recent review of compositional assessments conducted according to OECD guidelines that encompassed a total of seven biotechnology-derived crop varieties, nine countries, and eleven growing seasons concluded that incorporation of biotechnology-derived agronomic traits has had little impact on natural variation in crop composition; most compositional variation is attributable to growing region, agronomic practices and genetic background (Harrigan et al., 2010). Numerous scientific publications have further documented the extensive variability in the concentrations of crop nutrients and anti-nutrients that reflect the influence of environmental and genetic factors as well as extensive conventional breeding efforts to improve nutrition, agronomics and yield (Harrigan et al., 2010; Reynolds et al., 2005). Compositional equivalence between biotechnology-derived and conventional crops supports an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 1998). The OECD consensus documents emphasize quantitative measurements of essential nutrients and known anti-nutrients. This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and nutritional concerns. Levels of the components in harvested seed and forage of the biotechnology-derived crop are compared to: 1) corresponding levels in a conventional comparator, grown concurrently, under identical field conditions, and 2) natural ranges generated from an evaluation of commercial reference varieties grown concurrently and from data published in the scientific literature. The latter comparison places any potential differences between the assessed crop and its comparator in the context of the well-documented variation in the concentrations of crop nutrients and anti-nutrients.

VI.A. Compositional Equivalence of MON 87712 Seed and Forage to Conventional Soybean

Harvested seed and forage samples were collected from MON 87712 and the parental conventional soybean control A3525 grown in a 2009 U.S. field production. Three different conventional commercial reference varieties were included at each site of the field , with a total of sixteen unique references included in this study, production to provide data on natural variability of each compositional component analyzed. The field production was conducted at eight geographically diverse sites: Jackson County, Arkansas (ARNE); Parke County, Indiana (INRC); Clinton County, Illinois (ILCY); Madison County, Illinois (ILHI); Stark County, Illinois (ILWY); Boone County, Indiana (INSH); Pawnee County, Kansas (KSLA); and York County, Nebraska (NEYO). All soybean plants including MON 87712, the conventional control A3525, and conventional

commercial reference varieties were treated with maintenance pesticides as necessary throughout the growing season.

Compositional analyses were conducted to assess whether levels of key nutrients and anti-nutrients in MON 87712 were equivalent to levels in the conventional control A3525 and the composition of the conventional commercial reference varieties. A description of nutrients and anti-nutrients present in soybean is provided in the OECD consensus document on compositional considerations for soybean (OECD, 2001). Nutrients assessed in this study included proximates, fiber, amino acids, fatty acids, and vitamin E in harvested seed, and proximates and fiber in forage. The anti-nutrients assessed in harvested seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitors, and isoflavones.

In all, 63 different analytical components were measured (seven in forage and 56 in harvested seed). Due to statistical constraints, in order to proceed with the statistical analysis of any component in this study, at least 50% of the observed values for an analyte needed to be greater than the assay limit of quantitation (LOQ). Of the 63 components measured, 14 had more than 50% of the observations below the assay LOQ and thus were excluded from statistical analysis. Therefore, 49 components were statistically assessed using a mixed-model analysis of variance method. Values for all assessed components were reported on a dry weight basis with the exception of moisture, which was reported as % fresh weight and fatty acids (FA), which were reported as % of total FA.

For MON 87712, nine sets of statistical comparisons to the conventional control A3525 were conducted. One comparison was based on compositional data combined across all eight field sites (the combined-site analysis) and eight comparisons were conducted on data from each of the eight individual field sites. Statistical differences were identified at a 5% level of significance (α=0.05). Compositional data from the conventional commercial reference varieties were combined across all sites and used to calculate a 99% tolerance interval for each component to define the natural variability in soybean varieties that have a history of safe consumption and that were grown concurrently with MON 87712 and the conventional control A3525 in the same trial.

For the combined-site analysis, significant differences in nutrient and anti-nutrient components were evaluated further using considerations relevant to the safety and nutritional quality of MON 87712 when compared to the conventional control A3525. The evaluation included: 1) the relative magnitude of the significant difference in the mean values (mean difference as % of the conventional control A3525) of nutrient and anti-nutrient components of MON 87712 compared to the conventional control A3525, relative to natural variability 2) whether the MON 87712 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of conventional commercial reference varieties grown concurrently in the same trial, 3) analyses of the reproducibility of the significant combined-site component differences at individual sites, and 4) assessing the combined-site significant differences and reproducible individual site significant differences within the context of natural variability of commercial soybean composition published in the scientific

literature and/or in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2010).

This evaluation provides a comprehensive comparative assessment of the levels of key nutrients and anti-nutrients in harvested seed, and of key nutrients in forage of MON 87712 and the conventional control A3525 discussed in the context of natural variability in commercial conventional soybean. Results of the comparison indicate that the composition of the harvested seed and forage of MON 87712 is equivalent to that of the conventional control A3525, and within the natural variability of conventional commercial reference varieties.

VI.A.1. Nutrient Levels in Harvested Soybean Seed

In the combined-site analysis of harvested soybean seed, 22 of the 34 nutrient component comparisons were not significantly different between MON 87712 and the conventional control A3525.

The following components showed no significant differences in mean values between MON 87712 and the conventional control: four proximates (ash, carbohydrates by calculation, moisture, and total fat), two types of fiber (ADF and NDF), nine amino acids (histidine, leucine, lysine, methionine, proline, serine, threonine, tryptophan, and tyrosine), six fatty acids (18:1 oleic acid, 18:2 linoleic acid, 18:3 linolenic acid, and 20:0 arachidic acid, 20:1 eicosenoic acid, and 22:0 behenic acid), and vitamin E (Table VI-2).

The components that showed significant differences in mean values between MON 87712 and the conventional control A3525 were: one proximate (protein), nine amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, isoleucine, phenylalanine, and valine), and two fatty acids (16:0 palmitic acid and 18:0 stearic acid) (Table VI-1 and VI-2).

The significant differences in nutrients were further evaluated using considerations relevant to the safety and nutritional quality of MON 87712 when compared to the conventional control A3525:

1) All nutrient component significant differences observed in the combined-site statistical analysis, whether reflecting increased or decreased mean values for MON 87712 with respect to the conventional control A3525, had small relative magnitudes when compared to natural variability, and therefore these differences were not meaningful from a food/feed nutrition or safety perspective. The relative magnitude of the difference for protein was 1.09%, and relative magnitudes of the differences ranged from 1.22 to 3.07% for amino acids; and from 1.42 to 3.04% for fatty acids.

2) Mean values for all significantly different nutrient components from the combined-site analysis of MON 87712 were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial, and were, therefore, within the range of natural variability in commercial soybean varieties with a history of safe consumption.

3) Assessment of the reproducibility of the combined-site significant differences at the eight individual sites showed significant differences for: protein at one site; alanine, aspartic acid, isoleucine, and valine at two sites; arginine, glutamic acid, glycine, phenylalanine, and 18:0 stearic acid at three sites; and 16:0 palmitic acid at four sites. Cystine was not significantly different at any of the individual sites. Individual site mean values of MON 87712 for all nutrient components with significant differences were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial and were, therefore, within the range of natural variability in conventional commercial soybeans with a history of safe consumption.

4) All combined-site mean values of MON 87712 for all nutrient components were within the context of the natural variability of commercial soybean composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2010).

Overall, observed significant differences in protein and amino acid values between MON 87712 and the conventional control A3525 were not considered to be meaningful from a food and feed safety or nutritional perspective. Ten of the 12 significant differences in the harvested seed nutrient levels between MON 87712 and the conventional control A3525 observed in the combined-site data analysis were attributable to small differences in protein and nine amino acids (all expressed as % dw). The relative magnitude of the difference between the mean protein values for MON 87712 and the conventional control A3525 was small (an increase of 1.09% in the combined-site analysis for MON 87712), and the mean protein values for MON 87712 were significantly different from the conventional control A3525 at only one of the eight individual sites. Correspondingly, the relative magnitudes of the significant differences for all amino acid values were small, and significant differences in the combined-site data analysis were not consistently observed as significant differences at all individual sites. Eight of the nine amino acids observed to be different in the combined-site analysis were increased (1.22 – 2.26%) consistent with an increase in protein, one amino acid (cystine) was decreased (3.07%) relative to the conventional control A3525 and, as with protein, significant differences between MON 87712 and the conventional control A3525 were not consistently observed at all sites. Cystine, the amino acid observed to be significantly decreased (3.07%) in MON 87712 when compared to the conventional control A3525 in the combined-site analysis, was not significantly different at any of the individual sites. Thus, observed significant differences in protein and amino acid values between MON 87712 and the conventional control A3525 were not considered to be meaningful from a food and feed safety or nutritional perspective because they were not consistently reproduced at the individual sites, and the mean MON 87712 values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial.

Overall, observed significant differences in fatty acid values between MON 87712 and the conventional control A3525 were not considered to be meaningful from a food and feed safety and nutritional perspective. Two of the combined-site significant differences between MON 87712 and the conventional control A3525 were attributable to fatty acids

(expressed as % total FA). The relative magnitudes of the significant differences between the mean fatty acid values for MON 87712 and the conventional control A3525 in the combined-site analysis were low with a decrease of 1.42% for 16:0 palmitic acid, and a decrease of 3.04% for 18:0 stearic acid. Neither of these fatty acids were significantly different between MON 87712 and the conventional control A3525 at more than four of the eight individual sites. Thus, observed significant differences in fatty acid values between MON 87712 and the conventional control A3525 were not considered to be meaningful from a food and feed safety and nutritional perspective because they were not consistently reproduced at the individual sites, and the mean MON 87712 values were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial.

In summary, statistical analyses found no consistent significant differences between the levels of nutrient components in harvested seed from MON 87712 and the conventional control A3525. These findings supported the conclusion of compositional equivalence of MON 87712 to conventional soybean.

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

In the combined-site analysis, no significant differences were observed for any of the eight anti-nutrient component comparisons (lectin, phytic acid, raffinose, stachyose, trypsin inhibitor, daidzein, genistein, and glycitein) between MON 87712 and the conventional control A3525. Thus, the evaluation of anti-nutrient components in harvested seed supported the conclusion that MON 87712 is compositionally equivalent to conventional soybean.

VI.A.3. Nutrient Levels in Soybean Forage

In the combined-site analysis of forage, no significant differences were observed between MON 87712 and the conventional control A3525 for six of the seven nutrients. No significant differences were observed for ash, carbohydrates, moisture, protein, ADF or NDF (Table VI-4). One significant difference for total fat was observed between MON 87712 and the conventional control. The relative magnitude of the difference, with respect to the conventional control, was only 11.34%, and the value was within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial. Nutritionally, only ruminants would consume soybean forage and the major role of fat would be as a source of energy. If the gross energy of the soybean forage is calculated using Atwater Values (Merrill and Watt, 1973) and the composition observed in the combined-site analysis, it would result in only a 1% relative difference. This difference is not meaningful from a nutritional perspective and, in addition any diet would be balanced using other feedstuffs (NRC, 2001).

The single nutrient component significant difference between MON 87712 and the conventional control A3525 observed in the combined-site analysis was evaluated for reproducibility at the individual sites. Significant differences were observed between MON 87712 and the conventional control A3525 in total fat values at only two of the eight individual sites. The relative magnitudes of the significant differences were 12.96

and 39.10% lower, but all mean values at the individual sites that were significantly different were within the 99% tolerance interval established by conventional commercial reference varieties grown concurrently in the same trial.

Thus, an evaluation of nutrient components in forage supported the conclusion of compositional equivalence of MON 87712 to conventional soybean.

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in Combined-Site Analysis						
Seed Proximate (% dwt)						
Protein	40.93	40.49	1.09	0.005	38.87 - 42.79	35.06, 43.58
Seed Amino Acid (% dwt)						
Alanine	1.77	1.75	1.30	0.014	1.69 - 1.85	1.54, 1.88
Arginine	3.14	3.07	2.26	0.027	2.93 - 3.38	2.51, 3.33
Aspartic Acid	4.73	4.67	1.30	0.029	4.44 - 4.92	4.04, 5.07
Cystine	0.59	0.61	-3.07	<0.001	0.55 - 0.63	0.51, 0.67
Glutamic Acid	7.60	7.49	1.49	0.038	7.18 - 7.96	6.28, 8.18
Glycine	1.79	1.77	1.22	0.020	1.70 - 1.85	1.52, 1.90

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in Combined-Site Analysis						
Seed Amino Acid (% dwt)						
Isoleucine	1.90	1.87	1.57	0.032	1.78 - 1.98	1.62, 2.03
Phenylalanine	2.15	2.12	1.59	0.016	1.98 - 2.26	1.81, 2.33
Valine	1.99	1.95	2.02	0.013	1.88 - 2.08	1.71, 2.13
Seed Fatty Acid (% Total FA)						
16:0 Palmitic	11.48	11.64	-1.42	0.002	10.78 - 11.94	7.76, 13.14
18:0 Stearic	4.05	4.17	-3.04	0.004	3.54 - 4.72	3.06, 5.10
Forage Proximate (% dwt)						
Total Fat	6.23	7.03	-11.34	0.005	3.41 - 8.62	0.54, 13.11

Table VI-1 . Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Antinutrient						
Raffinose (% dwt) Site ARNE	0.82	0.73	13.42	<0.001	0.77 - 0.86	0.39, 1.01
Raffinose (% dwt) Site ILCY	0.78	0.70	10.76	0.006	0.72 - 0.83	0.39, 1.01
Raffinose (% dwt) Site ILHI	0.78	0.90	-13.27	0.002	0.76 - 0.80	0.39, 1.01
Raffinose (% dwt) Site KSLA	0.87	0.82	5.25	0.011	0.86 - 0.88	0.39, 1.01
Raffinose (% dwt) Site NEYO	0.66	0.71	-6.57	0.023	0.65 - 0.66	0.39, 1.01
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site ARNE	11.69	12.01	-2.66	0.001	11.54 - 11.80	7.76, 13.14
16:0 Palmitic Site ILHI	11.37	11.55	-1.56	0.002	11.30 - 11.49	7.76, 13.14

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Site KSLA	11.89	12.12	-1.88	0.002	11.84 - 11.94	7.76, 13.14
16:0 Palmitic Site NEYO	11.63	11.89	-2.17	0.012	11.59 - 11.71	7.76, 13.14
Seed Amino Acid (% dwt)						
Arginine Site ILCY	3.28	3.11	5.76	0.007	3.23 - 3.38	2.51, 3.33
Arginine Site KSLA	3.05	2.90	5.22	0.001	3.03 - 3.08	2.51, 3.33
Arginine Site NEYO	3.19	3.02	5.51	<0.001	3.16 - 3.24	2.51, 3.33
Glutamic Acid Site ILCY	7.78	7.57	2.76	0.042	7.63 - 7.87	6.28, 8.18
Glutamic Acid Site KSLA	7.24	7.03	2.96	0.020	7.19 - 7.36	6.28, 8.18

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean2	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Amino Acid (% dwt)						
Glutamic Acid Site NEYO	7.68	7.41	3.66	0.007	7.60 - 7.76	6.28, 8.18
Glycine Site ILCY	1.84	1.79	2.44	0.035	1.80 - 1.85	1.52, 1.90
Glycine Site KSLA	1.72	1.69	1.86	0.034	1.70 - 1.75	1.52, 1.90
Glycine Site NEYO	1.79	1.74	2.99	0.001	1.78 - 1.80	1.52, 1.90
Leucine Site ILCY	3.23	3.15	2.30	0.041	3.18 - 3.27	2.71, 3.38
Leucine Site KSLA	3.02	2.96	2.12	0.043	3.00 - 3.05	2.71, 3.38
Leucine Site NEYO	3.18	3.09	3.20	0.002	3.17 - 3.21	2.71, 3.38
Phenylalanine Site ILCY	2.22	2.15	3.24	0.022	2.17 - 2.26	1.81, 2.33

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87771 2 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Amino Acid (% dwt)						
Phenylalanine Site KSLA	2.04	1.98	2.80	0.049	1.98 - 2.07	1.81, 2.33
Phenylalanine Site NEYO	2.17	2.08	4.58	0.003	2.15 - 2.19	1.81, 2.33
Seed Fatty Acid (% Total FA)						
18:0 Stearic Site ARNE	3.85	4.16	-7.37	<0.001	3.78 - 3.89	3.06, 5.10
18:0 Stearic Site ILCY	3.77	4.00	-5.54	0.001	3.71 - 3.91	3.06, 5.10
18:0 Stearic Site INRC	3.67	3.96	-7.25	0.001	3.54 - 3.84	3.06, 5.10
18:2 Linoleic Site ARNE	51.50	53.62	-3.96	0.003	50.26 - 52.27	50.14, 57.81
18:2 Linoleic Site ILCY	54.93	54.34	1.09	0.045	54.84 - 55.03	50.14, 57.81

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
18:2 Linoleic Site INSH	52.72	53.25	-0.98	0.034	52.33 - 53.25	50.14, 57.81
Seed Amino Acid (% dwt)						
Alanine Site KSLA	1.70	1.67	2.20	0.031	1.69 - 1.72	1.54, 1.88
Alanine Site NEYO	1.78	1.73	3.01	0.007	1.76 - 1.80	1.54, 1.88
Aspartic Acid Site KSLA	4.56	4.45	2.30	0.037	4.51 - 4.62	4.04, 5.07
Aspartic Acid Site NEYO	4.77	4.64	2.75	0.005	4.74 - 4.81	4.04, 5.07
Histidine Site KSLA	1.04	1.01	3.05	0.021	1.03 - 1.06	0.91, 1.17
Histidine Site NEYO	1.09	1.06	2.34	0.001	1.08 - 1.09	0.91, 1.17

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Amino Acid (% dwt)						
Isoleucine Site ILCY	1.94	1.87	3.82	0.029	1.87 - 1.97	1.62, 2.03
Isoleucine Site NEYO	1.92	1.85	3.59	0.009	1.90 - 1.93	1.62, 2.03
Valine Site ILCY	2.05	1.96	4.20	0.040	1.95 - 2.08	1.71, 2.13
Valine Site NEYO	2.03	1.95	4.29	0.013	2.00 - 2.05	1.71, 2.13
Seed Fatty Acid (% Total FA)						
18:1 Oleic Site ARNE	24.18	21.03	14.99	0.001	23.08 - 25.71	17.37, 26.86
18:1 Oleic Site INSH	22.26	21.25	4.76	0.001	22.07 - 22.50	17.37, 26.86
20:0 Arachidic Site ARNE	0.29	0.32	-9.78	0.040	0.26 - 0.30	0.22, 0.39

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Seed Fatty Acid (% Total FA)						
20:0 Arachidic Site KSLA	0.30	0.26	12.23	0.006	0.29 - 0.30	0.22, 0.39
Seed Vitamin (mg/100g dwt)						
Vitamin E Site ARNE	1.78	2.16	-17.89	0.023	1.58 - 1.88	0.10, 2.85
Vitamin E Site NEYO	0.96	1.13	-15.03	0.027	0.94 - 0.99	0.10, 2.85
Seed Secondary Metabolite (% dwt)						
Stachyose Site ARNE	4.13	3.80	8.77	0.021	3.94 - 4.33	2.45, 5.34
Stachyose Site NEYO	4.27	4.11	3.89	0.009	4.24 - 4.29	2.45, 5.34
Forage Proximate (% dwt)						
Moisture (% fwt) Site ARNE	75.53	72.63	3.99	0.004	74.60 - 77.50	65.61, 80.67

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in More than One Individual Site						
Forage Proximate (% dwt)						
Moisture (% fwt) Site ILCY	72.85	74.70	-2.48	0.006	71.80 - 74.50	65.61, 80.67
Total Fat Site ILHI	8.15	9.36	-12.96	0.008	7.44 - 8.62	0.54, 13.11
Total Fat Site KSLA	3.74	6.14	-39.10	0.001	3.41 - 4.03	0.54, 13.11
Statistical Differences Observed in One Site						
Seed Proximate (% dwt)						
Carbohydrates Site ILWY	40.46	39.22	3.17	0.020	39.98 - 41.50	32.36, 41.63
Moisture (% fwt) Site INRC	8.04	7.45	7.86	0.021	7.91 - 8.11	5.41, 10.36
Protein Site NEYO	41.05	40.14	2.27	<0.001	40.85 - 41.36	35.06, 43.58
Total Fat Site ILWY	14.35	15.35	-6.52	0.039	13.91 - 14.60	13.15, 23.90

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in One Site						
Seed Fiber (% dwt)						
Acid Detergent Fiber Site ILCY	15.30	13.46	13.69	0.004	13.99 - 16.40	9.99, 22.21
Neutral Detergent Fiber Site ILCY	16.42	14.58	12.65	0.014	15.64 - 17.27	11.03, 23.27
Seed Amino Acid (% dwt)						
Lysine Site NEYO	2.68	2.62	2.20	0.004	2.66 - 2.68	2.33, 2.81
Proline Site NEYO	2.02	1.97	2.57	0.046	2.00 - 2.04	1.70, 2.13
Serine Site NEYO	2.19	2.12	3.47	0.044	2.17 - 2.24	1.86, 2.33
Tyrosine Site KSLA	1.45	1.40	3.39	0.035	1.41 - 1.48	1.28, 1.57
Seed Fatty Acid (% Total FA)						
18:3 Linolenic Site ARNE	8.06	8.39	-3.86	0.031	7.87 - 8.29	5.60, 11.61

Table VI-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	MON 87712 Mean[2]	Control[3] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[4]
Statistical Differences Observed in One Site						
Seed Fatty Acid (% Total FA)						
22:0 Behenic Site KSLA	0.29	0.23	22.35	0.017	0.28 - 0.30	0.18, 0.43
Seed Antinutrient						
Trypsin Inhibitor (TIU/mg dwt) Site ARNE	28.47	36.40	-21.78	0.029	24.54 - 34.92	20.97, 50.01
Seed Isoflavone (µg/g dwt)						
Glycitein Site ILWY	117.88	96.68	21.92	0.005	112.20 - 122.62	8.13, 299.67

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid; TIU = Trypsin Inhibitor Units.
[2]Mean = least-square mean.
[3]Control refers to the conventional control, A3525
[4]With 95% confidence,the interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table VI-2. Summary of Combined-site Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.99 (0.11) (4.48 - 6.36)	5.07 (0.11) (4.46 - 5.71)	-0.081 (0.069) (-0.78 - 1.42)	-0.22, 0.055	0.238	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	37.83 (0.57) (34.64 - 41.50)	37.96 (0.57) (34.72 - 40.86)	-0.14 (0.25) (-3.07 - 2.47)	-0.63, 0.35	0.582	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.01 (0.20) (5.72 - 9.13)	7.89 (0.20) (6.44 - 10.30)	0.12 (0.13) (-1.69 - 1.84)	-0.13, 0.38	0.345	5.41, 10.36 (5.43 - 9.86)
Protein	40.93 (0.35) (38.87 - 42.79)	40.49 (0.35) (37.73 - 42.14)	0.44 (0.15) (-1.65 - 2.56)	0.14, 0.75	0.005	35.06, 43.58 (35.11 - 42.16)
Total Fat	16.27 (0.44) (13.91 - 18.78)	16.48 (0.44) (13.91 - 19.05)	-0.21 (0.18) (-2.38 - 1.47)	-0.58, 0.15	0.250	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	15.54 (0.50) (12.94 - 19.11)	15.59 (0.49) (12.41 - 20.81)	-0.048 (0.45) (-4.82 - 2.81)	-1.01, 0.91	0.916	9.99, 22.21 (11.74 - 22.13)

Table VI-2. Summary of Combined-site Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	16.54 (0.42) (13.68 - 20.74)	16.76 (0.42) (13.40 - 20.37)	-0.23 (0.40) (-2.65 - 2.76)	-1.09, 0.63	0.579	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.77 (0.014) (1.69 - 1.85)	1.75 (0.014) (1.65 - 1.83)	0.023 (0.0081) (-0.055 - 0.073)	0.0053, 0.040	0.014	1.54, 1.88 (1.58 - 1.84)
Arginine	3.14 (0.036) (2.93 - 3.38)	3.07 (0.036) (2.85 - 3.31)	0.069 (0.028) (-0.20 - 0.23)	0.0089, 0.13	0.027	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.73 (0.043) (4.44 - 4.92)	4.67 (0.043) (4.36 - 4.89)	0.061 (0.025) (-0.16 - 0.24)	0.0069, 0.11	0.029	4.04, 5.07 (4.06 - 4.89)
Cystine	0.59 (0.0082) (0.55 - 0.63)	0.61 (0.0082) (0.56 - 0.66)	-0.019 (0.0043) (-0.072 - 0.030)	-0.027, -0.010	<0.001	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.60 (0.081) (7.18 - 7.96)	7.49 (0.080) (6.86 - 7.97)	0.11 (0.049) (-0.37 - 0.38)	0.0068, 0.22	0.038	6.28, 8.18 (6.40 - 7.94)

Table VI-2 . Summary of Combined-site Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.79 (0.015) (1.70 - 1.85)	1.77 (0.015) (1.66 - 1.84)	0.021 (0.0082) (-0.041 - 0.081)	0.0038, 0.039	0.020	1.52, 1.90 (1.54 - 1.85)
Histidine	1.07 (0.0080) (1.03 - 1.12)	1.06 (0.0079) (1.00 - 1.12)	0.0089 (0.0057) (-0.046 - 0.051)	-0.0033, 0.021	0.140	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.90 (0.019) (1.78 - 1.98)	1.87 (0.019) (1.70 - 1.97)	0.029 (0.012) (-0.073 - 0.13)	0.0028, 0.056	0.032	1.62, 2.03 (1.60 - 2.00)
Leucine	3.15 (0.029) (3.00 - 3.30)	3.11 (0.029) (2.90 - 3.26)	0.035 (0.017) (-0.12 - 0.13)	-0.00077, 0.071	0.054	2.71, 3.38 (2.77 - 3.29)
Lysine	2.66 (0.019) (2.54 - 2.74)	2.64 (0.019) (2.49 - 2.75)	0.013 (0.011) (-0.11 - 0.074)	-0.011, 0.038	0.263	2.33, 2.81 (2.36 - 2.74)
Methionine	0.57 (0.0037) (0.52 - 0.60)	0.57 (0.0036) (0.53 - 0.60)	-0.0034 (0.0039) (-0.054 - 0.036)	-0.012, 0.0050	0.403	0.51, 0.59 (0.51 - 0.60)

Table VI-2. Summary of Combined-site Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.15 (0.023) (1.98 - 2.26)	2.12 (0.023) (1.95 - 2.24)	0.034 (0.012) (-0.077 - 0.15)	0.0071, 0.060	0.016	1.81, 2.33 (1.81 - 2.25)
Proline	2.00 (0.018) (1.83 - 2.14)	1.99 (0.018) (1.85 - 2.11)	0.0060 (0.014) (-0.20 - 0.14)	-0.022, 0.034	0.667	1.70, 2.13 (1.69 - 2.09)
Serine	2.16 (0.019) (2.01 - 2.25)	2.15 (0.019) (2.01 - 2.34)	0.0080 (0.015) (-0.16 - 0.14)	-0.023, 0.039	0.593	1.86, 2.33 (1.90 - 2.30)
Threonine	1.59 (0.011) (1.51 - 1.66)	1.58 (0.011) (1.50 - 1.66)	0.015 (0.0098) (-0.089 - 0.13)	-0.0056, 0.036	0.137	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.44 (0.0052) (0.36 - 0.49)	0.45 (0.0051) (0.38 - 0.49)	-0.0074 (0.0062) (-0.094 - 0.10)	-0.021, 0.0060	0.256	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.48 (0.014) (1.40 - 1.57)	1.46 (0.014) (1.33 - 1.56)	0.013 (0.012) (-0.095 - 0.18)	-0.010, 0.037	0.267	1.28, 1.57 (1.28 - 1.55)

Table VI-2. Summary of Combined-site Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	1.99 (0.017) (1.88 - 2.08)	1.95 (0.017) (1.78 - 2.07)	0.039 (0.014) (-0.072 - 0.15)	0.0095, 0.069	0.013	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.48 (0.13) (10.78 - 11.94)	11.64 (0.13) (11.13 - 12.23)	-0.16 (0.045) (-0.44 - 0.091)	-0.26, -0.067	0.002	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	4.05 (0.099) (3.54 - 4.72)	4.17 (0.099) (3.78 - 4.65)	-0.13 (0.037) (-0.37 - 0.25)	-0.21, -0.046	0.004	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	20.82 (0.46) (18.72 - 25.71)	20.21 (0.46) (18.72 - 21.54)	0.61 (0.33) (-1.57 - 4.66)	-0.093, 1.31	0.083	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	54.01 (0.32) (50.26 - 55.60)	54.18 (0.32) (53.21 - 55.02)	-0.17 (0.25) (-3.63 - 1.50)	-0.71, 0.37	0.514	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	8.95 (0.27) (7.46 - 10.22)	9.07 (0.27) (7.77 - 10.31)	-0.12 (0.057) (-0.68 - 0.52)	-0.24, 0.0016	0.052	5.60, 11.61 (5.89 - 10.16)

Table VI-2. Summary of Combined-site Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.29 (0.0072) (0.25 - 0.34)	0.30 (0.0072) (0.25 - 0.34)	-0.010 (0.0060) (-0.077 - 0.049)	-0.023, 0.0028	0.117	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.14 (0.012) (0.075 - 0.19)	0.14 (0.012) (0.073 - 0.19)	-0.0080 (0.0057) (-0.082 - 0.071)	-0.019, 0.0033	0.163	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.26 (0.0099) (0.18 - 0.33)	0.28 (0.0098) (0.18 - 0.32)	-0.014 (0.012) (-0.11 - 0.096)	-0.039, 0.011	0.258	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	1.24 (0.15) (0.81 - 2.05)	1.37 (0.15) (0.79 - 2.23)	-0.13 (0.064) (-0.76 - 0.44)	-0.27, 0.0025	0.053	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the conventional control, A3525
[5]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial substances. Negative limits set were to zero.

Table VI-3. Summary of Combined-site Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	2.06 (0.14) (1.10 - 3.41)	2.22 (0.13) (0.58 - 4.15)	-0.17 (0.18) (-1.84 - 1.63)	-0.54, 0.21	0.363	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	1.18 (0.077) (0.65 - 1.81)	1.20 (0.077) (0.86 - 1.77)	-0.029 (0.043) (-0.58 - 0.42)	-0.12, 0.063	0.513	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.73 (0.030) (0.54 - 0.88)	0.72 (0.030) (0.52 - 0.93)	0.0095 (0.022) (-0.17 - 0.14)	-0.038, 0.057	0.674	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.28 (0.081) (3.77 - 4.76)	4.19 (0.081) (3.52 - 4.88)	0.092 (0.060) (-0.41 - 0.90)	-0.037, 0.22	0.147	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	32.94 (1.09) (24.54 - 40.86)	34.14 (1.07) (20.34 - 54.88)	-1.20 (1.53) (-23.03 - 14.32)	-4.23, 1.84	0.435	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	1089.62 (85.86) (549.34 1609.37)	1054.04 (85.80) -(537.10 - 1533.78)	35.58 (30.54) (-187.49 - 384.84)	-29.88, 101.04	0.263	0, 1756.99 (138.15 - 1548.98)

Table VI-3. Summary of Combined-site Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	867.61 (58.80) (503.75 - 1204.29)	832.31 (58.73) (508.77 - 1175.72)	35.30 (26.14) (-219.09 - 391.63)	-20.80, 91.41	0.198	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	102.13 (4.02) (75.71 - 131.79)	100.86 (3.98) (77.28 - 162.85)	1.27 (5.47) (-46.46 - 33.87)	-10.44, 12.99	0.819	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the conventional control, A3525
[5]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table VI-4. Summary of Combined-site Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	6.56 (0.31) (4.55 - 8.75)	6.47 (0.31) (4.79 - 8.74)	0.089 (0.17) (-2.60 - 1.62)	-0.27, 0.45	0.606	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	65.10 (0.78) (59.41 - 70.88)	64.76 (0.78) (58.14 - 70.63)	0.33 (0.34) (-3.97 - 5.67)	-0.35, 1.02	0.332	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	72.80 (0.92) (67.60 - 77.50)	72.01 (0.92) (66.60 - 75.50)	0.79 (0.42) (-3.50 - 4.70)	-0.099, 1.68	0.077	65.61, 80.67 (64.50 - 79.80)
Protein	22.13 (0.51) (18.45 - 26.20)	21.75 (0.51) (17.88 - 26.12)	0.39 (0.22) (-2.05 - 3.29)	-0.058, 0.83	0.086	13.77, 26.51 (16.56 - 27.76)
Total Fat	6.23 (0.56) (3.41 - 8.62)	7.03 (0.56) (4.57 - 9.80)	-0.80 (0.25) (-3.15 - 1.00)	-1.32, -0.27	0.005	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	29.72 (0.93) (24.36 - 38.10)	29.05 (0.93) (24.46 - 35.21)	0.67 (0.62) (-5.70 - 11.81)	-0.67, 2.01	0.301	23.12, 38.15 (22.60 - 41.29)

Table VI-4. Summary of Combined-site Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	33.30 (1.16) (26.56 - 41.73)	32.58 (1.15) (24.92 - 41.41)	0.72 (0.77) (-6.68 - 8.04)	-0.94, 2.37	0.368	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the conventional control, A3525
[5]With 95% confidence, the interval contains 99% of the values expressed in the population of commercial substances. Negative limits were set to zero.

Table VI-5. Literature and ILSI Database Ranges for Components in Soybean Forage and Seed

Seed Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Seed Nutrients		
Proximates (% dwt)		
Ash	4.61 – 6.32[a]; 4.32 – 5.88[b]	3.89 – 6.99
Carbohydrates by calculation	32.75 – 40.98[a]; 29.88 – 43.48[b]	29.6 – 50.2
Moisture (% fwt)	6.24 – 12.10[a]; 5.44 – 11.70[b]	4.7 – 34.4
Protein	34.78 – 43.35[a]; 32.29 – 42.66[b]	33.19 – 45.48
Total Fat	14.40 – 20.91[a]; 15.10 – 23.56[b]; 15.5 – 24.7[4]	8.10 – 23.56
Fiber (% dwt)		
Acid Detergent Fiber	9.22 – 26.26[a]; 11.81 – 19.45[b]	7.81 – 18.61
Neutral Detergent Fiber	10.79 – 23.90[a]; 13.32 – 23.57[b]	8.53 – 21.25
Amino Acids (% dwt)		
Alanine	1.62 – 1.89[a]; 1.43 – 1.93[b]	1.51 – 2.10
Arginine	2.57 – 3.34[a]; 2.15 – 3.05[b]	2.29 – 3.40
Aspartic acid	4.16 – 5.02[a]; 4.01 – 5.72[b]	3.81 – 5.12
Cystine/Cysteine	0.52 – 0.69[a]; 0.41 – 0.71[b]	0.37 – 0.81
Glutamic acid	6.52 – 8.19[a]; 5.49 – 8.72[b]	5.84 – 8.20
Glycine	1.59 – 1.90[a]; 1.41 – 1.99[b]	1.46 – 2.00
Histidine	0.96 – 1.13[a]; 0.86 – 1.24[b]	0.88 – 1.18
Isoleucine	1.59 – 2.00[a]; 1.41 – 2.02[b]	1.54 – 2.08
Leucine	2.79 – 3.42[a]; 2.39 – 3.32[b]	2.59 – 3.62
Lysine	2.36 – 2.77[a]; 2.19 – 3.15[b]	2.29 – 2.84
Methionine	0.45 – 0.63[a]; 0.39 – 0.65[b]	0.43 – 0.68
Phenylalanine	1.82 – 2.29[a]; 1.62 – 2.44[b]	1.63 – 2.35
Proline	1.83 – 2.23[a]; 1.63 – 2.25[b]	1.69 – 2.28
Serine	1.95 – 2.42[a]; 1.51 – 2.30[b]	1.11 – 2.48
Threonine	1.44 – 1.71[a]; 1.23 – 1.74[b]	1.14 – 1.86
Tryptophan	0.30 – 0.48[a]; 0.41 – 0.56[b]	0.36 – 0.50
Tyrosine	1.27 – 1.53[a]; 0.74 – 1.31[b]	1.02 – 1.61
Valine	1.68 – 2.11[a]; 1.50 – 2.13[b]	1.60 – 2.20
Fatty Acids (% total FA)		
8:0 Caprylic	not available	0.148 – 0.148
10:0 Capric	0.15 – 0.27[b]	not available
12:0 Lauric	not available	0.082 – 0.132
14:0 Myristic	0.063 – 0.11[b]	0.071 – 0.238
14:1 Myristoleic	not available	0.121 – 0.125
15:0 Pentadecanoic	not available	not available
15:1 Pentadecenoic	not available	not available
16:0 Palmitic	9.80 – 12.63[b]	9.55 – 15.77
16:1 Palmitoleic	0.055 – 0.14[b]	0.086 – 0.194
17:0 Heptadecanoic	0.076 – 0.13[b]	0.085 – 0.146
17:1 Heptadecenoic	0.019 – 0.064[b]	0.073 – 0.087
18:0 Stearic	3.21 – 5.63[b]	2.70 – 5.88
18:1 Oleic	16.69 – 35.16[b]	14.3 – 32.2
18:2 Linoleic	44.17 – 57.72[b]	42.3 – 58.8

Table VI-5. Literature and ILSI Database Ranges for Components in Soybean Forage and Seed (continued)

Seed Tissue Components[1]	Literature Range[2]	ILSI Range[3]
18:3 Gamma Linolenic	not available	not available
18:3 Linolenic	4.27 – 9.90[b]	3.00 – 12.52
20:0 Arachidic	0.35 – 0.57[b]	0.163 – 0.482
20:1 Eicosenoic	0.13 – 0.30[b]	0.140 – 0.350
20:2 Eicosadienoic	0.016 – 0.071[b]	0.077 – 0.245
20:3 Eicosatrienoic	not available	not available
20:4 Arachidonic	not available	not available
22:0 Behenic	0.35 – 0.59[b]	0.277 – 0.595
Vitamins (mg/100g dwt)		
Vitamin E	1.29 – 4.80[a]; 1.12 – 8.08[b]	0.19 – 6.17
Seed Anti-Nutrients		
Lectin (H.U./mg fwt)	0.45 – 10.87[a]; 0.090 – 11.18[b]	0.09 – 8.46
Trypsin Inhibitor (TIU/mg dwt)	20.79 – 59.03[a]; 18.14 – 42.51[b]	18.00 – 108.00
Phytic Acid (% dwt)	0.41 – 1.92[a]; 0.81 – 2.66[b]	0.63 – 1.96
Raffinose (% dwt)	0.26 – 0.84[a]; 0.43 – 1.85[b]	0.21 – 0.66
Stachyose (% dwt)	1.53 – 3.04[a]; 1.97 – 6.65[b]	1.21 – 3.50
Isoflavones	**(µg/g dwt)**	**(mg/kg dwt)**
Daidzein	224.03 – 1571.91[a]; 198.95 – 1458.24[b]	60.0 – 2453.5
Genistein	338.24 – 1488.89[a]; 148.06 – 1095.57[b]	144.3 – 2837.2
Glycitein	52.72 – 298.57[a]; 32.42 – 255.94[b]	15.3 – 310.0

Forage Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Forage Nutrients		
Proximate (% dwt)		
Ash	5.28 – 9.24[a]; 4.77 – 8.54[b]	6.72 – 10.78
Carbohydrates by calculation	62.25 – 72.30[a]; 60.61 – 77.26[b]	59.8 – 74.7
Moisture (% fwt)	68.50 – 78.40[a]; 62.76 – 80.20[b]	73.5 – 81.6
Protein	16.48 – 24.29[a]; 12.68 – 23.76[b]	14.38 – 24.71
Total Fat	2.65 – 9.87[a]; 2.96 – 7.88[b]	1.30 – 5.13
Fiber (% dwt)		
Acid Detergent Fiber	23.86 – 50.89[a]; 25.49 – 47.33[b]	not available
Neutral Detergent Fiber	19.61 – 43.70[a]; 30.96 – 54.55[b]	not available

[1]fw=fresh weight; dwt=dry weight; H.U. = hemagglutinating unit; TIU = trypsin inhibitor unit.
[2]Literature range references; [a](Lundry et al., 2008); [b](Berman et al., 2009).
[3](ILSI, 2010).
[4](OECD, 2001).

VI.B. Compositional Assessment of MON 87712 Conclusion

Detailed comparisons were conducted on nutrient and anti-nutrient levels identified by the OECD as important to understanding the safety and nutrition of soybean (OECD, 2001) in MON 87712 to corresponding levels in the parental conventional soybean control A3525. These compositional comparisons were made by analyzing the seed and forage harvested from plants grown at each of eight field sites in the U.S. during the 2009 field season. The composition analysis, conducted in accordance with OECD guidelines, also included measurement of these OECD defined soybean nutrients and anti-nutrients in the conventional commercial soybean varieties, to provide data on natural variability of each compositional component analyzed.

For MON 87712 compared to the conventional control A3525, the combined-site analysis of both harvested seed and forage showed no statistically significant differences (α=0.05) between MON 87712 and the control for 36 (73.5%) of the 49 mean value comparisons. Of the significant differences observed, 12 were from the seed analysis, and one was from the forage analysis. No differences in mean values between MON 87712 and the conventional control A3525 were observed in the combined-site analysis for anti-nutrients. Seed nutrient component differences were observed for mean values for protein, amino acids (nine components), and fatty acids (two components), and the forage nutrient component difference was in total fat and were evaluated using considerations relevant to the safety and nutritional quality of MON 87712 when compared to the conventional control:

1) All nutrient component differences observed in the combined-site statistical analysis, whether reflecting increased or decreased MON 87712 mean values with respect to the conventional control A3525, had small relative magnitudes when compared to natural variability, and therefore were not meaningful from a food/feed nutrition or safety perspective. The relative magnitude of the significant difference for protein when MON 87712 was compared to A3525 was 1.09%, and relative magnitudes of the differences ranged from 1.22 to 3.07% for amino acids, from 1.42 to 3.04% for fatty acids, and a relative magnitude of the difference of 11.34% was observed for forage total fat.

2) Mean values for all significantly different nutrient components from the combined-site analysis of MON 87712 were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial, and were, therefore, within the range of natural variability of that component in commercial soybean varieties with a history of safe consumption.

3) Assessment of the reproducibility of the combined-site significant differences at the eight individual sites showed significant differences for: protein at one site; alanine, aspartic acid, isoleucine, and valine at two sites; arginine, glutamic acid, glycine, phenylalanine, and 18:0 stearic acid at three sites; and 16:0 palmitic acid at four sites. Cystine was not significantly different at any of the individual sites. Individual site mean values of MON 87712 for all nutrient components with

statistically significant differences were within the 99% tolerance interval established from the conventional commercial reference varieties grown concurrently in the same trial and were, therefore, within the range of natural variability of that component in commercial soybean with a history of safe consumption..

4) All combined-site mean values of MON 87712 for all nutrient components were within the context of the natural variability of commercial soybean composition as published in the scientific literature and/or available in the ILSI Crop Composition Database (ILSI, 2010).

In summary, MON 87712 mean component values observed to be significantly different ($\alpha<0.05$) from that of the parental conventional control A3525 were generally shown to have relative magnitudes of difference that were less than 12% (most were <3%) from the control and not reproducible across sites. For MON 87712 compared to the conventional control A3525, none of the 49 components assessed were observed to be significantly different at all eight individual sites. All MON 87712 mean component values were within the 99% tolerance interval established from the conventional commercial references varieties grown concurrently at the same field sites. Additionally, the combined-site component values were within the range of values reported in the scientific literature and/or in the ILSI Crop Composition Database. Based on these data, it is concluded that harvested soybean seed and soybean forage produced from MON 87712 are compositionally equivalent to that of the conventional soybean and that the presence of BBX32 in MON 87712 does not have a meaningful impact on the composition and therefore on the food and feed safety or nutritional quality of MON 87712 compared to conventional soybean. In addition, these data are meaningful from a plant pest risk perspective because they support the concept of familiarity that the USDA recognizes as an important underlying concept in risk assessment.

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides an assessment of the phenotypic, agronomic, and environmental interaction characteristics including plant-symbiont associations, and volunteer potential and persistence outside cultivation. These assessments were comparative between MON 87712 and the parental conventional control A3525, a conventional soybean variety that has background genetics similar to MON 87712 but does not possess the *BBX32* gene. The data support a determination that MON 87712 is not different from conventional soybean as a plant pest risk or have a significant environmental impact compared to conventional soybean. These conclusions are based on the results of multiple evaluations.

Phenotypic, agronomic, and environmental interaction characteristics of MON 87712 were evaluated in a comparative manner to assess plant pest potential. These assessments included evaluation of seed germination characteristics, plant growth and development characteristics, observations of plant responses to abiotic stress, plant-disease and plant-arthropod interactions, pollen characteristics, plant-symbiont interaction associations, and volunteer potential, and persitence outside of cultivation characteristics. Results from the phenotypic, agronomic, and environmental interactions assessment demonstrate that MON 87712 does not possess a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods; or c) characteristics that would confer a plant pest risk or a significant environmental impact compared to the conventional control A3525.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 87712, data were collected to evaluate specific aspects of altered plant pest potential. A detailed description of the regulated article phenotype is requested as part of the petition for determination of nonregulated status in 7 CFR § 340. 6 including differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived…" As part of the characterization of MON 87712, data were collected to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 87712. A subset of these data were included in an evaluation of specific characteristics related to altered plant pest potential and adverse environmental impact.

The MON 87712 plant characterization and assessment encompassed seven general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) seed retention on the plant and lodging; 5) plant response to abiotic stress and interactions with diseases and arthropods; 6) plant-symbiont interactions and 7) volunteer potential and persistence outside of cultivation characteristics. An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (OECD, 1993) and were comprised of a combination of field, greenhouse, and laboratory studies conducted by scientists who are familiar with the production and evaluation of soybean. In each of these assessments, MON 87712 was compared to an appropriate conventional control that had a genetic background similar to MON 87712 but did not possess the *BBX32* gene. In addition, multiple commercial reference varieties (see Appendices G-L and Tables G-1, H-1, and J-1) were included to provide a range of comparative values that are representative of existing commercial soybean varieties for each measured phenotypic, agronomic, and environmental interaction characteristic. Commercial reference varieties are developed through a process of selecting and breeding for various desirable soybean characteristics and can provide a range of natural variability for characteristics and context for interpreting experimental results.

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

Data category	Characteristics measured (associated section where discussed)	Evaluation timing (setting of evaluation)[1]	Evaluation description (measurement endpoints)
Seed germination, dormancy, and emergence	Normal germinated (VII.C.1)	Day 5 and 8 (20/30°C) (laboratory)	Percentage of seed producing seedlings exhibiting normal developmental characteristics
	Abnormal germinated (VII.C.1)	Day 8 (20/30°C) (laboratory)	Percentage of seed producing seedlings that could not be classified as normal germinated
	Germinated (VII.C.1)	Day 5, 8, and 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that had germinated normally or abnormally
	Dead (VII.C.1)	Day 5 and 8 (10, 20, 30, 10/20, 10/30, and 20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that had visibly deteriorated and become soft to the touch (also included non-viable hard and non-viable firm-swollen seed)
	Viable hard (VII.C.1)	Day 8 (20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that did not imbibe water and remained hard to the touch (viability determined by a tetrazolium test[2])
	Viable firm-swollen (VII.C.1)	Day 8 (20/30°C); Day 13 (10, 20, 30, 10/20 and 10/30°C) (laboratory)	Percentage of seed that imbibed water and were firm to the touch but did not germinate (viability determined by a tetrazolium test[2])
	Early stand count (VII.C.2.1)	V2 - V4 (Field)	Number of emerged plants in two rows, standardized to 20 ft rows
	Final stand count (VII.C.2.1)	Maturity, R8 (Field)	Number of plants in two rows, standardized to 20 ft rows
Vegetative growth	Seedling vigor (VII.C.2.1)	V2 - V4 (Field)	Rated on a 1-9 scale, where 1 = excellent and 9 = poor vigor
	Growth stage assessment (VII.C.2.1)	Every 2-3 weeks, V2-R8 (Field)	Average soybean plant growth stage per plot
	Flower color (VII.C.2.1)	Flowering, R2 (Field)	Color of flowers: purple, white, or mixed
	Plant pubescence (VII.C.2.1)	Maturity, R8 (Field)	Pubescence on plants in each plot categorized as hairy or hairless
	Plant height (VII.C.2.1)	Maturity, R8 (Field)	Distance (in) from the soil surface to the uppermost node on the main stem of five representative plants per plot
Reproductive development	Days to 50% flowering (VII.C.2.1)	Flowering, R1-R2 (Field)	Days after January 1 in year of planting until 50% of the marked plants in each plot were flowering
	Pollen viability (VII.C.3)	Flowering, R1-R2 (laboratory)	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen morphology (VII.C.3)	Flowering, R1-R2 (laboratory)	Diameter (µm) of viable pollen grains
	Days to 50% end of flowering (VII.C.2.1)	Flowering, end of (Field)	Days after January 1 in year of planting until 50% of the marked plants in each plot have stopped flowering
	Days to 50% senescence (VII.C.2.1)	R6-R7 (Field)	Days after January 1 in year of planting until 50% of the marked plants in each plot have reached 50% senescence

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

Data category	Characteristics measured (associated section where discussed)	Evaluation timing (setting of evaluation)[1]	Evaluation description (measurement endpoints)
Reproductive development	Days to physiological maturity (VII.C.2.1)	R8 (Field)	Days after January 1 in year of planting until 50% of the marked plants in each plot have reach physiological maturity
	Seed moisture (VII.C.2.1)	Harvest (Field)	Percent moisture content of harvested seed
	100 seed weight (VII.C.2.1)	Harvest (Field)	Mass (g) of 100 harvested seed
	Yield (VII.C.2.1)	Harvest (Field)	Bushels of harvested seed per acre, adjusted to 13% moisture
Seed retention and lodging	Lodging (VII.C.2.1)	Maturity, R8 (Field)	Rated on 1-9 scale, where 1 = completely erect and 9 = completely flat or lodged
	Pod shattering (VII.C.2.1)	Maturity, R8 (Field)	Rated on 1-9 scale, where 1 = no shattering and 9 = completely shattered
Plant-environment interactions	Plant response to abiotic stress (VII.C.2.2)	Four times per growing season (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Disease damage (VII.C.2.2)	Four times per growing season (Field)	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Arthropod-related damage (VII.C.2.2)	Four times per growing season (Field)	Damage assessed on upper four nodes of 10 representative plants per plot using arthropod-specific 0-5 rating scales of increasing severity
	Arthropod abundance (VII.C.2.2)	Four times per growing season (Field)	Quantitative assessment of pest and beneficial arthropods
Plant-symbiont interactions	Biomass (VII.C.4)	6 weeks after emergence (Greenhouse)	Nodule, root, and shoot dry weight (g/plant)
	Nodule number (VII.C.4)	6 weeks after emergence (Greenhouse)	Nodule number
	Total nitrogen (VII.C.4)	6 weeks after emergence (Greenhouse)	Shoot total nitrogen (% and g/plant)
Volunteer Potential characteristics	Plant Counts (VII.C.5)	Every 2 weeks (Field)	Number of emerged plants per plot
Persistence Outside of Cultivation characteristics	Stand Count (VII.C.6)	Approximately every 14 days (Field)	Number of emerged plants per plot
	Growth Stage Monitoring (VII.C.6)	Approximately every 14 days (Field)	Average soybean plant growth stage per plot
	Vigor Monitoring (VII.C.6)	Approximately every 14 days up to R1 growth stage (Field)	Rated on a 1-9 scale, where 1 = excellent and 9 = poor vigor
	Number of Plants Producting Pods (VII.C.6)	R8 (Field)	Total number of plants in a plot which produced pods
	Number of Seeds Produced (VII.C.6)	R8 (Field)	Total number of seeds produced in a plot
	Seed Weight (VII.C.6)	R8 (Field)	Total weight of all seeds produced in a plot (g)

[1]Soybean plant growth stages were determined using descriptions and guidelines outlined in Soybean Growth and Development (Pedersen, 2004).
[2]Viability of hard and firm-swollen seed were determined by a tetrazolium test (AOSA, 2000).

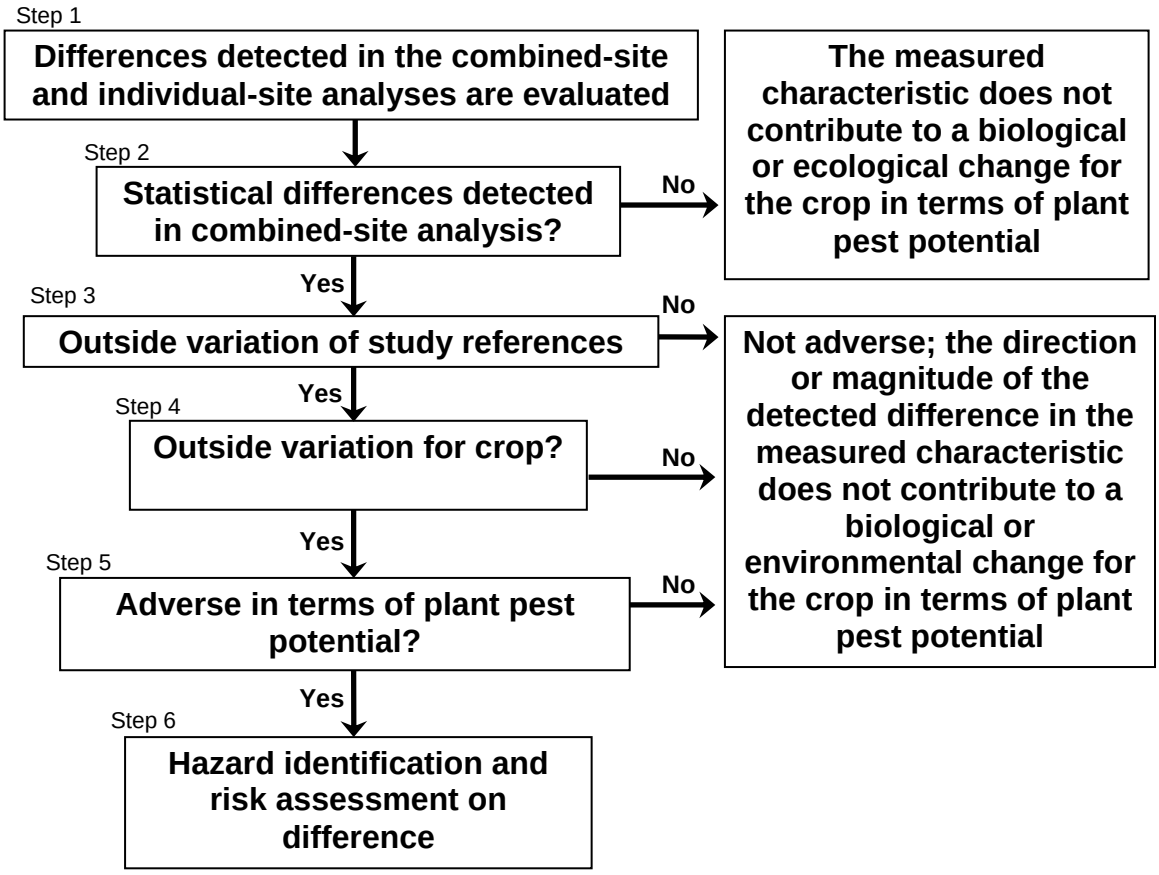
VII.B. Interpretation of Phenotypic and Environmental Interaction Data

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

Expert knowledge and experience with conventionally bred soybean was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for soybean. As such, MON 87712 was compared to the conventional control in the assessment of phenotypic, agronomic, and environmental interaction characteristics. An overview of the characteristics assessed is presented in Table VII-1. A subset of the data relating to well-understood weediness criteria (*e.g.*, seed dormancy, pre-harvest seed loss characteristics, and lodging) was used to assess whether there was an increase in weediness potential of MON 87712, an element of Animal and Plant Health Inspection Service's (APHIS) plant pest determination. Evaluation of environmental interaction characteristics (*e.g.*, plant abiotic stress, plant-disease, plant-arthropod, and plant-symbiont interactions) was also considered in the plant pest risk assessment. Based on all of the data collected, an assessment was made to determine if MON 87712 is likely to pose an increased plant pest risk compared to conventional soybean. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an unexpected plant response. No unexpected observations or issues were identified.

VII.B.1. Interpretation of Detected Differences Criteria

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



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

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

Steps 1 and 2 - Evaluate Detected Statistically Significant Differences

Data on each measured characteristic are statistically analyzed, where appropriate, within each individual-site and in a combined-site analysis, in which the data are pooled among sites. All statistically significant differences are evaluated and considered in the context of a change in plant pest potential. Differences detected in individual-site analyses that are not consistently observed across multiple environments in the combined-site analysis are considered not biologically meaningful in terms of plant pest potential and, therefore, are not further considered in subsequent steps. Any difference detected in the combined-site analysis is further assessed.

Step 3 - Evaluate Differences Relative to Commercial Reference Varieties Range

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

Step 4 - Evaluate Differences in the Context of the Crop

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

Step 5 - Plant Pest Potential

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

Step 6 - Conduct Risk Assessment on Identified Hazard

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

VII.C. Comparative Assessments of the Phenotypic, Agronomic, and Environmental Interaction Characteristics of MON 87712

This section provides the results of comparative assessments conducted in replicated laboratory, greenhouse, and/or multi-site field experiments to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 87712. The MON 87712 characteristics evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1), plant phenotypic, agronomic, and environmental interaction observations under field conditions (Section VII.C.2), pollen characteristics (Section VII.C.3), symbiont interactions (Section VII.C.4), volunteer potential characteristics (Section VII.C.5), and persistence outside of cultivation characteristics (Section VII.C.6). Additional details for each assessment are provided in Appendices G through L.

VII.C.1. Seed Dormancy and Germination Characteristics

USDA-APHIS considers the potential for weediness to constitute a plant pest risk (7 CFR § 340.6). Seed germination and dormancy mechanisms vary among species and their genetic basis tends to be complex. Seed dormancy (e.g., hard seed) is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; Lingenfelter and Hartwig, 2007). However, it is important to note that it is not uncommon to observe low levels of hard seed in soybean (Mullin and Xu, 2001; Potts et al., 1978). Standardized germination assays are available and routinely used to measure the germination characteristics of soybean seed. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature range of 20/30°C as optimal for germination of soybean (AOSA, 2007).

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 87712 and the conventional control A3525. In addition, ten commercial reference varieties were included to provide a range of comparative values that are representative of existing commercial soybean varieties. The seed lots for MON 87712, its conventional control A3525, and the commercial reference varieties were produced in replicated field trials at three sites during 2009 in Illinois and Missouri, geographical areas which represent environmentally relevant conditions for soybean production for this product. In addition to the AOSA recommended temperature range of 20/30°C, seed was tested at five additional temperature regimes of 10, 20, 30, 10/20, and 10/30°C to assess seed germination properties. The details of the materials, experimental methods, and germination data from all individual production sites are presented in Appendix G.

In a combined-site analysis, in which the data were pooled among the three production sites, no statistically significant differences ($\alpha = 0.05$) were detected between MON 87712 and the conventional control A3525 in any of the temperature regimes for any seed characteristic measured (Table VII-2). Within some temperature regimes, it was not possible to conduct an analysis of variance for percent viable firm swollen seed and

viable hard seed because there was no variability present in the data. For these data, the values of MON 87712 and the conventional control A3525 were all zero, indicating no biological differences.

The biological characteristics evaluated were used to characterize MON 87712 in the context of plant pest risk assessment. Based on the dormancy and germination characteristics assessed and the results of this study, particularly the lack of increased hard seed, it was concluded that there were no changes indicative of increased weediness or plant pest potential of MON 87712 compared to conventional soybean.

Table VII-2. Germination Characteristics of MON 87712 and the Conventional Control A3525

Temperature Regime	Germination Characteristic[1]	Mean % (S.E.)[2]		Reference Range[3]
		MON 87712	Control	Min. – Max.
10°C	Germinated	99.8 (0.1)	99.6 (0.1)	97.5-99.8
	Viable Hard[†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
	Dead	0.3 (0.1)	0.4 (0.1)	0.3-2.5
	Viable Firm-Swollen	0.0 (0.0)	0.0 (0.0)	0.0-0.5
20°C	Germinated	99.4 (0.2)	99.3 (0.2)	96.8-99.6
	Viable Hard [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
	Dead	0.6 (0.2)	0.7 (0.2)	0.4-3.3
	Viable Firm- Swollen [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
30°C	Germinated	99.3 (0.3)	99.5 (0.2)	97.0-99.8
	Viable Hard [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
	Dead	0.7 (0.3)	0.5 (0.2)	0.3-3.0
	Viable Firm- Swollen [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
10/20°C	Germinated	99.8 (0.1)	99.5 (0.2)	97.8-100.0
	Viable Hard [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
	Dead	0.2 (0.1)	0.5 (0.2)	0.0-2.3
	Viable Firm-Swollen [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
10/30°C	Germinated	99.5 (0.2)	99.5 (0.2)	96.0-100.0
	Viable Hard [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
	Dead	0.5 (0.2)	0.5 (0.2)	0.0-4.0
	Viable Firm-Swollen [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
20/30°C (AOSA)	Normal Germinated	96.8 (0.8)	96.6 (0.8)	89.8-99.0
	Abnormal Germinated	2.6 (0.7)	3.0 (0.7)	0.8-6.3
	Viable Hard[†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0
	Dead	0.7 (0.2)	0.4 (0.2)	0.1-4.0
	Viable Firm-Swollen [†]	0.0 (0.0)	0.0 (0.0)	0.0-0.0

Note: The data in this table are the combined-site results of the seeds from three 2009 field sites. The experimental design was a split-plot where whole-plot treatment was seed production site and the sub-plot treatment was seed material (*i.e.*, MON 87712, conventional control A3525, or commercial reference variety).

No statistically significant differences were detected (α=0.05) between MON 87712 and the conventional control A3525. S.E. = Standard Error.

[†] No statistical comparison could be made due to lack of variability in the data.

[1] Germinated seed in the AOSA temperature regime (20/30°C) were categorized as either normal germinated or abnormal germinated seed.

[2] Means based on 12 replicates (n = 12) of 100 seeds. In some instances, the total percentage for both MON 87712 and the conventional control A3525 did not equal exactly 100% due to numerical rounding of the means.

[3] Minimum and maximum means determined from among the commercial reference varieties.

VII.C.2. Field Phenotypic, Agronomic Characteristics, and Environmental Interactions

Phenotypic, agronomic, and environmental interactions were evaluated under field conditions as part of the plant characterization assessment of MON 87712. These data were developed to provide USDA-APHIS with a detailed description of MON 87712 relative to the parental conventional control A3525 and commercial reference varieties. According to 7 CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "a detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 87712 and the conventional control A3525 that may impact its pest potential. Although all data from the plant characterization is considered in the pest potential and environmental impact assessments, it is important to note that with some traits, phenotypic differences may be detected that are consistent with the product concept or mode of action of the biotechnology-derived trait (e.g. increased yield with a yield trait). Thus, the environmental assessment takes account of this when evaluating weediness, pest potential, or environmental impact. Certain growth, reproduction, and pre-harvest seed loss characteristics (*e.g.*, lodging, pod shattering) were used to assess whether there is an increase in weediness of MON 87712, an element of APHIS's plant pest risk determination. Environmental interactions were also assessed on an individual site basis, as an indirect indicator of phenotypic changes to MON 87712 compared to the same comparators described above and are also considered in the plant pest risk assessment.

Data were collected at 19 field locations in the U.S. during 2009 to evaluate phenotypic, agronomic, and environmental interaction characteristics. These 19 field sites provided a range of environmental and agronomic conditions representative of commercial soybean production areas in the U.S. (Table VII-3). The experimental design at each site was a randomized complete block with four replications. All plots of MON 87712, the parental conventional control A3525, and the commercial reference varieties within each site were uniformly managed in order to assess whether the introduction of BBX32 altered the phenotypic and agronomic characteristics or the environmental interactions of MON 87712 compared to the conventional control A3525. A description of the evaluated phenotypic and environmental interaction characteristics and the designated developmental stages when evaluations occurred are listed in Table VII-1. The methods and detailed results of the individual-site data comparisons are presented and discussed in Appendix H, while the combined-site analyses are summarized below. The results of this assessment demonstrated that the introduction of BBX32 protein did not alter MON 87712 compared to the conventional control A3525 in terms of weediness potential. The lack of differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance further support the conclusion that the introduction of BBX32 is not likely to result in increased plant pest potential or an adverse environmental impact from MON 87712 compared to conventional soybean.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

A total of 13 phenotypic and agronomic characteristics were evaluated (Table VII-4 and Table H-4). In a combined-site analysis in which the data were pooled among the sites, no statistically significant differences were detected ($\alpha = 0.05$) between MON 87712 and the conventional control A3525 for seedling vigor, days to 50% flowering, days to 50% end of flowering, plant height, lodging, pod shattering, grain moisture, or 100 seed weight (Table VII-4). Five statistically significant differences were detected between MON 87712 and the conventional control A3525 in the combined-site analysis. MON 87712 had higher early stand count (302.7 vs. 297.0 plants per plot), increased days to 50% senescence (267.7 vs. 265.0 days), increased days to physiological maturity (280.8 vs. 277.5 days), higher final stand count (296.8 vs. 286.4 plants per plot), and higher yield (52.6 vs. 49.0 bu/ac). Although significantly different from the conventional control A3525, the mean values of MON 87712 for early stand count and final stand count were small in magnitude and were within the range of commercial reference varieties for each characteristic and thus would not be adverse in terms of pest potential. Differences in 50% senescence, days to physiological maturity, and yield were consistent with the mode of action. The increase in yield is agronomically desirable and would not contribute to increased weediness potential of MON 87712 without changes in a combination of other characteristics associated with weediness (Baker, 1974). Furthermore, each difference, regardless of whether it is associated with the trait or not is assessed for pest potential and environmental impact. For each, it is unlikely that a difference in these characteristics (50% senescence, days of physiological maturity, and yield) would contribute to increased weediness potential of MON 87712 compared to the conventional control A3525.

Flower color and plant growth stage data were categorical and were not statistically analyzed; however, at each site, all plants of MON 87712 and the conventional control A3525 had purple flowers as expected. Additionally, MON 87712 and the conventional control A3525 were within the same range of plant growth stages for all growth stage observations among all sites (Table H-5). Thus, there were no biologically-meaningful differences in plant development observed between MON 87712 and the conventional control A3525.

The plant phenotypic and agronomic characteristics evaluated were used to provide a detailed description of MON 87712 compared to the conventional control A3525. A subset of these characteristics was useful to assess the weediness potential of MON 87712. Based on the assessed phenotypic and agronomic characteristics, the results support a determination that MON 87712 is no more weedy or likely to pose a plant pest risk or have a significant environmental impact than conventional soybean.

Table VII-3. Field Phenotypic Evaluation Sites for MON 87712 during 2009

Location	Location Code	Site Designation in Statistical Report	USDA-APHIS Notification Number
Jackson County, Arkansas	ARNE	YARNE	09-075-110n
Guthrie County, Iowa	IAJA	YIAJA	09-072-106n
Jefferson County, Iowa	IARL	YIARL	09-072-106n
Clinton County, Illinois	ILCY	YILCY	09-075-110n
Effingham County, Illinois	ILMS	YILMS	09-075-110n
Champaign County, Illinois	ILSE	YILSE	09-075-110n
Stark County, Illinois	ILWY	YILWY	09-075-110n
Parke County, Indiana	INRC	YINRC	09-100-102n
Boone County, Indiana	INSH	YINSH	09-075-110n
Pawnee County, Kansas	KSLA	YKSLA	09-075-110n
Macon County, Missouri	MOAN	YMOAN	09-072-106n
Shelby County, Missouri	MOCB	YMOCB	09-072-106n
Butler County, Missouri	MOFI	YMOFI	09-072-106n
Callaway County, Missouri	MOKI	YMOKI	09-072-106n
St Louis County, Missouri	MOSL	YMOSL	09-072-106n
Lincoln County, Missouri	MOWR	YMOWR	09-072-106n
York County, Nebraska	NEYO	YNEYO	09-072-106n
Berks County, Pennsylvania*	PAGR	YPAH2	09-075-110n
Berks County, Pennsylvania*	PAHM	YPAH1	09-075-110n

Note: Two additional sites, IAJE (Greene County, IA) and MOLP (Adair County, MO), were planted but multiple plots sustained substantial soybean plant damage due to frost and flood damage.

* Two sites were planted in Berks, Pennsylvania.

Table VII-4. Combined-Site Comparison of MON 87712 to Conventional Control A3525 During 2009 for Phenotypic and Agronomic Characteristics

Phenotypic Characteristic (units)	Mean (S.E.)		Reference Range[1]	
	MON 87712	Control	Minimum	Maximum
Early stand count (#/plot)	302.7 (5.6)*	297.0 (6.0)	239.5	345.7
Seedling vigor (1-9 scale)	2.9 (0.2)	3.0 (0.2)	1.7	4.8
Days to 50% flowering[2]	209.0 (1.0)	208.9 (1.0)	190.5	215.9
Days to 50% end of flowering[3]	231.7 (1.2)	231.5 (1.1)	215.3	239.0
Days to 50% senescence[4]	267.7 (0.8)*	265.0 (0.8)	251.6	270.8
Days to physiological maturity[5]	280.8 (0.9)*	277.5 (1.0)	262.4	281.6
Plant height (in)	31.6 (0.8)	31.1 (0.8)	23.1	34.6
Lodging (1-9 scale)	2.2 (0.2)	2.0 (0.2)	1.0	1.8
Pod shattering (1-9 scale)	1.2 (0.1)	1.3 (0.1)	1.0	2.1
Final stand count (#/plot)	296.8 (4.6)*	286.4 (4.7)	241.9	328.6
Grain moisture (%)	12.9 (0.2)	12.9 (0.2)	11.5	14.4
100 seed weight (g)	16.3 (0.2)	15.9 (0.2)	15.4	20.8
Yield (bu/ac)	52.6 (1.4)*	49.0 (1.2)	43.4	56.0

Note: The experimental design was a randomized complete block with four replications. S.E. = Standard Error. Means based on n = 76 for MON 87712 and the conventional control A3525 for all characteristics except for the following: for days to 50% senescence, n = 75 for MON 87712, for early stand count, n = 75 for the conventional control A3525, for final stand count, n = 74 for MON 87712 and n = 75 for the conventional control A3525, for plant height, n = 64 for MON 87712 and n = 66 for the conventional control A3525, for seedling vigor, n = 68 for both MON 87712 and the conventional control A3525., for days to 50% end of flowering, n = 71 for MON 87712 and n = 72 for the conventional control A3525., for days to physiological maturity, n = 75 for MON 87712. For reasons of excluding data please refer to Table H-3.

* Statistically significant differences were detected (α=0.05) between MON 87712 and the conventional soybean control A3525.

[1] Reference range = Minimum and maximum mean values among the 18 commercial reference varieties.

[2] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot were flowering.

[3] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot have stopped flowering.

[4] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot reached 50% senescence.

[5] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot reached physiological maturity.

VII.C.2.2. Environmental Interaction Characteristics

USDA-APHIS considers the environmental interaction of the biotechnology-derived crop compared to its conventional control to determine the potential for increased plant pest characteristics. Evaluations of environmental interactions were conducted as part of the plant characterization for MON 87712. In the 2009 U.S. field trials conducted for evaluation of phenotypic and agronomic characteristics of MON 87712, data were also collected on plant response to abiotic stress (drought, wind, nutrient deficiencies, etc), disease damage, arthropod-related damage, and arthropod abundance (Tables H-6, H-7, H-8, H-9 and H-10). These data were used as part of the environmental consequences (Section IX) to assess plant pest potential and provide an indication of potential effects of MON 87712 on non-target organisms (NTOs) and threatened and endangered species compared to the conventional control. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each assessed characteristic. The environmental interactions evaluation included data collected in the phenotypic studies (plant-insect, plant-disease, and plant-environmental interactions). Data was analyzed on an individual site basis; no combined site analysis was conducted. As expected, the results of the field evaluations showed that the presence of BBX32 did not meaningfully alter the assessed environmental interactions of MON 87712 compared to the conventional soybean A3525. The lack of significant biologically-meaningful differences in plant response to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance support the conclusion that the introduction of BBX32 protein is unlikely to result in an increased plant pest potential or cause an adverse environmental impact from MON 87712 compared to conventional soybean A3525.

In the 2009 field trials, the observations of plant response to abiotic stress, disease damage, and arthropod-related damage were performed four times during the growing season at all 19 sites, and arthropod abundance was assessed quantitatively from collections performed four times during the growing season at three of the 19 sites (*i.e.,* ARNE, ILSE, and MOCB). The assessed stressors (abiotic, diseases, and arthropods) were at natural levels as no artificial infestation or imposed abiotic stress was used and therefore, typically varied between observations at a site and among sites. Abiotic stress and disease damage data were collected from each plot using a 0 – 9 scale of increasing severity of observed damage. Damage data were collected numerically and then placed into one of the following categories: none, slight, moderate, or severe. These categorical data were not subjected to statistical analysis. The response of MON 87712 and the conventional control A3525 to an abiotic stress or disease were considered different on a particular observation date at a site if the range of injury severity to MON 87712 did not overlap with the range of injury severity to the control across all three replications (*e.g.,* "none" vs. "slight-moderate" rating). For each observation at a site, the range of injury severity across the commercial reference varieties provided assessment data that are representative of commercial soybean varieties. Arthropod-related damage was assessed from each plot on the upper four nodes of 10 representative plants using a 0 – 5 rating scale of increasing severity of observed damage. These numerical data along with the quantitative arthropod abundance data were subjected to statistical analysis.

In an assessment of plant response to abiotic stressors and disease damage, no statistically significant differences were detected between MON 87712 and the conventional control A3525 for any of the 384 comparisons which included 186 abiotic stressors and 198 disease damage comparisons (Tables H-6 and H-7).

In an assessment of arthropod-related damage, no statistically significant differences were detected between MON 87712 and the conventional control A3525 for 129 out of 137 comparisons among the observations across all sites (Table H-8). In addition, no numerical differences were observed for the 55 comparisons for which p-value could not be generated due to lack of variability in the data. The mean damage ratings were slightly higher than the respective reference ranges for eight detected differences. However, the differences detected were small in magnitude and these differences were not consistent across observations or sites. Thus, these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of an adverse environmental impact of MON 87712 compared to conventional soybean.

In an assessment of pest and beneficial arthropod abundance, no statistically significant differences were detected between MON 87712 and the conventional control A3525 for 106 out of 115 comparisons (including 59 pest arthropod comparisons and 56 beneficial arthropod comparisons) among the collections at the three sites (Tables H-9 and H-10). In addition, no numerical differences were observed for the eight comparisons (including six pest arthropod comparisons and two beneficial arthropod comparisons) for which p-values could not be generated due to lack of variability in the data. The mean abundance values from MON 87712 were within the respective reference range for five of the nine detected differences. For the remaining four differences, the mean abundance values for MON 87712 were outside of the reference range; however, these differences were not consistent across collections or sites. Thus, these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of an adverse environmental impact of MON 87712 compared to conventional soybean.

The results of the field evaluations showed that the presence of BBX32 did not alter the assessed environmental interactions of MON 87712 compared to the conventional control A3525. The lack of significant biological differences in plant responses to abiotic stress, disease damage, arthropod-related damage, and arthropod pest and beneficial insect abundance support the conclusion that the introduction of BBX32 in MON 87712 is unlikely to result in increased plant pest potential or an adverse environmental impact compared to conventional soybean.

VII.C.3. Pollen Characteristics

USDA-APHIS considers the potential for gene flow and introgression of the biotechnology-derived trait into other soybean varieties and wild relatives. Pollen morphology and viability information are pertinent to this assessment and, therefore, were assessed for MON 87712. In addition, characterization of pollen produced by MON 87712 and the conventional control A3525 is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87712 compared to the conventional control A3525.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from MON 87712 compared to that of the conventional control A3525. Pollen was collected from MON 87712, the conventional control A3525, and four commercial reference varieties grown under similar agronomic conditions in a field trial in Missouri. The trial was arranged in a randomized completed block design with four replications. A minimum of twenty flowers were collected from each plot. Pollen was extracted, combined among flowers collected from the same plot, and stained with Alexander's stain (Alexander, 1980). Pollen viability was evaluated for each sample, and pollen grain diameter was measured for ten representative viable pollen grains per replication. General morphology of the pollen was observed for each of the four replications of MON 87712, the conventional control A3525, and the commercial reference varieties (see Appendix I).

No statistically significant differences were detected between MON 87712 and the conventional control A3525 for percent viable pollen or pollen grain diameter (Table VII-5). Furthermore, no visual differences in general pollen morphology were observed between MON 87712 and the conventional control A3525. These results demonstrate that the introduction of BBX32 did not alter the overall morphology or viability of MON 87712 pollen compared to the conventional control A3525. The pollen characterization data contribute to the detailed phenotypic description of MON 87712 compared to the conventional control A3525. The result supports an overall conclusion that MON 87712 is no more likely to pose a plant pest risk than conventional soybean.

Table VII-5. Pollen Characteristics of MON 87712 Compared to the Conventional Control A3525

Pollen Characteristic	MON 87712 Mean (SE)[1]	Control Mean (SE)[1]	Reference Range[2] Minimum	Reference Range[2] Maximum
Viability (%)	98.7 (0.75)	99.4 (0.37)	98.8	99.7
Diameter (μm)	25.4 (0.45)	24.9 (0.90)	24.8	26.1

Note: No significant differences were detected between the test and the conventional control A3525 (α=0.05).
[1] Mean based on n=4. SE = Standard Error.
[2] Reference range is the minimum and maximum mean value observed among the four commercial reference varieties.

VII.C.4. Symbiont Interactions

As part of the plant pest risk assessment, USDA-APHIS considers the impact of the biotechnology-derived crop on plant pest potential and the environment as well as on agricultural or cultivation practices compared to its conventional counterpart. Potential changes in the symbiotic relationship with the rhizosphere-inhabiting bacteria *Rhizobiaceae* and *Bradyrhizobiaceae* could directly impact pest potential, the environment, or cultivation practices (*i.e.,* the need to add additional nitrogen to sustain soybean production). Thus, the purpose of this evaluation was to assess whether the introduction of BBX32 altered the symbiotic interaction of MON 87712 with *Bradyrhizobium japonicum* compared to that of the conventional control A3525.

Members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae* form a highly complex and specific symbiotic relationship with leguminous plants, including soybean (Gage, 2004). The nitrogen-fixing plant-microbe symbiosis results in the formation of root nodules, which provide 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. As a result of this relationship, nitrogen inputs are typically not necessary for agricultural production of soybeans.

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

MON 87712, parental conventional control A3525, and six commercial reference varieties were produced from seeds planted in pots containing nitrogen-deficient potting medium and grown in the greenhouse. Seeds were inoculated with a solution of *B. japonicum*. The pots were arranged in a randomized complete block design with eight replicates. At 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. Nodules were separated from roots prior to enumeration and determination of dry weight. MON 87712 was compared to the conventional control A3525 for key characteristics related to their association with the soybean - *B. japonicum* symbiosis. Detailed information on materials and methods used for the symbiont evaluation is presented in Appendix J.

No statistically significant differences were detected (α=0.05) between MON 87712 and the conventional control A3525 for each measured parameter, including nodule number, shoot percent total nitrogen, shoot total nitrogen (g), and dry weight of nodules, shoot material, and root material (Table VII-6).

Based on the assessed characteristics, the results support the conclusion that the introduction of BBX32 does not alter the symbiotic relationship between *B. japonicum* and MON 87712 compared to that of conventional soybean. Thus, these data further support a conclusion of no change in plant pest potential and no expected impact to cultivation practices relative to nitrogen inputs for MON 87712 compared to conventional soybean.

Table VII-6. Symbiont Interaction Assessment of MON 87712 and Conventional Control A3525

	Mean (S.E.)*			Reference Range[1]	
Measurements	MON 87712	A3525	p-Value	Minimum	Maximum
Nodule Number (per plant)	124 (34)	110 (23)	0.5731	69	134
Nodule Dry Wt (g/plant)	0.51 (0.06)	0.49 (0.04)	0.7954	0.41	0.59
Root Dry Wt (g)	0.94 (0.09)	0.93 (0.07)	0.9617	0.78	1.59
Shoot Dry Wt (g)	4.52 (0.60)	4.43 (0.36)	0.9061	4.03	6.62
Shoot Percent Total Nitrogen (% dwt)	3.07 (0.31)	3.18 (0.19)	0.5899	2.59	3.39
Shoot Total Nitrogen (g)	0.15 (0.03)	0.14 (0.02)	0.8043	0.13	0.18

Note: Pots were arranged in eight replicated blocks (n = 8) in a greenhouse using a randomized completed block design. S.E. = Standard Error.

* No significant differences were detected between MON 87712 and the conventional control A3525 (α=0.05).

[1] Reference range is the minimum and maximum mean value observed among six commercial reference varieties.

VII.C.5. Volunteer Potential Assessment

Volunteer potential can also play a role in determining whether a regulated article has increased weediness potential. In some crops, seed remaining in the field after harvest have the potential to over-winter and volunteer in the subsequent cropping season. The purpose of this study was to assess the volunteer potential of MON 87712 compared to the near isogenic conventional control A3525. In the fall of 2009, field trials were established at four locations to assess volunteer potential. Comparative assessments were conducted on MON 87712 and conventional control A3525. In addition, 4 commercial reference varieties were included as references at each site. It was determined that the seed were acceptable for evaluating volunteer potential based on standard viability testing. Normal seed germination rates were confirmed for MON 87712, the conventional control A3525, and commercial reference varieties, with two exceptions; two references at one site had germination rates of 42 and 31% respectively. The trials were established at each location as a randomized complete block design with four replications. Each plot was 5-8 ft wide by 20 ft long and was hand-seeded by uniformly scattering approximately 400 seeds on the soil surface. Seeds were then mechanically incorporated to a maximum depth of approximately 0.5 – 3 inches to avoid surface predation. Additional material and methods are provided in Appendix K.

Agronomic practices used to prepare each study site were characteristic of each region. No irrigation was applied to the study areas, and no plot management was required after seed was incorporated into the soil. Volunteer plant counts were taken every two weeks after planting until the environmental conditions were no longer conducive for germination and emergence. Monitoring resumed in the spring of 2010, when environmental conditions became favorable for soybean germination and emergence. Volunteer counts continued approximately every two weeks until mid-June for a total of seven observations at each site. Data was analyzed on an individual site basis; no combined site analysis was conducted.

No volunteer plants were observed at any site or observation time during the fall of 2009. No volunteer plants were observed at any observation time for two of the four sites during the spring of 2010. A small number of volunteers were observed at two of the four sites during the spring of 2010 (Table VII-7). However, the small number of volunteers observed in the study was not consistent across locations. Based on the assessed data, the results of this study support a conclusion that the introduction of BBX32 did not alter the ability of MON 87712 to volunteer compared to conventional soybean. Furthermore, these results demonstrate that BBX32 in MON 87712 confers no biologically meaningful change to the potential for soybean to persist in the environment.

Table VII-7. Observed Volunteer Soybean Plants of MON 87712 Compared to the Conventional Control A3525 and References in a 2009/2010 U.S. Field Trial

Site[1]	Season	Observation Dates[2]	Number of Volunteers		Reference range[3]	
			MON 87712	Control	Min.	Max.
ARAU	Fall	12/07/09, 12/21/09	0	0	0	0
	Spring	4/22/10, 5/06/10, 5/20/10, 6/03/10, 6/17/10	0	0	0	0
ARNE	Fall	12/07/09, 12/21/09	0	0	0	0
	Spring	4/22/10, 5/06/10, 5/20/10, 6/03/10, 6/17/10	0	0	0	0
ILWY	Fall	12/07/09	0	0	0	0
	Spring	4/08/10, 4/22/10, 5/06/10, 5/20/10, 6/03/10, 6/17/10	3	0	0	7
MOAN	Fall	None	-	-	-	-
	Spring	3/19/10, 4/02/10, 4/16/10, 4/28/10, 5/12/10, 5/26/10, 6/09/10	0	1	0	1
Mean Across			0.75	0.25	0.0	2.0

Note: No statistical analyses were performed due to lack of variability. Numbers shown are total numbers of volunteers for each substance across all four replications at each specific site (n=4).

[1] ARAU = Woodruff County, AR; ARNE = Jackson County, AR; ILWY = Stark County, IL; MOAN = Shelby County, MO.

[2] Observations were made approximately every two weeks.

[3] Minimum and maximum number of volunteers observed from among the soybean reference varieties.

4 Mean of volunteers observed for test control and references across all four sites and calculated using Microsoft Office Excel 2007.

VII.C.6. Persistence Outside of Cultivation Assessment

Weediness or invasiveness may also be indicated if soybean exhibited an increased rate of persistence outside of cultivation. The purpose of this study was to assess the ability of MON 87712 to establish and persist in unmanaged, competitive environments that are not cultivated for agricultural production. Four sites were established in 2009. Each site was unmanaged and received no agricultural inputs allowing MON 87712, the conventional control A3525, and the commercial reference varieties (four per site) to compete with existing vegetation and respond to abiotic and biotic stressors present in each environment. Additional materials and methods are provided in Appendix L.

Phenotypic and agronomic characteristics encompassing plant growth, development, and seed production were assessed for MON 87712, the parental conventional control A3525, and the commercial reference varieties in unmanaged environments. The experiment was established at each of four sites in a randomized complete block design with three replications. Stand count, growth stage, and plant vigor was evaluated at approximately 14 day intervals throughout the season and the number of plants producing pods per plot, number of seeds produced per plot, and weight of seeds produced per plot were collected at the end of the season. Data was analyzed on an individual site basis; no combined site analysis was conducted.

Additionally, replacement values were calculated and used to evaluate the ability of MON 87712, the conventional control A3525, and the commercial reference varieties to persist outside of cultivation. Each replacement value is the ratio of number of seeds produced to the number of seeds sown. A replacement value less than one means that fewer seeds were produced than sown. This is indicative of a population that will not replace itself and will not persist. A replacement value greater than one means that more seeds were produced than were sown and indicated a population that may potentially increase.

The replacement value at three of the four sites was zero. At the end of the first growing season, one site had a replacement value of 2.72 for MON 87712 and 2.63 for the conventional control A3525, which indicated that the plots produced more seeds than planted. However, no plants emerged in any of the plots during the second season at this site and thus, the replacement value was zero. This indicated that the populations were all in decline and would not persist in the unmanaged environments and did not demonstrate a competitive advantage in this study compared to conventional soybean. Therefore, the presence of BBX32 in MON 87712 confers no biologically meaningful change to the fitness, invasiveness, or potential for soybean to persist outside of managed agricultural environments.

VII.D. Conclusions for Phenotypic, Agronomic, and Environmental Interactions Evaluation

An extensive and robust set of information and data were used to assess whether the introduction of BBX32 altered the plant pest potential of MON 87712 compared to the parental conventional control A3525. Phenotypic, agronomic, and environmental interaction characteristics of MON 87712 were evaluated and compared to those of the conventional control A3525 and considered within the variation among commercial reference varieties. These assessments included seven general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) seed retention and lodging; 5) plant response to abiotic stress and interactions with diseases and arthropods; 6) plant-symbiont interactions and 7) volunteer potential and persistence outside of cultivation characteristics. Results from the phenotypic, agronomic, and environmental interactions assessment demonstrate that MON 87712 does not possess a) weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stress, disease, or arthropods; or c) characteristics that would confer a plant pest risk or significant environmental impact compared to conventional soybean.

VIII. U.S. AGRONOMIC PRACTICES

VIII.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4), impacts to agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. for producing soybean and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 87712. Discussions include soybean production, seed production, plant growth and development, general management practices during the season, management of weeds, insects and diseases, soybean rotational crops, and volunteer soybean management. Information presented in Section VIII.C.2 demonstrated that MON 87712 produced more yield per soybean plant and has slightly delayed maturity compared to conventional soybean. However, MON 87712 is no more susceptible to diseases or pests than conventional soybean and required no additional inputs to produce a crop from MON 87712, and no specific impacts were noted to agronomic practices employed for production of soybean. In the areas where there is potential for impact on agronomic practices from the deregulation of MON 87712, the scope and magnitude of those impacts will be discussed.

Soybean is grown as a commercial crop in over 35 countries, demonstrating its wide adaptation to varied soils and climate. The soil, moisture, and temperature requirements for producing soybean are generally similar to those for corn, and thus the two crops share a similar cultivation area. Proper seedbed preparation, selection of a variety of the appropriate maturity that is adapted to the local environment, appropriate planting dates and plant population, and good integrated pest management practices are important for optimizing the yield potential and economic return for soybean.

VIII.B Soybean Yield

Improvement in grain yield remains one of the major objectives for plant breeders. Gains in major crop yields over the years can be attributed to genetic improvement through traditional breeding. Breeders have crossed plants with different genetic backgrounds and selected traits resulting in higher yields, compositional improvements, and desirable production traits. Biotechnological approaches complement traditional soybean breeding efforts by targeting the same major characteristics as traditional breeding.

Crop yield results from a sequential growth and development process – first the plant grows vegetatively and produces photosynthetic tissue, followed by flowering and the production of seeds, and finally seed filling and maturation. Improvements in crop yield have been a primary focus of conventional breeding. The genetic changes that resulted in crop domestication and yield improvement in conventional varieties have been shown by modern molecular biology analysis to have been typically achieved through the selection and safe use of plant genes encoding transcriptional regulator proteins. Agricultural biotechnology provides the opportunity to further enhance crop yields through the introduction of new genetic elements that use or modify existing pathways in the plant.

World soybean production has increased steadily in the last decade, rising from 133 million metric tons in 1996 to 258.4 million metric tons (MMT) in 2010 (ASA, 2011), due to higher economic values of protein and oil contents, industrial uses and medicinal importance. Soybean crop yields have risen consistently in North America over the past half-century. In the U.S., soybean yield rose at an annual average rate of 0.35bu/A (0.8%) between 1924 – 2010 (Specht et al., 1999), and similar yield increases have been reported in Canada. A survey of soybean yield in Canada between 1934 – 1992 revealed an annual increase in yield of 0.5%, with evidence that since 1976 the rate of genetic improvement of seed yield is accelerating (Morrison et al., 1999; Voldeng et al., 1997).

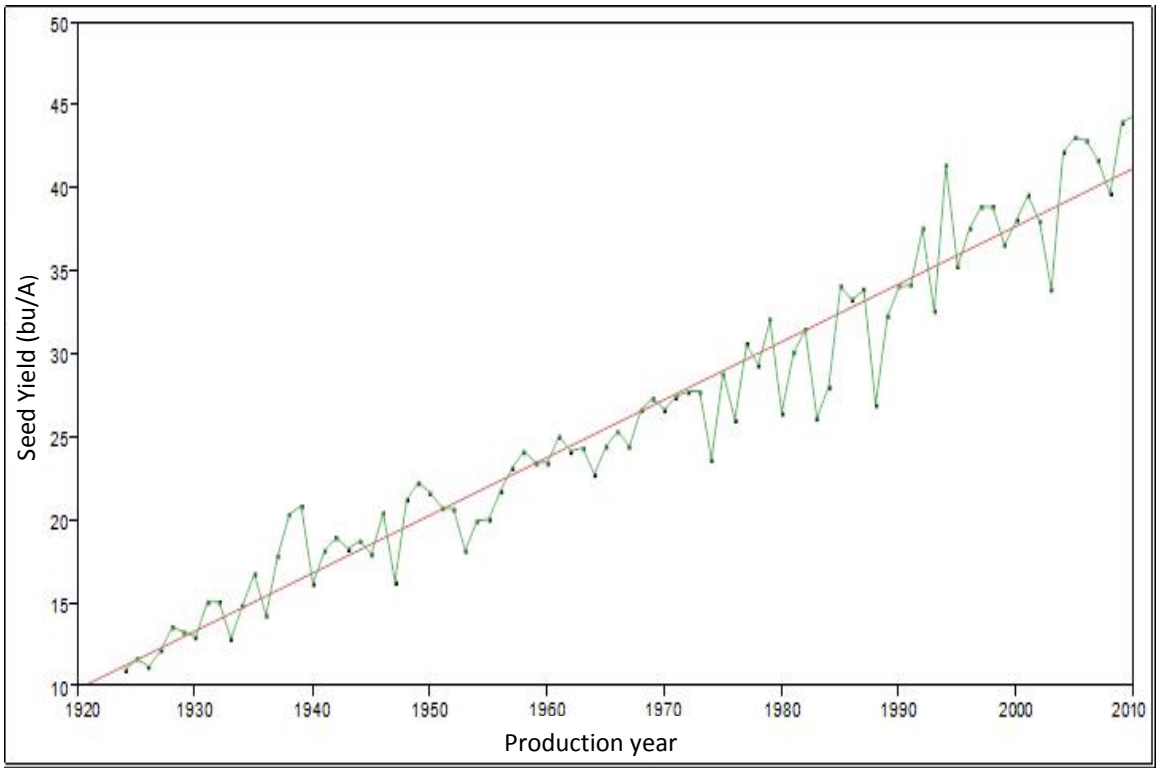


Figure VIII-1. U.S. soybean yield rose at an annual average rate of 0.35 bu/A between 1924 – 2010.
Linear regression analysis was conducted on data from the USDA National Agricultural Statistics Service (USDA-NASS, 2011b).

Soybean yield trends in the U.S. indicate that yield growth rates have not reached a plateau. Average soybean yield in 2010 was 43.5 bu/A (2900 kg/ha), but record yields reported from yield contests in the U.S. (Iowa, Missouri and Nebraska, 1966-1998) were greater than 67.5 bu/A (4500 kg/ha) and in one instance reached 100 bu/A (6660 kg/ha) (Specht et al., 1999), demonstrating that future yield growth is possible. The concept of yield potential of soybean is defined as the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting, and with pests, diseases, weeds, lodging, and other stresses effectively controlled (Evans and Fischer, 1999; Specht et al., 1999). Conventional soybean yield potential has been

estimated to be approximately 120 bu/A (8000 kg/ha) using crop simulation models (Specht et al., 1999). The U.S. average in 2010 of 43.5 bu/A (2900 kg/ha) was substantially below the current estimate of yield potential, suggesting that there is an opportunity to close the gap between average annual yield performance and yield potential. Actual yield performance is a complex outcome that is dependent on a number of genetic and environmental factors that influence a crop's opportunity to realize its full yield potential. Growers are accustomed to experiencing field-to-field and year-to-year yield variation based on environmental conditions and the genetics of the varieties they select for planting.

From 1924 to 2010, soybean acreage increased almost 50-fold and yield rose at an average annual rate of approximately 0.35 bu/A (0.8%) in the U.S. (USDA-NASS, 2011b). Annual improvement in U.S. soybean yield is attributed to rapid producer adoption of agricultural improvements such as genetic and agronomic innovations that provide producers with means for reducing "on-farm" yield constraints (Specht et al., 1999). Agricultural production depends on continuing infusions of genetic resources for yield stability and growth (Day-Rubenstein and Heisey, 2006).

Increased soybean production in the U.S. has been accomplished by both increasing the area under cultivation and through yield increases per unit area. However, based on recent trends in farm production and land area, most OECD countries, including the U.S. and Canada, are predicted to face the challenge of expanding agricultural output by raising productivity on a stable or reduced land area (OECD-FAO, 2008). Therefore, much of the projected expansion in soybean production in the future is expected to come from increased yield rather than increased area under production (OECD-FAO, 2008).

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

VIII.C.1. Soybean Production

Soybean first entered North America in the 18th century (Hoeft et al., 2000b). During the 1930s, soybean started to be processed industrially in the U.S. for edible oil and protein meal. In 2010, soybean represented 58% of world oilseed production, and approximately 35% of world soybean production in 2010 were produced in the U.S. (ASA, 2011). In 2010, the U.S. exported 1.59 billion bushels (43.27 million metric tons) of soybean, which accounted for 44% of the world's soybean exports (ASA, 2011). In total, the U.S. exported over $23 billion worth of soybean and soybean products globally in 2010 (ASA, 2011). China was the largest export market for U.S. soybean with purchases totaling over $11 billion. Mexico was the second largest export market with purchases of $2.0 billion.

The productivity of soybean is highly dependent upon soil and climatic conditions. In the U.S., the soil and climatic requirements for growing soybean are very similar to corn. The soils and climate in the Midwestern, Eastern, and portions of the Great Plains regions of the U.S. provide sufficient water under normal climatic conditions to produce a soybean crop. The general water requirement for a high-yielding soybean crop is approximately 20 inches of water during the growing season (Hoeft et al., 2000b). Soil

texture and structure are key components determining water availability in soils, where medium-textured soils hold more water, allowing soybean roots to penetrate deeper in medium-textured soils than in clay soils. Irrigation is used on approximately 9% of the soybean acreage to supplement the water supply during dry periods in the Western and Southern soybean growing regions (USDA-ERS, 2008).

Most of the soybean acreage is grown as a full-season crop. Approximately 8% of the soybean acres are planted in a double-crop system following winter wheat south of 35º North latitude (Wilcox, 2004). However, this percentage can vary significantly from year to year. The decision to plant double-crop soybean is influenced by both agronomic and economic factors. Agronomic factors include harvest date of the wheat crop, which determines the double-crop soybean planting date, and available soil moisture. Economic factors include expected soybean price and anticipated economic return (Heatherly and Elmore, 2004).

The U.S. soybean acreage in the past 10 years has varied from approximately 64.7 to 77.5 million acres, with the lowest acreage recorded in 2007 and the highest in 2009 (Table VIII-1). Average soybean yields have varied from 33.9 to 44.0 bushels per acre over this same time period. Annual soybean production ranged from 2.45 to 3.19 billion bushels over the past ten years. According to data from USDA-NASS (2011d), soybean was planted on approximately 76.6 million acres in the U.S. in 2010, producing 3.32 billion bushels of soybean (Table VIII-1). Soybean acreage and production in 2008 was up from 2007, mainly due to a decrease in corn acreage. The value of soybean reached $38.9 billion in the U.S. in 2010 (USDA-NASS, 2011a). In comparison, corn and wheat values in 2010 were $66.65 and $12.99 billion, respectively (USDA-NASS, 2011a).

For purposes of this agronomic practices discussion, soybean production is divided into three major soybean growing regions Midwest/Great Plains region (IL, IN, IA, KS, KY, MI, MN, MO, NE, ND, OH, SD, and WI), Southeast region (AL, AR, GA, LA, MS, NC, SC, and TN) and the Eastern Coastal region (DE, MD, NJ, NY, PA, and VA) (Table VIII-2). The vast majority of soybean was grown in the Midwest region, representing 83.8% of the total U.S. acreage. The Southeast and Eastern Coastal regions represented 13.5% and 2.7% of the acreage, respectively. Among the three regions, the Midwest region produced the highest average yield at 43.9 bushels per acre in 2010, and average state yields in this region ranged from 32.5 to 52.5 bushels per acre. The average yield in the Southeast region was 31.0 bushels per acre, with states within this region averaging from 26.0 to 41.0 bushels per acre. The average yield in the Eastern Coastal region was 34.0 bushels per acre, with individual state averages ranging from 24.0 to 48.0 bushels per acre.

Managing input costs is a major component to the economics of producing a soybean crop (Helsel and Minor, 1993). Key decisions on input costs include choosing what soybean varieties to plant, amounts of fertilizer to apply, and what herbicide program to use. The total operating cost for producing soybean in the U.S. in 2010 was $132.29 per acre, according to statistics compiled by the USDA-Economic Research Service (USDA-ERS, 2011). The value of the production less operating cost was reported to be $317.03

per acre. A summary of potential production costs and returns are presented in Table VIII-3.

In the short term, individual growers' decisions about whether to plant and which crop to plant are typically based on the relationship between operating costs and expected prices; i.e., on expected crop profitability (Ash et al., 2006). Managing input costs and managing the crop for yield are major components to the economics of producing a soybean crop. Growers' costs include both overhead costs and operating costs. Overhead costs are those that are not associated with a particular crop and/or that are present whether or not a crop is grown, such as the cost of land and the depreciation of equipment. Operating costs are those associated with growing a particular crop in a given year, such as seed and fertilizer. A producer's cost of growing a particular crop includes a proportional part of the overhead of his or her entire farming operation, plus all the operating costs associated with that crop.

Figure VIII-2 shows the average per acre net value of soybean production in the U.S. from 1975 to 2010, based on data compiled by the USDA Economic Research Service (ERS). The net value is the value of the soybeans produced less all costs of production, both the allocated overhead and the operating costs. For comparison, corn and wheat are also shown. USDA's data does not include crop subsidies. Overhead costs represent well over half the total costs (up to 69%), with the "opportunity cost of land (rental rate)" and the capital recovery cost of machinery and equipment representing the bulk of the overhead costs. The largest single operating cost is seed (USDA-ERS, 2011). As the data show, farming is often not profitable when all costs, including land value costs, are included. For example, USDA reports that in 2004, 70 percent of soybean-producing farm operations were considered profitable, not considering government payments. The percent profitable rises to 76 when government payments are included (Ash et al., 2006). While an individual grower typically makes planting decisions based on the relationship between operating costs and expected prices (Ash et al., 2006), many factors influence both operating costs and expected prices. Government price supports can have a large effect on costs, and supply and demand governs prices.

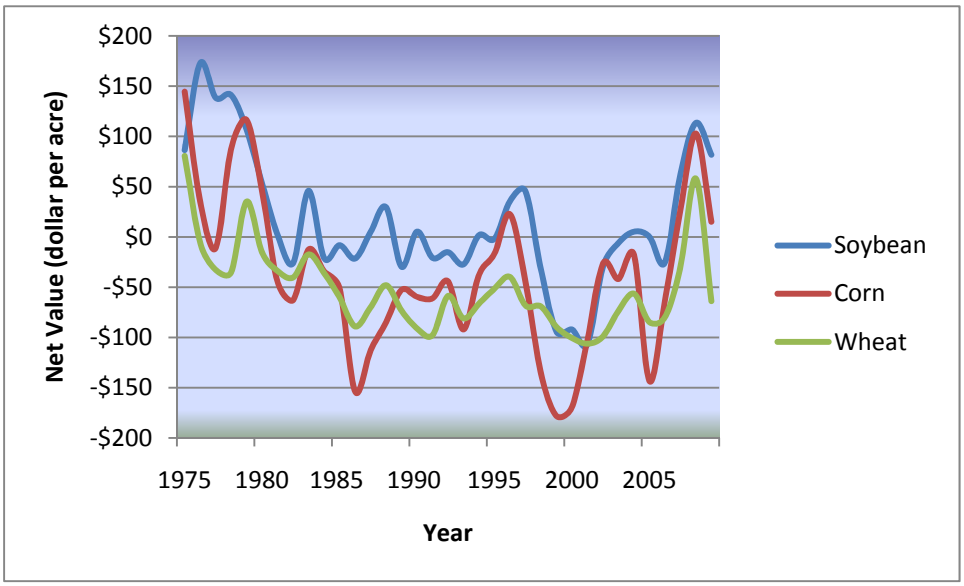


Figure VIII-2. U.S. Net Value of Soybean Production, Dollars Per Acre
Inflation Adjusted (to 2011) Values from 1975-2009, and does not include Government Subsidies
Sources: (USDA-ERS, 2011), U.S. Bureau of Labor Statistics 2011

Table VIII-1. Soybean Production in the U.S, 2001 – 2010[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
2010	77,404	76,616	43.5	3,329,341	38.92
2009	77,451	76,372	44.0	3,359,011	33.60
2008	75,718	74,641	39.6	2,959,174	27.40
2007	64,741	64,146	41.7	2,677,117	26.97
2006	75,522	74,602	42.7	3,188,247	20.42
2005	72,142	71,361	43.3	3,086,432	16.93
2004	75,208	73,958	42.2	3,123,686	17.89
2003	73,404	72,476	33.9	2,453,665	18.01
2002	73,963	72,497	38.0	2,756,147	15.25
2001	74,075	72,975	39.6	2,890,682	12.61
Ave.	73,969	72,875	40.0	2,936,536	21.00

[1]Source is USDA-NASS(USDA-NASS, 2011c).

Table VIII-2. U.S. Soybean Production by Region and State in 2010

Region/State	Acres Planted (thousands)	Acres Harvested (thousands)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
Midwest Region					
Illinois	9,100	9,050	51.5	466,075	5.78
Indiana	5,350	5,330	48.5	258,505	3.05
Iowa	9,800	9,730	51.0	496,230	5.81
Kansas	4,300	4,250	32.5	138,125	1.66
Kentucky	1,400	1,390	34.0	47,260	0.57
Michigan	2,050	2,040	43.5	88,740	1.01
Minnesota	7,400	7,310	45.0	328,950	3.72
Missouri	5,150	5,070	41.5	210,405	2.55
Nebraska	5,150	5,100	52.5	267,750	3.03
North Dakota	4,100	4,070	34.0	138,380	1.56
Ohio	4,600	4,590	48.0	220,320	2.60
South Dakota	4,200	4,140	38.0	157,320	1.76
Wisconsin	1,640	1,630	50.5	82,315	0.94
Region Totals	**64,240**	**63,700**	**43.9**	**2,900,375**	**34.04**
Southeast Region					
Alabama	350	345	26.0	8,970	0.10
Arkansas	3,190	3,150	35.0	110,250	1.25
Georgia	270	260	26.0	6,760	0.07
Louisiana	1,030	1,020	41.0	41,820	0.46
Mississippi	2,000	1,980	38.5	76,230	0.85
North Carolina	1,580	1,550	26.0	40,300	0.50
South Carolina	465	455	23.0	10,465	0.12
Tennessee	1,450	1,410	31.0	43,710	0.51
Region Totals	**10,335**	**10,170**	**31.0**	**338,496**	**3.86**
Eastern Coastal Region					
Delaware	175	173	32.0	5,536	0.07
Maryland	470	465	34.0	15,810	0.19
New Jersey	94	92	24.0	2,208	0.02
New York	280	279	48.0	13,392	0.15
Pennsylvania	500	495	42.0	20,790	0.25
Virginia	560	540	26.0	14,040	0.17
Region Totals	**2097**	**2044**	**34.0**	**71,776**	**0.85**

USDA-NASS (2011d; 2011a)

Table VIII-3. U.S. Soybean Production Costs and Returns in 2010[1]

Production Cost or Return Category	Itemized Costs	Return per Planted Acre ($ USD)
Total Gross Value of Production		**449.32**
Operating Costs:	Seed	59.2
	Fertilizer	17.87
	Chemicals	17.04
	Custom operations	6.52
	Fuel, lube and electricity	16.75
	Repairs	13.46
	Purchased irrigation water	0.14
	Interest on operating capital	1.31
Total, operating costs		**132.29**
Allocated overhead:	Hired labor	2.11
	Opportunity cost of unpaid grower's labor	17.33
	Capital recovery of machinery and equipment	77.51
	Opportunity cost of land (rental rate)	148.34
	Taxes and insurance	9.41
	General farm overhead	14.86
Total, allocated overhead		**269.56**
Total cost listed		**401.85**
Value of production less total cost listed		**47.47**
Value of production less operating costs		**317.03**

Supporting Information: Yield = 43 bushels/acre, Price = $10.48/bushel, Enterprise size = 303 planted acres, Irrigated = 9%, Dry land = 91%.
[1]Source is USDA-ERS (2011).

VIII.C.2. Variety Development

Crop domestication and improvement through breeding has been largely achieved through selection of genes that regulate the expression of desirable traits, such as those associated with higher yields or disease resistance. Once plants with the desired traits have been selected, a population of those plants with similar characteristics are classified as varieties. Historically breeders have developed desirable varieties by retaining for further breeding those plants that possess the desirable traits, as determined by visual inspection or by testing. In recent years breeders have used the more direct methods of molecular breeding techniques, such as marker assisted breeding, to accelerate the process of identifying breeding lines containing a desired set of positive traits. These techniques rely on inventories of genomic regions or genetic markers that have been positively associated with the desirable traits. Once the genetic markers associated with the desired traits have been identified, molecular breeders can quickly select the offspring inheriting the genes for further development (Voosen, 2009).

Hundreds of soybean varieties are tested each year in performance trials (variety trials) conducted by universities and private companies in all the major soybean growing states. The following information can typically be obtained from the results of variety trials: maturity group, disease resistance, insecticide seed treatment, yield, maturity date, percent lodging (plants fallen over on the ground), height, and herbicide resistance (Tylka et al., 2010). In different parts of the country and/or in other trials, additional characteristics may be identified, such as iron deficiency tolerance or protein or oil content {Pedersen, 2008 #56136}.

Soybean is a self pollinating crop and the vast majority of commercial soybean currently under production was produced through a process referred to as forward breeding. Soybean varieties are developed by seed companies through conventional forward breeding that involves the following steps:

1. Initial Crosses to generate genetic variability
2. Advance generations through self pollination
3. Bulk production of a segregating population of plants
4. Selection of plants with a desired phenotype within the bulk population
5. Harvesting of the self pollinating seed from selected plants
6. Planting of the selected seed and bulk production through self pollination
7. Harvest of self pollinating seed

A typical breeding cycle takes roughly 6 generations once a desired phenotype has been selected to produce commercial seed.

VIII.C.3. Soybean Seed Production

Standardized seed production practices are responsible for maintaining high-quality seed stocks, an essential basis for U.S. agriculture. By the early 20th century, agronomists learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties that were distributed to

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

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

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

U.S soybean production for all purposes has varied from approximately 64.7 to 77.5 million acres in the past ten years (USDA-NASS, 2011c). To plant this area of soybean acreage requires 105 to 125 million units (50 lbs/unit) of soybean seed. This seed volume includes allowances for seed losses due to weather, poor yields, and quality issues. Additional allowances are included for distribution excess, seed returns, replants, and potential increases in soybean acreage. Assuming an average soybean yield of 45 bushels, or 54 units

(50 lbs/unit) per acre, 1.9 to 2.3 million acres would be required to produce this volume of commercial certified soybean seed each year.

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

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

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

The field operations and management practices for producing soybean seed are similar to normal soybean production. However, special attention is needed in certain areas to produce seed with high quality, high germination rates, and high genetic purity (Helsel and Minor, 1993). General guidelines specific for seed production are discussed below. Importantly, the seed production field should not have been planted with soybean in the previous crop season in order to avoid potential volunteer soybean plants (even though the risk of soybean volunteer plants is negligible) and to ensure genetic purity.

Very early planting is typically avoided because the seed produced from early planting often results in poorer quality seed (Helsel and Minor, 1993). Every effort must be made to eliminate weeds in a seed field through the use of herbicides and cultivation practices to prevent weed seed in the harvested soybean seed. Fields are scouted frequently for insect pests and insecticides are applied when insect pest infestations reach economical threshold levels. Foliar-applied fungicides should be considered when disease infestations are

predicted in the area. Harvest should occur as soon as the mature soybean seed reaches 13% moisture content. Harvesting soybean seed with less than 13% moisture can cause damage to the seed coat and result in split soybean seed that can affect germination and viability. Harvesting equipment must be adjusted to minimize or avoid seed damage. Harvesting equipment must be cleaned before entering the seed fields to assure genetic purity. Certain seed handling equipment, such as auger elevators, should be avoided because they can increase seed damage.

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

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

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

When deregulated, MON 87712 seed will be produced in the same manner as commercially certified soybean seed, such that it will meet all state and federal seed standards and labeling requirements.

VIII.D. Production Management Considerations

VIII.D.1. Pre-Season

Well in advance of planting a soybean crop, decisions are made regarding the planned crop rotation, the tillage system and row spacing that will be implemented, the planting equipment that will be used, the seed or variety that will be planted, and soil fertility

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

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

Most field crops, including soybean, respond well to fertilizer when planted in soils with low fertility levels. Soybean requires 16 essential elements for growth and development. Deficiencies in any of these elements can reduce yields (Hoeft et al., 2000b). The primary or major essential nutrients are nitrogen, phosphorus and potassium. The soybean plant is a member of the legume family, like alfalfa and clover, and fixes a significant portion of its own nitrogen through the symbiotic relationship with the nitrogen-fixing Bradyrhizobia bacteria (*Bradyrhizobium japonicum*) that live in the nodules on its roots. Bradyrhizobia are unicellar, microscopic bacteria that invade the soybean plant through its root hairs (Hoeft et al., 2000b). The plant responds to this invasion by forming nodules which contain colonies of bacteria. Once established on the soybean root, bacteria in the nodule take gaseous nitrogen from the atmosphere and fix it in forms easily used by the soybean plant. Since these bacteria are not native to U.S. soils and would not normally be found in these soils, inoculation of the soybean seed with these bacteria is recommended when soybean has not been grown in a field for three to five years. Nitrogen fertilizer applications at planting generally do not improve yield and decrease nodulation while increasing the plant's dependency on the soil for nitrogen (Pedersen, 2008f). Therefore, nitrogen fertilizer is seldom applied prior to planting a soybean crop.

Soil tests are the only reliable way to determine the pH, phosphorus, and potassium levels in the soil. Liming and fertilizer requirements subsequently are determined based on soil test results. Ideal soil test results for corn are also ideal for soybean (Scott and Aldrich, 1970). In corn-soybean rotations in the Midwest, phosphorus and potassium fertilizers are applied

prior to a corn crop in accordance with soil test recommendations, but are seldom applied prior to a soybean crop. However, in some of the southern growing areas, differences in crop rotations and soil types may require a fertilizer application prior to planting soybean.

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

Because soybean growth is so dependent on day/night length, different varieties are developed for different latitudes. In the U.S. ten geographically-designated "maturity groups" originally defined by Scott and Aldrich (1970) are widely used. Maturity groups are often designated by Arabic rather than Roman numerals, so the sequence is 00 to 8, and there are subdivisions within the major maturity groups. These are designated by a decimal value. For example, a variety with maturity group designation 2.9 would be at the northern end of Group II (2). These maturity groups are mapped as bands extending from north to south, beginning with Group 00 at the far north and ending with Group VIII in the far south.[4] Groups 00 and 0 are the earliest maturity groups and are adapted best to the area north of latitude 46º North. Succeeding groups are adapted further south with Groups I and II within latitudes 41º and 46º North, and Group III within latitudes 38º and 41º North. Group 00 through Group IV soybean varieties are planted in the Midwest and Eastern Coastal regions. Groups II, III and IV, which extend from approximately the northern border of Iowa to the southern tip of Illinois, account for approximately 76% (24%, 36%, and 16%, respectively) of the soybean planted in the U.S. (T. Schlueter, personal communication, August 2008). Because day length delays maturity, a soybean cultivar suited to a southern maturity group would mature too late if planted too far north. Conversely, a northern cultivar would mature too early if planted in the south (Heatherly and Elmore, 2004).

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

VIII.D.2. Planting and Early Season

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

[4] They were originally designated north to south as Groups I through VIII. Groups 0 and 00 were later added to the north.

the impact of certain weather conditions, insect pests, and diseases on soybean yield is dependent on growth stage. The system of soybean growth stages divides plant development into vegetative (V) and reproductive (R) stages (Pedersen, 2004). The vegetative stages begin with VE, which designates emergence. V stages continue and are numbered according to how many fully developed trifoliate leaves are present (*i.e.*, V1, V2, etc.). The reproductive (R) stages begin at flowering (R1) and include pod development and plant maturation. Full maturity is designated as R8.

The time of onset and the duration of the various growth stages in soybean are highly dependent on photoperiod (hours of daylight and darkness) and temperature (Major et al., 1975), and therefore, for the same soybean plant grown at different latitudes, the onset and duration of the growth stages and the total time from planting to maturity would be different. Also, in contrast to most other temperate-season crops, soybean is a "short-day" plant, meaning that maturity is delayed by longer day length (Major, 1980). In soybeans, flowering is initiated only after the night is longer (and days grow shorter) than a critical length (Holshouser, 2010). Once flowering begins, temperature controls the duration of flowering time (Heatherly and Elmore, 2004).

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

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

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

Soybean has the ability to produce good yield over a wide range of plant populations. Most soybean varieties have the ability to branch and adjust the number of pods on branches to compensate for large differences in seeding rate. Maximum yields generally require planting rates that result in about 2.5 to 5 plants per square foot (Hoeft et al., 2000b). Therefore, a full stand of soybean is approximately eight to ten plants per foot of row at harvest for 40-inch rows, six to eight plants per foot of row in 30-inch rows, four to six plants in 20-inch rows, and two to three plants in 10-inch rows. This translates to 109,000 to 218,000 plants per acre at harvest. Higher populations are recommended in narrow rows for maximum yields because plants are more uniformly spaced in narrow rows. Seeding rates are generally 10 to 25% higher than the desired harvest population, especially in no-till fields, to account for the losses in germination, emergence, and seedling diseases. The accuracy of the planting equipment also can impact the decision on seeding rate. Soybean seed traditionally has been sold by weight. Therefore, the grower must know the number of seeds per pound for the particular soybean varieties being planted for accurate seeding rates.

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

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

VIII.D.3. Mid to Late Season

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

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

progress upward. Flowering on determinate plants begins at the eight or tenth node and progresses both downward and upward.

The first appearance of flowers signals the beginning of the reproductive stage, namely the R1 stage (Hoeft et al., 2000b). The reproductive period consists of flowering, pod set, and seed formation. Climatic conditions such as temperature and moisture supply during the flowering period will affect the number of flowers. The soybean plant does not form a pod from each flower. It is common for the soybean plant to have 75% of the flowers fail to develop a pod (Scott and Aldrich, 1970). This characteristic makes soybean less susceptible than corn to short periods of adverse weather during flowering. Under normal conditions, pod set occurs over about a three week period. Good soil moisture is most critical during the pod-filling stages to prevent pod abortion and to ensure high yields (Hoeft et al., 2000b). Another critical requirement during the seed-filling stages is a high rate of photosynthesis to maximize yield. High humidity and temperatures during seed development and maturity can result in poor seed quality because these conditions promote the development of reproductive-stage diseases.

VIII.D.4. Harvest Season

When dry matter accumulation ends, the plant is considered to be physiologically mature. The seed moisture content is approximately 55 to 60% at this stage (Hoeft et al., 2000b). 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., 2000b). Soybean can be harvested when the moisture content drops below 15%. However, soybean should be at 13% moisture to be stored without artificial drying (Scott and Aldrich, 1970). Moisture content below 12% may increase seed cracking and seed coat damage.

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

A larger than normal crop can stress the storage and transportation system for the crop. Because of the very high variability in crop production, storage facilities are not always adequate. Soybeans and other grain must sometimes be stored in temporary structures or in other existing buildings if storage facilities are overloaded, and this may result in additional costs for constructing or renting temporary facilities and/or potential losses from exposure (Dorn, 2011; Hellevang, 1998). The same conditions can result when prices are low and growers want to hold on to their crops in the hopes of selling at higher prices (Maier and Wilcke, 2011). Soybeans can also compete with corn for available storage space; and while corn can be stored on the ground, soybeans rarely are (Hurburgh, 2005). University extensions provide practical guidelines for temporary storage of soybeans and other crops (Dorn, 2011; Harner et al., 1998; Hellevang, 1998; Hurburgh, 2005; Maier and Wilcke, 2011).

Growers decide where to sell their soybeans based on the cost of delivering soybeans to their customer, usually an elevator or processing facility (USSEC, 2009). Growers usually deliver their soybeans to the sale point using their own trucks. From the elevator or processing facility, the soybeans or oil and meal are shipped by rail, barge or truck. Approximately 24% of soybeans are transported by rail, although higher percentages of meal and soybean oil are transported by rail (STC, 2010). More than half of U.S. soybean exports are first shipped by barge on the Mississippi River (Ash et al., 2006). USDA reported in 2006 that recent record-large soybean harvests have tested the capacity of the U.S. bulk transportation system; however, no specifics were provided (Ash et al., 2006). Large crops can result in greater shipping competition and higher shipping costs, which translates to lower prices offered to growers (Ash et al., 2006).

VIII.E. Management of Insects

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

Characterizing soybean responses to insect injury is essential in establishing economic injury levels (Way, 1994). Most often, soybean insects are categorized or defined by the plant parts they injure, namely root-feeding, stem-feeding, leaf-feeding, or pod-feeding insects. The root- and stem-feeding insect groups are often the hardest to scout and typically are not detected until after they have caused their damage. The leaf-feeding insects comprise the biggest group of soybean insect pests, but not necessarily the most economically damaging insects. Research on defoliation has determined that a major effect of leaf injury is to reduce light interception by the soybean canopy which in turn can have a significant effect on yield (Way, 1994). Soybean has an extraordinary capacity to withstand considerable defoliation early in the season without significant yield loss. By contrast, defoliation during the flowering and pod filling stages poses a greater threat to yield because the soybean plant has less time to compensate for injury compared to other growth stages. Research indicates that the soybean plant can sustain a 35% leaf loss prior to the pre-bloom period without lowering yield (NDSU, 2002). However, from pod-set to maturity, the plant can tolerate only a 20% defoliation level before yield is impacted.

VIII.F. Management of Diseases and Other Pests

More than 100 pathogens are known to affect soybean, of which 35 are considered to be of economic importance (Bowers and Russin, 1999). The estimated yield losses to soybean diseases in the U.S. were 10.9, 11.9, and 14.0 million metric tons in 1996, 1997, and 1998, respectively (Wrather et al., 2001), which equated to 16.7%, 16.0% and 18.6% of total soybean production, respectively. Pathogens can affect all parts of the soybean plant,

resulting in reduced quality and yield. The extent of losses depends upon the pathogen, the state of plant development and health when infection occurs, the severity of the disease on individual plants, and the number of plants affected (Bowers and Russin, 1999).

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

According to field surveys conducted in fifteen soybean-producing states during 1996 to 1998, soybean cyst nematode (SCN), *Heterodera gylcines*, caused the greatest soybean yield losses (Wrather et al., 2001). *Phytophthora* root and stem rot (*Phytophthora sojae*), brown stem rot (*Phialophora gregata*), *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*), and seedling diseases followed in economical importance. As expected, yield losses varied by region. *Sclerotinia* stem rot caused yield losses in several Northern states, but not in other states. *Rhizoctonia* foliar blight losses were greatest in Arkansas, Louisiana, and Texas where humidity and temperature conditions are suitable for disease development.

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

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

High-quality seed is essential for controlling seedling diseases. The most important seedling diseases in soybean are *Phytophthora sp*., *Pythium sp*., *Rhizoctonia sp*., and *Fusarium sp*. (Pedersen, 2008e). Many soybean varieties demonstrate resistance to specific

taxonomic races of *Phytophthora*. Treating soybean seed with a fungicide (*e.g.*, metalaxyl or mefenoxam) is effective against damping-off disease (seedling blight) caused by common soil fungi, such as *Phytophthora sp.* and *Pythium sp*. Fungicide seed treatments are recommended where there is a history of these seedling diseases.

Asian soybean rust is a foliar fungal disease that typically infests soybean during reproductive stages of development and can cause defoliation and reduce yields significantly in geographies such as Brazil (Dorrance et al., 2007b). Soybean rust is caused by the fungus *Phakopsora pachyrhizi*. This disease in the U.S. was first detected in Louisiana in 2004 (LSU, 2010). At this time, foliar application of fungicides is the standard disease-management practice to limit yield losses due to soybean rust.

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

VIII.G. Weed Management

Annual weeds are perceived to be the greatest pest problem in soybean production, followed by perennial weeds (Aref and Pike, 1998b). Soybean insects and diseases are rated less problematic but may reach economic thresholds requiring treatment. Weed control in soybean is essential to optimizing yields. Weeds compete with soybean for light, nutrients, and soil moisture. Weeds can harbor insects and diseases, and also can interfere with harvest, causing extra wear on harvest equipment (Pedersen, 2007). The primary factors affecting soybean yield loss from weed competition are the weed species, weed density, and the duration of the competition. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001b). Generally, the competition between crops and weeds increases with higher levels of weed density. The time period that weeds compete with the soybean crop influences the level of yield loss. In general, early season weed competition will have the greatest negative impact on yield (Dalley et al., 2001b). Although, soybean plants withstand early-season weed competition longer than corn without affecting yield, and the canopy closes earlier in soybean than corn. In addition, canopy closure is much sooner when soybean is drilled or planted in narrow rows.

Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in soybean. Foxtail spp. (*Setaria spp.*), pigweed (*Amaranthus spp*.), velvetleaf (*Abutilon theophrasti*), lambsquarters (*Chenopodium album*), and cocklebur (*Xanthium strumarium*) are common weeds in Midwest corn and soybean fields. However, growers consider giant ragweed (*Ambrosia artemisiifolia*), lambsquarters, Canada thistle (*Cirsium arvense)*, cocklebur, and velvetleaf to be the top five most problematic weeds in corn and soybean because of difficulty controlling these weeds (Nice and Johnson, 2005). In a recent survey of growers utilizing glyphosate-tolerant crops, pigweed, morningglory (*Ipomoea spp*.), Johnsongrass (*Sorghum halepense*), ragweed spp. (*Ambrosia spp*.), foxtail, and velvetleaf were mentioned as the most problematic weeds, depending on the state and

cropping system (Kruger et al., 2009). With the exception of morningglory and pigweed, the weed species identified as problematic were present and problematic before glyphosate-tolerant crops were introduced, but then were to a reduced degree after implementing glyphosate-tolerant cropping systems (Kruger et al., 2009). Common waterhemp (*Amaranthus rudis*) and ragweed were the most frequently mentioned problematic weeds in glyphosate-tolerant crops in Illinois, Indiana and Iowa.

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

Cultural and mechanical weed control practices can be important components of an effective weed management program (Loux et al., 2010). Crop rotation, narrow row spacing and planting date are a few of the crop management practices that are implemented to provide the crop with a competitive edge over weeds. Although the primary purpose of tillage is for seedbed preparation, tillage is still used to supplement weed control with selective herbicides in soybean production. Approximately 98% of the soybean acreage received an herbicide application in 2006, indicating the importance of excellent weed control in maximizing soybean yield (USDA-NASS, 2007).

Herbicide-tolerant soybean was introduced to provide growers with additional options to improve crop safety and/or improve weed control. The Roundup Ready soybean system (planting Roundup Ready soybean and applying glyphosate in crop to provide primary weed control) was introduced in 1996 and has become the standard weed control program in U.S. soybean production and is utilized on 91% of U.S. soybean acreage (USDA-NASS, 2009).

VIII.H. Crop Rotation Practices in Soybean

The well-established farming practice of crop rotation is still a key management tool for growers. The purpose of growing soybean in rotation is to improve yield and profitability of one or both crops over time, decrease the need for nitrogen fertilizer on the crop following soybean, increase residue cover, mitigate or break disease, insect, and weed cycles, reduce soil erosion, increase soil organic matter, improve soil tilth and soil physical properties, and reduce runoff of nutrients, herbicides, and insecticides (Al-Kaisi et al., 2003; Heatherly and Elmore, 2004). According to the USDA Economic Research Service, 95% of the soybean-planted acreage has been in some form of a crop rotation system since 1991 (USDA-ERS, 2001). Corn- and wheat-planted acreage has been rotated at a slightly lower level of 75% and 70%, respectively. Although the benefits of crop rotations can be substantial, the grower must make cropping decisions by evaluating both the agronomic and economic returns of various cropping systems. Crop rotations also afford growers the opportunity to diversify farm production in order to minimize market risks.

Continuous soybean production is not a common practice in the Midwest and is discouraged by most extension soybean specialists to reduce the risk of damage from diseases and

nematodes (Al-Kaisi et al., 2003; Hoeft et al., 2000b). 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. The yield of both corn and soybean is approximately 10% higher when grown in rotation than when either crop is grown continuously (Hoeft et al., 2000b).

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

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

VIII.I. Soybean Volunteer Management

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

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

major rotational crops. These results indicate that herbicides which are effective for the control of volunteer soybean will control MON 87712.

Table VIII-4. Ratings for Postemergence Control of Volunteer Soybean in Labeled Rotational Crops[1]

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

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

VIII.J. Stewardship of MON 87712

Monsanto Company develops effective products and technologies and is committed to assuring that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship℠ (ETS) Program[5]. These policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides[6] that are distributed annually to growers who utilize Monsanto branded traits.

As an integral action of fulfilling this commitment, Monsanto will seek biotechnology regulatory approvals for MON 87712 in all key soybean import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. These actions will be consistent with the Biotechnology Industry Organization (BIO) Policy on Product Launch[7]. Monsanto continues to monitor other countries that are key importers of soybean from the U.S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions.

Monsanto also commits to best industry practices on seed quality assurance and control to ensure the purity and integrity of MON 87712 seed. As with all of Monsanto's products, before commercializing MON 87712 in any country, a MON 87712 detection method will be made available to soybean producers, processors, and buyers.

VIII.K. Impact of the Introduction of MON 87712 on Agricultural Practices

Introduction of MON 87712 is expected to have minimal impact on current cultivation and management practices for soybean. MON 87712 has been shown to be no different from conventional soybean in its agronomic, and compositional characteristics (refer to Sections VI and VII), and has the same levels of susceptibility to insects and diseases as commercial soybean. The increase in yield and small delay in senescence is expected to have minimal impacts if any on the agricultural practices farmers use to produce a soybean crop. MON 87712 did not require any additional inputs to produce a crop and varieties that contain MON 87712 are similarly not expected to require additional inputs. Farmers understand the value of increased yield for their farm's productivity and profitability, and are accustomed to the incremental yield improvements for varieties obtained through traditional breeding. Growers are also accustomed to field-to-field or year-to-year yield variation based on environmental conditions and the varieties they

---

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

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

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

select for planting. Therefore, farmers are capable of adjusting harvesting and storage equipment to handle increased yields. MON 87712 will provide another option for farmers to pursue better yielding varieties for their farm. MON 87712 offers the potential to improve productivity in the U.S. soybean production system, thereby helping to meet the growing global demand for soybean.

IX. ENVIRONMENTAL CONSEQUENCES

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 87712 and its impact on current agronomic practices. USDA-APHIS has responsibility, under the Plant Protection Act (PPA) (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition may be granted, thereby allowing unrestricted introduction of the article.

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

The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the article in question is not likely to pose a plant pest risk. The approach used to assess the plant pest potential of MON 87712 is a weight of the evidence approach based primarily on eight lines of evidence: 1) insertion of a single functional copy of the BBX32 expression cassette, 2) characterization of BBX32 protein expressed in MON 87712, 3) safety of BBX32 in MON 87712, 4) compositional equivalence of harvested MON 87712 seed and forage to conventional soybean, 5) phenotypic, agronomic, and environmental interaction characteristics demonstrating no increased plant pest potential compared to conventional soybean, 6) negligible risk to Non Target Organisms (NTO) and threatened or endangered species, 7) familiarity with soybean as a cultivated crop and the inherently low plant pest potential of soybean, and (8) no unexpected impact to agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than conventional soybean.

Using the assessment above, the data and analysis presented in this petition lead to a conclusion that MON 87712 is unlikely to be a plant pest and, therefore, should no longer be subject to regulation under 7 CFR § 340.

In 2008, APHIS proposed amendments to 7 CFR Part 340 that included provisions to utilize its noxious weed authority in regulating genetically engineered plants (73 FR 60008). Because the data presented in this petition demonstrate that MON 87712 has no potential to cause injury or damage to protected interests under the noxious weed authority, MON 87712 would not be considered a "noxious weed" as defined by the Plant Protection Act.

IX.B. Plant Pest Assessment of MON 87712 Insert and Expressed Protein

This section summarizes the details of the genetic insert, characteristics of the genetic modification, and safety and expression of the BBX32 protein in MON 87712 used in food, feed, and environmental safety evaluation of MON 87712.

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

IX.B.1.1. Genetic Insert

MON 87712 was developed through *Agrobacterium tumefaciens*-mediated transformation of conventional soybean A3525 meristem tissue utilizing transformation plasmid vector PV-GMAP5779, a binary vector containing two transfer DNA (T-DNA I and T-DNA II) (Figure III-1). Each T-DNA in PV-GMAP5779 is delineated by Left and Right Border regions to facilitate transformation. T-DNA I contains the *BBX32* coding sequence under regulation of the *e35S* promoter and the *E6* 3′ untranslated region while T-DNA II contains the *cp4 epsps* coding sequence under the regulation of the *FMV/EF-1α* promoter, *EF-1α* leader, *EF-1α* intron, *CTP2* targeting sequence, and the *E9* 3′ untranslated region. During transformation, both T-DNAs were inserted into the soybean genome (Section III.B) where T-DNA II, containing the *cp4 epsps* expression cassette, functioned as a marker gene for the selection of transformed plantlets. Subsequently, conventional self-pollinated breeding methods and segregation, along with a combination of analytical techniques, were used to isolate those plants that contain the *BBX32* expression cassette (T-DNA I) but not the *cp4 epsps* expression cassette (T-DNA II) resulting in the production of marker-free MON 87712. The promoter, leader, and border sequences of T-DNA I are not known to cause plant disease. Furthermore, these sequences are well characterized, are noncoding regions, and will not cause MON 87712 to promote plant disease.

Molecular characterization of MON 87712 by Southern blot analyses confirmed that one copy of the *BBX32* expression cassette was integrated into the soybean genome at a single locus. No T-DNA II or backbone DNA sequences from plasmid vector PV-GMAP5779 were detected in MON 87712. Additionally, the data confirmed the organization and sequence of the insert and demonstrated the stability of the insert over several generations. These data demonstrated that there are no unintended changes in the MON 87712 genome as a result of the insertion of the *BBX32* expression cassette, and supports the overall conclusion that MON 87712 is unlikely to be a plant pest.

IX.B.1.2. Protein Safety

MON 87712 is a biotechnology-derived soybean that has been demonstrated to provide increased yield (See Appendix B), due to the insertion of the *Arabidopsis thaliana BBX32* gene. *Arabidopsis thaliana* is generally not considered an allergenic or toxic source organism. Although *Arabidopsis thaliana* contains homologs of proteins previously described as allergens in other plant species (e.g., germins, lipid transfer protein, profilins, and small molecular weight calcium binding proteins), no Arabidopsis proteins have been reported in a peer-reviewed database of known allergens.

BBX32 is a member of a family of B-box-containing proteins. B-box zinc finger family is found in many plant species including soybean. Additionally, bioinformatic searches using the BBX32 amino acid sequence as a query yield homologous sequences from several different plant species, including the food crops citrus, grape, apple, soybean, rice, lettuce, and corn. Overall protein sequence identity of BBX32 to homologs in these food species range from ~43-31%. Further, the overall protein sequence identity of BBX32 to its homolog in canola is 66%, indicating that *Brassicaceae* species contain proteins very similar to BBX32.

The levels of BBX32 in various tissues of MON 87712 that are relevant to the risk assessment were determined by western blot analysis. BBX32 protein levels in MON 87712 across tissue types ranged from <LOD to 110 ng/g dwt. The western blot method developed was highly sensitive as indicated by the low LODs established for each tissue (Table V-1). In spite of the high sensitivity of the western technique BBX32 protein levels in many samples, including all seed, forage and OSL-4 samples, were <LOD or not detected, indicating that the expression levels in these tissues are very low.

Taken together, the low level of BBX32 protein expressed in MON 87712 tissues along with the presence of homologous sequences of BBX32 in several plant species including soybean, support the conclusion that food and feed products containing or derived from MON 87712 are as safe for human and animal consumption as soybean currently on the market. Therefore, unintended environmental effects are not anticipated from dietary exposure to BBX32 in MON 87712, and support the overall conclusion that MON 87712 is unlikely to be a plant pest

IX.B.2. Compositional Characteristics

Detailed compositional analyses in accordance with OECD guidelines were conducted to assess whether levels of key nutrients and anti-nutrients in MON 87712 were comparable to levels present in the conventional control A3525 and several conventional commercial reference varieties. Seed and forage were harvested from eight individual sites in which MON 87712, the conventional control A3525, and a range of conventional commercial reference varieties were concurrently grown in the same field trial. The conventional commercial reference varieties were used to establish a range of natural variability for the key nutrients and anti-nutrients in conventional soybean varieties that have a history of safe consumption.

The combined site analysis was conducted to determine statistically significant differences (5% level of significance) between MON 87712 and the conventional control A3525. The results from the combined-site data were reviewed using considerations relevant to food and feed safety and nutritional quality including the relative magnitudes of the difference in the mean values of nutrient and anti-nutrient components of MON 87712 and the conventional control A3525, whether the MON 87712 component mean value was within the range of natural variability of that component as represented by the 99% tolerance interval of the conventional commercial reference varieties grown concurrently in the same field trial, and analyses of the reproducibility of the statistically significant combined-site component differences at individual sites.

Assessment of the analytical results confirmed that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 87712 soybeans. In addition, the levels of assessed components in MON 87712 were compositionally equivalent to the conventional control A3525 and within the range of variability of conventional commercial soybeans that were grown concurrently in the same field trial. These results support the overall conclusion that MON 87712 is unlikely to alter plant pest potential.

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

Comparative plant characterization data was used to assess whether the introduction of the BBX32 protein altered the plant pest potential of MON 87712 compared to the conventional control A3525 (Section VII). Phenotypic, agronomic, and environmental interaction characteristics of MON 87712 were evaluated and compared to those of the conventional control (Section VII.B). As described below, these assessments included: seed dormancy and germination characteristics; plant growth and development characteristics; observations for abiotic stress response, disease damage, arthropod-related damage; pollen characteristics; arthropod abundance; plant-symbiont interaction; volunteer potential and persistence outside of cultivation characteristics. Results from the phenotypic, agronomic, and environmental interaction assessments, as well as the volunteer potential and persistence outside of cultivation assessments demonstrated that MON 87712 possess neither weedy characteristics, nor increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors, or altered symbiont interactions compared to conventional soybean. Taken together, the results of the analysis support a determination that MON 87712 is no more likely to pose a plant pest risk or have a biologically meaningful change in environmental impact than conventional soybean.

IX.B.3.1. Seed Dormancy and Germination

Seed dormancy and germination characterization demonstrated that MON 87712 seed had germination characteristics not different to those of the conventional control (Section VII.C). In particular, the lack of hard seed, a well-accepted characteristic often associated with plants that are weeds, supports a conclusion of no increased weediness or plant pest potential of MON 87712 compared to conventional soybean.

IX.B.3.2. Plant Growth and Development

Evaluations of plant growth and development characteristics in the field are useful for assessing potential weediness characteristics such as lodging and pod shattering (Section VIII). No statistically significant differences were detected ($\alpha = 0.05$) between MON 87712 and the conventional control A3525 for seedling vigor, days to 50% flowering, days to 50% end of flowering, plant height, lodging, pod shattering, grain moisture, or 100 seed weight (Table VII-4). Five statistically significant differences were detected between MON 87712 and the conventional control A3525 for early stand count, days to 50% senescence, days to physiological maturity, final stand count, and yield. Although significantly different from the conventional control A3525, the mean values of MON 87712 for early stand count and final stand count were within the range of

commercial reference varieties for each characteristic and thus would not be adverse in terms of pest potential. Differences in days to 50% senescence, days to physiological maturity, and yield were consistent with the mode of action. The increase in yield is agronomically desirable and would not contribute to increased weediness potential of MON 87712 without changes in a combination of other characteristics associated with weediness (Baker, 1974). Thus, the observed differences are not considered to be biologically meaningful in terms of increased weediness or plant pest potential of MON 87712 compared to conventional soybean.

IX.B.3.3. Response to Abiotic Stressors

No biologically meaningful differences were observed during comparative field observations between MON 87712 and the conventional control A3525 and responses to abiotic stressors, such as drought, mineral and nutrient toxicity, and temperature stress (Section VIII). The lack of significant biologically meaningful differences in the MON 87712 response to abiotic stress support the conclusion that the introduction of the BBX32 protein is unlikely to result in increased weediness or plant pest potential compared to conventional soybean.

IX.B.3.4. Pollen Morphology and Viability

Evaluations of pollen morphology and viability from field-grown plants provide information useful in a plant pest assessment as it relates to the potential for gene flow and introgression of the biotechnology-derived trait into other soybean varieties and wild relatives. Pollen morphology and viability evaluations demonstrated no statistically significant differences between MON 87712 and the conventional control A3525. These comparative assessments indicate that MON 87712 is not likely to have increased plant pest potential compared to conventional soybean.

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

Evaluation of MON 87712 for potential adverse impacts on NTOs is a component of the plant pest risk assessment. Since MON 87712 is a product with no pesticidal activity, all organisms that interact with MON 87712 are considered to be NTOs. In a 2009 U.S. phenotypic and agronomic assessment, observational data on environmental interactions were collected for MON 87712 and the conventional control A3525. In addition, multiple commercial reference varieties were included in the analysis to establish a range of natural variability for each characteristic. The environmental interactions assessment (Section VIII) included data collected on plant-arthropod and plant-disease interactions. The results of this assessment indicated that the presence of BBX32 did not meaningfully alter plant-arthropod interactions, including beneficial arthropods and arthropod pests, nor did it alter disease susceptibility of MON 87712 compared to conventional soybean. The lack of meaningful differences in disease damage, arthropod-related damage, and pest and beneficial arthropod abundance demonstrate that the introduction of the BBX32 in MON 87712 is unlikely to be biologically meaningful in terms of increased plant pest potential.

In the field, soybean forms a complex symbiotic relationship with members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae*. This symbiosis results in the formation of root nodules in which the bacteria reduce or fix atmospheric nitrogen-producing ammonia that can be used by the plant. MON 87712 was assessed for changes in the symbiotic relationship with *B. japonicum* relative to the conventional control A3525 by evaluating shoot total nitrogen, nodule number, and nodule, root, and shoot dry weights (Section VIII). No statistically significant differences were detected between MON 87712 and the conventional control A3525 for the parameters measured, indicating no impact on either the symbiotic relationship or the symbiotic nitrogen-fixing bacteria. These data support a conclusion of no change in plant pest potential and no expected impact to cultivation practices relative to nitrogen inputs for MON 87712 compared to conventional soybean.

The potential for MON 87712 to affect NTOs was evaluated using a combination of biochemical information and experimental data. The biochemical information and experimental data included molecular characterization, safety assessments of BBX32, results from the environmental assessment described above, and the demonstration of compositional, agronomic and phenotypic equivalence to conventional soybean. Taken together, these data support the conclusion that MON 87712 is unlikely to adversely affect NTOs, or pose an additional risk to threatened and endangered species above those posed by the cultivation of conventional soybean.

Furthermore, according to APHIS, the only listed threatened or endangered animal that occupies a habitat where it is likely to include soybean fields, and that might feed on soybean, is the federally endangered Delmarva Peninsula Fox Squirrel (*Sciurus niger cinereus*), found in areas of the mid-Atlantic Eastern seaboard (USDA-APHIS, 2007). It is known to utilize certain agricultural lands readily, but its diet includes acorns; nuts/seeds of hickory, beech, walnut, and loblolly pine; buds and flowers of trees; fungi; insects; fruit; and an occasional bird egg (NatureServe, 2010). The safety of the BBX32 protein in MON 87712, the compositional, agronomic and phenotypic equivalence of MON 87712 to conventional soybean, and the diversity of the Fox Squirrel diet, support a conclusion that no biologically significant changes to the habitat or diet of the Delmarva Peninsula Fox Squirrel are expected. Consequently, the planting of MON 87712 is not expected to affect the Delmarva Peninsula Fox Squirrel.

IX.B.3.4. Volunteer Potential and Persistence Outside of Cultivation

Evaluations of volunteer potential and persistence outside of cultivation from field-grown plants provide information useful in assessing potential weediness characteristics of MON 87712 compared to the conventional control A3525 (Section VII.C). Volunteer potential evaluations demonstrated no statistically significant differences between MON 87712 and the conventional control A3525. The persistence outside of cultivation evaluations demonstrated a few statistically significant differences between MON 87712 and the conventional control A3525, however these differences were not seen across the individual sites and were small in magnitude. Taken together, these comparative assessments indicate that MON 87712 is not likely to have increased weediness or plant pest potential compared to conventional soybean.

IX.C. Weediness Potential of MON 87712

The commercial *Glycine* species in the U.S. (*Glycine max L.*) does not exhibit weedy characteristics and is not effective in invading established ecosystems. Soybean is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Soybean does not possess any of the attributes commonly associated with weeds (Baker, 1974), such as the ability to disperse, invade, and become a dominant species in new or diverse landscapes or the ability to compete well with native vegetation. Due to the lack of dormancy, which is a trait that has been removed from soybean through commercial breeding, soybean seed can germinate quickly under adequate temperature and moisture conditions, and potentially grow as volunteer plants. However, the volunteer potential evaluation demonstrated that plants of MON 87712 were killed by frost during autumn or winter of the year they were produced. If they did become established, volunteer plants would not compete well with the succeeding crop, and could be controlled readily by either mechanical or chemical means (OECD, 2000). In addition, since wild populations of *Glycine* species are not known to exist in the U.S., the potential does not exist for MON 87712 to outcross to wild or weedy relatives and to alter their weediness potential.

In comparative studies between MON 87712 and the conventional control A3525, phenotypic, agronomic, and environmental interaction data were evaluated (Section VII) for changes that would impact the plant pest potential and, in particular, plant weediness potential. Results of these evaluations show that there is no biologically meaningful difference between MON 87712 and the conventional control A3525 for characteristics potentially associated with weediness. Furthermore, comparative field observations between MON 87712 and its conventional control A3525 in their response to abiotic stressors, such as drought, mineral and nutrient toxicity, and temperature stress, indicated no biologically meaningful differences and, therefore, no increased weediness potential. Data on environmental interactions also indicate that MON 87712 does not confer any biologically meaningful increased susceptibility or tolerance to specific diseases or insect pests. Collectively, these findings support the conclusion that MON 87712 has no increased weediness potential compared to conventional soybean.

IX.D. Potential for Pollen Mediated Gene Flow

Gene introgression is a process whereby one or more genes successfully integrate into the genome of a recipient plant population. Introgression is affected by many factors, including the frequency of the initial pollination event, environmental factors, sexual compatibility of pollen donor and recipient plants, pollination biology, flowering phenology, hybrid stability and fertility, selection, and the ability to backcross repeatedly. Because gene introgression is a natural biological process, it does not constitute an environmental risk in and of itself (Sutherland and Poppy, 2005). Gene introgression must be considered in the context of the transgene(s) inserted into the biotechnology-derived plant, and the likelihood that the presence of the transgene(s) and their subsequent transfer to recipient plants will result in increased plant pest potential. The potential for gene introgression from MON 87712 is discussed below.

The assessment for gene introgression from MON 87712 with other cultivated or wild relatives of soybean, discussed in detail below, indicates that MON 87712 is no more likely to become a weed than conventional soybean, and MON 87712 is expected to be similar to conventional soybean regarding its potential for and impacts from gene flow. Soybean lacks sexually-compatible relatives in the U.S.; therefore, the only pollen-mediated gene flow would be within cultivated soybean.

IX.D.1. Hybridization with Cultivated Soybean

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

Numerous studies on soybean cross-pollination have been conducted, and the published results, with and without supplemental pollinators, are summarized in Table IX-1. Under natural conditions, cross-pollination among adjacent plants in a row or among plants in adjacent rows ranged from 0 to 6.3%. In experiments where supplemental pollinators (usually bees) were added to the experimental area, cross-pollination ranged from 0.5 to 7.74% in adjacent plants or adjacent rows. However, cross-pollination does not occur at these levels over long distances. Cross-pollination rates decrease to less than 1.5% beyond one meter from the pollen source, and rapidly decrease with greater distances from the source. The following cross-pollination rates at extended distances have been reported: 0.05% at 5.4 meters (Ray et al., 2003), 0% at 6.5 meters (Abud et al., 2003), 0% at 10.5 meters (Yoshimura et al., 2006), and 0.004% at 13.7 meters of separation (Caviness, 1966).

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

The consequence of introgression of BBX32 from MON 87712 into other soybean is negligible since soybean gene flow is naturally low; therefore the presence of BBX32 confers no increased plant pest potential to cultivated soybean.

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

The subgenus *Soja* includes the cultivated soybean *Glycine max* and the wild annual species *Glycine soja*. *Glycine soja* is found in China, Taiwan, Japan, Korea, and Russia (Hymowitz, 2004; Lu, 2004). Hybridization between female *G. soja* and male *G. max* was less successful than hybridization in the opposite direction (Dorokhov et al., 2004), where frequency of spontaneous cross pollination in reciprocal combinations of *G. max*

and *G. soja* varied from 0.73 (♀ *G. soja* × ♂ *G. max*) to 12.8% (♀ *G. max* × ♂ *G. soja*). Species relationships in the subgenus *soja* indicated that F_1 hybrids of *G. max* and *G. soja* carry similar genomes and are fertile (Singh and Hymowitz, 1989). Abe et al. (1999) note that "natural hybrids between *G. max* and *G. soja* are rare and hybrid swarms involving both species have never been reported." This is also supported by work from Kuroda et al. (2008) in which molecular markers were used and no gene flow from *G. max* to *G. soja* was detected. Many barriers to natural hybridization exist between soybean and wild relatives, including the highly selfing nature of both plants, required proximity of wild soybean to cultivated soybean, synchrony of flowering, and presence of pollinators. As such, it is highly unlikely that naturally occurring, pollen-mediated gene flow and transgene introgression into wild soybean relatives from incidentally released biotechnology-derived soybean will occur at any meaningful frequency.

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

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

Wild perennial species of the *Glycine* subgenus occur in Australia; West, Central and South Pacific Islands; China; Papua New Guinea; Philippines; and Taiwan (Hymowitz et al., 1992; Hymowitz and Singh, 1992). Therefore, the only opportunities for inter-subgeneric hybridization would occur in areas where those species are endemic. Nonetheless, the likelihood of interspecific hybridization between *G. max* and the wild perennial *Glycine* species is extremely low because they are genomically dissimilar (Hymowitz, 1970; Lu, 2004) and pod abortion is common. From time to time, immature seeds of the crosses have been germinated aseptically *in vitro*, but the resulting F1 hybrids are slow-growing, morphologically weak, and completely sterile. Their sterility is due to poor chromosome pairing. Furthermore, species distantly related usually produce nonviable F1 seeds that either have premature death of the germinating seedlings or suffer from seedling and vegetative lethality (Kollipara et al., 1993). In North and South America, it is not possible for gene transfer to occur between cultivated soybean and wild perennial species of *Glycine* subgenera because these wild species do not exist in these regions.

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

Monsanto is unaware of any reports regarding the unaided transfer of genetic material from soybean species to other sexually-incompatible plant species. The likelihood for horizontal gene flow to occur is exceedingly small. Therefore, potential ecological risk

associated with horizontal gene flow from MON 87712 is not expected. The consequence of horizontal gene flow of the *BBX32* from MON 87712 into other plants that are sexually-incompatible is negligible since, as data presented in this petition confirm, the gene and trait confer no increased plant pest potential to soybean. Thus in the highly unlikely event that horizontal gene transfer were to occur, the presence of *BBX32* would not be expected to increase pest potential in the recipient species.

IX.E. Potential Impact on Soybean Agronomic Practices

An assessment of current soybean agronomic practices was conducted to determine whether the cultivation of MON 87712 has the potential to impact current soybean and weed management practices. Soybean fields are typically highly managed agricultural areas that are dedicated to crop production. MON 87712 is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 87712 to growers. Cultivation of MON 87712 is not expected to differ from typical soybean cultivation.

MON 87712 is comparable to conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics and has levels of resistance to insects and diseases comparable to conventional soybean. Therefore, no significant impacts on current cultivation and management practices for soybean are expected following the introduction of MON 87712. Based on this assessment, the introduction of MON 87712 is expected to have minimal impact on current U.S. soybean cultivation practices or weed management practices.

IX.F. Summary of Plant Pest Assessments

Plant pests are defined in the Plant Protection Act as certain living organisms that can directly or indirectly injure, cause damage to, or cause disease to any plant or plant product (7 U.S.C. § 7702[14]). Characterization data presented in Sections IV through VIII of this petition confirm that MON 87712 is not different from conventional soybean in terms of pest potential in its phenotypic, agronomic, and environmental interaction characteristics. Monsanto is not aware of any study results or observations associated with MON 87712 that would suggest an increased plant pest potential would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 87712 compared to conventional soybean, followed by a risk assessment on detected differences. The risk assessment considered various factors including: 1) insertion of a single functional copy of the *BBX32* expression cassette, 2) characterization of BBX32 protein expressed in MON 87712, 3) safety of BBX32 in MON 87712, 4) compositional equivalence of harvested MON 87712 seed and forage to conventional soybean, 5) phenotypic, agronomic, and environmental interaction characteristics demonstrating no increased plant pest potential compared to conventional soybean, 6) negligible risk to Non Target Organisms (NTO) and threatened or endangered species, 7) familiarity with soybean as a cultivated crop and the inherently

low plant pest potential of soybean, and 8) no unexpected impact to agronomic practices, including land use, cultivation practices, or the management of weeds diseases, and insects than conventional soybean.

Based on the data and information presented in this petition, it is concluded that, like conventional soybean and currently deregulated biotechnology-derived soybean, MON 87712 is highly unlikely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87712 and any progeny derived from crosses between MON 87712 and other commercial soybean be granted nonregulated status under 7 CFR Part 340.

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

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

X. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto knows of no results or observations associated with MON 87712 or the BBX32 protein indicating that there would be an adverse environmental consequence from the introduction of MON 87712. MON 87712 contains BBX32 protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night processes and results in increased availability of assimilates in the plant resulting in increased yield of MON 87712 when compared to a comparator without the introduced gene. As demonstrated by field results and laboratory tests, the difference between MON 87712 and conventional soybean is the increased yield opportunity in MON 87712.

The data and information presented in this petition demonstrate that MON 87712 is unlikely to pose an increased plant pest risk or have an adverse environmental consequence compared to conventional soybean. This conclusion is based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional soybean, followed by risk assessment on detected differences. The characterization evaluations included molecular analyses, which confirmed the insertion of a single functional copy of the *BBX32* expression cassette at a single locus within the soybean genome. In addition, protein expression analysis demonstrates that BBX32 was detected in leaf and root of MON 87712, and was expressed at levels below the limit of detection in seed and forage. The BBX32 protein produced in MON 87712 is not novel and it has sequence homology with several different plant species, including the food crops citrus, grape, apple, soybean, rice, lettuce, and corn, where a history of safe use is established. Compositional analysis of key nutrients and antinutrients from seed and forage demonstrate that MON 87712 is compositionally equivalent to conventional soybean. Finally, extensive characterization of the plant phenotype and environmental interactions indicate that MON 87712 is comparable to conventional soybean. Therefore, based on the lack of increased pest potential or adverse environmental consequences compared to conventional soybean, the risks for humans, animals, and other NTOs from MON 87712 are negligible under the conditions of use. Additionally, the introduction of MON 87712 will not adversely impact cultivation practices or the management of weeds, diseases, and insects in soybean production systems. Moreover, the increased yield performance of MON 87712 is expected to have minimal impact on any of the agricultural practices farmers use to produce a soybean crop.

Introduction of MON 87712 offers the opportunity to increase soybean yield, which is beneficial to growers and has potential to help global efforts to provide an adequate supply of soybeans, and thus, help sustain a robust domestic and global livestock market for soybean and soybean products.

REFERENCES

AAFCO. 2011. Official names and definitions of feed ingredients. Pages 437-438 in 2011 Official Publication. Association of American Feed Control Officials Incorporated, Charleston, West Virginia.

Abe, J., A. Hasegawa, H. Fukushi, T. Mikami, M. Ohara and Y. Shimamoto. 1999. Introgression between wild and cultivated soybeans of Japan revealed by RFLP analysis for chloroplast DNAs. Economic Botany 53:285-291.

Abrams, R.I., C.R. Edwards and T. Harris. 1978. Yields and cross-pollination of soybeans as affected by honey bees and alfalfa leafcutting bees. American Bee Journal 118:555-558.

Abud, S., P.I. Mello de Souza, C.T. Moreira, S.R.M. Andrade, A.V. Ulbrich, G.R. Vianna, E.L. Rech and F.J. Lima Aragão. 2003. Gene flow in transgenic soybean in the Cerrado region, Brazil. Pesquisa Agropecuária Brasileira 38:1229-1235.

Ahrent, D.K. and C.E. Caviness. 1994. Natural cross-pollination of twelve soybean cultivars in Arkansas. Crop Science 34:376-378.

Al-Kaisi, M. 2001. Value of crop rotation in nitrogen management. Iowa State University, Ames, Iowa. http://www.ipm.iastate.edu/ipm/icm/2001/4-23-2001/valuen.html [Accessed May 12, 2010].

Al-Kaisi, M., M. Hanna and M. Tidman. 2003. Crop rotation considerations for 2004 management season rotation. Iowa State University, Ames, Iowa. http://www.ipm.iastate.edu/ipm/icm/2003/12-15-2003/croprotation.html [Accessed June 19, 2006].

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

Anderson, W.P. 1996. Weed ecology. Pages 27-38 in Weed Science: Principles and Applications. Third Edition. West Publishing Company, St. Paul, Minnesota.

AOSA. 2000. Tetrazolium testing handbook. Contribution no. 29. Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2007. Rules for testing seeds. Association of Official Seed Analysis, Ithaca, New York.

AOSCA. 2009. Seed certification handbook: Including genetic and crop standards, procedures, and AOSCA service programs. Association of Official Seed Certifying Agencies, Moline, Illinois.

Appunu, C. and B. Dhar. 2006. Differential symbiotic response of phage-typed strains of *Bradyrhizobium japonicum* with soybean cultivars. Journal of Microbiology 44:363-368.

Aref, S. and D.R. Pike. 1998a. Midwest farmer's perceptions of crop pest infestations. Agron. J. 90:819-825.

Aref, S. and D.R. Pike. 1998b. Midwest farmers' perceptions of crop pest infestation. Agronomy Journal 90:819-825.

ASA. 2008. Soy Stats 2008. American Soybean Association, St. Louis, Missouri, http://www.soystats.com/2008.

ASA. 2011. Soy stats introduction. American Soybean Association, St. Louis, Missouri. http://www.soystats.com/2011/Default-frames.htm [Accessed June 06, 2011].

Ash, M., J. Livezey and E. Dohlman. 2006. Soybean backgrounder. U.S. Department of Agriculture, Economic Research Service, Washington, D.C.

Axelos, M., C. Bardet, T. Liboz, A. Le Van Thai, C. Curie and B. Lescure. 1989. The gene family encoding the *Arabidopsis thaliana* translation elongation factor EF-1α: Molecular cloning, characterization and expression. Molecular and General Genetics 219:106-112.

Baker, H.G. 1974. The evolution of weeds. Annual Review of Ecology and Systematics 5:1-24.

Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Molecular Biology 2:335-350.

Barry, G.F., G.M. Kishore, S.R. Padgette and W.C. Stallings. 1997. Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. Patent 5,633,435, U.S. Patent Office, Washington, D.C.

Barry, G.F., G.M. Kishore, S.R. Padgette and W.C. Stallings. 2001. Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. Patent 6,248,876, U.S. Patent Office, Washington, D.C.

Beard, B.H. and P.F. Knowles. 1971. Frequency of cross-pollination of soybeans after seed irradiation. Crop Science 11:489-492.

Berman, K.H., G.G. Harrigan, S.G. Riordan, M.A. Nemeth, C. Hanson, M. Smith, R. Sorbet, E. Zhu and W.P. Ridley. 2009. Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. Journal of Agricultural and Food Chemistry 57:11360-11369.

Bond, J.A. and T.W. Walker. 2009. Control of volunteer glyphosate-resistant soybean in rice. Weed Technology 23:225-230.

Bowers, G.R. and J.S. Russin. 1999. Soybean disease management. Pages 233-271 in Soybean Production in the Midsouth. L.G. Heatherly and H.F. Hodges (eds.). CRC Press, Inc., Boca Raton, Florida.

Bradford, K.J. 2006. Methods to maintain genetic purity of seed stocks. Publication 8189. University of California, Oakland, California.

Carpenter, J.E., A. Felsot, T. Goode, M. Hammig, D. Onstad and S. Sankula. 2002. Comparative environmental impacts of biotechnology-derived and traditional soybean, corn, and cotton crops. Council for Agricultural Science and Technology, Ames, Iowa.

CAST. 2007. Implications of gene flow in the scale-up and commercial use of biotechnology-derived crops: Economic and policy considerations. Issue paper 37. Council for Agricultural Science and Technology, Ames, Iowa.

Caviness, C.E. 1966. Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science 6:211-212.

CFIA. 1996. The biology of *Glycine max* (L.) Merr. (Soybean). BIO1996-10. Canadian Food Inspection Agency, Ottawa, Ontario.

Chiang, Y.C. and Y.T. Kiang. 1987. Geometric position of genotypes, honeybee foraging patterns and outcrossing in soybean. Botanical Bulletin of Academia Sinica 28:1-11.

Codex Alimentarius. 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome, Italy. ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf [Accessed May 12, 2010].

Codex Alimentarius. 2009. Foods derived from modern biotechnology. World Health Organization, Food and Agriculture Organization of the United Nations, Rome, Italy.

Coruzzi, G., R. Broglie, C. Edwards and N.-H. Chua. 1984. Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1, 5-bisphosphate carboxylase. EMBO Journal 3:1671-1679.

Crockett, L. 1977. Contents. Pages vii-ix in Wildly Successful Plants: A Handbook of North American Weeds. Macmillan Publishing Co., Inc., New York, New York.

CTIC. 2000. Top 10 conservation tillage benefits: Conservation for agriculture's future. Conservation Technology Information Center, West Lafayette, Indiana.

http://www.ctic.purdue.edu/Core4/CT/CTSurvey/10Benefits.html [Accessed February 22, 2001].

CTIC. 2007. 2007 amendment to the national crop residue management survey summary. Conservation Technology Information Center, West Lafayette, Indiana. http://www.conservationinformation.org/pdf/National_Summary_2007_Amendment.pdf [Accessed June 14, 2010].

Curie, C., T. Liboz, C. Bardet, E. Gander, C. Medale, M. Axelos and B. Lescure. 1991. *Cis* and *trans*-acting elements involved in the activation of *Arabidopsis thaliana* A1 gene encoding the translation elongation factor EF-lα. Nucleic Acids Research 19:1305-1310.

Cutler, G.H. 1934. A simple method for making soybean hybrids. Journal of the American Society of Agronomy 26:252-254.

Dalley, C.D., K.A. Renner and J.J. Kells. 2001a. Weed competition in Roundup Ready soybean and corn. Michigan State University, Dept of Crop and Soil Science.

Dalley, C.D., K.A. Renner and J.J. Kells. 2001b. Weed competition in Roundup Ready soybean and corn. Michigan State University, Department of Crop and Soil Science, East Lansing, Michigan. http://www.ipm.msu.edu/CAT05_fld/pdf/5-26FactSheet.pdf [Accessed June 15, 2010].

Day-Rubenstein, K. and P. Heisey. 2006. Crop genetic resources. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/publications/arei/eib16/Chapter3/3.1/ [Accessed June 7, 2011].

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski and H.M. Goodman. 1982. Nopaline synthase: Transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics 1:561-573.

Dillon, T.W., R.C. Scott, N.D. Pearrow and K.A. Meins. 2006. Effect of sulfonylurea rice herbicides on soybeans. Page 1 in Southern Weed Science Society Proceedings, Little Rock, Arkansas.

Dorn, T.W. 2011. Computations on grain stored in various configurations. University of Nebraska - Lincoln, Lincoln, Nebraska. http://lancaster.unl.edu/ag/crops/inservice/outside.htm [Accessed July 7, 2011].

Dorokhov, D., A. Ignatov, E. Deineko, A. Serjapin, A. Ala and K. Skryabin. 2004. Potential for gene flow from herbicide-resistant GM soybeans to wild soya in the Russian Far East. Pages 151-161 in Introgression from Genetically Modified Plants into Wild Relatives. H.C.M. den Nijs, D. Bartsch, and J. Sweet (eds.). CAB International, Wallingford, United Kingdom.

Dorrance, A.E., M.A. Draper and D. Hershman. 2007a. Using foliar fungicides to manage soybean rust. Ohio State University and Univesity of Kentucky.

Dorrance, A.E., M.A. Draper and D. Hershman. 2007b. Using foliar fungicides to manage soybean rust. The Ohio State University, Colombus, Ohio. http://oardc.osu.edu/soyrust/2007edition/fungisoyrust.pdf [Accessed May 12, 2010].

Erickson, E.H. 1975. Honey bees and soybeans. American Bee Journal 115:351-353.

Evans, L.T. and R.A. Fischer. 1999. Yield potential: Its definition, measurement, and significance. Crop Science 39:1544-1551.

Facciola, S. 1998. Brassicaceae. Pages 52-61 in Cornucopia II: A Source Book of Edible Plants. Kampong Publications, Vista, California.

FARRP. 2011. Allergen database. University of Nebraska, Food Allergy Research and Resource Program, Lincoln, Nebraska. http://www.allergenonline.org/databasebrowse.shtml [Accessed February 14, 2011].

Fling, M.E., J. Kopf and C. Richards. 1985. Nucleotide sequence of the transposon Tn*7* gene encoding an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase. Nucleic Acids Research 13:7095-7106.

Gage, D.J. 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing Rhizobia during nodulation of temperate legumes. Microbiology and Molecular Biology Reviews 68:280-300.

Garber, R.J. and T.E. Odland. 1926. Natural crossing in soybeans. Journal of the American Society of Agronomy 18:967-970.

Giza, P.E. and R.C.C. Huang. 1989. A self-inducing runaway-replication plasmid expression system utilizing the Rop protein. Gene 78:73-84.

Gupta, M., W. Nirunsuksiri, G. Schulenberg, T. Hartl, S. Novak, J. Bryan, N. Vanopdorp, J. Bing and S. Thompson. 2008. A non-PCR-based Invader ® assay quantitatively detects single-copy genes in complex plant genomes. Molecular Breeding 21:173-181.

Gurley, W.B., A.G. Hepburn and J.L. Key. 1979. Sequence organization of the soybean genome. Biochimica et Biophysica Acta 561:167-183.

Harner, J.P., T.J. Herrman and C. Reed. 1998. Temporary grain storage considerations. MF-2362. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Pittsburg, Kansas.

Harrigan, G.G., D. Lundry, S. Drury, K. Berman, S.G. Riordan, M.A. Nemeth, W.P. Ridley and K.C. Glenn. 2010. Natural variation in crop composition and the impact of transgenesis. Nature Biotechnology 28:402-404.

Harrison, L.A., M.R. Bailey, M.W. Naylor, J.E. Ream, B.G. Hammond, D.L. Nida, B.L. Burnette, T.E. Nickson, T.A. Mitsky, M.L. Taylor, R.L. Fuchs and S.R. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126:728-740.

Heatherly, L.G. and R.W. Elmore. 2004. Managing inputs for peak production. Pages 451-536 in Soybeans: Improvement, Production, and Uses. H.R. Boerma and J.E. Specht (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Heatherly, L.G. and H.F. Hodges. 1999. Pages 273-275 in Soybean production in the Midsouth. CRC Press, Boca Raton, Florida.

Hellevang, K.J. 1998. Temporary grain storage. North Dakota State University Extension, Fargo, North Dakota. http://www.ag.ndsu.edu/pubs/ageng/grainsto/ae84-1.htm [Accessed July 7, 2011].

Helsel, Z.R. and H.C. Minor. 1993. G4410: Soybean production in Missouri. University of Missouri Extension, Columbia, Missouri. http://extension.missouri.edu/explore/agguides/crops/g04410.htm [Accessed November 11, 2009].

Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. Plant Cell 7:907-919.

Higley, L.G. and D.J. Boethel. 1994. Handbook of Soybean Insect Pests. The Entomological Society of America, Lanham, Maryland.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000a. Soybean as a crop. Pages 31,36,38,39,41,43,47,86,89,93,96,120,208 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000b. Soybean as a crop. Pages Pages 31, 36, 38, 39, 40, 41, 47, 86, 87, 89, 90, 93, 94, 96, 97,120,121, 208, 209 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Holm, L., J.V. Pancho, J.P. Herberger and D.L. Plucknett. 1979. *Glycine* L. Papilionaceae (*Leguminosiae*). Page 171 in A Geographical Atlas of World Weeds. John Wiley & Sons, Inc., New York, New York.

Holshouser, D.L. 2010. Days to soybean physiological maturity. 3009-1459. Virginia Cooperative Extension, Blacksburg, Virginia.

Holtan, H.E., S. Bandong, C.M. Marion, L. Adam, S. Tiwari, Y. Shen, J.N. Maloof, D.R. Maszle, M.-a. Ohto, S. Preuss, R. Meister, M. Petracek, P.P. Repetti, T.L. Reuber, O.J. Ratcliffe and R. Khanna. 2011. BBX32, an *Arabidopsis* B-box protein, functions in light signaling by suppressing HY5-regulated gene expression and interacting with STH2. Plant Physiology, American Society of Plant Biologists, Hayward, California. http://www.plantphysiol.org/content/early/2011/06/01/pp.111.177139.abstract.

Hurburgh, C.R. 2005. Grain storage challenges in Iowa for the 2005 crop. Iowa State University Cooperative Extension, Ames, Iowa. http://www.extension.iastate.edu/agdm/articles/others/HurOct05.htm [Accessed July 7, 2011].

Hymowitz, T. 1970. On the domestication of the soybean. Economic Botany 24:408-421.

Hymowitz, T. 2004. Speciation and cytogenetics. Pages 97-136 in Soybeans: Improvement, Production, and Uses. Third Edition. H.R. Boerma and J.E. Specht (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Hymowitz, T. and C.A. Newell. 1981. Taxonomy of the genus *Glycine*, domestication and uses of soybeans. Economic Botany 35:272-288.

Hymowitz, T., R.G. Palmer and R.J. Singh. 1992. Cytogenetics of the genus *Glycine*. Pages 53-63 in Chromosome Engineering in Plants: Genetics, Breeding and Evolution. T. Tsuchiya and P.K. Gupta (eds.). Elsevier Science Publishers, Amsterdam, Netherlands.

Hymowitz, T. and R.J. Singh. 1992. Species in the genus *Glycine* Willd., three letter code, somatic chromosome number, genome symbol, and distribution, 1991. Biosystems of the Genus *Glycine*, Urbana, Illinois.

ILSI. 2010. Crop Composition Database, Version 4.1. International Life Sciences Institute, Washington, D.C. http://www.cropcomposition.org/.

ISO. 2009. Selection and use of the ISO 9000 family of standards. International Standards Organization, Geneva, Switzerland.

Israel, D.W., J.N. Mathis, W.M. Barbour and G.H. Elkan. 1986. Symbiotic effectiveness and host-strain interactions of *Rhizobium fredii* USDA 191 on different soybean cultivars. Applied and Environmental Microbiology 51:898-903.

Jaycox, E.R. 1970a. Ecological relationships between honey bees and soybeans. I. Introduction. American Bee Journal 110:306-307.

Jaycox, E.R. 1970b. Ecological relationships between honey bees and soybeans: II the plant factors. American Bee Journal 110:343-345.

Jaycox, E.R. 1970c. Ecological relationships between honey bees and soybeans: III the honey-bee factors. American Bee Journal 110:383-385.

John, M.E. 1996. Structural characterization of genes corresponding to cotton fiber mRNA, E6: Reduced E6 protein in transgenic plants by antisense gene. Plant Molecular Biology 30:297-306.

Kay, R., A. Chan, M. Daly and J. McPherson. 1987. Duplication of CaMV 35S Promoter sequences creates a strong enhancer for plant genes. Science 236:1299-1302.

Khanna, R., B. Kronmiller, D.R. Maszle, G. Coupland, M. Holm, T. Mizuno and S.-H. Wu. 2009. The *Arabidopsis* B-box zinc finger family. Plant Cell 21:3416-3420.

Klee, H.J., Y.M. Muskopf and C.S. Gasser. 1987. Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: Sequence analysis and manipulation to obtain glyphosate-tolerant plants. Molecular and General Genetics 210:437-442.

Kollipara, K.P., R.J. Singh and T. Hymowitz. 1993. Genomic diversity in aneudiploid (2n=38) and diploid (2n=40) *Glycine tomentella* revealed by cytogenetic and biochemical methods. Genome 36:391-396.

Kruger, G.R., W.G. Johnson, S.C. Weller, M.D.K. Owen, D.R. Shaw, J.W. Wilcut, D.L. Jordan, R.G. Wilson, M.L. Bernards and B.G. Young. 2009. U.S. grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. Weed Technology 23:162-166.

Kuroda, Y., A. Kaga, N. Tomooka and D.A. Vaughan. 2008. Gene flow and genetic structure of wild soybean (*Glycine soja*) in Japan. Crop Science 48:1071-1079.

Lackey, J.A. 1981. Tribe 10. Phaseoleae DC. (1825). Advances in Legume Systematics. R.M. Polhill and P.H. Raven (eds.). Kew Publishing, Surrey, United Kingdom.

Lersten, N.R. and J.B. Carlson. 2004. Vegetative morphology. Pages 15-57 in Soybeans: Improvement, Production and Uses. Third Edition. H.R. Boerma and J.E. Specht (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Lingenfelter, D.D. and N.L. Hartwig. 2007. Introduction to weeds and herbicides. Penn State University Agricultural Research and Cooperative Extension, University Park, Pennsylvania.

Loux, M.M., D. Doohan, A.F. Dobbels, W.G. Johnson, G.R.W. Nice, T.N. Jordan and T.T. Bauman. 2010. Weed control guide for Ohio and Indiana. Bulletin 789. Purdue University and Ohio State University Extension, West Lafayette, Indiana.

LSU. 2010. Asian soybean rust: Background. Louisiana State University, Baton Rouge, Louisiana. http://www.agctr.lsu.edu/en/crops_livestock/crops/soybeans/Asian_Soybean_Rust/ [Accessed June 17, 2010].

Lu, B.-R. 2004. Conserving biodiversity of soybean gene pool in the biotechnology era. Plant Species Biology 19:115-125.

Lundry, D.R., W.P. Ridley, J.J. Meyer, S.G. Riordan, M.A. Nemeth, W.A. Trujillo, M.L. Breeze and R. Sorbet. 2008. Composition of grain, forage, and processed fractions from second-generation glyphosate-tolerant soybean, MON 89788, is equivalent to that of conventional soybean (*glycine max* L.). Journal of Agricultural and Food Chemistry 56:4611-4622.

Maier, D.E. and W.F. Wilcke. 2011. Temporary grain storage considerations. Purdue University, Grain Quality Task Force, Purdue, Indiana. http://www.extension.purdue.edu/extmedia/GQ/GQTF38/GQTF-38.html [Accessed July 7, 2011].

Major, D.J. 1980. Photoperiod response characteristics controlling flowering of nine crop species. Canadian Journal of Plant Science 60:777-784.

Major, D.J., D.R. Johnson, J.W. Tanner and I.C. Anderson. 1975. Effects of daylength and temperature on soybean development. Crop Science 15:174-179.

Martinell, B.J., L.S. Julson, C.A. Emler, Y. Huang, D.E. McCabe and E.J. Williams. 2002. Soybean agrobacterium transformation method. Patent 6,384,301, U.S. Patent Office, Washington, D.C.

Meinke, D.W., J.M. Cherry, C. Dean, S.D. Rounsley and M. Koornneef. 1998. *Arabidopsis thaliana*: A model plant for genome analysis. Science 282:662-682.

Merrill, A.L. and B.K. Watt. 1973. Energy value of foods... Basis and derivation. Agricultural Handbook No. 74. US Department of Agriculture, Agricultural Research Service, Washington, D.C.

Morrison, M.J., H.D. Voldeng and E.R. Cober. 1999. Physiological changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. Agronomy Journal 91:685-689.

Mullin, W.J. and W. Xu. 2001. Study of soybean seed coat components and their relationship to water absorption. Journal of Agricultural and Food Chemistry 49:5331-5335.

NatureServe. 2010. NatureServe Explorer Version 6.2: An online encyclopedia of life. NatureServe, Arlington, Virginia. http://www.natureserve.org/explorer/ [Accessed August 12, 2010].

NDSU. 2002. Soybean production field guide for North Dakota and northwestern Minnesota. North Dakota State University Extension Service and North Dakota Soybean Council, Fargo, North Dakota. http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a1172.pdf [Accessed November 11, 2009].

Nice, G. and B. Johnson. 2005. Indiana's top ten most problematic weeds. Purdue Extension, West Lafayette, Indiana. http://www.btny.purdue.edu/WeedScience/2005/topten05.pdf [Accessed November 11, 2009].

NRC. 2001. Nutrient composition of feeds. Pages 281-306 in Nutrient Requirements of Dairy Cattle. Seventh Edition. National Research Council, National Academy Press, Washington, D.C.

Odell, J.T., F. Nagy and N.-H. Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313:810-812.

OECD-FAO. 2008. Agricultural outlook 2008-2017. Organisation for Economic Cooperation and Development, Paris, France.

OECD. 1993. Safety considerations for biotechnology: Scale-up of crop plants. Organisation for Economic Co-operation and Development, Paris, France. www.biosafety.be/CU/BSL_Ressources/PDF/M00034525.pdf [Accessed January 9, 2009].

OECD. 1998. Report of the OECD workshop on the toxicological and nutritional testing of novel foods. Aussois, France, March 1997. SG/ICGB(1998)1/FINAL. Organisation for Economic Development, Paris, France.

OECD. 2000. Consensus document on the biology of *Glycine max* (L.) merr. (soybean). ENV/JM/MONO(2000)9. Series on harmonization of regulatory oversight in biotechnology No.15. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2001. Consensus document on compositional considerations for new varieties of soybean: Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2001)15. Series on the Safety of Novel Foods and Feeds No.2. Organisation for Economic Co-operation and Development, Paris, France.

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichholtz, X. Delannay, R.L. Fuchs, G.M. Kishore and R.T. Fraley. 1996a. New weed control opportunities: Development of soybeans with a Roundup Ready™ gene. Pages 53-84 in Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects. S.O. Duke (ed.). CRC Press, Inc., Boca Raton, Florida.

Padgette, S.R., N.B. Taylor, D.L. Nida, M.R. Bailey, J. MacDonald, L.R. Holden and R.L. Fuchs. 1996b. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. Journal of Nutrition 126:702-716.

Pattison, R.J. and A. Amtmann. 2009. N-glycan production in the endoplasmic reticulum of plants. Trends in Plant Science 14:92-99.

Pedersen, P. 2004. Soybean growth and development. Iowa State University, Ames, Iowa.

Pedersen, P. 2007. Soybean does not handle early season weed competition. Iowa State University Extension, Des Moines, Iowa. http://extension.agron.iastate.edu/soybean/articles/ISRspring2006.html [Accessed January 26, 2011].

Pedersen, P. 2008a. Planting and tillage. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/production_planting.html [Accessed July 14, 2011].

Pedersen, P. 2008b. Soybean variety selection. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/production_varietyselect.html [Accessed April 19, 2011].

Pedersen, P. 2008c. Fungicide seed treatments. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/seedfungtmt.html [Accessed July 14, 2011].

Pedersen, P. 2008d. Soybean cyst nematode. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/diseases_SCNbiology.html [Accessed July 14, 2011].

Pedersen, P. 2008e. Damping off and seed decay. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/diseases_dampoff.html [Accessed July 14, 2011].

Pedersen, P. 2008f. Soil fertility. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/production_soilfert.html [Accessed July 14, 2011].

Pedersen, P. 2008g. Row spacing is important to maximize your yield. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/production_spacing.html [Accessed June 5, 2008].

Pilgeram, A.L., D.C. Sands, D. Boss, N. Dale, D. Wichman, P. Lamb, C. Lu, R. Barrows, M. Kirkpatrick, B. Thompson and D.L. Johnson. 2007. *Camelina sativa*, a Montana omega-3 and fuel crop. Pages 129-131 in Issues in New Crops and New Uses. ASHS Press, Alexandria, Virginia.

Potts, H.C., J. Duangpatra, W.G. Hairston and J.C. Delouche. 1978. Some influences of hardseededness on soybean seed quality. Crop Science 18:221-224.

Putterill, J., F. Robson, K. Lee, R. Simon and G. Coupland. 1995. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80:847-857.

Ray, J.D., T.C. Kilen, C.A. Abel and R.L. Paris. 2003. Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research 2:133-138.

Reynolds, T.L., M.A. Nemeth, K.C. Glenn, W.P. Ridley and J.D. Astwood. 2005. Natural variability of metabolites in maize grain: Differences due to genetic background. Journal of Agricultural and Food Chemistry 53:10061-10067.

Richins, R.D., H.B. Scholthof and R.J. Shepherd. 1987. Sequence of figwort mosaic virus DNA (caulimovirus group). Nucleic Acids Research 15:8451-8466.

Salomon, S. and H. Puchta. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO Journal 17:6086-6095.

Sandring, S., M.-A. Riihimaki, O. Savolainen and J. Ågren. 2007. Selection on flowering time and floral display in an alpine and a lowland population of *Arabidopsis lyrata*. Journal of Evolutionary Biology 20:558-567.

SAS. 2008. SAS/STAT software version 9.2. SAS Institute, Inc., Cary, North Carolina.

Scott, W.O. and S.R. Aldrich. 1970. Modern soybean production. The Farm Quarterly: 16-152.

Singh, R.J. and T. Hymowitz. 1989. The genomic relationships among *Glycine soja* Sieb. and Zucc., *G. max* (L.) Merr. and '*G. gracilis*' Skvortz. Plant Breeding 103:171-173.

Singh, R.J. and T. Hymowitz. 1999. Soybean genetic resources and crop improvement. Genome 42:605-616.

Singh, R.J., H.H. Kim and T. Hymowitz. 2001. Distribution of rDNA loci in the genus *Glycine* Willd. Theoretical and Applied Genetics 103:212-218.

Skorupska, H., M.C. Albertsen, K.D. Langholz and R.G. Palmer. 1989. Detection of ribosomal RNA genes in soybean, *Glycine max* (L.) Merr., by *in situ* hybridization. Genome 32:1091-1095.

Specht, J.E., D.J. Hume and S.V. Kumudini. 1999. Soybean yield potential - A genetic and physiological perspective. Crop Science 39:1560-1570.

Stalker, D.M., C.M. Thomas and D.R. Helinski. 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK2. Molecular and General Genetics 181:8-12.

STC. 2010. Volume moved by railroads - Total U.S. Soy Transporation Coalition, Ankeny, Iowa.

Suarez-Lopez, P., K. Wheatley, F. Robson, H. Onouchi, F. Valverde and G. Coupland. 2001. *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410:1116-1120.

Sundstrom, F.J., J. Williams, A.V. Deynze and K.J. Bradford. 2002. Identity preservation of agricultural commodities. Publication 8077. University of California, Division of Agricultural and Natural Resources, Oakland, California.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Pages 77-90 in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, New York.

Sutherland, J.P. and G.M. Poppy. 2005. Quantifying exposure. Pages 186-212 in Gene Flow from GM Plants. G.M. Poppy and M.J. Wilkinson (eds.). Blackwell Publishing, Ames, Iowa.

Tylka, G.L., G.D. Gebhart, C.C. Marett, M.P. Mullaney and S.N. Wiggs. 2010. Evaluation of soybean verieties resistant to soybean cyst nematode in Iowa - 2010. Iowa State University Extension, Ames, Iowa.

U.S. EPA. 2000. Assigning values to non-detected/non-quantified pesticide residues in human health food exposure assessments. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, D.C.

U.S. FDA. 1992. Statement of policy: Foods derived from new plant varieties. Federal Register: 22984-23005.

USDA-APHIS. 2006. Soybean. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C. http://www.aphis.usda.gov/br/soybean.html [Accessed March 2, 2006].

USDA-APHIS. 2007. Finding of no significant impact. Animal and Plant Health Inspection Service. Petition for non-regulated status for soybean line MON 89788 (APHIS 06-178-01p). U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C. http://www.aphis.usda.gov/brs/aphisdocs2/06_17801p_com.pdf [Accessed August 11, 2010].

USDA-ERS. 2001. How much U.S. cropland acreage is under crop residue management. Briefing Room. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/Briefing/AgChemicals/soilmangement.htm [Accessed November 14, 2009].

USDA-ERS. 2008. Data sets: U.S. and regional cost and return estimates in soybean for 2008. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/data/CostsandReturns/testpick.htm [Accessed January 18, 2010].

USDA-ERS. 2011. US Soybean production costs and returns. US Department of Agricultural - Economic Research Service, Washington, D.C. http://www.ers.usda.gov/Data/CostsAndReturns/testpick.htm#historic1 [Accessed June 20, 2011].

USDA-NASS. 2007. Agricultural chemical usage - 2006 field crops summary. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2011a. Crop values: 2010 summary, February 2011. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2011b. Quick stats: Soybean. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://quickstats.nass.usda.gov/results/0CDD3A22-756D-3595-B9E1-D36B8F4423D5 [Accessed May 25, 2011].

USDA-NASS. 2011c. Crop production: Historical track records, April 2011. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2011d. Crop production: 2010 summary, January 2011. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USSEC. 2009. Transportation logistics. Pages 36-41 in How the Global Oilseed & Grain Trade Works. U.S. Soybean Export Council, United Soybean Board, Chesterfield, Missouri.

Valverde, F., A. Mouradov, W. Soppe, D. Ravenscroft, A. Samach and G. Coupland. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303:1003-1006.

Vitale, A. and J. Denecke. 1999. The endoplasmic reticulum - Gateway of the secretory pathway. Plant Cell 11:615-628.

Voldeng, H.D., E.R. Cober, D.J. Hume, C. Gillard and M.J. Morrison. 1997. Fifty-eight years of genetic improvement of short-season soybean cultivars in Canada. Crop Science 37:428-431.

Voosen, P. 2009. Molecular breeding makes crops hardier and more nutrutious: Markers, knockouts and other technical advances improved breeding without modifying genes. Scientific American, London, United Kingdom. http://www.scientificamerican.com/article.cfm?id=molecular-breeding-crops-genetics-rice-soy-corn-wheat [Accessed April 20, 2011].

Way, M.O. 1994. Status of soybean insect pests in the United States. Pages 15-16 in Handbook of Soybean Insect Pests. L.G. Higley and D.J. Boethel (eds.). The Entomological Society of America, Lanham, Maryland.

Weber, C.R. and W.D. Hanson. 1961. Natural hybridization with and without ionizing radiation in soybeans. Crop Science 1:389-392.

Webster, T.M., M. Patterson, J. Everest, K. Smith, D. Oliver, J. Norsworthy, N. Burgos, B. Scott, J. Byrd, C. Fristoe, J. Ferrell, B. Brecke, P. Minogue, A.S. Culpepper, E.P. Prostko, D. Moorhead, J.M. Moore, J.D. Green, J.R. Martin, B. Williams, D. Stephenson, D. Miller, D. Sanders, J. Griffin, K. Bradley, A. York, D. Jordan, L. Fisher, D. Murray, M. Marshall, S. Hagood and C. Johnson. 2009. Weed survey – Southern states 2009: Broadleaf crops subsection (cotton, peanut, soybean, tobacco, and forestry). Pages 509-524 in 2009 Southern Weed Science Society Annual Meeting, Las Cruces, New Mexico.

Wilcox, J.R. 2004. World distribution and trade of soybean. Pages 1-13 in Soybeans: Improvement, Production and Uses. Third Edition. H.R. Boerma and J.E. Specht (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Woodworth, C.M. 1922. The extent of natural cross-pollination in soybeans. Journal of the American Society of Agronomy 14:278-283.

Wrather, J.A., W.C. Stienstra and S.R. Koenning. 2000. Soybean disease loss estimates for the United States from 1996 to 1998. Can. J. Plant Pathol. 23:122-131.

Wrather, J.A., W.C. Stienstra and S.R. Koenning. 2001. Soybean disease loss estimates for the United States from 1996 to 1998. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 23:122-131.

Yates, B., A. De Soyza, R. Harkawat and C. Stenton. 2008. Occupational asthma caused by *Arabidopsis thaliana*: A case of laboratory plant allergy. European Respiratory Journal 32:1111-1112.

York, A.C., J.B. Beam and A.S. Culpepper. 2005. Control of volunteer glyphosate-resistant soybean in cotton. Journal of Cotton Science 9:102-109.

Yoshimura, Y., K. Matsuo and K. Yasuda. 2006. Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environmental Biosafety Research 5:169-173.

Zambryski, P., A. Depicker, K. Kruger and H.M. Goodman. 1982. Tumor induction by *Agrobacterium tumefaciens*: Analysis of the boundaries of T-DNA. Journal of Molecular and Applied Genetics 1:361-370.

Zollinger, R., M. McMullen, J. Knodel, J. Gray, D. Jantzi, G. Kimmet, K. Hagemeister and C. Schmitt. 2009. Statewide pesticide usage for North Dakota. Page 23 in Pesticide Use and Pest Management Practices in North Dakota 2008. North Dakota State University Extension Service, North Dakota Agricultural Experiment Station, Fargo, North Dakota.

APPENDICES

Appendix A: USDA Notifications

Field trials of MON 87712 have been conducted in the U.S. since 2006. The protocols for these trials include field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to the MON 87712 phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2009-2010 seasons, are still in preparation. A list of trials conducted under USDA notifications and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications and PermitsApproved for MON 87712 and Status of Trials Conducted under These Notifications

USDA No.	Effective Date	Release Site (State)	Trial Status
2006 Field Trials			
06-146-102n	6/27/2006	PR	Submitted to USDA
2007 Field Trials			
07-018-103n	2/17/2007	IL (8), IN(3)	Submitted to USDA
07-024-101n	3/18/2007	IA (4), KS(4)	Submitted to USDA
07-094-104n	5/4/2007	IA (2)	Submitted to USDA
07-261-101n	10/18/2007	PR	Submitted to USDA
2008 Field Trials			
08-030-103n	2/28/2008	PR	Submitted to USDA
08-039-107n	3/9/2008	IA (5), IN (2), KS (4), MO	Submitted to USDA
08-043-107n	3/13/2008	IA (3), IL (9), OH, IN	Submitted to USDA
07-352-101rm	3/26/2008	IA (6), IL (6), IN (3), KS (3)	Submitted to USDA
08-064-105n	4/3/2008	IL, IN	Submitted to USDA
08-182-101n	8/1/2008	PR	Submitted to USDA
08-253-101n	10/7/2008	PR (2)	Submitted to USDA
08-316-101n	12/16/2008	PR	Submitted to USDA
2009 Field Trials			
09-007-106n	2/25/2009	PR	Submitted to USDA
08-357-101rm	3/17/2009	IA (7), IL (7), IN (3), KS (3)	
09-050-116n	3/21/2009	IN, MO (2), NE	Submitted to USDA
09-072-106n	4/12/2009	IA (3), MO (7), NE	Submitted to USDA
09-075-110n	4/15/2009	AR, IL (4), IN, KS, PA (2)	Submitted to USDA
09-090-105n	4/30/2009	AR, IA, IL (3), IN (2), MO, KS, NE	Submitted to USDA
09-100-102n	5/10/2009	IN	Submitted to USDA
09-124-102n	6/3/2009	PR	Submitted to USDA
09-162-106n	7/11/2009	PR	Submitted to USDA
09-162-105n	7/11/2009	PR	Submitted to USDA
09-177-110n	7/26/2009	PR	Submitted to USDA
09-222-101n	9/9/2009	PR (2)	Submitted to USDA
09-261-105n	10/18/2009	AR (2), IL, MO	Submitted to USDA
09-247-101rm	11/17/2009	PR	In Progress
09-292-107rm	12/16/2009	MO	In Progress
2010 Field Trials			
09-355-101n	1/20/2010	PR	In Progress
09-351-101rm	3/10/2010	IA (8), IL (7), IN (3), MO (2), KS (5)	In Progress
10-068-112n	3/31/2010	IA (2), IL (2), IN (3),	In Progress
10-067-101n	4/4/2010	IL (2), IN, MS, NE	In Progress
10-073-103n	4/9/2010	AR, IA, IL (3), IN, KS, MO, NE, PA	In Progress

Table A-1. USDA Notifications and PermitsApproved for MON 87712 and Status of Trials Conducted under These Notifications (continued)

USDA No.	Effective Date	Release Site (State)	Trial Status
10-073-104n	4/10/2010	AL (2), AR (2), KS (4), MS, TX	In Progress
10-074-101n	4/11/2010	IA (2)	In Progress
10-074-109n	4/14/2010	IA	In Progress
10-082-106n	4/18/2010	FL, IL (3), KY	In Progress
10-083-103n	4/22/2010	IA	In Progress
10-089-104n	4/24/2010	AR, IA (4), IN (4), MO (2), NE, PA	In Progress
10-085-107n	4/25/2010	KS, MN, MO, OH	In Progress
10-089-102n	4/28/2010	IL (5), NE, OH	In Progress
10-090-102n	4/29/2010	TN	In Progress
10-091-101rm	5/21/2010	PR	In Progress
10-175-102n	7/23/2010	PR	In Progress
10-257-101rm	11/9/2010	PR	In Progress
10-334-102n	12/30/2010	PR	In Progress

Appendix B. Mode of Action of BBX32 Protein in MON 87712

Crop yield results from a sequential growth and development process – first the plant grows vegetatively and produces photosynthetic tissue, followed by flowering and the production of seeds, and finally seed filling and maturation (Pedersen et al., 2007). Yield is a complex trait that is dependent on a number of genetic and environmental factors, that influence a crop's opportunity to realize its full yield potential. Improvements in crop yield have been a primary focus of conventional breeding. The genetic changes that resulted in crop domestication and yield improvement in conventional varieties have been shown by modern molecular biology analysis to have been typically achieved through the selection and safe use of plant genes encoding transcriptional regulator proteins (Doebley et al., 2006). Agricultural biotechnology provides the opportunity to further enhance crop yields through the introduction of new genetic elements that use or modify existing pathways in the plant. The yield increase in MON 87712 is achieved using the *BBX32* gene from the plant *Arabidopsis thaliana* that produces a protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night processes and results in increased availability of assimilates in MON 87712 compared to a near isogenic comparator without this gene. Plant nutrient assimilation and utilization are known to be critical processes to drive yield improvement (Kumudini, 2002; Sinclair et al., 2004). Increased assimilate availability in MON 87712 is supported by the measurement of factors indicative of an extended period of photosynthetic activity in MON 87712 and evidence of changes in diurnal metabolism during the reproductive phase of the soybean plant, as well as by the significantly higher yield of MON 87712 when compared to control, as observed in multisite field studies in the U.S.

To understand the mode-of-action of BBX32 protein in MON 87712, it is important to understand the biological processes associated with yield and the impact of diurnal biology on those processes in soybean. Analysis of yield improvement and yield determination in conventional soybean has shown that the same basic principles and mechanisms apply for MON 87712. Through highlighting yield limitation in conventional soybean as a function of carbon and nitrogen availability that are controlled by diurnal processes, we demonstrate how the plant BBX32 protein affects these existing diurnal processes in soybean to increase yield in MON 87712.

B.1. Improvement in Grain Yield is a Major Objective for Soybean Breeders

Soybean crop yields have risen consistently in North America since the 1920s. In the U.S., soybean yield rose at an average annual rate of approximately 0.35 bu/A (0.8%) between 1924 – 2010 (Figure B-1), and similar yield increases have been reported in Canada. A survey of soybean yield in Canada between 1934 – 1992 revealed an average increase in yield of 0.5%, with evidence that since 1976 the rate of genetic improvement of seed yield is accelerating (Voldeng et al. 1997; Morrison et al., 1999). Annual improvement in soybean yields is attributable to rapid producer adoption of repetitive waves of agricultural innovation in the form of genetic and agronomic improvement that provide producers improved means for reducing "on-farm" yield constraints (Specht et al, 1999).

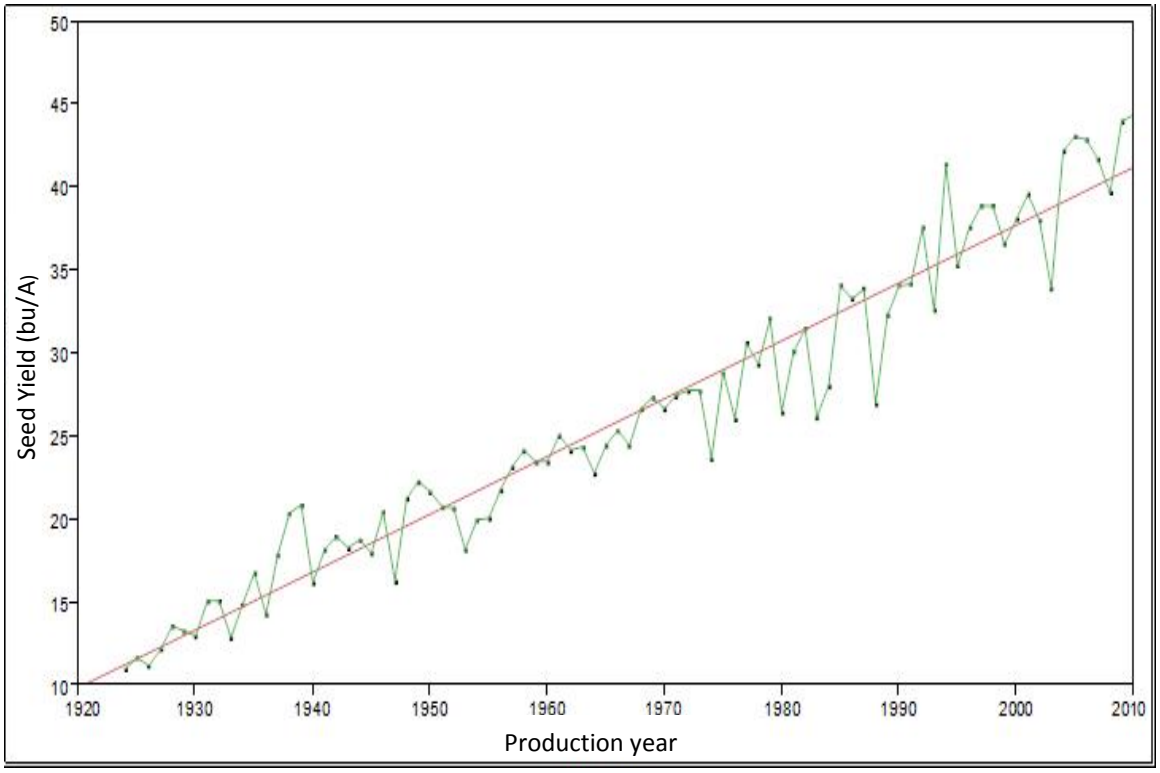


Figure B-1. U.S. soybean yield rose at an annual average rate of 0.35 bu/A between 1924 – 2010. Linear regression analysis was conducted on data from the USDA National Agricultural Statistics Service (USDA-NASS, 2011, http://www.nass.usda.gov).

Soybean yield trends in the U.S. indicate that yield growth rates have not reached a plateau. Average soybean yield in 2010 was 43.5 bu/A (2900 kg/ha), but record yields reported from yield contests in the U.S. (Iowa, Missouri and Nebraska, 1966-1998) were greater than 67.5 bu/A (4500 kg/ha) and in one instance reached 100 bu/A (6660 kg/ha) (Specht et al., 1999), demonstrating that future yield growth is possible. The concept of yield potential of soybean is defined as the yield of a cultivar when grown in environments where it is adapted, with nutrients and water non-limiting, and with pests, diseases, weeds, lodging, and other stresses effectively controlled (Evans and Fisher, 1999; Specht et al., 1999). Conventional soybean yield potential has been estimated to be approximately 120 bu/A (8000 kg/ha) using crop simulation models (Specht et al., 1999). The U.S. average in 2010 of 43.5 bu/A (2900 kg/ha) was substantially below the current estimate of yield potential, suggesting that there is an opportunity to close the gap between average annual yield performance and yield potential.

B.2. Soybean Yield is Frequently Limited by the Availability of Assimilates

Plants derive nutrition through critical processes such as photosynthesis (carbon assimilation) and absorption of raw materials such as nitrogen (nitrogen assimilation) to drive yield improvement (Kumudini, 2002; Sinclair et al., 2004). Soybean yield is the net result of metabolic assimilate availability within the plant (the source) and translocation of these assimilates to the developing seed (the sink) where these assimilates are used to synthesize storage compounds such as protein, oil and starch (Egli, 1999). Soybean yield can be limited by the activity of the source or by the ability of the seed to utilize the available assimilate produced by the source and convert into dry weight (Egli, 1999). This division recognizes the two major developmental processes involved in the accumulation of yield, the production of assimilate in the leaves (the source) and utilization of this assimilate by the developing seed (the sink).

Soybean plant development can be separated into two major generally overlapping developmental phases: vegetative and reproductive. The duration of these phases is controlled primarily by genetics, temperature, and day length (Pedersen, 2007). Soybean producers influence the duration of these phases through variety selection, geographic location, and planting date. Yield production begins with vegetative growth when the formation of organs for nutrient absorption and photosynthesis provides the machinery to produce yield. The reproductive phase is typically the most important for yield determination and is divided into eight reproductive (R) stages (Fehr and Caviness, 1981) (Table B-1). Fehr and Caviness (1981) classify reproductive development based on flowering, pod development, seed development and plant maturation stages. The first two stages (R1 and R2) refer to flowering stages. The next two stages (R3 and R4) refer to pod development. Seed development begins when the pod approaches its maximum size. The R5 and R6 stages refer to seed development phases, whereas the R7 and R8 stages refer to phases of plant maturation.

Table B-1. Soybean reproductive stages as defined by Fehr and Caviness (1981)

Stage ID	Description of Developmental Stage
R1	**Beginning bloom** — One open flower at any node on the main stem.
R2	**Full bloom** — An open flower at one of the two uppermost nodes on the main stem with a fully developed leaf.
R3	**Beginning pod** — Pods are 3/16 inch (5 mm) at one of the four uppermost nodes on the main stem with a fully developed leaf.
R4	**Full pod** — Pods are 3/4 inch (2 cm) at one of the four uppermost nodes on the main stem with a fully developed leaf.
R5	**Beginning seed** — Pod at one of the four uppermost nodes on the main stem contains seeds that are 1/8 inch (3 mm) long.
R6	**Full seed** — Pod at one of the four uppermost nodes on the main stem contains green seeds that fill the pod cavity.
R7	**Beginning maturity** — One normal pod on the main stem has reached its mature pod color.
R8	**Full maturity** — 95 percent of the pods have reached their full mature color.

Final crop yield is a function of the number and size of seeds produced (Figure B-2). The period from R1 to R6 stages is critical for yield, because this is when both pod and seed number are set. The period between the R5 stage and onset of the R7 stage is important in setting seed weight. Because pod development begins at the R3 stage and seed growth ends at the R7 stage, conditions that limit growth during this period can impact yield by limiting seed number, seed weight, or both (Pedersen, 2007). During the reproductive phase, the number and size of seeds is limited by the capacity and efficiency of the soybean canopies to produce and translocate assimilate (Egli, 1999). Canopy-level photosynthetic rates provide the best estimate of assimilate availability at a given time (Long, 2006). The rate of canopy photosynthesis is determined by leaf area index (Westgate, 2001), the photosynthetic capacity of the leaves, and environmental conditions.

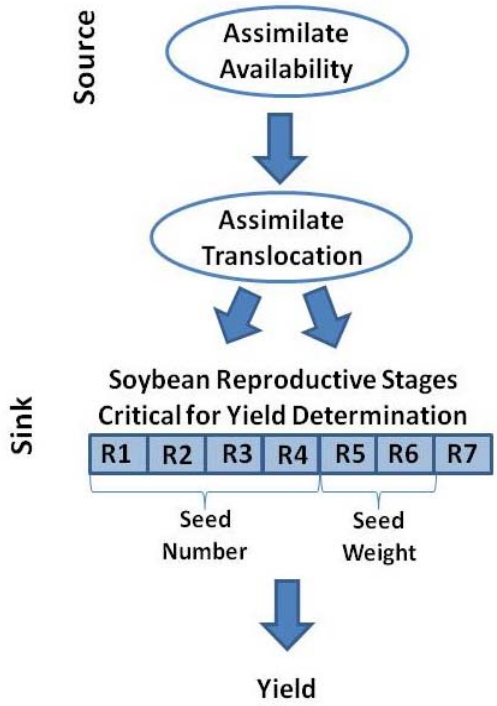


Figure B-2. Determination of yield in conventional soybean.
Soybean yield is a function of the number and size of seeds. The period from the R1 stage (flower initiation) to the R6 stage (full seed) is critical for yield determination, because this is when both seed number and seed weight are determined. The developing seed (the sink) receives assimilates from the plant (the source).

Soybean plants are able to compensate for canopy gaps by increasing branch production per plant, improving leaf area index, therefore even large increases in plant stand may result in little to no yield increase (Board 2000; Carpenter and Board 1997). A wide range of seeding rates (100,000-200,000 plants/acre) that are used agronomically have been shown to have relatively little influence on soybean yield (Board 2000; Butler et al. 2010; De Bruin and Pedersen 2009). Specifically, in a multi-year and multi-site study, less than 5% difference in soybean yield was reported for a final plant stand increase of 80% (De Bruin and Pedersen 2008). Thus, minor differences in plant stand would not be expected to substantially affect yield when plant stands are near agronomically acceptable levels.

Under a broad range of environmental conditions, soybean yield is more frequently limited by the amount of translocated assimilates from the source than from sink limitation (Egli, 1999). Reducing source capacity through shading or partial defoliation during reproductive growth stages is known to result in decreased yield (Board and Tan, 1995; Board et al., 1995; Egli and Zhen-wen, 1991). Reducing source capacity early in the reproductive growth cycle (R3 to R4) decreases seed number while reducing source capacity during later growth stages (R5 to R6.5) decreases individual seed size (Board

and Tan, 1995). Thus, increasing the availability of assimilates at the source is a potential mechanism to increase yield in soybean.

B.3. Diurnal Processes Control Assimilate Availability in Plants

Plant growth and development responds to the diurnal cycling of light and dark. This is manifested both at the physiological level, with changes in plant metabolism and assimilate availability, and at the molecular level, with expression of some genes occurring only at certain times of the day (Harmer et al, 2000; Schaffer et al, 2001). The day/night cycling of plant processes is called a diurnal rhythm and is achieved primarily by two mechanisms: first, by light, and second, by an internal circadian clock (Schaffer et al, 2001). Coordination of diurnal processes such as nitrogen and carbon metabolism which affect assimilate availability and plant growth have been shown to be necessary for maintaining plant productivity (Smith and Stitt, 2007; Gutierrez et al, 2008; Graf et al, 2010).

The first mechanism to consider in diurnal processes is light signaling. Light is one of the most important environmental factors for plants as it provides the source of energy for plant life. The ability of plants to respond to light is achieved through photoreceptors (Jiao et al, 2007). Plants detect a range of light intensities and wavelengths via photoreceptors, and subsequently convert the light signal into physiological responses through transcriptional regulation (Jiao et al, 2007). Light-responsive transcription factors, such as LONG HYPOCOTYL5 (HY5), mediate light signaling through the coordinated activation and repression of specific plant genes (Lee et al, 2007). The ability to sense a light signal allows the plant to respond to recurring diurnal cycles and thus control growth and development throughout vegetative and reproductive stages (Jiao et al, 2007).

The second mechanism in plant diurnal processes is the circadian clock, which oscillates with an approximate 24-h period in the absence of external stimuli and thus allows plants to anticipate daily changes in the environment, such as the onset of dawn (Eckhardt, 2005; Graf et al, 2010). Such anticipation allows plants to time internal biological processes to the part of the 24-h cycle that would most benefit from interaction with the external stimuli occurring at any given time of day (Millar, 2004). Thus, the circadian clock acts at an interface in the signaling network between environmental response pathways and internal programs (Millar, 2004). The circadian clock is continually modified by light via a mechanism involving the HY5 transcription factor, which helps the plant to regulate diurnal processes to maximize productivity in any given environment (Devlin and Kay, 2001; Eckhardt, 2005; Li, 2011). The core components of the circadian clock are two closely related transcription factor proteins CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), and a response regulator protein TIMING OF CAB EXPRESSION 1 (TOC1) (Salome and McClung, 2004). Diurnal regulation of CCA1, LHY and TOC1 proteins are responsible for generating self sustained rhythmicity in the plant. Genes regulated by diurnal output pathway have been shown to peak in expression at different times of the day/night and are associated with circadian biology, nitrogen and carbon metabolism (Harmer et al, 2000).

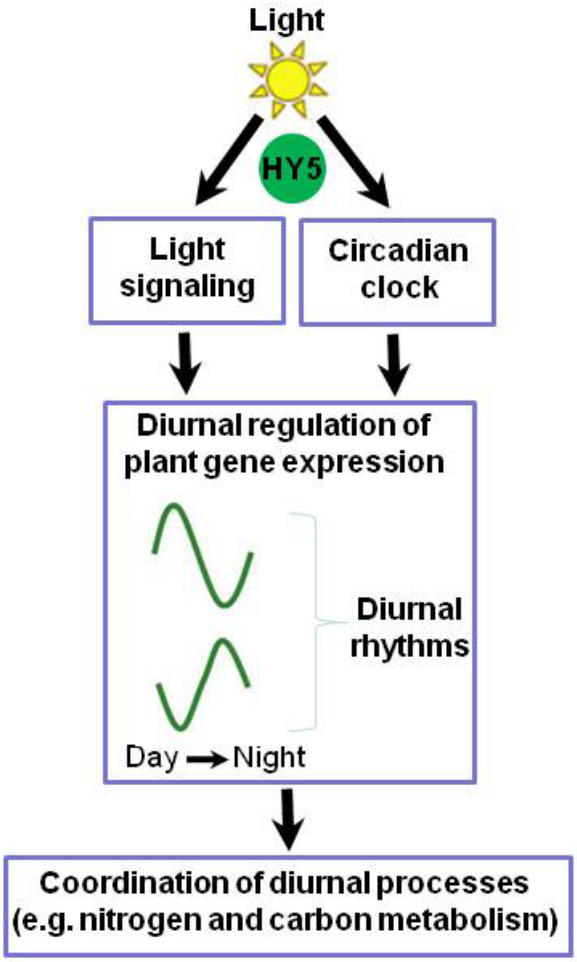


Figure B-3. Diurnal cycling of processes such as nitrogen and carbon metabolism in plants is achieved through light signaling and the circadian clock
The day/night cycling of plant processes is called a diurnal rhythm and is achieved by light signaling and the circadian clock. The circadian clock is continually modified by light input, which helps the plant optimize diurnal processes. Both light signaling and the circadian clock are regulated, in part, by the ELONGATED HYPOCOTYL5 (HY5) transcription factor. Genes regulated by the diurnal pathway have been shown to peak in expression at different times of the day/night and control biological processes such as nitrogen and carbon metabolism.

Light and the circadian clock control diurnal processes such as carbon metabolism. During the day, plants absorb light and stomates open to facilitate CO_2 assimilation and water transpiration (Blasing et al., 2005). Carbon dioxide is fixed into sugars by photosynthesis during the day to support metabolism, storage, and plant growth (Blasing et al., 2005). The entire supply of assimilated carbon is not used immediately for growth.

Newly assimilated carbon provides for both the immediate demand and, via the accumulation of temporary storage compounds such as starch in leaves, an anticipated demand during the following night (Smith and Stitt, 2007). Changes in environmental conditions result in adjustments of assimilation and storage that serve to maintain adequate carbon supply throughout the day and night (Smith and Stitt, 2007). For example, changes in day length result in alterations in both the partitioning of assimilate between starch and sucrose during the day, and the rate of starch degradation to sucrose for remobilization at night (Smith and Stitt, 2007). In soybean, the rate of starch synthesis is inversely related to day length so that the proportion of assimilate partitioned as starch for use at night is greater when the day length is shortened (Chatterton and Silvius 1979). This concept is demonstrated when soybean plants are grown under different light/dark cycles. Conventional soybean plants grown with a 14h light period partitioned 60% of assimilate into starch whereas plants grown with a 7h light period partitioned 90% of assimilate into starch (Chatterton and Silvius 1979). Thus, the control of assimilate supply for growth is a major function of diurnal mechanisms in plants.

Soybean reproductive development responds to day/night length after flowering (Summerfield et al, 1998; Kantolic and Slafer, 2005). Under field conditions, Kantolic and Slafer (2005) showed that artificially extended day length post-flowering extends the R3–R6 growth phase in conventional soybean. Exposing plants to extended photoperiod increased the number of nodes per plant and improved node fertility, thus increasing the number of pods and seeds produced per unit area. Average seed weight tended to be reduced by 20% in plants exposed to extended photoperiod while seed number was increased by more than 75% (Kantolic and Slafer, 2005). The effect on seed number and seed size is hypothesized to include mechanisms related to carbon metabolism and assimilate availability (Kantolic and Slafer, 2005). Alteration of diurnal processes through genetic means therefore offers the potential for future yield improvements through optimization of post-flowering reproductive development.

B.4. Crop Domestication and Improvement through Breeding has been Largely Achieved Through Selection and Safe Use of Transcriptional Regulator Proteins

Introduced proteins that modulate plant gene expression operate through regulation of endogenous plant pathways and processes. Normally occurring variations in these pathways are likely to occur in response to genetic and environmental factors (Kier and Petrick, 2008). In addition, molecular biology analysis has recently shown that modulation of regulatory processes has been fundamental to crop domestication and breeding of plant varieties. Several of the genetic changes that control important domestication traits in maize and rice are transcription factors, as are some of the genes controlling varietial differences (Doebley et al, 2006). For example, conventional wheat varieties with specific alterations in the transcription factor Rht-1 are shorter and show increased yield (Peng et al., 1999). Thus, the safety of food or feed from biotechnology-derived crops that have a mechanism of action based on gene expression modulation should be considered in the context of the history of safe consumption of food and feed derived from conventionally bred plants grown under a range of environmental conditions (Kier and Petrick, 2008).

B.5. Plant BBX32 Protein Expression Affects Diurnal Processes in MON 87712 Leading to Increased Yield

The MON 87712 soybean line was produced through the insertion of the *Arabidopsis thaliana BBX32* gene in a parental conventional soybean plant with genetic background A3525. *BBX32* expression in *Arabidopsis thaliana* is known to regulate plant gene expression and repress plant responses to the transition from dark to light (Holtan et al, 2011). In this section, we demonstrate how the plant BBX32 protein affects existing diurnal processes in soybean to increase yield in MON 87712. Mode-of-action studies were conducted to understand BBX32 function in MON 87712 using two controls: the parental conventional control soybean variety, A3525, and a near isogenic negative segregant control line [MON 87712(-)]. The MON 87712(-) control was included to allow most precise determination of a trait effect while reducing to the minimum any variability associated with the background genetics of A3525. In a field study across multiple sites in the U.S. soybean production area in 2009, a statistically significant increase in harvested seed weight per area was observed for MON 87712 compared to both controls. Given the importance of controlling variability when measuring impacts on yield, our primary focus for the determination of the trait effect on yield was on the comparison of MON 87712 with the near isogenic negative segregant control MON 87712(-). Furthermore, analysis of leaf area, light interception, and leaf photosynthetic rate demonstrates that MON 87712 has higher canopy-level assimilate availability during the seed-filling period.

To examine the effect of BBX32 protein expression on diurnal processes in MON 87712, targeted analysis was conducted on a group of soybean genes and metabolites associated with diurnal biology and primary carbon and nitrogen metabolism. BBX32 protein expression is shown to modulate the response of MON 87712 to the plant's transition from night to day by repressing light signaling. Temporal-specific differences in levels of metabolites involved in carbon and nitrogen metabolism, metabolites indicative of source capacity, and the activity of an enzyme involved in carbon metabolism were observed when MON 87712 was compared to A3525 and MON 87712(-) controls. Increased plant assimilate availability is associated with improved yield in conventional soybean and the mode-of-action for MON 87712 is thus similar to that observed for historical increases in soybean yield achieved using traditional breeding methods.

B.5.1. MON 87712 Delivered Significantly Increased Yield when Compared to Conventional Soybeans of the Same Genetic Background

Soybean seed yield is expressed as a function of the primary yield components: final stand count per area, seed number per plant, and individual seed weight. According to Mullen (1996) yield is expressed as:

$$\text{Yield}\left(\frac{weight}{area}\right) = Stand\ count\left(\frac{plants}{area}\right) x\ \text{Seed number per plant}\left(\frac{seeds}{plant}\right) x\ \text{Individual seed weight}\left(\frac{weight}{seed}\right)$$

Yield and yield component characteristics of MON 87712 were compared to two controls: the parental conventional control population, A3525, and a near isogenic

negative segregant control line [MON 87712(-)] in U.S. 2009 field trials (Table III-2 and Table III-3) (Appendix C). Soybean variety A3525 is the parental line to MON 87712 and was used as the conventional soybean comparator in the safety assessment of MON 87712, including the plant pest risk evaluation. The MON 87712(-) control line was identified at the R1 generation during the MON 87712 breeding process and was specifically included in the 2009 study as the primary comparator for yield endpoints to allow determination of a trait effect while reducing the variability associated with the background genetics of mixed populations of A3525.

Nineteen sites across the U.S. soybean production area were planted at a constant seeding rate of approximately 156,816 seeds per acre, and yield and yield components were measured. Three statistically significant differences were detected ($p<0.05$) between MON 87712 and the MON 87712(-) control in the combined-site analysis (Table B-2). Consistent with the intended phenotype, a statistically significant increase in yield was observed for MON 87712 compared to MON 87712(-). Corresponding increases for MON 87712 compared to MON 87712(-) were observed in the primary yield components. A statistically significant increase in final stand count and numerical increases in individual seed weight and seed number per plant were observed for MON 87712 compared to MON 87712(-). In addition, numerical increases for MON 87712 compared to MON 87712(-) were observed in the calculated yield characteristics seed number per plot and seed weight per plant. Similar results were observed when comparing MON 87712 and the A3525 parental conventional control (Table B-3).

Results of this study across a range of sites support the conclusion that the introduced trait in MON 87712 significantly increased yield when compared to near isogenic control soybeans concurrently grown under the same conditions. Yield is defined as the seed weight produced per unit area, and MON 87712 produced more seed weight per unit area when compared to control. Corresponding numerical increases in yield components are consistent with the statistically significant increase in yield.

Table B-2. Combined-Site Least Square Means, P-values, and Percent Differences of Yield and Yield Components of MON 87712 Compared to the Negative Segregant Control [MON 87712 (-)] from U.S. 2009 Field Trials (19 sites)

Characteristic (units)	Least Squares Mean		P-Value	% Difference
	MON 87712	MON 87712(-)		
Yield (bu/ac)	52.6*	47.9	0.0054	11.41%
Final Stand Count (plants/plot)	292.1*	283.2	0.0076	3.69%
Seed Number Per Plant (seeds/plant)	69.6	66.8	0.2622	4.74%
Individual Seed Weight (g)	0.163	0.160	0.0506	3.60%
Seed Weight Per Plot (g)	3284.7	2994.5	0.0054	11.41%
Seed Number Per Plot (seeds/plot)	20235.	18866.8	0.0627	7.02%
Seed Weight Per Plant (g)	11.3	10.7	0.0752	8.24%

* Indicates a statistically significant difference between MON 87712 and MON 87712(-) (α=0.05).

Table B-3. Combined-Site Least Square Means, P-values, and Percent Differences of Yield and Yield Components of MON 87712 Compared to the Conventional Control, A3525 from U.S. 2009 Field Trials (19 sites)

Characteristic (units)	Least Squares Mean		P-Value	% Difference
	MON 87712	A3525		
Yield (bu/ac)	52.6*	49.0	0.0100	7.29%
Final Stand Count (plants/plot)	292.1	288.1	0.1370	1.37%
Seed Number Per Plant (seeds/plant)	69.6	67.7	0.3430	2.75%
Individual Seed Weight (g)	0.163	0.159	0.0596	2.43%
Seed Weight Per Plot (g)	3284.7*	3061.6	0.0100	7.29%
Seed Number Per Plot (seeds/plot)	20235.1	19302.0	0.0635	4.83%
Seed Weight Per Plant (g)	11.3	10.8	0.0905	5.43%

* Indicates a statistically significant difference between MON 87712 and A3525 (α=0.05).

Regarding the statistical difference in stand count observed between MON 87712 and controls, it is important to highlight that soybean plants are able to compensate for canopy gaps by increasing branch production per plant and thus that minor differences in soybean stand are unlikely to be a significant cause of yield differences at typical plant densities. Indeed, a wide range of seeding rates (100,000-200,000 plants/acre) have relatively little influence on soybean yield (Board 2000; Butler et al. 2010; De Bruin and Pedersen 2009). The means of the final stand counts of MON 87712 and the control plots ranged from 123,379 plants/acre (283.2 plants/plot) to 127,234 plants/acre (292.1 plants/plot). These values are within the wide range of seeding rates and stand counts that have relatively little influence on soybean yield (De Bruin and Pedersen, 2009). Based on the compensatory aspect of soybean growth, the increase in yield for MON 87712 observed in the U.S. field study in 2009 was greater than would be expected from differences in stand count alone; thus, seed number and seed weight have clearly contributed to the yield increase. The data for seed number and seed weight are consistent with the overall increase in yield.

As MON 87712 exhibited statistically significant differences in yield compared to control (Tables B-2 and B-3), an assessment was made in Section VII to evaluate whether any of the differences observed between MON 87712 and the conventional control A3525 would indicate that MON 87712 may be more likely to become a weed than conventional soybean. The phenotypic, agronomic and environmental interactions assessment (Section VII) showed few statistically significant differences between MON 87712 and the conventional control A3525. Small increases in early plant stand (302.7 vs. 297.0 plants per plot) and increases in final plant stand (296.8 vs. 286.4 plants per plot) were observed. These differences were of small relative magnitude when compared to commercial conventional reference varieties grown concurrently, therefore, there were no changes indicative of increased weediness or plant pest potential (Baker, 1974). Not unexpectedly from the trait, there were statistically significant increases in yield (52.6 vs. 49.0 bu/ac). Although the yield difference is biologically meaningful from a grower perspective, the increase in yield is not indicative of increased weediness or plant pest potential for environmental risk safety.

B.5.2. Increased Canopy Level Source Capacity in MON 87712, Resulting in Increased Assimilate Availability

Genetic improvement in soybean yield through traditional breeding methods have been shown to be associated with increased canopy photosynthesis (assimilate availability) during the reproductive period (Kumudini, 2002). The source capacity of a crop canopy is linearly related to the ability of a canopy to intercept solar radiation, and the ability to convert intercepted light into biomass (Monteith, 1977). The ability of the sink to utilize the assimilates produced at the source are related to the ability of the crop to partition its resources into seed (Beale and Long, 1995; Monteith, 1977). Light interception efficiency is measured directly by measuring the photosynthetic photon flux density above and below the canopy and calculating the proportion of light interception. Light interception is closely related to leaf area index, defined as the amount of leaf area per

unit land area measured in m^2 leaf area per m^2 land area. Conversion efficiency of intercepted light into biomass is typically estimated based on light-saturated photosynthetic rate at a single stage of crop development, however it is better to estimate conversion efficiency based on integrated whole-canopy photosynthesis (Long et al, 2006). Measurement of the parameters that determine the canopy-level photosynthetic rate for MON 87712 and its near isogenic control provides an indirect indication of the difference in assimilate availability in the plant, as a result of the action of BBX32 in MON 87712.

Measurements of leaf area index, light interception efficiency, and photosynthetic rate were collected during the seed-fill period (R_4, R_5 and R_6 stages) in order to understand the canopy-level source capacity of MON 87712 compared to the conventional control, A3525, and to the MON 87712(-) control during the most important growth stages for yield determination in soybean (Figure B-4 and Figure B-5) (Appendix C). MON 87712 had significantly higher leaf area index than the MON 87712(-) at the R_4 stage, and significantly higher leaf area index than both controls at the R_5 and R_6 stages ($p<0.05$ in all cases) (Figure B-4). MON 87712 had significantly higher light interception than the MON 87712(-) at the R_5 stage, and had higher light interception than both controls at the R_6 stage. At the R_4 growth stage, the increased leaf area index of MON 87712 is counteracted by a decrease in midday leaf photosynthetic rate (Figure B-5). At midday of the R_5 growth stage, MON 87712 had significantly higher photosynthetic rate. At the R_6 growth stage, MON 87712 had significantly higher photosynthetic rate than both controls at all of the measurement timepoints at the R_6 stage (Figure B-5).

Taken together, the increased leaf area index and light interception combined with increased leaf photosynthetic rate late in crop development indicate that MON 87712 had higher canopy-level assimilate availability during the seed-filling period (R_5-R_6/R_7) that is critical to yield determination in soybean. Increased source capacity has been associated with improved yield in conventional soybean and the mode of action for MON 87712 is thus similar to that observed for historical increases in soybean yield achieved using traditional breeding methods (Kumudini, 2002).

Apart from the intended effect on increased yield, increased canopy level assimilate availability in MON 87712 is related to two statistically significant differences observed between MON 87712 and the parental conventional control A3525 for phenotypic and agronomic data characteristics. Small increases in days to 50% senescence (267.7 vs. 265.0 days) and increases in days to physiological maturity (280.8 vs. 277.5 days) were observed. Although these differences could be expected and are biologically meaningful with regard to the increased source capacity of MON 87712, they were of small relative magnitude when compared to natural variability and are therefore not indicative of increased weediness or plant pest potential from an environmental risk perspective, as discussed in Section VII.

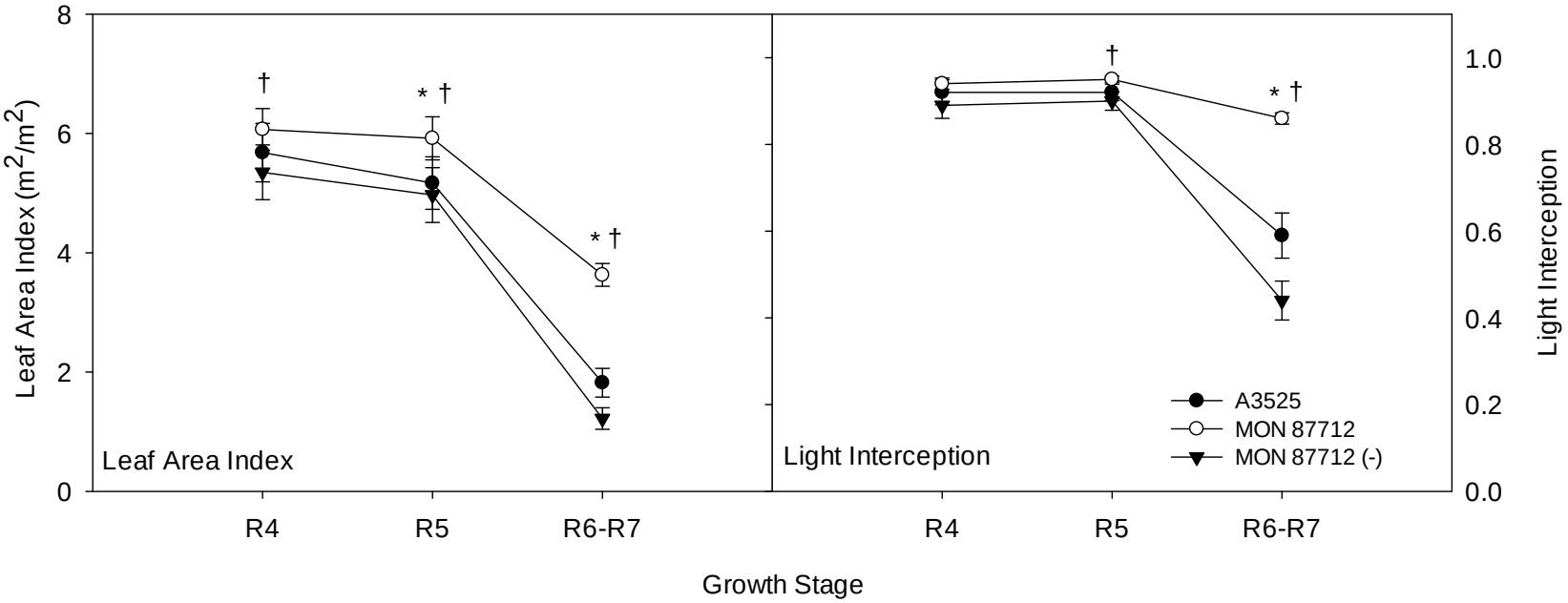


Figure B-4. Leaf Area Index and Light Interception

Leaf Area Index and Light Interception Efficiency data for MON 87712 (open circles), A3525 (dark circles), and MON 87712 (-) (dark triangles) at the R4, R5 and R6-R7 growth stages within the thinned treatment. Subsamples of 2 measurements per plot were collected on each of the measurement dates. Values represent arithmetic means ± 1 SE. Significant differences between MON 87712 and MON 87712 (-) (α=0.05) at a given measurement date are denoted by †. Significant differences between MON 87712 and A3525 (α=0.05) at a given measurement date are denoted by *.

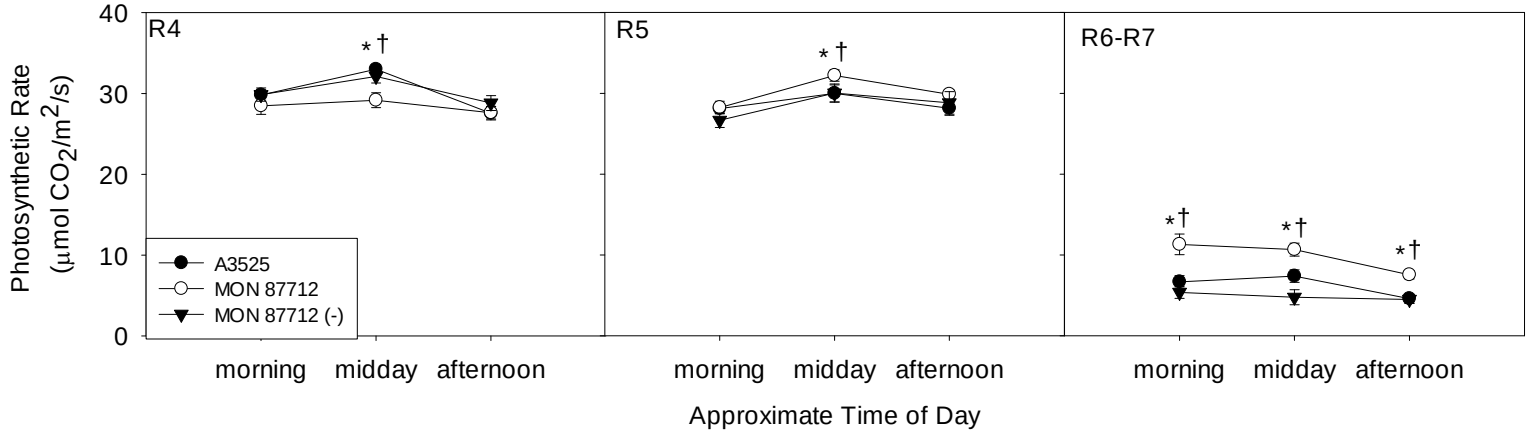


Figure B-5. Leaf Photosynthetic Rate Data

Leaf photosynthetic rate of MON 87712 (open circles), parental conventional control A3525 (dark circles) and MON 87712(-) (dark triangles) measured at morning, midday and afternoon timepoints at the R4, R5 and R6-R7 stages. Values represent arithmetic means ± 1 SE. Significant differences between MON 87712 and the MON 87712 negative segregant ($p<0.05$) at a given measurement date are denoted by †. Significant differences between MON 87712 and the conventional control A3525 ($p<0.05$) at a given measurement date are denoted by *.

B.5.3. Plant BBX32 Protein Modulates Diurnally Regulated Gene Transcription

Plant BBX32 is a member of the B-box zinc finger family from *Arabidopsis thaliana*. This family represents a subgroup of zinc finger proteins that contain one or more B-box domains with specialized tertiary structures that are stabilized by binding zinc ions. The B-box domain is predicted to be involved in protein-protein interactions (Khanna et al., 2009). BBX32 contains a single annotated protein domain, the B-Box B1 domain (Khanna et al., 2009). The B-box zinc finger family is found in many plant species; for example, the soybean B-box family contains 61 genes (Preuss et al., submitted for publication). Homologs of BBX32 are found in many agronomically-important species, suggesting that the function of BBX32 in its source plant, *Arabidopsis thaliana*, is conserved in other plant species.

Transcriptional regulation of eukaryotic genes is an orchestrated process that requires the concerted functions of multiple proteins. In addition to the ubiquitous general transcription factors, such as RNA polymerase II, there are target-specific DNA-binding transcription regulators which modulate gene expression in response to developmental or environmental cues, as well as co-regulator (*syn* accessory) proteins that interact with transcription factors to affect their activity (Martinez, 2002). In *Arabidopsis thaliana*, BBX32 participates in the regulation of genes involved in plant diurnal processes. BBX32 protein is a repressor of light signaling in *Arabidopsis thaliana*, functioning as an accessory protein involved in plant gene transcription (Holtan, et al. 2011). Transcriptional accessory proteins such as BBX32 assist the function of another protein through protein-protein interactions, and, while not directly involved in contacting DNA, aid transcriptional regulation (Martin, 1991).

Mode of action analyses in *Arabidopsis thaliana* show that BBX32 expression represses plant responses to the transition from dark to light via interaction with the HY5 transcriptional complex (Holtan et al, 2011). The relationship between BBX32 and repression of the HY5 function is evidenced by the phenotypic similarity between *Arabidopsis thaliana* seedlings overexpressing BBX32 and a *hy5* loss of function mutant. Both BBX32 overexpression lines and *hy5* mutants exhibit elongated hypocotyls when grown in the light (Holtan et al, 2011). Expression levels of specific genes targeted by HY5 for induction are reduced when BBX32 is overexpressed in *Arabidopsis thaliana*, which correlates with BBX32 repression of the HY5 complex (Holtan et al. 2011).

To investigate the function of BBX32 in MON 87712, targeted analysis of a group of soybean genes was conducted to determine the impact of expression of BBX32 on the mRNA level of certain diurnally regulated genes in MON 87712. The genes analyzed were selected based on their potential involvement in the mode of action for BBX32 leading to increased yield, and included genes associated with circadian biology, carbon and nitrogen metabolism, and phytohormone function. Expression analysis was conducted on tissue samples taken from growth chamber and field grown plants, and the expression level of each gene in MON 87712 was compared to two soybean controls, the parental conventional control A3525 and the negrative segregant control MON 87712(-). The MON 87712(-) is a near isogenic soybean line that does not contain the MON 87712 T-DNA insert. Sampling times in both the field and the growth chamber studies were

selected to evaluate the temporal specific impact of BBX32 protein expression on the expression of the analyzed soybean genes.

The function of BBX32 protein in soybean is reflected in significant differences ($p<0.05$) in the expression level of two examples of diurnally regulated genes at dawn. For example, the LHY gene, encoding a component of the circadian clock, and the *BBX13* gene, a B-box gene with similarity to CO-like genes in *Arabidopsis thaliana* (Preuss et al., submitted for publication), both show changes in expression in MON 87712 compared to the controls at the onset of daylight (Figure B-6). *LHY* mRNA levels were significantly reduced (2.5 fold) one hour pre-dawn, whereas *BBX13* mRNA levels were significantly increased (2.6 fold) one hour post-dawn in MON 87712 compared to controls, indicating that BBX32 activity can result in either up-regulation or down-regulation of endogenous genes. These observations support a role for BBX32 in modulating the response of MON 87712 to the plant's transition from night to day by repressing light signaling. At other time points, expression patterns of the soybean *LHY* and *BBX13* genes in MON 87712 were not significantly different from the expression pattern observed in control soybean, thus demonstrating that the introduced BBX32 protein functions within an existing framework of gene regulation in soybean.

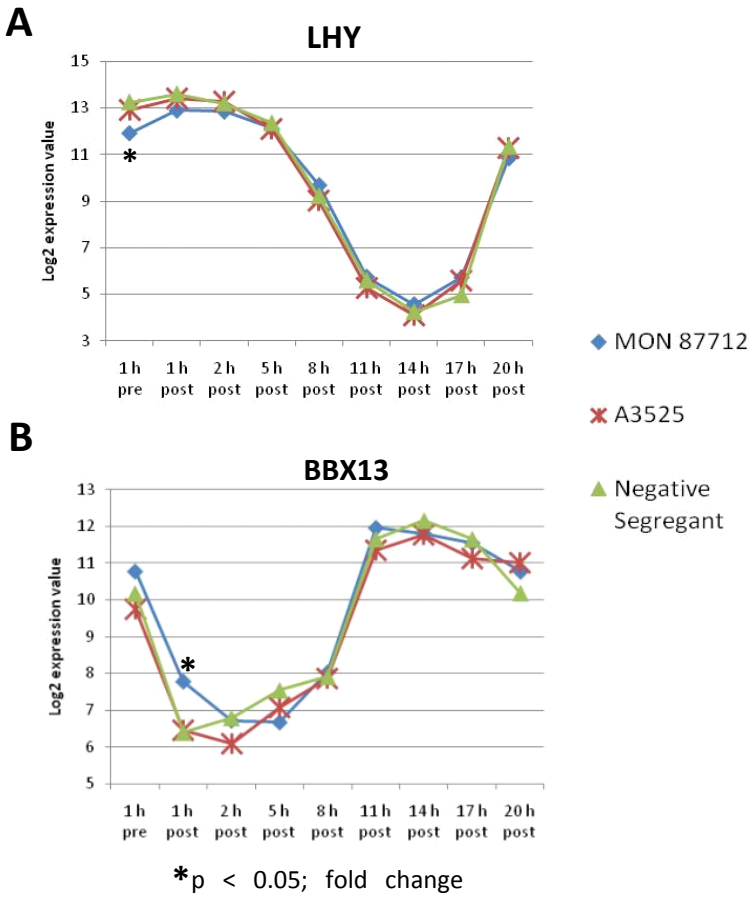


Figure B-6. Gene Expression Patterns for two Examples of Diurnally Regulated Genes in MON 87712, MON 87712(-) and A3525
Gene expression pattern of (A) *LHY* and (B) *BBX13* mRNA from V3 stage plants grown in the growth chamber. Time points "pre" and "post" are relative to dawn.

B.5.4. BBX32 Impacts Existing Metabolic Processes in MON 87712, Improving Assimilate Availability

The impact of BBX32 expression in MON 87712 on selected metabolite and enzyme activities was investigated. Leaf samples from the 2009 growing season were collected at two developmental stages (R1 and R6) at three different time points throughout the day: 1 h predawn, 1 h post dawn, and 9 h post dawn. The sampling times were intended to understand the immediate impact of BBX32 activity and downstream differences in metabolite values between MON 87712 and the two controls. It was not expected that the timing of the differences in metabolite levels would correlate to the timing of transcript level differences between MON 87712 and the controls because enzyme and metabolite profiles represent an integration, over time, of faster but more transient transcript level changes (Gibon, 2006). The levels of 41 metabolites were determined for each sample,

as well as the activities of six enzymes (Appendix D). Metabolite analyses included sixteen free amino acids, starch, eight organic acids, seven sugars, eight sugar phosphates, and two ureides. Enzyme activity analyses included allantoate amidohydrolase, glutamine synthetase, malate dehydrogenase, Nicotinamide Adenine Dinucleotide Phosphate (NADP) Malic Enzyme, Phosphoenolpyruvate (PEP) Carboxylase, and Sucrose Phosphate Synthase (SPS). Metabolites and enzymes selected for evaluation are generally associated with diurnal biology and primary carbon and nitrogen metabolism resulting in increased capacity for growth and reproductive development or correlated with yield or senescence (Morandi et al., 1990; Wingler et al., 1998; Yazdi-Samadi et al., 1977; Rainbird et al., 1984). Apart from starch these metabolites and most represent a minor fraction of soybean seed biomass. A statistical summary of percent differences was generated, and metabolites where statistically significant differences ($p<0.05$) were observed for both comparisons (MON 87712 vs. MON 87712(-) and MON 87712 vs. A3525) are reported (Table B-4 and Table B-5).

Table B-4: Summary of significant differences between MON 87712 soybean and control (MON 87712(-) and A3525) leaf tissue at the R1 developmental stage
Data is shown as percent change from control, and represents metabolites that exhibited significant differences that were consistent across comparisons to both controls.

	MON 87712 vs. MON 87712(-) R1 Leaf				MON 87712 vs. A3525 R1 Leaf		
	1 hour predawn	**1 hour post dawn**	**9 hours post dawn**		**1 hour predawn**	**1 hour post dawn**	**9 hours post dawn**
Glutamine	-12.96	47.44	**28.47***		149.09	28.05	**47.73***
GABA	**49.66***	42.54	9.04		**52.12***	17.24	34.66
Glycine	11.17	12.38	**18.18***		10.78	-4.13	**27.48***
Shikimic Acid	25.73	23.79	**40.52***		17.52	47.71	**121.18***
Fructose	-20.91	22.25	**22.63***		-3.2	20.75	**30.19***
Allantoic acid	29.12	82.35	**131.38***		109.82	43.48	**206.87***

*** Significantly different ($p < 0.05$) across comparisons to both controls.**

Shaded boxes indicate differences that were not significant across comparisons to both controls.

Table B-5: Summary of significant differences between MON 87712 soybean and control (MON 87712(-) and A3525) leaf tissue collected at the R6 developmental stage

Data is shown as percent change from control, and represents enzyme/metabolites that exhibited significant differences that were consistent across comparisons to both controls.

	MON 87712 vs. MON 87712(-) R6 Leaf				**MON 87712 vs.A3525 R6 Leaf**		
	1 hour predawn	**1 hour post dawn**	**9 hours post dawn**		**1 hour predawn**	**1 hour post dawn**	**9 hours post dawn**
Sucrose Phosphate Synthase	**55.56[a]**	**236.91[a]**	**51.59[a]**		N/A	N/A	N/A
Isoleucine	-19.18	-39.02	**-33.56***		-21.14	-13.06	**-28.81***
Leucine	-23.11	-29.61	**-42.71***		-18.55	6.38	**-37.32***
Valine	**-23.71***	-18.62	-23.87		**-22.98***	-15.53	-13.24
Malic Acid	-23.16	**-22.47***	-19.79		-7.45	**-21.87***	-10.81
Starch	**22.46***	24.08	14.88		**26.06***	11.64	12.71

*** Significantly different ($p < 0.05$) across comparisons to both controls.**

[a] Significantly different ($p < 0.05$ compared to A3525 where no data exists for MON 87712(-).

Shaded boxes indicate differences that were not significant across comparisons to both controls.

N/A = Data not available

Evaluation of the overall dataset indicates temporal-specific differences in levels of metabolites involved in carbon and nitrogen metabolism, metabolites indicative of source capacity, and the activity of an enzyme involved in carbon metabolism were observed when MON 87712 was compared to controls. At R1, significant differences were observed for values of three amino acids, shikimic acid, fructose, and allantoic acid. Glutamine and glycine levels were higher in MON 87712 than the controls at 9 hours post dawn, and γ-amino butyric acid (GABA) levels were significantly higher at 1 hour predawn. Shikimic acid, fructose, and allantoic acid were also higher in MON 87712 than the controls at 9 hours post dawn. Allantoic acid is a predominant form of nitrogen translocated from nodules to other parts of the soybean plant (Salisbury and Ross 1992), while glutamine is an initial product of ammonia (NH_3) assimilation (Reynolds et al., 1982). The increases in these metabolites in MON 87712 indicate changes in nitrogen metabolism in the soybean plant at R1, a developmental stage where increased nitrogen has been shown to increase yield (Gan et al., 2003). The increase in glycine levels is indicative of alterations in both carbon and nitrogen metabolism, as glycine is involved not only in protein biosynthesis, but also is one of the main sources of one-carbon units in higher plants, and therefore, forms the basis of carbon metabolism (Bourguignon et al., 1999) Fructose and shikimic acid are downstream of glycine, and levels in MON 87712 are also are likely increased as a result of alterations in carbon metabolism. GABA has also been hypothesized to play a role in carbon and nitrogen metabolism, possibly as storage and/or transport of nitrogen by assimilating carbons from glutamate to generate C:N fluxes that enter the tricarboxylic acid cycle, which is at the center of aerobic metabolism (Bouche and Fromm 2004). These temporal-specific metabolite changes at the R1 growth stage indicate changes in carbon and nitrogen metabolism, which are associated with increased seed number and increased yield.

At R6, significant differences were observed for values of three amino acids (isoleucine, leucine, and valine), one organic acid (malate), sucrose phosphate synthase (SPS), and starch. Isoleucine and leucine were both lower in MON 87712 when compared to the controls at the nine hour post-dawn timepoint, while valine was lower in MON 87712 when compared to the controls at the one hour predawn timepoint. The pathways synthesizing these three amino acids are considered biochemically parallel, as the enzymes performing the synthesis steps possess dual substrate specificities (Lea, 1997). These temporal-specific differences indicate changes in carbon and nitrogen metabolism, and support the hypothesis of increased transport of the amino acids from the source tissues to the sink (seed), leading to increased yield. The lower level of malate observed in MON 87712 compared to the controls one hour post dawn supports the observation of altered carbon metabolism, while the increased activitiy of SPS observed at all time points, coupled with higher starch levels at one hour predawn in MON 87712 compared to controls, relate to increased canopy carbon assimilation in MON 87712 to support the increased enzyme activity and production of carbon storage compounds. Taken together, these metabolite differences observed at R6, a critical seed-filling time for soybean, indicated altered carbon and nitrogen metabolism associated with increased individual seed weight to increased yield.

These temporal-specific differences in levels of metabolites involved in carbon and nitrogen metabolism, and indicative of source capacity, and the increased activity of SPS

enzyme observed in MON 87712 are consistent with the proposed mode of action hypothesis for the increased yield in MON 87712 when compared to conventional soybean with the same genetic background. Specifically, the expression of BBX32 in MON 87712 increases carbon and nitrogen assimilate availability and translocation to reproductive parts during development stages when yield is determined. All differences in metabolites occurred at discrete times of day; and with the exception of SPS, no significant differences were present at all three sampling time points, supporting the proposal that these differences in carbon and nitrogen metabolites were temporal-specific. These temporal differences in metabolism observed at select timepoints during the plant's reproductive phase are consistent with the ultimate increased yield phenotype of MON 87712 and the associated numerical increases in seed number and seed weight observed when compared to the conventional control (Table B-2), but they did not affect composition of the seed from a food and feed safety or nutritional perspective (section VII). Very few significant differences were observed when the composition of MON 87712 seed was compared to the conventional control A3525, although small increases in protein (1.09% relative increase), small increases in amino acids (1.22 - 2.22% relative increase), a small decrease in cystine (3.07% relative decrease), and small decreases in two fatty acids (palmitic acid and stearic acid, 1.42 and 3.04% relative decreases, respectively) were observed. These few significant differences were of small relative magnitude when compared to natural variability, and thus indicated that, although the small changes in metabolism observed in the green tissues increased seed number and seed weight, the changes did not affect composition of the seed from a food and feed safety or nutritional perspective.

B.5.5. BBX32 Protein Function: Conclusion

MON 87712 showed increased yield compared to its near isogenic negative segregant control MON 87712(-) of the same genetic background but without the introduced *BBX32* gene. The increase in yield results from higher canopy-level assimilate availability, as measured by factors associated with increased photosynthetic rate at canopy level and a result of altered diurnal plant metabolism. Plant BBX32-mediated changes in soybean gene expression impact existing diurnal processes such as carbon and nitrogen metabolism. Increased assimilate availability for translocation to the reporoductive organs, with consequent increases in seed number and/or seed weight, the major components of soybean yield.

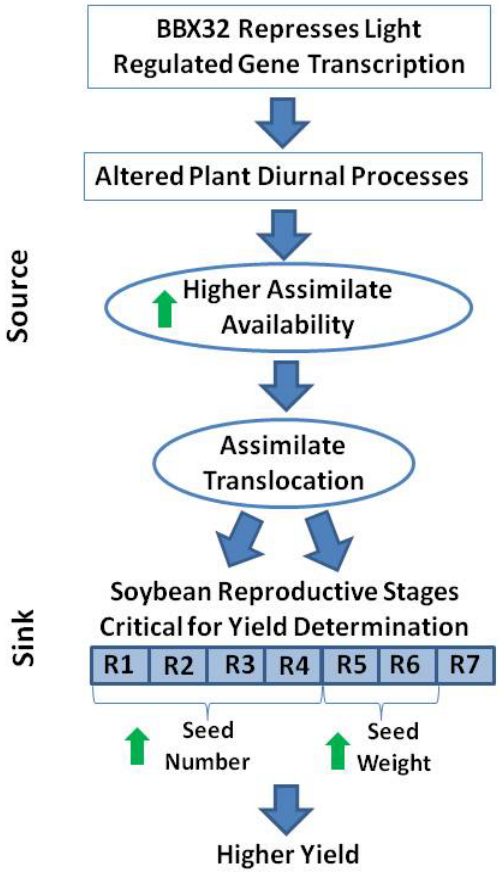


Figure B-7: BBX32 expression in MON 87712 leads to increased yield

References for Appendix B

Baker, H.G. 1974. The evolution of weeds. Annual Review of Ecology and Systematics 5:1-24.

Beale, C.V. and S.P. Long. 1995. Can perennial C_4 grasses attain high efficiencies of radiant energy conversion in cool climates? Plant, Cell and Environment 18:641-650.

Blasing, O.E., Y. Gibon, M. Gunther, M. Hohne, R. Morcuende, D. Osuna, O. Thimm, B. Usadel, W.R. Scheible and M. Stitt. 2005. Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in *Arabidopsis*. The Plant Cell 17:3257-3281.

Board, J.E. 2000. Light interception efficiency and light quality affect yield compensation of soybean at low plant populations. Crop Science 40:1285-1294.

Board, J.E. and Q. Tan. 1995. Assimilatory capacity effects on soybean yield components and pod number. Crop Science 35:846-851.

Board, J.E., A.T. Wier and D.J. Boethel. 1995. Source strength influence on soybean yield formation during early and late reproductive development. Crop Science 35:1104-1110.

Bouché, N. and H. Fromm. 2004. GABA in plants: Just a metabolite? TRENDS in Plant Science 9:110-115.

Bourguignon J, Rebeille F, and Douce, R. 1999. Serine and Glyvine Metabolism in Higher Plants. In: Plant Amino Acids. Marcel Dekker, Inc Basel, Switzerland. Chapter 3, p.111.

Butler, J.A., J.L. De Bruin and P. Pedersen. 2010. Plant density effect on reduced linolenic acid soybean cultivars. Agronomy Journal 102:348-354.

Carpenter, A.C. and J.E. Board. 1997. Branch yield components controlling soybean yield stability across plant populations. Crop Science 37:885-891.

Chatterton, N.J. and J.E. Silvius. 1979. Photosynthate partitioning into starch in soybean leaves: I. Effects of photoperiod *versus* photosynthetic period duration. Plant Physiology 64:749-753.

De Bruin, J.L. and P. Pedersen. 2008. Effect of row spacing and seeding rate on soybean yield. Agronomy Journal 100:704-710.

De Bruin, J.L. and P. Pedersen. 2009. New and old soybean cultivar responses to plant density and intercepted light. Crop Science 49:2225-2232.

Devlin, P.F. and S.A. Kay. 2001. Circadian photoperception. Annual Review of Physiology 63:677-694.

Doebley, J.F., B.S. Gaut and B.D. Smith. 2006. The molecular genetics of crop domestication. Cell 127:1309-1321.

Eckhardt, N.A. 2005. Temperature entrainment of the *Arabidopsis* circadian clock. Plant Cell 17:645-647.

Egli, D.B. 1999. Variation in leaf starch and sink limitations during seed filling in soybean. Crop Science 39:1361-1368.

Egli, D.B. and Y. Zhen-wen. 1991. Crop growth rate and seeds per unit area in soybean. Crop Science 31:439-442.

Evans, L.T. and R.A. Fischer. 1999. Yield potential: Its definition, measurement, and significance. Crop Science 39:1544-1551.

Fehr, W.R. and C.E. Caviness. 1981. Reproductive stages. Pages 6-7 in Stages of Soybean Development. Iowa State University Cooperative Extension, Ames, Iowa.

Gan, Y., I. Stulen, H. van Keulen and P.J.C. Kuiper. 2003. Effect of N fertilizer top-dressing at various reproductive stages on growth, N_2 fixation and yield of three soybean (*Glycine max* (L.) Merr.) genotypes. Field Crops Research 80:147-155.

Gibon, Y., B. Usadel, O.E. Blaesing, B. Kamlage, M. Hoehne, R. Trethewey and M. Stitt. 2006. Ingegration of metabolite with transcript and enzyme activity profiling during diurnal cycles in *Arabidopsis* rosettes. Genome Biology 7:R76.01-R76.23.

Graf, A., A. Schlereth, M. Stitt and A.M. Smith. 2010. Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. Proceedings of the National Academy of Sciences of the United States of America 107:9458-9463.

Gutierrez, R.A., T.L. Stokes, K. Thum, X. Xu, M. Obertello, M.S. Katari, M. Tanurdzic, A. Dean, D.C. Nero, C.R. McClung and G.M. Coruzzi. 2008. Systems approach identifies an organic nitrogen-responsive gene network that is regulated by the master clock control gene *CCA1*. Proceedings of the National Academy of Sciences of the United States of America 105:4939-4944.

Harmer, S.L., J.B. Hogenesch, M. Straume, H.-S. Chang, B. Han, T. Zhu, X. Wang, J.A. Kreps and S.A. Kay. 2000. Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290:2110-2113.

Holtan, H.E., S. Bandong, C.M. Marion, L. Adam, S. Tiwari, Y. Shen, J.N. Maloof, D.R. Maszle, M.-a. Ohto, S. Preuss, R. Meister, M. Petracek, P.P. Repetti, T.L. Reuber, O.J. Ratcliffe and R. Khanna. 2011. BBX32, an *Arabidopsis* B-box protein, functions in light

signaling by suppressing HY5-regulated gene expression and interacting with STH2. Plant Physiology, American Society of Plant Biologists, Hayward, California. http://www.plantphysiol.org/content/early/2011/06/01/pp.111.177139.abstract.

Jiao, Y.L., O.S. Lau and X.W. Deng. 2007. Light-regulated transcriptional networks in higher plants. Nature Reviews Genetics 8:217-230.

Kantolic, A.G. and G.A. Slafer. 2005. Reproductive development and yield components in indeterminate soybean as affected by post-flowering photoperiod. Field Crops Research 93:212-222.

Kier, L.D. and J.S. Petrick. 2008. Safety assessment considerations for food and feed derived from plants with genetic modifications that modulate endogenous gene expression and pathways. Food and Chemical Toxicology 46:2591-2605.

Khanna, R., B. Kronmiller, D.R. Maszle, G. Coupland, M. Holm, T. Mizuno and S.-H. Wu. 2009. The *Arabidopsis* B-box zinc finger family. Plant Cell 21:3416-3420.

Kumudini, S. 2002. Trials and tribulations: A review of the role of assimilate supply in soybean genetic yield improvement. Field Crops Research 75:211-222.

Lea, P.J. 1997. Primary nitrogen metabolism. Pages 300-302 in Plant Biochemistry. P.M. Dey and J.B. Harborne (eds.). Academic Press, London, United Kingdom.

Lee, J., H. Kun, V. Stolc, H. Lee, P. Figueroa, Y. Gao, W. Tongprasit, H. Zhao, I. Lee and X.W. Deng. 2007. Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell 19:731-749.

Li, G., H. Siddiqui, Y. Teng, R. Lin, X.-Y. Wan, J. Li, O.-S. Lau, X. Ouyang, M. Dai, J. Wan, P.F. Devlin, X.W. Deng and H. Wang. 2011. Coordinated transcriptional regulation underlying the circadian clock in *Arabidopsis*. Nature Cell Biology 13:616-622.

Long, S.P., X.-G. Zhu, S.L. Naidu and D.R. Ort. 2006. Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment 29:315-330.

Martin, K.J. 1991. The interactions of transcription factors and their adaptors, coactivators and accessory proteins. BioEssays 13:499-503.

Martinez, E. 2002. Multi-protein complexes in eukaryotic gene transcription. Plant Molecular Biology 50:925-947.

Millar, A.J. 2004. Input signals to the plant circadian clock. Journal of Experimental Botany 55:277-283.

Monteith, J.L. and C.J. Moss. 1977. Climate and the efficiency of crop production in Britain [and discussion]. Philosophical Transactions of the Royal Society of London B, Biological Sciences 281:277-294.

Morandi, E.N., J.R. Schussler and M.L. Brenner. 1990. Photoperiodically induced changes in seed growth rate of soybean as related to endogenous concentrations of ABA and sucrose in seed tissues. Annals of Botany 66:605-611.

Morrison, M.J., H.D. Voldeng and E.R. Cober. 1999. Physiological changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. Agronomy Journal 91:685-689.

Mullen, R.E. 1996. Rate of growth. Page 72 in Crop Science: Principles and Practice. Burgess Publishing Company, Minneapolis, Minnesota.

Pedersen, P. 2007. Soybean does not handle early season weed competition. Iowa State University Extension, Des Moines, Iowa. http://extension.agron.iastate.edu/soybean/articles/ISRspring2006.html [Accessed January 26, 2011].

Peng, J., D.E. Richards, N.M. Hartley, G.P. Murphy, K.M. Devos, J.E. Flintham, J. Beales, L.J. Fish, A.J. Worland, F. Pelica, D. Sudhakar, P. Christou, J.W. Snape, M.D. Gale and N.P. Harberd. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256-261.

Rainbird, R.M., J.H. Thorne and R.W.F. Hardy. 1984. Role of amides, amino acids, and ureides in the nutrition of developing soybean seeds. Plant Physiology 74:329-334.

Reynolds, P.H.S., M.J. Boland, D.G. Blevins, K.R. Schubert and D.D. Randall. 1982. Enzymes of amide and ureide biogenesis in developing soybean nodules. Plant Physiology 69:1334-1338.

Salisbury, F.R. and C.W. Ross. 1992. Assimilation of nitrogen and sulfur. Page 293 in Plant Physiology. Fourth Edition. Wadsworth Publishing Company, Belmont, California.

Salome, P.A. and C.R. McClung. 2004. The *Arabidopsis thaliana* clock. Journal of Biological Rhythms 19:425-435.

Schaffer, R., J. Landgraf, M. Accerbi, V. Simon, M. Larson and E. Wisman. 2001. Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. Plant Cell 13:113-123.

Sinclair, T.R., L.C. Purcell and C.H. Sneller. 2004. Crop transformation and the challenge to increase yield potential. TRENDS in Plant Science 9:70-75.

Smith, A.M. and M. Stitt. 2007. Coordination of carbon supply and plant growth. Plant, Cell & Environment 30:1126-1149.

Specht, J.E., D.J. Hume and S.V. Kumudini. 1999. Soybean yield potential - A genetic and physiological perspective. Crop Science 39:1560-1570.

Summerfield, R.J., H. Asumadu, R.H. Ellis and A. Qi. 1998. Characterization of the photoperiodic response of post-flowering development in maturity isolines of soyabean [*Glycine max* (L.) Merrill] 'Clark'. Annals of Botany 82:765-771.

USDA-NASS. 2011. Quick stats. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://quickstats.nass.usda.gov/results/9BA0D40F-CB7D-3C72-AA86-4BAB8498D8C3 [Accessed May 25, 2011].

Voldeng, H.D., E.R. Cober, D.J. Hume, C. Gillard and M.J. Morrison. 1997. Fifty-eight years of genetic improvement of short-season soybean cultivars in Canada. Crop Science 37:428-431.

Westgate, M.E. 2001. Physiology of high yielding corn and soybeans. Iowa State University Department of Agronomy, Ames, Iowa.

Wingler, A., A. von Schaewen, R.C. Leegood, P.J. Lea and W.P. Quick. 1998. Regulation of leaf senescence by cytokinin, sugars, and light: Effects on NADH-dependent hydroxypyruvate reductase. Plant Physiology 116:329-335.

Yazdi-Samadi, B., R.W. Rinne and R.D. Seif. 1977. Components of developing soybean seeds: Oil, protein, sugars, starch, organic acids, and amino acids. Agronomy Journal 69:481-486.

Appendix C: Materials, Methods, and Results for Molecular Analyses of MON 87712

C.1. Materials

The genomic DNA used in molecular analyses was isolated from leaf tissue of the R3 generation of MON 87712 and the conventional control A3525. For generational stability analysis, genomic DNA was extracted from leaf tissue of the R4, R5, R6, and R7 generations of MON 87712. The reference substance, PV-GMAP5779 (Figure III-1), was used as a positive hybridization control in Southern blot analyses. Probe templates generated from PV-GMAP5779 were used as additional positive hybridization controls. As additional reference standards, the 1 Kb DNA Extension Ladder and λ DNA/*Hin*d III Fragments from Invitrogen (Carlsbad, CA) were used for size estimations on Southern blots and agarose gels. The 1 Kb DNA Ladder from Invitrogen was used for size estimations on agarose gels for PCR analyses.

C.2. Characterization of the Materials

The identity of the source materials was verified by methods used in molecular characterization to confirm the presence or absence of MON 87712. The stability of the genomic DNA was confirmed by observation of interpretable signals from digested DNA samples on ethidium bromide stained agarose gels and/or specific PCR products, and the samples did not appear visibly degraded on the ethidium bromide stained gels.

C.3. DNA Isolation for Southern Blot and PCR Analyses

MON 87712 and conventional control genomic DNA samples were isolated from soybean leaf tissue. Prior to extraction, leaf tissue was processed to a fine powder by mortar and pestle using liquid nitrogen. Genomic DNA was extracted using a hexadecyltrimethylammonium bromide (CTAB) based method. Briefly, 5 ml of CTAB buffer (1.5% w/v CTAB, 75 mM Tris HCl, 100 mM EDTA, 1.05 M NaCl, and 0.75% w/v PVP) and 50 µg RNase A were added to approximately 1 ml of ground leaf tissue and incubated at 60-70°C for 40-50 minutes with intermittent mixing. Approximately 5 milliliters (ml) of chloroform was added to the samples and mixed by hand for 2-3 minutes, then centrifuged at 10,300 × *g* for 8-10 minutes. The upper aqueous phase was put into a clean tube and the chloroform step was repeated twice. After the last chloroform step, the aqueous phase was put into a clean tube and the DNA was precipitated with approximately 4 ml of 100% ethanol. The sample was centrifuged at 5,100 × *g* for 5-7 minutes to pellet the precipitated DNA. The DNA pellets were washed with 10-12 ml of 70% ethanol by centrifuging the samples at 5,100 × *g* for 5-7 minutes. The DNA pellets were air dried, then resuspended in 500 µl of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). For further purification of the DNA samples, polyethylene glycol (PEG) precipitation was performed. An equal volume of 20% (w/v) PEG precipitation buffer was added to the extracted DNA sample. The PEG/DNA sample mixture was incubated at 37°C for ~15 minutes and then centrifuged at 15,000 × *g* for 15 minutes. After the supernatant was removed, the pellets were washed at least twice with 80% ethanol and then air dried. The pellets were resuspended

by adding ~1 ml of TE buffer and incubating at 60-70°C. To obtain the DNA, the samples were centrifuged at 15,000 × *g* for 15 minutes and the supernatant was transferref to a clean tube. All extracted DNA was stored in a 4°C refrigerator or a 20°C freezer.

C.4. Quantification of DNA

Genomic DNA was quantified using a DyNA Quant 200 Fluorometer (Hoefer, Inc., Holliston, MA). Molecular Size Marker IX (Roche, Indianapolis, IN) was used as the calibration standard.

C.5. Restriction Enzyme Digestion of DNA

Approximately 10 micrograms (µg) of genomic DNA extracted from MON 87712 and conventional control was digested with restriction enzyme *Nco* I (Fermentas, Glen Burnie, MD or New England Biolabs, Beverly, MA) and a combination of restriction enzymes *EcoR* I and *Spe* I (New England Biolabs). All *Nco* I digests were conducted in 1X Tango buffer (Fernentas) and all *EcoR* I and *Spe* I digests were conducted in 1X NEBuffer 2 (New England Biolabs.). All digests were performed at 37°C in a total volume of ~500 microliter (µl) with ~50 units of each restriction enzyme. For the purpose of running positive hybridization controls, ~10 µg of genomic DNA extracted from the conventional control was digested with the restriction enzyme *Nco* I and the appropriate positive hybridization control(s) were added to these digests prior to loading the agarose gel.

C.6. Agarose Gel Electrophoresis

Digested DNA was resolved on 0.8% (w/v) agarose gels. For all Southern blot analyses except insert stability, individual digests containing ~10 µg each of MON 87712 and conventional control genomic DNA were loaded on the same gel in a long run/short run format. The long run allows for greater resolution of large molecular weight DNA, whereas the short run allowed the detection of small molecular weight DNA. The positive hybridization controls were only run in the short run format. For the insert stability analysis, individual digests of ~10 µg of genomic DNA extracted from leaf tissue across five generations of MON 87712 were loaded on the agarose gel in a single run format.

C.7. DNA Probe Preparation for Southern Blot Analyses

Probe templates were prepared by PCR amplification using PV-GMAP5779 as the template. The PCR products were separated on an agarose gel by electrophoresis and purified from the gel using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's instruction. The probe templates were designed based on the nucleotide composition (% GC) of the sequence, in order to optimize the detection of DNA sequences during hybridization. When possible, probes possessing a similar melting temperature (Tm) were combined in the same Southern blot hybridization. Approximately 25 ng of each probe template were radiolabeled with [α-^{32}P] deoxycytidine triphosphate (dCTP) (6000 Ci/mmol) using the random priming method

(Invitrogen). Probe locations relative to the genetic elements in PV-GMAP5779 are depicted in Figure III-1.

C.8. Southern Blot Analyses of DNA

Digested genomic DNA isolated from test and control substances was evaluated using Southern blot analyses (Southern, 1975). In Southern blots hybridized with a single probe, ~0.1 and ~1.0 genome equivalent of PV-GMAP5779 DNA previously digested with *Nco* I (Fermentas) was added to digested conventional control genomic DNA to serve as a positive hybridization control. In Southern blots hybridized with multiple probes, ~1.0 genome equivalent of the PV-GMAP5779 DNA previously digested with *Nco* I (Fermentas), as well as ~0.1 and ~1.0 genome equivalent of the appropriate probe templates were added to digested conventional control genomic DNA to serve as positive hybridization controls. The DNA was then separated by agarose gel electrophoresis and transferred onto a nylon membrane. Southern blots were hybridized and washed at 55°C or 60°C, depending on the calculated Tm of the probes used. Table C-1 lists the hybridization and radiolabeling conditions of the probes used in this study. Multiple exposures of each blot were then generated using Kodak Biomax MS film (Eastman Kodak, Rochester, NY) in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

Table C-1. Hybridization Conditions of Utilized Probes

Probe	DNA Probe	Probe labeled with dNTP (^{32}P)	Hybridization/ Wash Temperature (°C)
1	T-DNA I Probe 1	dCTP	60
2	T-DNA I Probe 2	dCTP	60
3	T-DNA II Probe 1	dCTP	55
4	T-DNA II Probe 2	dCTP	55
5	T-DNA II Probe 3	dCTP	55
6	Backbone Probe 1	dCTP	60
7	Backbone Probe 2	dCTP	60
8	Backbone Probe 3	dCTP	60
9	Backbone Probe 4	dCTP	60

C.9. DNA Sequence Analyses of the Insert

Overlapping PCR products, denoted as Product A, Product B, and Product C, were generated that span the insert and adjacent 5′ and 3′ flanking DNA sequences in MON 87712 (Figure IV-9). These products were sequenced to determine the nucleotide sequence of the insert in MON 87712, as well as the nucleotide sequence of the DNA flanking the 5′ and 3′ ends of the insert.

The PCR analyses for Product A, Product B, and Product C were conducted using 100 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 of each dNTP, and 0.05 units/µl of Accuprime *Taq* DNA Polymerase High Fidelity (Invitrogen).

The amplification of Product A and Product C were performed under the following cycling conditions: 1 cycle at 95°C for 30 seconds; 35 cycles at 95°C for 30 seconds, 58°C for 30 seconds, 68°C for 2 minutes; 1 cycle at 68°C for 10 minutes. The amplification of Product B was performed under the following cycling conditions: 1 cycle at 95°C for 30 seconds; 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 68 C for 1.5 minutes; 1 cycle at 68°C for 10 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium staining to verify that the products were the expected size. Prior to sequencing, each verified PCR product was purified using the QIAquick PCR Purification Kit (Qiagen) according to manufacturer's instruction. The purified PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry (Applied Biosystems, Foster City, CA).

A consensus sequence was generated by compiling multiple sequencing reactions performed on the overlapping PCR products. This consensus sequence was aligned to the PV-GMAP5779 sequence to determine the integrity and organization of the integrated DNA and the 5′ and 3′ insert-to-flank junctions in MON 87712.

C.10. PCR and DNA Sequence Analysis to Examine the MON 87712 Insertion Site

To examine the MON 87712 insertion site in conventional soybean, PCR and sequence analyses were performed on genomic DNA from both MON 87712 and conventional soybean (Figure IV-10). The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 87712. A forward primer specific to the DNA sequence flanking the 5' end of the insert was paired with a reverse primer specific to the DNA sequence flanking the 3' end of the insert.

The PCR reactions were conducted using 100 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 of each dNTP, and 0.05 units/µl of Accuprime *Taq* DNA Polymerase High Fidelity (Invitrogen). The amplification was performed under the following cycling conditions: 1 cycle at 95°C for 30 seconds; 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 68°C for 15 seconds; 1 cycle at 68°C for 10 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium staining to verify that the PCR products were the expected size prior to sequencing. Only the verified PCR product from the conventional control was purified with the QIAquick PCR Purification Kit (Qiagen) according to manufacturer's instruction. The purified PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry.

A consensus sequence was generated by compiling multiple sequencing reactions performed on the verified PCR product. This consensus sequence was aligned to the 5' and 3' sequences flanking the MON 87712 insert to determine the integrity and organization of the insertion site.

References for Appendix C

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98:503-517.

Appendix D: Materials, Methods and Results for Characterization of BBX32 Protein Produced in MON 87712

D.1. Characterization of BBX32 Protein in MON 87712

D.1.1. Materials

BBX32 was isolated from MON 87712 as a component of a cation exchange (CEX)-fractionated leaf extract (lot G-865162A), as described in Appendix D.1.3. Throughout this appendix, the BBX32-containing sample will be described as the MON 87712 CEX pool. MON 87712 leaf tissue (Orion lot 11295830) was produced under production plan PPN-10-450. The MON 87712 CEX pool was stored in a -80 °C freezer in a buffer solution containing 1 M NaCl, 40mM MES, 4mM TCEP, pH 6.2, 2 mM Benzamidine, 10 µM Bestatin, 20µM E64, 500 µM AEBSF. The records describing the preparation of the MON 87712 CEX pool will be retained in the Monsanto Regulatory Archives (notebook G-865151).

As a control, the CEX-enriched pool prepared from the non-transgenic A3525 leaf (lot G-865172A), was also analyzed. Throughout this appendix, this sample will be described as the A3525 CEX pool. The A3525 leaf (Orion lot 11295829) was produced under production plan PPN-10-450. The A3525 CEX pool was produced and stored under the same conditions as the MON 87712 CEX pool. The records describing the preparation of the A3525 CEX pool will be retained in the Monsanto Regulatory Archives (notebook G-865151).

The *E. coli*-produced BBX32 reference protein (Orion lot 11267091) was purified from the fermentation of *E. coli* transformed with plasmid pMON102114. The DNA sequence encoding this BBX32 reference protein was confirmed both prior to and following fermentation of *E. coli*. The *E. coli*-produced BBX32 was previously characterized. Records pertaining to the purification and the characterization of this *E. coli*-produced reference protein are archived under Orion lot 11267091 in the Monsanto Regulatory Archives.

D.1.2. Description of Assay Control

Protein molecular weight standards (Precision Plus Dual Color Standards, Bio-Rad, Hercules, CA) were used to verify protein transfer to nitrocellulose membranes, and to approximate the position of BBX32 on the western blots. BSA (Pierce, Rockford, IL) was used to generate a standard curve for total protein determination.

D.1.3. BBX32 Protein Purification

The MON 87712 CEX pool was isolated from the ground and processed soy leaf of MON 87712 using a combination of extraction, centrifugation and filtration, diafiltration, and cation exchange chromatography. A brief description of the isolation process is below.

The MON 87712 and the A3525 leaf tissues were harvested and immediately transferred to containers and frozen on dry ice. Leaf tissue processing was carried out by the Monsanto Regulatory Sample Management team in St. Louis, Missouri. The frozen leaf tissue was ground in the presence of dry ice, and was then stored frozen at -80 °C.

Aliquots of the ground leaf tissue were used as starting material for the isolation process. Approximately 135 g of processed ground MON 87712 leaf tissue was resuspended with 1 liter of chilled (4 °C) extraction buffer [50 mM Tris-HCl, pH 8.0, 150mM NaCl, 0.50% Igepal-630 (v/v), 4mM TCEP, 5mM sodium fluoride, 5mM sodium orthovanadate, 1 mM magnesium chloride, 2 mM Benzamidine, 10 µM Bestatin, 20µM E64, 500 µM AEBSF]. The leaf tissue suspension was thoroughly mixed by stirring for 10 minutes with a magnetic stir bar at 4 °C. After mixing, the pH of the extract was adjusted to 7.5 +/- 0.1 with sodium hydroxide. The extract was then homogenized with a 10-35GT Polytron homogenizer equipped with a medium grind probe at 12,000 rpm for 4 min at room temperature (RT). After homogenization the extract pH was measured and adjusted to 7.5 +/- 0.1 with sodium hydroxide. The extract was returned to cold room (4 °C) and incubated for 2 h with stirring. After 2 h the extract was centrifuged at 18,600 ×g for 45 minutes at 4 °C. The clarified extract supernatant was carefully decanted from pellet and the resultant supernatant poured through Miracloth (EMD Chemicals, Gibbstown, NJ) into a beaker chilled on ice to remove any loose pellet. The supernatant was then filtered through a Whatman 0.8/0.2µm 820cm^2 EFA Polycap filter (Whatman Inc., Piscataway, NJ). The filtered extract supernatant was kept chilled on ice.

The filtered extract supernatant of approximately 950 ml was concentrated to approximately 200 ml using a Millipore Pellicon-2 unit equipped with 2 x 0.1 m^2 10,000 kDa molecular weight cutoff (MWCO) cartridges (Millipore, Billerica, MA). Following concentration, the filtrate was diafiltered with approximately 6 volumes (1.2 liters) of SP column buffer (40mM MES, 4mM TCEP, pH 6.2, 2 mM Benzamidine, 10 µM Bestatin, 20µM E64, 500 µM AEBSF). This operation was performed at RT and the concentration unit and buffer reservoirs were chilled on ice during the operation. Following diafiltration and rinsing of the cartridges with SP column buffer, the dialfiltered extract volume was approximately 350 ml. This diafiltered extract was kept chilled on ice until subjected to chromatography.

The HP SP Sepharose chromatographic step was performed in the cold room (4 °C). Three 5ml HiTrap SP HP Sepharose cartridges (GE Healthcare, Piscataway, NJ) connected in series were equilibrated with SP column buffer. The equilibrated column was charged with the dialfiltered extract and then washed and equilibrated with 10 column volumes of the SP column buffer. The column was then washed with a 0.10 – 0.50M NaCl gradient in SP column buffer over 5 column volumes to remove contaminating proteins. The MON 87712 BBX32 pool was then eluted with a 1M NaCl step gradient in SP column buffer over 5 column volumes. BBX32-containing fractions from the 1M elution step were pooled (approximately 20ml, based on 280 nm absorbance) to give the MON 87712 CEX pool. The MON 87712 CEX pool was then aliquoted and frozen on dry ice. The frozen aliquots were stored at -80 °C.

The A3525 CEX pool (lot G-865172A) was prepared and stored using the same methods used for the isolation of the MON 87712 CEX pool, except that A3525 leaf tissue was used as the starting material for purification.

D.1.4. Western Blot Analysis-Immunoreactivity

D.1.4.1. Methods

SDS-PAGE and western blot analysis were performed to confirm the identity, and to estimate the molecular weight and purity of MON 87712 BBX32.

Aliquots of the MON 87712 CEX pool (lot G-865162A), or the A3525 CEX pool (G-865172A), were mixed with 5 × loading buffer (LB) [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 deionized water to give a final total protein concentration of 2 µg/µl in 1 × LB [62.4 mM Tris-HCl, 4% (v/v) 2-mercaptoethanol, 2% (w/v) SDS, 0.005% (w/v) bromophenol blue, 10% (v/v) glycerol, pH 6.8].

The *E. coli*-produced BBX32 reference protein (Orion lot 11267091) was serially diluted into matrix buffer (1.2 ml A3525 CEX pool, 0.3 ml 5 × LB) to a purity-corrected working concentration of 30 ng BBX32/µl, and then was further diluted in matrix buffer to generate a standard curve (10, 40, 80, 120 pg BBX32). All samples, including the MON 87712 CEX pool, the A3525 CEX pool, and the reference protein-spiked samples, were loaded at 40 µg of total protein per lane. Molecular weight markers (Precision Plus Dual Color, Bio-Rad, Hercules, CA) were loaded to estimate the size of the immunoreactive bands observed, and to verify electrotransfer of the proteins to the membrane. Samples were heated at 99°C for four minutes, and were loaded onto a pre-cast 10% polyacrylamide Bis-Tris, 10-well gel (Invitrogen, Carlsbad CA). Electrophoresis was performed at a constant 170 V for 50 minutes in 1 × MES, 4 mM TCEP, pH 7.1 buffer.

Electrotransfer to a 0.45 µm nitrocellulose membrane (Invitrogen) was performed for 120 minutes at a constant 25 V in 1 × Transfer Buffer [12 mM Tris-HCl, 96 mM Glycine, 20% (v/v) Methanol, pH 8.3] (Invitrogen).

Western blot analysis was performed to confirm the identity of MON 87712 BBX32, to compare the migrations of MON 87712-produced and *E. coli*-produced BBX32, and to estimate the purity of BBX32 in the MON 87712 CEX pool. For immunodetection, the membrane was incubated for 14 h at 4 °C with 10% (w/v) non-fat dried milk (NFDM) in 1 × Tris-buffered saline containing 0.05% (v/v) Tween-20 (TBST). The membrane was then probed with a 1:1,000 dilution of purified mouse monoclonal anti-BBX32 antibody (lot A0112690) in 5% (w/v) NFDM in TBST for 90 min. Excess antibody was removed using six 15 min washes with TBST. The membrane was then incubated with horseradish peroxidase (HRP)-conjugated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) at a dilution of 1:15,000 in 5% (w/v) NFDM in TBST for 90 min. Excess HRP-conjugate was removed using six 15 min washes with TBST. All antibody incubations and TBST washes were performed at RT. Immunoreactive bands were

visualized using the ECL (Enhanced Chemiluminescence) detection system (GE Healthcare) and exposed to Amersham Hyperfilm (GE Healthcare). The film was developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

Analysis of the blot was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One® software (version 4.4.0). Immunoreactive bands at ~25 kDa in the MON 87712 CEX pool and the *E. coli*-produced BBX32 reference protein-spiked lanes were quantified using Quantity One software. The mass of BBX32 present in the duplicate lanes of the MON 87712 CEX pool was quantified against the standard curve of the *E.coli*-produced BBX32 reference protein using densitometric analysis of the blot film. The purity of the BBX32 in the MON 87712 CEX pool was calculated by dividing the average computed BBX32 mass in the relevant lanes by the mass of total protein loaded in each lane (40 µg).

D.1.4.2. Results of MON 87712 BBX32 Western Blot Analysis - Immunoreactivity

The relative apparent molecular weights of the BBX32 in the MON 87712 CEX pool and the *E. coli*-produced BBX32 reference protein were evaluated by western blot and shown to be equivalent based on visual analysis (Figure D-1). Purity of BBX32 in the MON 87712 CEX pool relative to total protein loaded was calculated using the mass computed from densitometric analysis of the western blot and the total protein loaded per lane (Table D-1).

On the western blot (Figure D-1, lanes 2 and 3), the mouse anti-BBX32 antibody recognized two bands unique to the MON 87712 CEX pool, relative to the A3525 CEX pool. The major unique immunoreactive band comigrated with the spiked E. coli-produced BBX32 reference protein band at the expected apparent molecular weight of BBX32, ~25 kDa. The second band, which migrated at ~16 kDa, could be due to some degradation of the BBX32 protein. No additional bands unique to the MON 87712 CEX pool were observed. This western blot analysis confirmed the identity of the MON 87712 BBX32 protein.

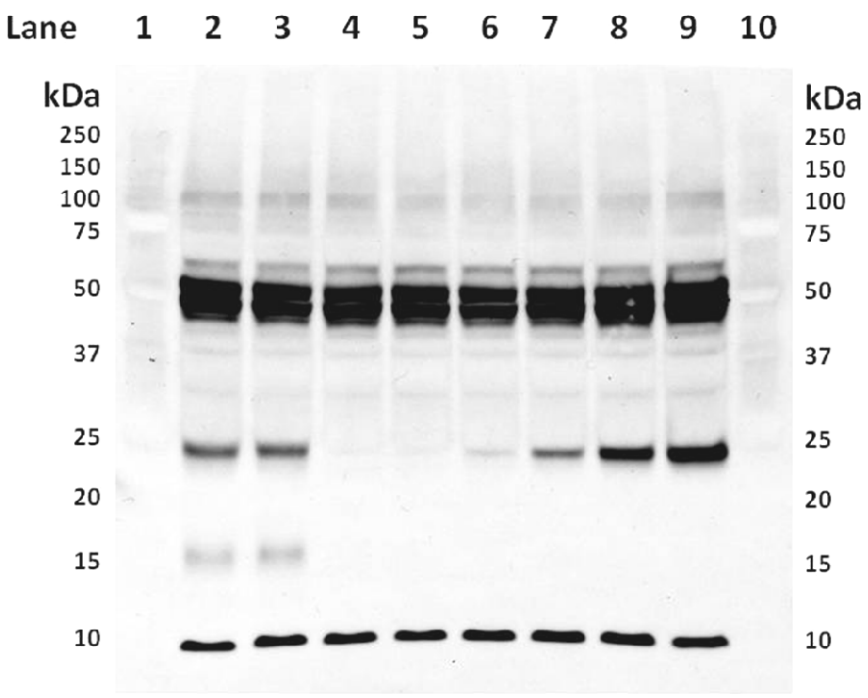


Figure D-1. Western Blot Analysis of MON 87712- and *E. coli* -produced BBX32 Proteins

The MON 87712 CEX pool and the A3525 CEX pool were each loaded in duplicate. The reference protein, *E. coli*-produced BBX32, was added in the indicated amounts into the A3525 CEX pool to provide both a comparison for BBX32 migration and a standard curve for determining BBX32 amount in the MON 87712 CEX pool. Samples were separated by SDS-PAGE and electrotransferred to a nitrocellulose membrane. The membrane was incubated with an anti-BBX32 antibody and immunoreactive bands were visualized using an ECL system. Approximate MWs (in kDa) are shown on the left and right sides and correspond to the markers loaded in lanes 1 and 10. The 45 sec exposure is shown.

Lane	Sample	Total Protein Load (ug)
1	Precision plus dual color MW marker	N/A
2	MON 87712 CEX pool	40
3	MON 87712 CEX pool	40
4	A3525 CEX pool	40
5	A3525 CEX pool	40
6	10 pg *E.coli* BBX32 in A3525 CEX pool	40
7	40 pg *E.coli* BBX32 in A3525 CEX pool	40
8	80 pg *E.coli* BBX32 in A3525 CEX pool	40
9	120 pg *E.coli* BBX32 in A3525 CEX pool	40
10	Precision plus dual color MW marker	N/A

Table D-1. Purity of the MON 87712-Produced BBX32 Protein

The purity of MON 87712-produced BBX32 was calculated using densitometric analysis of western blot shown in Figure D-1, and the total protein loaded.

Total Protein Loaded per lane	Mass of BBX32 (pg)		BBX32 Purity[1] (pg BBX32/ µg total protein)
	Lane 2	Lane 3	
40 µg	44.1	40.9	~1.1

[1]Purity of the MON 87712-produced BBX32 protein is calculated based on the average mass of BBX32 in the duplicate lanes of the MON 87712 CEX pool.

D.1.5. BBX32 Coding Sequence Comparison

Sequence alignment showed that translation of the BBX32 coding sequence from the vector used to transform conventional soybean to produce MON 87712, PV-GMAP5779, and from the *E. coli* BBX32 expression vector, pMON102114, resulted in the same amino acid sequence (Figure D-2). As reported in Section IV.D, the DNA sequence of the T-DNA I region in MON 87712 is identical to the corresponding T-DNA I sequence of PV-GMAP5779; thus the pMON102114 BBX32 coding sequence is identical to the BBX32 coding sequence in MON 87712.

```
PLANT_V MVSFCELCGAEADLHCAADSAFLCRSCDAKFHASNFLFARHFRRVICPNC
        :::::::::::::::::::::::::::::::::::::::::::::::::::
ECOLI_V MVSFCELCGAEADLHCAADSAFLCRSCDAKFHASNFLFARHFRRVICPNC

PLANT_V KSLTQNFVSGPLLPWPPRTTCCSESSSSSCCSSLDCVSSSELSSTTRDVN
        :::::::::::::::::::::::::::::::::::::::::::::::::::
ECOLI_V KSLTQNFVSGPLLPWPPRTTCCSESSSSSCCSSLDCVSSSELSSTTRDVN

PLANT_V RARGRENRVNAKAVAVTVADGIFVNWCGKLGLNRDLTNAVVSYASLALAV
        :::::::::::::::::::::::::::::::::::::::::::::::::::
ECOLI_V RARGRENRVNAKAVAVTVADGIFVNWCGKLGLNRDLTNAVVSYASLALAV

PLANT_V ETRPRATKRVFLAAAFWFGVKNTTTWQNLKKVEDVTGVSAGMIRAVESKL
        :::::::::::::::::::::::::::::::::::::::::::::::::::
ECOLI_V ETRPRATKRVFLAAAFWFGVKNTTTWQNLKKVEDVTGVSAGMIRAVESKL

PLANT_V ARAMTQQLRRWRVDSEEGWAENDNV
        :::::::::::::::::::::::::
ECOLI_V ARAMTQQLRRWRVDSEEGWAENDNV
```

Figure D-2. BBX32 Coding Sequence Comparison
The regions of *E. coli* plasmid pMON102114 (ECOLI_V) and plant vector PV-GMAP5779 (PLANT_V) containing the BBX32 coding sequence were translated. The resulting amino acid sequences were aligned.

Appendix E: Materials and Methods Used for the Analysis of the Levels of BBX32 Protein in MON 87712

E.1. Materials

Over-season leaf (OSL-1 - OSL-4), root (OSR-3), forage (forage-1), and seed tissue samples from MON 87712 and the conventional control A3525 were harvested from eight field sites in the U.S. during the 2009 growing season from starting seed lots 11223539 and 11223542, respectively. An *E. coli*-produced BBX32 protein (lot 11267091) was used as the analytical reference standard.

E.2. Characterization of the Materials

The identities of MON 87712 and the conventional control samples were confirmed by event specific polymerase chain reaction (PCR) analyses that were conducted on the harvested seed from each site. The PCR analyses and the resulting Verification of Identities were archived in the Monsanto Regulatory Archives under the starting seed lot numbers.

E.3. Field Design and Tissue Collection

Field trials were initiated during the 2009 planting season to generate MON 87712 samples at various soybean growing locations in the U.S. The OSL-1 - OSL-4, OSR-3, forage-1 and seed tissue samples from the following field sites were analyzed: Jackson County, Arkansas; Parke County, Indiana; Clinton County, Illinois; Madison County, Illinois; Stark County, Illinois; York County, Nebraska; Boone County, Indiana; and Pawnee County, Kansas. These field sites were representative of soybean producing regions suitable for commercial production. At each site, four replicated plots of plants containing MON 87712 and the conventional control A3525 were planted using a randomized complete block field design. OSL-1 - OSL-4, OSR-3, forage-1, and seed samples were collected from each replicated plot at all field sites. See Table V-1 for a detailed description of when the samples were collected.

E.4. Tissue Processing and Protein Extraction

Tissue samples were shipped to Monsanto, St. Louis and prepared by the Monsanto Sample Management Team. The prepared tissue samples were stored in a -80 °C freezer until transferred on dry ice to the analytical facility, except forage conventional control A3525 which were stored in a -20 °C freezer.

BBX32 protein was extracted from all tissues samples at 10:1 buffer:sample ratio (v/w) using a Polytron (Kinematica AG, Lucerne, Switzerland) with the appropriate amount of BBX32 extraction buffer [50 mM Tris-HCl buffer, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% (v/v) IGEPAL CA-630, 5 mM NaF, and 5 mM $NaVO_4$, supplemented with 4 mM TCEP, neutralized with 0.65 M Na_2HPO_4 at 20:1 ratio (v/v)] containing protease inhibitor cocktail (1 mM Benzamidine, 0.1 µM Bestatin, 10 µM E64 and 250 µM AEBSF)]. After homogenization each sample had 8, ¼" chrome steel beads added, and

was rotated at 4°C for 120 minutes. Each extract was clarified by centrifugation for 20 minutes x 10,000 × *g* at 4°C. The supernatant was collected, aliquoted and either analyzed immediately, or stored at -80 °C.

E.5. BBX32 Antibody

Production of the BBX32 monoclonal antibody was performed by Harlan Laboratories (Indianapolis, IN). Mouse monoclonal antibody from cell line SW2 8G7.16 (cell line I.D. Number 80212742, mouse IgG1 isotype), produced against the BBX32 protein, was purified from mouse ascites fluid using Protein G affinity chromatography and was used as the primary antibody in the BBX32 western blot. The antibody was then aliquoted and assigned lot number, G 863954. The concentration of the purified monoclonal antibody was determined to be 1.60 mg/ml by spectrophotometric methods. The purified antibody was stored in a phosphate buffered saline (pH 7.2) (1 × PBS) buffer, and 15 mM sodium azide. Horse anti mouse IgG conjugated to horseradish peroxidase (Cell Signaling Technology, Boston, MA. Lot 22) was used as the secondary antibody.

E.6. BBX32 Western Blot Method

Extracts were analyzed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) using a Bis-Tris 10% polyacrylamide Midi Gel (Invitrogen, Carlsbad, CA, Cat # WG1202BOX). Prior to loading, the samples including the conventional controls were diluted in 2 × Laemmli Buffer (Bio Rad, Hercules, CA, Cat # 161 0737) and heated at 95 °C for 5 minutes. Sample extracts were loaded on the gels with the appropriate reference standards. The reference standards were prepared in tissue-specific conventional control extracts and diluted in 1 × Laemmli buffer.

Preliminary analysis indicated that the BBX32 protein was not detectable in seed and forage tissues. Therefore, two different approaches for running samples and reference standards were taken depending on the expression level.. For tissues where the protein was detectable (leaf and root), the reference standards were diluted in a conventional tissue extract and loaded at concentrations ranging from 4 pg to 0 pg per lane. Leaf and root samples were loaded in triplicate and at least two bands were used for densitometry and quantification of the BBX32 protein. For seed and forage, only a single reference standard, diluted in conventional tissue extract, was loaded at 2 pg/lane for forage and 5 pg/lane for seed. Forage and seed samples were loaded in a single well per sample. The conventional control extracts used for diluting the reference standard for each tissue type were produced from a non-systematically selected tissue specific sample. Additionally, the Precision Plus Protein Dual Color Standards (Bio Rad, Cat # 161 0374) was loaded on the gel to demonstrate the transfer of protein to the membrane and for the approximate molecular weight determination. Electrophoresis was conducted at 100 V for approximately 120 minutes in MES running buffer (Invitrogen, Cat# NP0002), pH 7.0, with 4 mM TCEP (Thermo Scientific, Cat # 20491).

Proteins separated by SDS PAGE were electrophoretically transferred to 0.45 µm Invitrolon Polyvinylidene fluoride (PVDF) membrane (Invitrogen, Cat # LC2007) using 1 × NuPage transfer buffer (Invitrogen, Cat # NP0006 1) containing 20% methanol at 300

mA for approximately one hour. After transfer, nonspecific sites on the membrane were blocked overnight at 4 °C using 5% (w/v) non fat dried milk (NFDM) (Bio Rad, Hercules, CA, Cat # 170 6404) in 1 × Phosphate Buffered Saline with 0.05% (v/v) Tween 20 (1 × PBST). The membrane was probed for the presence of the BBX32 protein with a 1:1000 dilution of purified mouse monoclonal antibody (lot G 863954) in 1 × PBST with 5% (w/v) NFDM. Unbound antibody was removed by washing four times for 10 minutes each in 1 × PBST. Bound antibody was probed with a 1:5000 dilution of horse anti-mouse IgG antibody conjugated to horseradish peroxidase (Cell Signaling Technology, Cat # 7076, lot 22) in 1 × PBST with 5% (w/v) NFDM. Unbound antibody was removed by washing four times for 10 minutes each in 1 × PBST. The SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, Rockford, IL, Cat # 34076) comprised of SuperSignal western Dura Luminol Enhancer solution and the SuperSignal Western Dura Stable Peroxide Buffer were added to the membrane at a 1:2 ratio according to the manufacturer's instructions. The membrane was exposed to Amersham Hyperfilm enhanced chemiluninescence (ECL) (GE Healthcare, Cat # 28906839) and the film was developed using a Konica SRX 101A automated film processor (Tokyo, Japan). Different exposures were taken and a single exposure was scanned using a Bio Rad GS 800 densitometer with the supplied Quantity One software (version 4.4.0).

E.7. Moisture Analysis

Tissue moisture content was determined in all tissue types that expressed BBX32 at detectable levels using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). A homogeneous tissue-specific site pool (TSSP) was prepared consisting of samples of a given tissue type grown at a given site. The average percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$\text{DWCF} = 1 - \left(\frac{\text{Mean\% TSSP Moisture}}{100}\right)$$

The DWCF was used to convert protein levels assessed on a ng/g fresh weight (fwt) basis into levels reported on a ng/g dry weight (dwt) basis using the following calculation:

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

The protein levels that were reported to be less the limit of detection (LOD) on a fresh weight basis were not reported on a dry weight basis.

E.8. Data Analyses

The standards present on the developed film were used to establish a standard curve. At least three standard points were utilized to generate the standard curve for sample quantification. Anti BBX32 antibodies detected the MON 87712 produced BBX32, a ~25 kDa immunoreactive band not observed in the extract from the conventional control, A3525. For each sample where the presence of BBX32 was detected and clearly differentiated from the cross-reactive bands present in the conventional control, the levels were quantified by interpolation on the standard curve. The coefficient of variation (CV) met the pre-set criteria of ≤33% for the samples. Of the triplicate loads of each detectable sample either all three sample replicates, or a minimum of two, were used for densitometry and quantification. If only duplicate bands were visible for a sample they were used if each value was within 33% of the average. For leaf and root tissues where BBX32 was not detected, the levels were reported as below the limit of detection (<LOD). LOD is the protein amount corresponding to the lowest detectable band across all of the blots run for that tissue type. Thus samples reported as <LOD were not detected by western blot. For leaf and root tissues, LOQ corresponds to the range of all lowest detectable bands across all blots run for the specific tissue type.

Following the interpolation from the standard curve, the amount of protein (pg) in the tissue was reported on a "ng/g fwt" basis for data that were greater than or equal to the lowest detectable point on the standard curve of the western blot on which it was run. This conversion utilized the sample dilution factor and the tissue to buffer ratio. The protein values in "ng/g fwt" were also converted to "ng/g dwt" by applying the DWCF. Microsoft Excel 2007 (Microsoft, Redmond, WA) was used to calculate the BBX32 protein levels in soybean tissues. The sample means, standard deviations (SDs), and ranges were also calculated by Microsoft Excel 2007. All protein expression levels were rounded to two significant figures.

For forage and seed samples each extract was loaded in a single well and a single reference standard was used. Western blot analysis confirmed that the BBX32 protein was not detected in forage and seed. Separately, the LOD of the BBX32 protein in forage and seed tissues was determined by serially spiking the BBX32 protein reference standard in conventional control forage and seed extracts. The LOD of the BBX32 protein in MON 87712 seed and forage were analyzed using the aforementioned materials and methods with the exception of the standard curve preparation which was done as follows: Prior to loading, the samples were diluted in 2 × Laemmli Buffer (Bio-Rad, Hercules, CA, Cat # 161-0737). The seed tissue extract was further diluted 2-fold with 1× Laemmli Buffer to reduce matrix effects. The BBX32 protein reference standard (lot 11267091) was serially diluted into the diluted extracts to generate six protein concentrations to be loaded on the gel of 4.0, 2.0, 1.0, 0.5, 0.25, 0 pg for forage tissue extracts, and 20, 10, 5.0, 2.5, 1.25, 0 pg for seed tissue extracts. The spiked protein standards were heated at 95 °C for 5 minutes prior to loading, cooled, and then 10 µl of each sample was loaded on to the gel in triplicate. The LOD of BBX32 on the western blots was defined by the lowest amount of BBX32 protein that resulted in a visible band on the western blot.

Appendix F: Materials, Methods, and Individual-Site Results for Compositional Analysis of MON 87712 Soybean Seed and Forage

Compositional comparisons between MON 87712 and the conventional soybean control A3525 were performed using the principles and analytes outlined in the OECD consensus documents for soybean composition (OECD, 2001). These principles are accepted globally and have been employed previously in assessments of soybean products derived through biotechnology. The compositional assessment was conducted on seed and forage samples harvested from a single growing season conducted in the U.S. during 2009 under typical agronomic practices.

The materials and methods for compositional analysis, as well as the individual-site results (Tables F-4 to F-27), are provided below.

F.1. Materials

Forage and harvested seed from MON 87712, a conventional soybean control A3525 that has similar genetic background to that of MON 87712, and sixteen conventional commercial reference varieties were compositionally assessed. The conventional commercial reference varieties are listed in Table F-1.

Table F-1. Commercial Reference Soybean Varieties

Material Name	Orion ID	Field Site Codes
Midland 363	10001570	ARNE, KSLA
Pioneer 93B15	10001304	ARNE
Hoegemeyer 333	10001590	ARNE
Hoffman Seed HS387	11225760	ILCY, ILHI
Maverick	11225761	ILCY, ILHI
Maverick	10001507	KSLA
Williams 82	11225762	ILCY, ILHI
FS 3591	10001448	INRC, INSH
Garst 3585N	10001517	INRC, INSH
Dwight	10001434	INRC, INSH
Crows 37003N	10001508	ILWY
Crows C3908	10001074	ILWY
Lewis 391	10001476	ILWY
QualityPlus 365C	10001129	KSLA
NuPride 3202	11225763	NEYO
NuTech 315	11225764	NEYO
Pioneer 93M14	11225765	NEYO

F.2. Characterization of the Materials

The identities of the seed and forage samples from the T/C/R substances were verified by the Study Director prior to conducting the study by confirming the chain-of-custody documentation supplied with the seed and forage harvested from the field plots. The harvested seed of MON 87712, the conventional control, and the commercial reference varieties were characterized by event-specific polymerase chain reaction (PCR) analysis, for the presence or absence of the MON 87712 event.

F.3. Field Production of the Samples

Harvested seed and forage samples of MON 87712, the conventional control A3525, and conventional commercial reference varieties were collected from eight replicated sites in the U.S. during the 2009 growing season. The sites were: Jackson County, Arkansas (ARNE), Parke County, Indiana (INRC), Clinton County, Illinois (ILCY), Madison County, Illinois (ILHI), Stark County, Illinois (ILWY), Boone County, Indiana (INSH), Pawnee County, Kansas (KSLA), and York County, Nebraska (NEYO). The starting seeds were planted in a randomized complete block design with four blocks per site. MON 87712, the conventional control, and the commercial reference varieties were grown under normal agronomic field conditions for their respective geographic regions. Seed and forage samples were harvested from all plots and shipped on dry ice (forage) or at ambient temperature (harvested seed) to Monsanto Company, St. Louis, MO. A subsample for compositional analysis was obtained from each tissue sample collected. These subsamples were ground and stored in a freezer set to maintain –20°C until their shipment on dry ice to Covance Laboratories, Inc. (Madison, WI) for analysis.

F.4. Summary of Analytical Methods and Reference Standards

Nutrients assessed in this study included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), fiber (ADF and NDF), amino acids (18 components), fatty acids (FA, C8-C22), and vitamin E (α-tochopherol) in seed, and proximates (ash, carbohydrates by calculation, moisture, protein, and fat) and fiber (ADF and NDF) in forage. Anti-nutrients assessed in seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitors, and isoflavones (daidzein, genistein, and glycitein).

All compositional analyses were performed at Covance Laboratories, Inc. (Madison, WI). Methods for analysis were based on internationally-recognized procedures and literature publications. Brief descriptions of the methods utilized for the analyses are described below.

F.4.1 Acid Detergent Fiber

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. The lignocellulose fraction was collected on the frit and determined gravimetrically (Goering and Van Soest, 1970). The limit of quantitation (LOQ) for this study was 0.100%.

F.4.2. Amino Acid Composition

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 (AOAC-International, 2005a). The limit of quantitation for this study was 0.100 mg/g.

Reference Standards:

- ThermoScientific, Amino Acid Standard H, 2.5±0.1 µmol/mL per constituent except cystine (1.25±0.1 µmol/mL), Lot Number KG137091
- Sigma-Aldrich, L-Norvaline, 100%, Lot Number 087K1954
- Sigma-Aldrich, L-Tryptophan, 100%, Lot Number 097K0119
- Sigma-Aldrich, L-Cysteic Acid Monohydrate, 99.5%, Lot Number 1305674
- Sigma-Aldrich, L-Methionine Sulfone, 100%, Lot Number 047K1321

F.4.3. Ash

The sample was placed in an electric furnace at 550°C and ignited. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash (AOAC-International, 2005b). The limit of quantitation for this study was 0.100%.

F.4.4. Carbohydrates

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation (Merrill and Watt, 1973):

$$\% \text{ carbohydrates} = 100\ \% - (\% \text{ protein} + \% \text{ fat} + \% \text{ moisture} + \% \text{ ash})$$

The limit of quantitation for this study was 0.100%.

F.4.5. Fat by Acid Hydrolysis

The sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted with ether and hexane. The extract was evaporated on a steambath, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again on a steambath under nitrogen, dried, and weighed (AOAC-International, current edition). The limit of quantitation for this study was 0.100%.

F.4.6. Fat by Soxhlet Extraction

The sample was weighed into a cellulose thimble containing 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 (AOAC-International, 2007a). The limit of quantitation for this study was 0.100%.

F.4.7. Fatty Acids

The lipid was extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in 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 (AOAC-International, 2005c; AOCS, 1997a). The limit of quantitation was 0.0200%.

Reference Standards:

Component	Lot Number	Component	Weight (%)	Purity (%)
Nu-Chek Prep GLC Reference Standard Hazelton No. 1	MA30-U	Methyl Octanoate	16.66	99.6
		Methyl Decanoate	16.66	99.5
		Methyl Laurate	16.66	99.8
		Methyl Myristate	16.66	99.8
		Methyl Palmitoleate	16.66	99.7
		Methyl Linolenate	16.66	99.4
Nu-Chek Prep GLC Reference Standard Hazelton No. 1	JY20-U	Methyl Octanoate	16.66	99.6
		Methyl Decanoate	16.66	99.6
		Methyl Laurate	16.66	99.8
		Methyl Myristate	16.66	99.8
		Methyl Palmitoleate	16.66	99.7
		Methyl Linolenate	16.66	99.5
Nu-Chek Prep GLC Reference Standard Hazelton No. 2	AU24-T	Methyl Arachidate	33.33	99.6
		Methyl 11-Eicosenoate	33.33	99.5
		Methyl Arachidonate	33.33	99.6
Nu-Chek Prep GLC Reference Standard Hazelton No. 2	AU16-U	Methyl Arachidate	33.33	99.6
		Methyl 11-Eicosenoate	33.33	99.5
		Methyl Arachidonate	33.33	99.6
Nu-Chek Prep GLC Reference Standard Hazelton No. 3	JY17-T	Methyl Myristoleate	12.5	99.6
		Methyl Pentadecanoate	12.5	99.6
		Methyl 10-Pentadecenoate	12.5	99.5
		Methyl Heptadecanoate	12.5	99.7
		Methyl 10-Heptadecenoate	12.5	99.6
		Methyl 11-14 Eicosadienoate	12.5	99.6
		Methyl Behenate	12.5	99.8
		Methyl 11-14-17 Eicosatrienoate	12.5	99.5
Nu-Chek Prep GLC Reference Standard Hazelton No. 3	JY28U	Methyl Myristoleate	12.5	99.5
		Methyl Pentadecanoate	12.5	99.6
		Methyl 10-Pentadecenoate	12.5	99.5
		Methyl Heptadecanoate	12.5	99.6
		Methyl 10-Heptadecenoate	12.5	99.5
		Methyl 11-14 Eicosadienoate	12.5	99.6
		Methyl Behenate	12.5	99.8
		Methyl 11-14-17 Eicosatrienoate	12.5	99.5
Nu-Chek Prep GLC Reference Standard Hazelton No. 4	MA30-U	Methyl Palmitate	27.0	99.6
		Methyl Stearate	19.0	99.5
		Methyl Oleate	27.0	99.8
		Methyl Linoleate	27.0	99.8

- Nu Chek Prep, Methyl Gamma Linolenate, >99%, Lot Number U-63M-08-T
- Nu Chek Prep, Methyl Tridecanoate, >99%, Lot Number N-13M-MA25-T

F.5.8. Isoflavones Analysis

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 detection and was compared to an external standard curve of known standards for quantitation (Pettersson and Kiessling, 1984; Seo and Morr, 1984). The limit of quantitation for each component was 10.0 ppm (µg/g).

Reference Standards:

- Chromadex, daidzein, 97.5%, Lot number 00004007-121
- Indofine, glycitein, 97%, Lot number 0803103
- Indofine, genistein, 99.35%, Lot number 0604043

F.5.9. Lectin

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 measured on 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 (Klurfeld and Kritchevsky, 1987; Liener, 1955). The limit of quantitation for this study was 0.10 H.U./mg.

F.5.10. Moisture

The sample was dried in a vacuum oven at approximately 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture (AOAC-International, 2007b). The limit of quantitation for this study was 0.100%.

F.5.11. Neutral Detergent Fiber, Enzyme Method

The sample was 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 (AACC, 1998; Goering and Van Soest, 1970). The limit of quantitation for this study was 0.100%.

F.5.12. Phytic Acid

The sample was extracted using 0.5M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer high-performance liquid chromatography column PRP-1, 5µm (150 x 4.1mm) with a refractive index detector (Lehrfeld, 1989; Lehrfeld, 1994). The limit of quantitation for this study was 0.100%.

Reference Standard:

- Sigma-Aldrich, Phytic Acid, Sodium Salt Hydrate, 96%, Lot Number 089K0159

F.5.13. Protein

The protein and other organic nitrogen in the sample were converted to ammonia by digesting the sample with sulfuric acid containing a catalyst mixture. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25 (AOAC-International, 2005d; AOCS, 1997a). The limit of quantitation for this study was 0.100%.

F.5.14. Raffinose and Stachyose

Sugars in the sample are extracted with a 50:50 water:methanol solution. Aliquots are taken, dried under inert gas, and then reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl-β-D-glucopyranoside as the internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector (Brobst, 1972; Mason and Slover, 1971). The quantitation limit for this study was 0.0500%.

Reference Standards:

- Sigma-Aldrich, D-(+)-Raffinose Pentahydrate, 99% Lot Number 037K1059
- Sigma-Aldrich, Stachyose Hydrate, 98%, Lot Number 049K3800

F.5.15. Trypsin Inhibitor

The sample was ground and defatted with petroleum ether. A sample of matrix was extracted with 0.01N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoy1-DL-arginine~p~nitroanilide 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 centrifuged, then the absorbance was determined at 410 nm. Trypsin inhibitor activity was determined by photometrically measuring the inhibition of trypsin's reaction with benzoyl-DL-arginine~p~nitroanilide hydrochloride (Kakade et al, 1997). The limit of quantitation for this study was 1.00 Trypsin Inhibitor Units (TIU)/mg.

F.5.16. Vitamin E

The sample was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column (Cort, et al., 1983; McMurray, et al., 1980; Speek, et al., 1985). The limit of quantitation for this study was 0.500 mg/100g.

Reference Standard:

- USP, Alpha Tocopherol, 98.9%, Lot Number N0F068

F.6. Data Processing and Statistical Analysis

After compositional analyses were performed, data spreadsheets were forwarded to Monsanto Company. The data were reviewed, formatted, and sent to Certus International, Inc. (Chesterfield, MO) for statistical analysis.

The following formulas were used for re-expression of soybean composition data for statistical analysis (Table F-2):

Table F-2. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture), Fiber, Phytic Acid, Raffinose, Stachyose	% fwt	% dwt	X/d
Isoflavones	µg/g fwt	µg/g dwt	X/d
Lectin	H.U./mg fwt	H.U./mg dwt	X/d
Trypsin Inhibitor	TIU/mg fwt	TIU/mg dwt	X/d
Vitamin E	mg/100g fwt	mg/100g dwt	X/d
Amino Acids (AA)	mg/g fwt	% dwt	X/(10d)
Fatty Acids (FA)	% fwt	% Total FA	$(100)X_j/\Sigma X$, for each FA_j where ΣX is over all the FA

[1]'X' is the individual sample value; 'd' is the fraction of the sample that is dry matter.

In order to complete a statistical analysis for a component in this study, at least 50% of the values for an analyte had to be greater than the assay limit of quantitation (LOQ). Analytes with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 14 analytes with more than 50% of observations below the assay LOQ 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 acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid.

If less than 50% of the observations for a component were below the LOQ, individual analyses that were below the LOQ were assigned a value equal to one-half the LOQ. The following analyte was assigned a value (Table F-3):

Table F-3. Component with Observations Below the Assay Limit of Quantification Not Excluded from Statistical Analysis

Component	Units	Obs. Below LOQ		Total	LOQ	Value
		N	(%)	N		Assigned
Seed Fatty Acid						
20:1 Eicosenoic	% fwt	25	13.2	190	0.020	0.010

The data were assessed for potential outliers using a studentized PRESS residuals calculation. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme data points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. No values were removed from analysis based on this assessment.

All soybean compositional components were statistically analyzed using a mixed model analysis of variance. The eight replicated sites were analyzed both separately and combined. Individual replicated site analyses used model *(1)*.

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

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 model *(2)*.

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

where Y_{ijk} = unique individual observation, U = overall mean, T_i = substance effect, L_j = random site effect, $B(L)_{jk}$ = random block within site effect, LT_{ij} = random site by substance interaction effect, and e_{ijk} = residual error.

A range of observed values from the reference varieties was determined for each analytical component. Additionally, the reference varieties 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 reference varieties. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

SAS® software was used to generate all summary statistics and perform all analyses. Report tables present p-values from SAS as either <0.001 or the actual value truncated to three decimal places.

® SAS is a registered trademark of SAS Institute, Inc.

Table F-4. Summary of Site ARNE Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.90 (0.13) (4.71 - 5.24)	5.20 (0.13) (4.86 - 5.66)	-0.30 (0.18) (-0.78 - -0.060)	-0.75, 0.15	0.152	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	36.05 (0.51) (34.74 - 37.51)	35.66 (0.51) (34.72 - 36.61)	0.40 (0.65) (-0.087 - 0.91)	-1.21, 2.00	0.566	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	7.57 (0.23) (7.21 - 7.89)	7.94 (0.23) (7.54 - 8.21)	-0.37 (0.31) (-0.68 - -0.11)	-1.13, 0.39	0.282	5.41, 10.36 (5.43 - 9.86)
Protein	41.06 (0.25) (40.44 - 41.58)	40.87 (0.25) (40.39 - 41.75)	0.19 (0.32) (-0.42 - 1.09)	-0.59, 0.97	0.571	35.06, 43.58 (35.11 - 42.16)
Total Fat	17.96 (0.32) (17.35 - 18.78)	18.28 (0.32) (17.54 - 19.05)	-0.31 (0.41) (-0.45 - -0.19)	-1.32, 0.69	0.471	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	17.99 (0.87) (17.24 - 19.11)	18.71 (0.87) (17.80 - 19.68)	-0.72 (1.19) (-2.40 - 0.71)	-3.63, 2.19	0.566	9.99, 22.21 (11.74 - 22.13)

Table F-4. Summary of Site ARNE Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	19.42 (0.54) (18.32 - 20.74)	19.39 (0.54) (17.80 - 20.37)	0.033 (0.76) (-1.83 - 1.47)	-1.82, 1.89	0.967	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.77 (0.015) (1.76 - 1.79)	1.76 (0.015) (1.73 - 1.81)	0.0092 (0.021) (-0.024 - 0.039)	-0.042, 0.060	0.672	1.54, 1.88 (1.58 - 1.84)
Arginine	3.08 (0.025) (3.05 - 3.12)	3.03 (0.025) (2.96 - 3.11)	0.053 (0.036) (0.0071 - 0.16)	-0.035, 0.14	0.189	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.81 (0.037) (4.73 - 4.91)	4.72 (0.037) (4.63 - 4.79)	0.090 (0.053) (-0.0027 - 0.24)	-0.039, 0.22	0.139	4.04, 5.07 (4.06 - 4.89)
Cystine	0.60 (0.013) (0.56 - 0.63)	0.62 (0.013) (0.61 - 0.63)	-0.027 (0.018) (-0.052 - -0.0029)	-0.072, 0.018	0.199	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.68 (0.062) (7.53 - 7.81)	7.54 (0.062) (7.44 - 7.67)	0.14 (0.088) (0.056 - 0.36)	-0.071, 0.36	0.152	6.28, 8.18 (6.40 - 7.94)

Table F-4. Summary of Site ARNE Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.80 (0.012) (1.77 - 1.81)	1.78 (0.012) (1.76 - 1.81)	0.015 (0.017) (-0.013 - 0.050)	-0.027, 0.056	0.422	1.52, 1.90 (1.54 - 1.85)
Histidine	1.07 (0.0077) (1.06 - 1.08)	1.07 (0.0077) (1.05 - 1.08)	0.0053 (0.011) (-0.0058 - 0.030)	-0.021, 0.032	0.645	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.93 (0.025) (1.91 - 1.97)	1.90 (0.025) (1.82 - 1.97)	0.028 (0.035) (-0.058 - 0.089)	-0.058, 0.11	0.459	1.62, 2.03 (1.60 - 2.00)
Leucine	3.16 (0.022) (3.12 - 3.20)	3.13 (0.022) (3.08 - 3.19)	0.031 (0.031) (-0.023 - 0.11)	-0.045, 0.11	0.355	2.71, 3.38 (2.77 - 3.29)
Lysine	2.67 (0.016) (2.65 - 2.70)	2.67 (0.016) (2.63 - 2.70)	0.0057 (0.022) (-0.031 - 0.069)	-0.049, 0.060	0.806	2.33, 2.81 (2.36 - 2.74)
Methionine	0.56 (0.012) (0.52 - 0.60)	0.57 (0.012) (0.56 - 0.58)	-0.0071 (0.017) (-0.031 - 0.017)	-0.048, 0.034	0.682	0.51, 0.59 (0.51 - 0.60)

Table F-4. Summary of Site ARNE Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.17 (0.018) (2.15 - 2.19)	2.14 (0.018) (2.10 - 2.17)	0.029 (0.026) (-0.016 - 0.082)	-0.034, 0.093	0.301	1.81, 2.33 (1.81 - 2.25)
Proline	2.01 (0.020) (1.99 - 2.06)	1.99 (0.020) (1.95 - 2.04)	0.025 (0.029) (-0.046 - 0.088)	-0.046, 0.095	0.423	1.70, 2.13 (1.69 - 2.09)
Serine	2.16 (0.016) (2.11 - 2.20)	2.16 (0.016) (2.13 - 2.20)	0.0022 (0.023) (-0.065 - 0.071)	-0.053, 0.057	0.924	1.86, 2.33 (1.90 - 2.30)
Threonine	1.60 (0.016) (1.57 - 1.63)	1.56 (0.016) (1.50 - 1.59)	0.034 (0.023) (-0.0077 - 0.13)	-0.021, 0.090	0.177	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.44 (0.0089) (0.41 - 0.46)	0.45 (0.0089) (0.44 - 0.46)	-0.012 (0.013) (-0.043 - 0.021)	-0.043, 0.019	0.367	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.48 (0.016) (1.45 - 1.52)	1.49 (0.016) (1.47 - 1.51)	-0.014 (0.022) (-0.045 - 0.021)	-0.069, 0.040	0.550	1.28, 1.57 (1.28 - 1.55)

Table F-4. Summary of Site ARNE Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	1.99 (0.029) (1.95 - 2.02)	1.96 (0.029) (1.85 - 2.02)	0.030 (0.041) (-0.069 - 0.13)	-0.069, 0.13	0.484	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.69 (0.043) (11.54 - 11.80)	12.01 (0.043) (11.96 - 12.07)	-0.32 (0.057) (-0.44 - -0.21)	-0.46, -0.18	0.001	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	3.85 (0.033) (3.78 - 3.89)	4.16 (0.033) (4.07 - 4.23)	-0.31 (0.031) (-0.34 - -0.22)	-0.38, -0.23	<0.001	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	24.18 (0.40) (23.08 - 25.71)	21.03 (0.40) (20.80 - 21.18)	3.15 (0.53) (2.07 - 4.66)	1.85, 4.45	0.001	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	51.50 (0.33) (50.26 - 52.27)	53.62 (0.33) (53.44 - 53.90)	-2.12 (0.46) (-3.63 - -1.28)	-3.25, -0.99	0.003	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	8.06 (0.089) (7.87 - 8.29)	8.39 (0.089) (8.17 - 8.59)	-0.32 (0.12) (-0.68 - -0.064)	-0.61, -0.040	0.031	5.60, 11.61 (5.89 - 10.16)

Table F-4. Summary of Site ARNE Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.29 (0.0084) (0.26 - 0.30)	0.32 (0.0084) (0.30 - 0.34)	-0.031 (0.012) (-0.077 - -0.0061)	-0.060, -0.0018	0.040	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.19 (0.0040) (0.18 - 0.19)	0.18 (0.0040) (0.17 - 0.19)	0.0010 (0.0057) (-0.011 - 0.019)	-0.013, 0.015	0.863	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.25 (0.019) (0.22 - 0.31)	0.29 (0.019) (0.24 - 0.32)	-0.049 (0.026) (-0.092 - -0.00071)	-0.11, 0.014	0.105	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	1.78 (0.091) (1.58 - 1.88)	2.16 (0.091) (2.00 - 2.23)	-0.39 (0.13) (-0.64 - -0.12)	-0.70, -0.073	0.023	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-5. Summary of Site ARNE Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	2.53 (0.24) (2.23 - 3.17)	2.99 (0.24) (2.53 - 3.66)	-0.45 (0.32) (-1.43 - 0.031)	-1.23, 0.32	0.204	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	1.14 (0.087) (0.86 - 1.45)	1.22 (0.087) (0.97 - 1.32)	-0.075 (0.12) (-0.46 - 0.15)	-0.37, 0.23	0.565	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.82 (0.016) (0.77 - 0.86)	0.73 (0.016) (0.69 - 0.75)	0.098 (0.0097) (0.082 - 0.14)	0.074, 0.12	<0.001	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.13 (0.078) (3.94 - 4.33)	3.80 (0.078) (3.52 - 3.99)	0.33 (0.11) (0.077 - 0.53)	0.068, 0.60	0.021	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	28.47 (2.28) (24.54 - 34.92)	36.40 (2.28) (31.47 - 42.82)	-7.93 (2.78) (-15.39 - -1.56)	-14.74, -1.11	0.029	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	603.87 (30.50) (549.34 - 687.57)	646.83 (30.50) (537.10 - 728.12)	-42.96 (38.60) (-178.78 - 63.51)	-137.42, 51.49	0.308	0, 1756.99 (138.15 - 1548.98)

Table F-5. Summary of Site ARNE Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	532.00 (35.50) (503.75 - 559.33)	610.70 (35.50) (508.77 - 778.42)	-78.70 (43.31) (-219.09 - 39.80)	-184.67, 27.27	0.119	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	116.05 (10.94) (106.50 - 131.79)	116.47 (10.94) (86.28 - 162.85)	-0.42 (14.75) (-46.46 - 23.21)	-36.51, 35.67	0.978	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-6. Summary of Site ARNE Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	6.42 (0.38) (5.35 - 7.91)	5.94 (0.38) (5.32 - 6.54)	0.48 (0.43) (-1.19 - 1.62)	-0.57, 1.53	0.305	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	66.80 (0.56) (65.75 - 67.72)	67.35 (0.56) (65.78 - 69.37)	-0.55 (0.79) (-2.29 - 1.94)	-2.49, 1.39	0.516	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	75.53 (0.54) (74.60 - 77.50)	72.63 (0.54) (71.60 - 73.70)	2.90 (0.65) (0.90 - 3.90)	1.30, 4.50	0.004	65.61, 80.67 (64.50 - 79.80)
Protein	22.25 (0.31) (21.60 - 22.99)	21.38 (0.31) (20.35 - 22.32)	0.87 (0.40) (0.12 - 1.62)	-0.10, 1.85	0.071	13.77, 26.51 (16.56 - 27.76)
Total Fat	4.41 (0.31) (3.64 - 4.96)	5.39 (0.31) (5.04 - 6.10)	-0.97 (0.44) (-2.46 - -0.076)	-2.05, 0.10	0.068	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	32.48 (1.30) (30.71 - 34.07)	31.88 (1.30) (26.14 - 35.21)	0.60 (1.36) (-2.21 - 6.09)	-2.73, 3.93	0.673	23.12, 38.15 (22.60 - 41.29)

Table F-6. Summary of Site ARNE Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	37.01 (1.72) (33.46 - 41.73)	36.71 (1.72) (33.69 - 39.79)	0.30 (1.99) (-4.41 - 6.39)	-4.56, 5.16	0.884	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-7. Summary of Site ILCY Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.86 (0.14) (4.48 - 5.27)	5.05 (0.14) (4.64 - 5.24)	-0.19 (0.19) (-0.73 - 0.62)	-0.66, 0.28	0.357	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	37.26 (0.54) (35.09 - 38.66)	37.66 (0.54) (37.17 - 38.16)	-0.40 (0.76) (-3.07 - 1.01)	-2.26, 1.45	0.614	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.34 (0.27) (7.78 - 9.13)	8.06 (0.27) (7.44 - 8.40)	0.29 (0.38) (-0.18 - 0.73)	-0.63, 1.20	0.476	5.41, 10.36 (5.43 - 9.86)
Protein	41.34 (0.37) (39.62 - 42.09)	40.95 (0.37) (40.59 - 41.27)	0.39 (0.51) (-1.65 - 1.22)	-0.85, 1.64	0.465	35.06, 43.58 (35.11 - 42.16)
Total Fat	16.56 (0.41) (14.77 - 17.60)	16.34 (0.41) (15.83 - 16.75)	0.22 (0.58) (-1.88 - 1.47)	-1.19, 1.63	0.717	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	15.30 (0.51) (13.99 - 16.40)	13.46 (0.51) (12.87 - 14.08)	1.84 (0.42) (1.13 - 2.47)	0.82, 2.86	0.004	9.99, 22.21 (11.74 - 22.13)

Table F-7. Summary of Site ILCY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	16.42 (0.46) (15.64 - 17.27)	14.58 (0.46) (13.40 - 15.37)	1.84 (0.54) (1.28 - 2.76)	0.52, 3.17	0.014	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.81 (0.016) (1.78 - 1.85)	1.76 (0.016) (1.74 - 1.78)	0.049 (0.020) (0.038 - 0.069)	-0.00087, 0.099	0.052	1.54, 1.88 (1.58 - 1.84)
Arginine	3.28 (0.034) (3.23 - 3.38)	3.11 (0.034) (3.03 - 3.20)	0.18 (0.045) (0.11 - 0.23)	0.070, 0.29	0.007	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.84 (0.035) (4.77 - 4.91)	4.75 (0.035) (4.69 - 4.83)	0.097 (0.049) (0.078 - 0.13)	-0.023, 0.22	0.096	4.04, 5.07 (4.06 - 4.89)
Cystine	0.61 (0.0072) (0.59 - 0.62)	0.63 (0.0072) (0.61 - 0.66)	-0.024 (0.010) (-0.051 - 0.00050)	-0.049, 0.0011	0.058	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.78 (0.065) (7.63 - 7.87)	7.57 (0.065) (7.46 - 7.66)	0.21 (0.081) (0.17 - 0.29)	0.010, 0.41	0.042	6.28, 8.18 (6.40 - 7.94)

Table F-7. Summary of Site ILCY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.84 (0.011) (1.80 - 1.85)	1.79 (0.011) (1.77 - 1.81)	0.044 (0.016) (0.029 - 0.061)	0.0041, 0.083	0.035	1.52, 1.90 (1.54 - 1.85)
Histidine	1.10 (0.0083) (1.08 - 1.12)	1.08 (0.0083) (1.07 - 1.09)	0.024 (0.012) (0.011 - 0.033)	-0.0043, 0.053	0.082	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.94 (0.018) (1.87 - 1.97)	1.87 (0.018) (1.84 - 1.91)	0.071 (0.025) (0.029 - 0.13)	0.010, 0.13	0.029	1.62, 2.03 (1.60 - 2.00)
Leucine	3.23 (0.021) (3.18 - 3.27)	3.15 (0.021) (3.12 - 3.20)	0.073 (0.028) (0.059 - 0.087)	0.0038, 0.14	0.041	2.71, 3.38 (2.77 - 3.29)
Lysine	2.71 (0.018) (2.66 - 2.74)	2.68 (0.018) (2.66 - 2.70)	0.036 (0.026) (-0.0052 - 0.053)	-0.027, 0.098	0.211	2.33, 2.81 (2.36 - 2.74)
Methionine	0.58 (0.0049) (0.57 - 0.59)	0.59 (0.0049) (0.58 - 0.60)	-0.0082 (0.0067) (-0.025 - 0.012)	-0.025, 0.0082	0.265	0.51, 0.59 (0.51 - 0.60)

Table F-7. Summary of Site ILCY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.22 (0.016) (2.17 - 2.26)	2.15 (0.016) (2.13 - 2.17)	0.070 (0.023) (0.039 - 0.083)	0.014, 0.13	0.022	1.81, 2.33 (1.81 - 2.25)
Proline	2.04 (0.029) (1.91 - 2.09)	2.00 (0.029) (1.95 - 2.06)	0.036 (0.040) (-0.15 - 0.14)	-0.063, 0.13	0.409	1.70, 2.13 (1.69 - 2.09)
Serine	2.22 (0.028) (2.21 - 2.22)	2.20 (0.028) (2.18 - 2.24)	0.018 (0.035) (-0.024 - 0.039)	-0.069, 0.10	0.632	1.86, 2.33 (1.90 - 2.30)
Threonine	1.63 (0.013) (1.58 - 1.66)	1.62 (0.013) (1.59 - 1.65)	0.011 (0.019) (-0.0065 - 0.040)	-0.035, 0.056	0.591	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.43 (0.015) (0.36 - 0.47)	0.46 (0.015) (0.44 - 0.49)	-0.036 (0.022) (-0.094 - 0.010)	-0.089, 0.017	0.146	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.50 (0.031) (1.42 - 1.57)	1.45 (0.031) (1.40 - 1.51)	0.057 (0.044) (-0.090 - 0.18)	-0.050, 0.16	0.242	1.28, 1.57 (1.28 - 1.55)

Table F-7. Summary of Site ILCY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	2.05 (0.022) (1.95 - 2.08)	1.96 (0.022) (1.92 - 2.01)	0.083 (0.032) (0.018 - 0.15)	0.0052, 0.16	0.040	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.79 (0.044) (11.73 - 11.86)	11.85 (0.044) (11.76 - 11.97)	-0.065 (0.038) (-0.17 - 0.013)	-0.16, 0.029	0.141	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	3.77 (0.049) (3.71 - 3.91)	4.00 (0.049) (3.94 - 4.14)	-0.22 (0.039) (-0.25 - -0.18)	-0.32, -0.13	0.001	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	19.99 (0.29) (19.88 - 20.04)	20.31 (0.29) (19.60 - 21.45)	-0.32 (0.34) (-1.57 - 0.44)	-1.15, 0.51	0.380	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	54.93 (0.21) (54.84 - 55.03)	54.34 (0.21) (53.49 - 54.78)	0.59 (0.23) (0.059 - 1.50)	0.017, 1.16	0.045	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	8.89 (0.15) (8.69 - 9.13)	8.76 (0.15) (8.17 - 9.09)	0.13 (0.17) (-0.38 - 0.52)	-0.29, 0.55	0.469	5.60, 11.61 (5.89 - 10.16)

Table F-7. Summary of Site ILCY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.27 (0.0090) (0.25 - 0.29)	0.30 (0.0090) (0.29 - 0.31)	-0.027 (0.013) (-0.042 - -0.018)	-0.058, 0.0045	0.081	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.14 (0.012) (0.077 - 0.16)	0.16 (0.012) (0.16 - 0.17)	-0.027 (0.017) (-0.082 - -0.0059)	-0.068, 0.014	0.155	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.23 (0.021) (0.18 - 0.28)	0.29 (0.021) (0.29 - 0.30)	-0.062 (0.029) (-0.11 - -0.012)	-0.13, 0.0094	0.077	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	1.03 (0.056) (0.89 - 1.14)	1.18 (0.056) (1.09 - 1.28)	-0.15 (0.079) (-0.28 - -0.0056)	-0.34, 0.043	0.106	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-8. Summary of Site ILCY Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	2.24 (0.44) (1.71 - 2.56)	2.60 (0.44) (1.42 - 3.69)	-0.36 (0.39) (-1.35 - 0.94)	-1.32, 0.60	0.394	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	1.22 (0.038) (1.10 - 1.28)	1.25 (0.038) (1.22 - 1.30)	-0.029 (0.054) (-0.20 - 0.048)	-0.16, 0.10	0.610	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.78 (0.016) (0.72 - 0.83)	0.70 (0.016) (0.68 - 0.72)	0.076 (0.019) (0.036 - 0.12)	0.030, 0.12	0.006	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.02 (0.091) (3.77 - 4.43)	4.24 (0.091) (4.18 - 4.31)	-0.22 (0.12) (-0.41 - 0.12)	-0.52, 0.070	0.111	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	32.64 (2.52) (30.15 - 36.92)	33.68 (2.52) (29.00 - 37.06)	-1.04 (3.56) (-6.91 - 4.12)	-9.76, 7.68	0.780	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	1056.89 (32.01) (980.67 - 1126.05)	1049.27 (32.01) (943.09 - 1120.78)	7.63 (45.27) (-140.11 - 182.96)	-103.14, 118.40	0.871	0, 1756.99 (138.15 - 1548.98)

Table F-8. Summary of Site ILCY Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	915.27 (22.00) (877.25 - 957.41)	863.79 (22.00) (802.44 - 912.95)	51.48 (30.69) (-31.11 - 142.13)	-23.62, 126.58	0.144	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	92.70 (6.02) (82.10 - 107.24)	97.78 (6.02) (89.62 - 121.18)	-5.08 (8.52) (-23.24 - 16.92)	-25.93, 15.77	0.573	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-9. Summary of Site ILCY Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	7.68 (0.20) (7.49 - 7.88)	7.22 (0.20) (7.10 - 7.45)	0.47 (0.23) (0.28 - 0.70)	-0.11, 1.04	0.093	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	65.33 (0.77) (63.14 - 66.31)	65.29 (0.77) (64.37 - 66.28)	0.039 (0.91) (-1.35 - 1.94)	-2.18, 2.26	0.967	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	72.85 (0.43) (71.80 - 74.50)	74.70 (0.43) (73.80 - 75.50)	-1.85 (0.46) (-3.50 - -1.00)	-2.97, -0.73	0.006	65.61, 80.67 (64.50 - 79.80)
Protein	22.12 (0.59) (21.06 - 24.04)	22.05 (0.59) (21.72 - 22.49)	0.069 (0.74) (-1.04 - 1.55)	-1.74, 1.88	0.928	13.77, 26.51 (16.56 - 27.76)
Total Fat	4.83 (0.30) (4.69 - 4.93)	5.39 (0.30) (4.57 - 6.15)	-0.56 (0.42) (-1.33 - 0.12)	-1.58, 0.45	0.224	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	29.44 (0.60) (28.24 - 30.21)	28.68 (0.60) (28.09 - 30.32)	0.76 (0.85) (-0.11 - 1.68)	-1.31, 2.83	0.403	23.12, 38.15 (22.60 - 41.29)

Table F-9. Summary of Site ILCY Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	33.99 (1.04) (33.35 - 34.71)	31.13 (1.04) (28.38 - 32.98)	2.86 (1.48) (1.20 - 5.66)	-0.76, 6.47	0.101	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-10. Summary of Site ILHI Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.67 (0.12) (4.56 - 4.91)	4.80 (0.12) (4.46 - 5.34)	-0.13 (0.17) (-0.73 - 0.22)	-0.54, 0.29	0.480	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	35.62 (0.34) (34.64 - 36.63)	35.84 (0.34) (35.50 - 36.10)	-0.22 (0.48) (-1.27 - 1.13)	-1.39, 0.94	0.657	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.34 (0.16) (8.00 - 8.49)	8.20 (0.16) (7.89 - 8.49)	0.14 (0.21) (-0.020 - 0.38)	-0.37, 0.64	0.536	5.41, 10.36 (5.43 - 9.86)
Protein	42.14 (0.36) (40.98 - 42.79)	41.39 (0.36) (41.09 - 42.12)	0.75 (0.50) (-1.15 - 1.56)	-0.49, 1.98	0.188	35.06, 43.58 (35.11 - 42.16)
Total Fat	17.56 (0.23) (16.83 - 18.14)	17.97 (0.23) (17.70 - 18.28)	-0.41 (0.22) (-0.88 - 0.13)	-0.94, 0.12	0.108	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	14.48 (0.58) (13.21 - 16.72)	16.40 (0.58) (15.89 - 16.91)	-1.91 (0.82) (-3.70 - 0.83)	-3.92, 0.10	0.059	9.99, 22.21 (11.74 - 22.13)

Table F-10. Summary of Site ILHI Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	15.77 (0.35) (14.35 - 16.83)	16.45 (0.35) (15.96 - 17.05)	-0.68 (0.50) (-1.61 - 0.83)	-1.90, 0.53	0.218	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.81 (0.018) (1.76 - 1.82)	1.79 (0.018) (1.75 - 1.80)	0.019 (0.025) (-0.041 - 0.073)	-0.043, 0.081	0.482	1.54, 1.88 (1.58 - 1.84)
Arginine	3.21 (0.060) (3.05 - 3.31)	3.16 (0.060) (3.11 - 3.26)	0.046 (0.084) (-0.20 - 0.19)	-0.16, 0.25	0.605	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.84 (0.060) (4.68 - 4.92)	4.73 (0.060) (4.65 - 4.83)	0.11 (0.085) (-0.15 - 0.24)	-0.10, 0.31	0.260	4.04, 5.07 (4.06 - 4.89)
Cystine	0.58 (0.0096) (0.57 - 0.60)	0.59 (0.0096) (0.57 - 0.60)	-0.011 (0.011) (-0.031 - 0.0059)	-0.038, 0.017	0.375	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.79 (0.11) (7.49 - 7.96)	7.62 (0.11) (7.49 - 7.74)	0.17 (0.15) (-0.25 - 0.38)	-0.19, 0.54	0.291	6.28, 8.18 (6.40 - 7.94)

Table F-10. Summary of Site ILHI Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.82 (0.018) (1.77 - 1.84)	1.80 (0.018) (1.77 - 1.81)	0.025 (0.025) (-0.041 - 0.062)	-0.037, 0.086	0.369	1.52, 1.90 (1.54 - 1.85)
Histidine	1.10 (0.015) (1.06 - 1.11)	1.07 (0.015) (1.05 - 1.09)	0.021 (0.021) (-0.024 - 0.051)	-0.030, 0.073	0.351	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.94 (0.025) (1.88 - 1.98)	1.92 (0.025) (1.89 - 1.96)	0.022 (0.035) (-0.063 - 0.073)	-0.064, 0.11	0.553	1.62, 2.03 (1.60 - 2.00)
Leucine	3.23 (0.038) (3.12 - 3.30)	3.18 (0.038) (3.12 - 3.24)	0.046 (0.053) (-0.12 - 0.12)	-0.085, 0.18	0.424	2.71, 3.38 (2.77 - 3.29)
Lysine	2.68 (0.025) (2.62 - 2.72)	2.66 (0.025) (2.62 - 2.69)	0.029 (0.036) (-0.073 - 0.068)	-0.059, 0.12	0.454	2.33, 2.81 (2.36 - 2.74)
Methionine	0.57 (0.0074) (0.56 - 0.59)	0.57 (0.0074) (0.56 - 0.58)	-0.00079 (0.0086) (-0.011 - 0.012)	-0.022, 0.020	0.929	0.51, 0.59 (0.51 - 0.60)

Table F-10. Summary of Site ILHI Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.22 (0.033) (2.13 - 2.26)	2.17 (0.033) (2.14 - 2.20)	0.044 (0.047) (-0.073 - 0.11)	-0.070, 0.16	0.380	1.81, 2.33 (1.81 - 2.25)
Proline	2.03 (0.038) (1.96 - 2.14)	2.05 (0.038) (1.99 - 2.11)	-0.013 (0.039) (-0.095 - 0.056)	-0.11, 0.082	0.745	1.70, 2.13 (1.69 - 2.09)
Serine	2.21 (0.024) (2.16 - 2.25)	2.16 (0.024) (2.11 - 2.23)	0.044 (0.035) (-0.019 - 0.14)	-0.040, 0.13	0.248	1.86, 2.33 (1.90 - 2.30)
Threonine	1.63 (0.019) (1.59 - 1.65)	1.59 (0.019) (1.55 - 1.66)	0.035 (0.027) (-0.074 - 0.083)	-0.031, 0.10	0.239	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.46 (0.011) (0.44 - 0.49)	0.46 (0.011) (0.44 - 0.48)	0.00096 (0.016) (-0.037 - 0.028)	-0.038, 0.040	0.954	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.49 (0.027) (1.43 - 1.56)	1.48 (0.027) (1.43 - 1.50)	0.010 (0.038) (-0.063 - 0.072)	-0.082, 0.10	0.790	1.28, 1.57 (1.28 - 1.55)

Table F-10. Summary of Site ILHI Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	2.03 (0.027) (1.96 - 2.07)	2.00 (0.027) (1.97 - 2.05)	0.028 (0.039) (-0.063 - 0.087)	-0.067, 0.12	0.503	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.37 (0.055) (11.30 - 11.49)	11.55 (0.055) (11.46 - 11.72)	-0.18 (0.036) (-0.23 - -0.070)	-0.27, -0.092	0.002	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	4.37 (0.073) (4.16 - 4.58)	4.50 (0.073) (4.32 - 4.64)	-0.12 (0.074) (-0.36 - 0.0014)	-0.30, 0.058	0.147	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	21.58 (0.25) (21.05 - 21.95)	20.84 (0.25) (20.62 - 20.98)	0.74 (0.34) (0.26 - 1.32)	-0.083, 1.57	0.070	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	54.24 (0.23) (53.94 - 54.60)	54.41 (0.23) (54.19 - 54.61)	-0.16 (0.32) (-0.44 - 0.052)	-0.94, 0.62	0.629	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	7.65 (0.10) (7.46 - 8.01)	7.92 (0.10) (7.77 - 8.17)	-0.27 (0.13) (-0.53 - 0.23)	-0.59, 0.044	0.079	5.60, 11.61 (5.89 - 10.16)

Table F-10. Summary of Site ILHI Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.32 (0.011) (0.28 - 0.34)	0.32 (0.011) (0.30 - 0.34)	-0.0063 (0.014) (-0.043 - 0.047)	-0.041, 0.028	0.674	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.17 (0.0035) (0.16 - 0.18)	0.17 (0.0035) (0.16 - 0.18)	0.0014 (0.0047) (-0.0097 - 0.019)	-0.010, 0.013	0.780	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.30 (0.017) (0.23 - 0.33)	0.29 (0.017) (0.23 - 0.32)	0.0039 (0.024) (-0.073 - 0.096)	-0.055, 0.063	0.876	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	1.65 (0.16) (1.30 - 2.05)	1.85 (0.16) (1.61 - 2.06)	-0.20 (0.21) (-0.76 - 0.44)	-0.73, 0.32	0.376	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-11. Summary of Site ILHI Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	1.84 (0.34) (1.49 - 2.46)	2.66 (0.34) (1.88 - 4.15)	-0.81 (0.37) (-1.69 - 0.035)	-1.71, 0.080	0.067	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	0.89 (0.072) (0.65 - 1.11)	1.14 (0.072) (1.02 - 1.23)	-0.25 (0.10) (-0.58 - 0.091)	-0.50, 0.0038	0.052	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.78 (0.017) (0.76 - 0.80)	0.90 (0.017) (0.84 - 0.93)	-0.12 (0.024) (-0.17 - -0.047)	-0.18, -0.061	0.002	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.50 (0.11) (4.44 - 4.58)	4.21 (0.11) (3.69 - 4.48)	0.29 (0.16) (0.051 - 0.90)	-0.097, 0.68	0.115	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	35.70 (2.98) (32.45 - 40.72)	32.16 (2.98) (26.40 - 35.84)	3.54 (4.22) (-3.39 - 14.32)	-6.79, 13.86	0.433	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	911.92 (40.49) (811.93 - 1017.39)	946.01 (40.49) (827.27 - 1050.16)	-34.09 (57.27) (-150.09 - 190.12)	-174.22, 106.04	0.573	0, 1756.99 (138.15 - 1548.98)

Table F-11. Summary of Site ILHI Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	750.00 (30.31) (679.71 - 829.24)	754.26 (30.31) (661.17 - 849.09)	-4.26 (37.35) (-76.63 - 122.53)	-95.65, 87.13	0.912	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	86.98 (8.44) (78.13 - 99.75)	96.44 (8.44) (77.73 - 115.62)	-9.47 (11.94) (-32.22 - 17.24)	-38.68, 19.75	0.458	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-12. Summary of Site ILHI Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	5.99 (0.20) (5.66 - 6.28)	5.55 (0.20) (4.79 - 6.11)	0.44 (0.21) (-0.11 - 0.87)	-0.081, 0.96	0.084	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	63.53 (0.73) (61.74 - 64.50)	62.90 (0.73) (61.46 - 64.97)	0.63 (0.48) (-0.66 - 1.52)	-0.54, 1.80	0.235	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	69.00 (0.54) (67.60 - 70.20)	68.35 (0.54) (66.60 - 69.90)	0.65 (0.58) (-1.30 - 2.30)	-0.76, 2.06	0.302	65.61, 80.67 (64.50 - 79.80)
Protein	22.29 (0.55) (21.30 - 23.56)	22.15 (0.55) (20.96 - 23.15)	0.14 (0.28) (-0.45 - 0.83)	-0.53, 0.82	0.626	13.77, 26.51 (16.56 - 27.76)
Total Fat	8.15 (0.27) (7.44 - 8.62)	9.36 (0.27) (9.06 - 9.80)	-1.21 (0.32) (-1.89 - -0.63)	-1.98, -0.44	0.008	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	26.24 (0.68) (24.36 - 27.87)	25.53 (0.68) (24.69 - 25.95)	0.71 (0.97) (-1.58 - 2.45)	-1.66, 3.07	0.492	23.12, 38.15 (22.60 - 41.29)

Table F-12. Summary of Site ILHI Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	29.16 (0.84) (28.30 - 30.60)	26.91 (0.84) (24.92 - 29.59)	2.25 (0.93) (-0.96 - 4.19)	-0.023, 4.52	0.051	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-13. Summary of Site ILWY Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	5.41 (0.22) (4.98 - 6.36)	5.33 (0.22) (4.94 - 5.60)	0.084 (0.31) (-0.45 - 1.42)	-0.67, 0.84	0.794	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	40.46 (0.46) (39.98 - 41.50)	39.22 (0.46) (37.73 - 40.20)	1.24 (0.40) (-0.024 - 2.47)	0.27, 2.21	0.020	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.38 (0.15) (7.95 - 8.70)	8.16 (0.15) (7.72 - 8.55)	0.22 (0.19) (-0.32 - 0.94)	-0.25, 0.69	0.291	5.41, 10.36 (5.43 - 9.86)
Protein	39.78 (0.26) (38.87 - 40.53)	40.09 (0.26) (39.57 - 40.42)	-0.31 (0.35) (-1.55 - 0.39)	-1.17, 0.54	0.407	35.06, 43.58 (35.11 - 42.16)
Total Fat	14.35 (0.35) (13.91 - 14.60)	15.35 (0.35) (14.41 - 16.73)	-1.00 (0.38) (-2.38 - 0.15)	-1.94, -0.066	0.039	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	16.59 (1.06) (15.47 - 17.49)	16.54 (1.06) (14.65 - 20.81)	0.047 (1.43) (-4.82 - 2.76)	-3.44, 3.54	0.974	9.99, 22.21 (11.74 - 22.13)

Table F-13. Summary of Site ILWY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	16.76 (0.89) (15.22 - 19.93)	17.50 (0.89) (16.84 - 18.31)	-0.75 (1.26) (-1.62 - 1.61)	-3.83, 2.34	0.576	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.73 (0.016) (1.72 - 1.75)	1.73 (0.016) (1.68 - 1.76)	-0.0013 (0.015) (-0.028 - 0.039)	-0.039, 0.036	0.933	1.54, 1.88 (1.58 - 1.84)
Arginine	3.03 (0.044) (2.93 - 3.08)	3.04 (0.044) (2.96 - 3.10)	-0.015 (0.036) (-0.065 - 0.058)	-0.10, 0.072	0.693	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.55 (0.051) (4.44 - 4.64)	4.59 (0.051) (4.45 - 4.70)	-0.038 (0.049) (-0.16 - 0.093)	-0.16, 0.082	0.463	4.04, 5.07 (4.06 - 4.89)
Cystine	0.56 (0.0064) (0.56 - 0.56)	0.57 (0.0064) (0.57 - 0.58)	-0.015 (0.0082) (-0.021 - -0.0036)	-0.035, 0.0053	0.122	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.34 (0.091) (7.18 - 7.46)	7.44 (0.091) (7.18 - 7.70)	-0.10 (0.083) (-0.37 - 0.097)	-0.31, 0.10	0.262	6.28, 8.18 (6.40 - 7.94)

Table F-13. Summary of Site ILWY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.74 (0.013) (1.72 - 1.76)	1.76 (0.013) (1.72 - 1.78)	-0.015 (0.013) (-0.041 - 0.013)	-0.046, 0.016	0.279	1.52, 1.90 (1.54 - 1.85)
Histidine	1.04 (0.012) (1.03 - 1.05)	1.06 (0.012) (1.03 - 1.09)	-0.017 (0.0098) (-0.045 - -0.00034)	-0.041, 0.0065	0.125	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.82 (0.021) (1.79 - 1.85)	1.84 (0.021) (1.80 - 1.88)	-0.020 (0.025) (-0.072 - 0.024)	-0.082, 0.041	0.452	1.62, 2.03 (1.60 - 2.00)
Leucine	3.05 (0.028) (3.00 - 3.09)	3.08 (0.028) (2.99 - 3.13)	-0.026 (0.027) (-0.082 - 0.026)	-0.091, 0.040	0.378	2.71, 3.38 (2.77 - 3.29)
Lysine	2.59 (0.026) (2.54 - 2.63)	2.62 (0.026) (2.55 - 2.69)	-0.038 (0.022) (-0.11 - 0.0034)	-0.092, 0.017	0.145	2.33, 2.81 (2.36 - 2.74)
Methionine	0.55 (0.0054) (0.54 - 0.56)	0.56 (0.0054) (0.55 - 0.57)	-0.0028 (0.0077) (-0.026 - 0.016)	-0.022, 0.016	0.731	0.51, 0.59 (0.51 - 0.60)

Table F-13. Summary of Site ILWY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.09 (0.027) (2.05 - 2.11)	2.10 (0.027) (2.04 - 2.15)	-0.014 (0.022) (-0.062 - 0.025)	-0.069, 0.040	0.547	1.81, 2.33 (1.81 - 2.25)
Proline	1.98 (0.035) (1.83 - 2.04)	1.94 (0.035) (1.85 - 1.98)	0.037 (0.037) (-0.025 - 0.079)	-0.053, 0.13	0.350	1.70, 2.13 (1.69 - 2.09)
Serine	2.08 (0.031) (2.01 - 2.13)	2.12 (0.031) (2.05 - 2.19)	-0.042 (0.024) (-0.13 - 0.0028)	-0.10, 0.017	0.135	1.86, 2.33 (1.90 - 2.30)
Threonine	1.57 (0.022) (1.51 - 1.61)	1.55 (0.022) (1.52 - 1.61)	0.017 (0.026) (-0.0063 - 0.046)	-0.047, 0.082	0.534	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.42 (0.010) (0.39 - 0.44)	0.43 (0.010) (0.40 - 0.46)	-0.011 (0.015) (-0.047 - 0.042)	-0.047, 0.024	0.468	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.45 (0.027) (1.40 - 1.47)	1.44 (0.027) (1.37 - 1.49)	0.0089 (0.037) (-0.027 - 0.080)	-0.081, 0.098	0.815	1.28, 1.57 (1.28 - 1.55)

Table F-13. Summary of Site ILWY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	1.93 (0.023) (1.89 - 1.96)	1.95 (0.023) (1.91 - 2.00)	-0.017 (0.027) (-0.072 - 0.035)	-0.083, 0.049	0.548	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.23 (0.031) (11.21 - 11.26)	11.28 (0.031) (11.22 - 11.33)	-0.049 (0.044) (-0.12 - -0.0020)	-0.16, 0.059	0.306	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	4.09 (0.062) (3.99 - 4.21)	4.27 (0.062) (4.11 - 4.44)	-0.18 (0.075) (-0.29 - -0.075)	-0.36, 0.0023	0.052	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	20.03 (0.21) (19.88 - 20.16)	19.84 (0.21) (19.41 - 20.17)	0.19 (0.30) (-0.0059 - 0.54)	-0.54, 0.92	0.549	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	54.42 (0.19) (54.31 - 54.55)	54.42 (0.19) (53.98 - 54.81)	0.0039 (0.26) (-0.26 - 0.32)	-0.64, 0.65	0.988	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	9.61 (0.078) (9.45 - 9.78)	9.50 (0.078) (9.34 - 9.71)	0.11 (0.11) (-0.087 - 0.44)	-0.15, 0.37	0.327	5.60, 11.61 (5.89 - 10.16)

Table F-13. Summary of Site ILWY Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.29 (0.0070) (0.27 - 0.31)	0.30 (0.0070) (0.28 - 0.32)	-0.014 (0.0099) (-0.042 - 0.013)	-0.038, 0.010	0.203	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.081 (0.016) (0.080 - 0.083)	0.12 (0.016) (0.080 - 0.16)	-0.036 (0.020) (-0.077 - 0.0032)	-0.085, 0.012	0.118	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.25 (0.023) (0.21 - 0.30)	0.28 (0.023) (0.22 - 0.30)	-0.024 (0.032) (-0.082 - 0.060)	-0.10, 0.055	0.485	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	0.89 (0.11) (0.81 - 0.94)	1.19 (0.11) (1.02 - 1.37)	-0.30 (0.15) (-0.44 - -0.13)	-0.68, 0.069	0.093	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-14. Summary of Site ILWY Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	1.91 (0.23) (1.37 - 2.23)	1.99 (0.23) (1.02 - 2.43)	-0.082 (0.33) (-1.05 - 1.07)	-0.89, 0.72	0.812	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	1.37 (0.072) (1.33 - 1.48)	1.33 (0.072) (1.14 - 1.44)	0.038 (0.10) (-0.12 - 0.34)	-0.21, 0.29	0.719	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.68 (0.023) (0.63 - 0.69)	0.66 (0.023) (0.64 - 0.70)	0.013 (0.032) (-0.065 - 0.057)	-0.065, 0.091	0.689	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.29 (0.055) (4.14 - 4.37)	4.21 (0.055) (4.15 - 4.31)	0.084 (0.078) (-0.025 - 0.20)	-0.11, 0.27	0.322	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	35.39 (4.06) (32.35 - 39.32)	37.57 (4.06) (27.58 - 53.09)	-2.18 (4.78) (-20.74 - 8.70)	-13.87, 9.52	0.664	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	1397.02 (59.70) (1226.73 - 1609.37)	1252.03 (59.70) (1126.30 - 1362.69)	144.98 (71.95) (-20.09 - 384.84)	-31.08, 321.05	0.090	0, 1756.99 (138.15 - 1548.98)

Table F-14. Summary of Site ILWY Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	1039.09 (47.68) (929.19 - 1204.29)	933.81 (47.68) (810.28 - 1044.37)	105.29 (61.87) (-14.02 - 266.93)	-46.10, 256.67	0.139	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	117.88 (4.74) (112.20 - 122.62)	96.68 (4.74) (85.40 - 110.11)	21.19 (5.09) (9.72 - 33.87)	8.75, 33.64	0.005	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-15. Summary of Site ILWY Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	6.18 (0.18) (5.82 - 6.53)	6.24 (0.18) (5.90 - 6.51)	-0.059 (0.26) (-0.38 - 0.35)	-0.69, 0.57	0.825	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	65.05 (0.44) (63.87 - 66.05)	64.69 (0.44) (64.08 - 66.17)	0.36 (0.47) (-0.37 - 1.97)	-0.79, 1.51	0.473	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	72.18 (0.81) (71.30 - 72.90)	70.08 (0.81) (66.60 - 71.60)	2.10 (1.07) (1.00 - 4.70)	-0.52, 4.72	0.097	65.61, 80.67 (64.50 - 79.80)
Protein	21.74 (0.46) (20.70 - 22.67)	21.90 (0.46) (21.11 - 22.57)	-0.16 (0.49) (-1.87 - 0.92)	-1.35, 1.03	0.751	13.77, 26.51 (16.56 - 27.76)
Total Fat	7.09 (0.30) (6.94 - 7.35)	7.19 (0.30) (6.69 - 8.16)	-0.091 (0.35) (-1.12 - 0.44)	-0.94, 0.76	0.802	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	29.54 (1.33) (26.09 - 32.03)	29.48 (1.33) (27.01 - 31.44)	0.052 (0.99) (-2.34 - 1.96)	-2.38, 2.49	0.960	23.12, 38.15 (22.60 - 41.29)

Table F-15. Summary of Site ILWY Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	30.73 (0.99) (29.85 - 31.55)	32.78 (0.99) (29.47 - 35.33)	-2.05 (1.40) (-4.21 - 2.08)	-5.49, 1.39	0.194	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-16. Summary of Site INRC Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.69 (0.19) (4.52 - 5.04)	4.92 (0.17) (4.58 - 5.46)	-0.23 (0.17) (-0.68 - -0.028)	-0.68, 0.22	0.246	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	38.77 (0.46) (38.33 - 39.39)	39.38 (0.40) (38.13 - 40.43)	-0.61 (0.58) (-1.87 - 0.72)	-2.09, 0.87	0.335	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.04 (0.14) (7.91 - 8.11)	7.45 (0.12) (7.17 - 7.95)	0.59 (0.18) (-0.040 - 0.94)	0.13, 1.04	0.021	5.41, 10.36 (5.43 - 9.86)
Protein	41.36 (0.31) (40.37 - 42.01)	40.71 (0.28) (39.86 - 41.17)	0.65 (0.33) (0.42 - 1.29)	-0.19, 1.50	0.103	35.06, 43.58 (35.11 - 42.16)
Total Fat	15.11 (0.39) (15.02 - 15.31)	14.99 (0.34) (13.91 - 16.42)	0.12 (0.46) (-1.19 - 0.58)	-1.07, 1.30	0.811	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	14.25 (0.70) (13.93 - 14.69)	14.96 (0.61) (13.25 - 16.31)	-0.71 (0.84) (-2.38 - 1.19)	-2.87, 1.45	0.435	9.99, 22.21 (11.74 - 22.13)

Table F-16. Summary of Site INRC Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	15.95 (0.64) (15.53 - 16.43)	16.12 (0.55) (14.23 - 17.57)	-0.17 (0.84) (-1.40 - 1.30)	-2.35, 2.00	0.844	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.76 (0.012) (1.75 - 1.77)	1.76 (0.011) (1.74 - 1.80)	0.00036 (0.016) (-0.055 - 0.034)	-0.042, 0.042	0.983	1.54, 1.88 (1.58 - 1.84)
Arginine	3.09 (0.037) (3.02 - 3.18)	3.10 (0.033) (3.02 - 3.14)	-0.011 (0.042) (-0.12 - 0.086)	-0.12, 0.096	0.808	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.70 (0.031) (4.65 - 4.73)	4.71 (0.027) (4.66 - 4.75)	-0.00071 (0.041) (-0.031 - 0.069)	-0.11, 0.11	0.986	4.04, 5.07 (4.06 - 4.89)
Cystine	0.59 (0.011) (0.59 - 0.60)	0.60 (0.0095) (0.56 - 0.63)	-0.010 (0.013) (-0.034 - 0.030)	-0.045, 0.025	0.491	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.55 (0.063) (7.49 - 7.60)	7.54 (0.054) (7.43 - 7.62)	0.0074 (0.083) (-0.058 - 0.16)	-0.21, 0.22	0.932	6.28, 8.18 (6.40 - 7.94)

Table F-16. Summary of Site INRC Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.78 (0.010) (1.77 - 1.78)	1.77 (0.0089) (1.76 - 1.80)	0.0050 (0.014) (-0.023 - 0.029)	-0.030, 0.040	0.728	1.52, 1.90 (1.54 - 1.85)
Histidine	1.06 (0.0089) (1.04 - 1.08)	1.07 (0.0077) (1.05 - 1.09)	-0.014 (0.012) (-0.046 - 0.022)	-0.044, 0.016	0.287	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.87 (0.016) (1.83 - 1.90)	1.87 (0.014) (1.83 - 1.90)	-0.0016 (0.022) (-0.073 - 0.051)	-0.057, 0.054	0.943	1.62, 2.03 (1.60 - 2.00)
Leucine	3.13 (0.018) (3.10 - 3.15)	3.14 (0.016) (3.10 - 3.16)	-0.0098 (0.024) (-0.053 - 0.043)	-0.072, 0.052	0.700	2.71, 3.38 (2.77 - 3.29)
Lysine	2.68 (0.017) (2.67 - 2.69)	2.69 (0.015) (2.65 - 2.73)	-0.015 (0.023) (-0.055 - 0.038)	-0.075, 0.044	0.533	2.33, 2.81 (2.36 - 2.74)
Methionine	0.57 (0.011) (0.56 - 0.57)	0.56 (0.0095) (0.53 - 0.59)	0.00094 (0.015) (-0.033 - 0.036)	-0.036, 0.038	0.950	0.51, 0.59 (0.51 - 0.60)

Table F-16. Summary of Site INRC Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.13 (0.019) (2.08 - 2.17)	2.15 (0.016) (2.11 - 2.16)	-0.020 (0.025) (-0.077 - 0.054)	-0.083, 0.044	0.460	1.81, 2.33 (1.81 - 2.25)
Proline	2.00 (0.025) (1.94 - 2.06)	2.03 (0.022) (1.99 - 2.08)	-0.036 (0.032) (-0.077 - 0.075)	-0.12, 0.046	0.309	1.70, 2.13 (1.69 - 2.09)
Serine	2.17 (0.027) (2.13 - 2.21)	2.20 (0.024) (2.16 - 2.25)	-0.021 (0.036) (-0.12 - 0.033)	-0.11, 0.071	0.579	1.86, 2.33 (1.90 - 2.30)
Threonine	1.59 (0.020) (1.55 - 1.63)	1.58 (0.017) (1.56 - 1.61)	0.0074 (0.026) (-0.060 - 0.064)	-0.060, 0.075	0.788	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.44 (0.0096) (0.43 - 0.45)	0.46 (0.0084) (0.45 - 0.47)	-0.023 (0.013) (-0.040 - -0.0074)	-0.055, 0.010	0.137	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.48 (0.018) (1.47 - 1.49)	1.50 (0.016) (1.49 - 1.52)	-0.020 (0.024) (-0.054 - -0.0066)	-0.082, 0.041	0.435	1.28, 1.57 (1.28 - 1.55)

Table F-16. Summary of Site INRC Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	1.97 (0.019) (1.93 - 1.99)	1.95 (0.016) (1.91 - 1.97)	0.015 (0.025) (-0.040 - 0.085)	-0.049, 0.079	0.569	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.23 (0.067) (11.10 - 11.35)	11.24 (0.063) (11.13 - 11.44)	-0.0071 (0.049) (-0.093 - 0.091)	-0.13, 0.12	0.889	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	3.67 (0.11) (3.54 - 3.84)	3.96 (0.11) (3.78 - 4.21)	-0.29 (0.048) (-0.37 - -0.24)	-0.41, -0.16	0.001	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	19.32 (0.43) (18.72 - 20.01)	19.60 (0.41) (18.72 - 20.50)	-0.27 (0.27) (-0.49 - -0.0016)	-0.97, 0.43	0.361	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	55.27 (0.36) (54.66 - 55.60)	54.55 (0.33) (53.75 - 55.02)	0.72 (0.34) (0.46 - 0.91)	-0.15, 1.58	0.085	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	9.85 (0.17) (9.49 - 10.22)	9.98 (0.16) (9.66 - 10.31)	-0.14 (0.072) (-0.31 - 0.0045)	-0.32, 0.049	0.116	5.60, 11.61 (5.89 - 10.16)

Table F-16. Summary of Site INRC Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.27 (0.0097) (0.25 - 0.29)	0.29 (0.0085) (0.27 - 0.31)	-0.020 (0.011) (-0.023 - -0.017)	-0.049, 0.0085	0.129	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.13 (0.019) (0.079 - 0.16)	0.12 (0.017) (0.079 - 0.16)	0.0068 (0.020) (-0.0043 - 0.00068)	-0.045, 0.059	0.750	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.25 (0.026) (0.19 - 0.29)	0.26 (0.023) (0.18 - 0.29)	-0.0089 (0.035) (-0.014 - 0.0073)	-0.098, 0.080	0.806	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	0.96 (0.050) (0.90 - 1.03)	0.86 (0.043) (0.79 - 0.95)	0.092 (0.066) (0.077 - 0.13)	-0.077, 0.26	0.219	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-17. Summary of Site INRC Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	2.03 (0.45) (1.22 - 3.41)	2.35 (0.39) (1.43 - 3.05)	-0.32 (0.59) (-1.84 - 1.21)	-1.85, 1.20	0.608	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	1.08 (0.065) (0.96 - 1.17)	1.17 (0.057) (1.03 - 1.24)	-0.089 (0.075) (-0.12 - -0.066)	-0.28, 0.10	0.291	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.63 (0.046) (0.54 - 0.70)	0.59 (0.042) (0.52 - 0.66)	0.035 (0.049) (0.015 - 0.062)	-0.090, 0.16	0.500	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.59 (0.12) (4.46 - 4.76)	4.70 (0.11) (4.48 - 4.88)	-0.11 (0.13) (-0.17 - -0.019)	-0.45, 0.23	0.437	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	31.45 (2.42) (32.21 - 32.86)	32.94 (2.15) (24.37 - 38.89)	-1.49 (2.65) (-6.64 - -1.61)	-8.31, 5.33	0.598	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	1036.00 (48.75) (978.39 - 1120.78)	878.45 (42.22) (770.23 - 1050.52)	157.54 (64.50) (-72.13 - 350.56)	-8.25, 323.33	0.058	0, 1756.99 (138.15 - 1548.98)

Table F-17. Summary of Site INRC Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	918.56 (57.59) (852.43 - 974.97)	759.54 (49.88) (583.34 - 924.50)	159.02 (76.19) (-72.07 - 391.63)	-36.83, 354.87	0.091	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	97.52 (5.54) (90.56 - 104.90)	89.93 (4.80) (80.16 - 102.96)	7.59 (7.17) (-3.52 - 24.74)	-10.85, 26.02	0.338	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-18. Summary of Site INRC Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	6.65 (0.19) (6.38 - 6.93)	6.17 (0.17) (5.63 - 6.57)	0.47 (0.25) (-0.089 - 0.97)	-0.16, 1.10	0.113	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	60.79 (0.98) (59.41 - 62.73)	61.41 (0.93) (58.14 - 63.43)	-0.63 (0.70) (-2.60 - 1.27)	-2.42, 1.16	0.408	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	73.13 (0.48) (72.90 - 73.60)	73.35 (0.41) (72.90 - 74.20)	-0.22 (0.63) (-1.30 - 0.50)	-1.84, 1.41	0.745	65.61, 80.67 (64.50 - 79.80)
Protein	24.85 (0.76) (23.06 - 26.20)	23.91 (0.74) (21.87 - 26.12)	0.94 (0.45) (0.075 - 1.35)	-0.23, 2.10	0.092	13.77, 26.51 (16.56 - 27.76)
Total Fat	7.87 (0.37) (7.60 - 8.18)	8.51 (0.32) (7.84 - 9.22)	-0.64 (0.45) (-1.61 - 0.34)	-1.80, 0.51	0.212	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	27.04 (1.79) (25.61 - 29.37)	29.14 (1.57) (24.46 - 31.30)	-2.10 (2.11) (-5.70 - -0.94)	-7.54, 3.33	0.365	23.12, 38.15 (22.60 - 41.29)

Table F-18. Summary of Site INRC Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	31.87 (1.00) (29.59 - 34.10)	32.74 (0.86) (30.22 - 34.42)	-0.87 (1.32) (-3.39 - 0.76)	-4.25, 2.52	0.539	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-19. Summary of Site INSH Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.65 (0.081) (4.56 - 4.73)	4.77 (0.081) (4.55 - 4.94)	-0.12 (0.11) (-0.21 - 0.0049)	-0.40, 0.16	0.348	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	38.06 (0.64) (37.20 - 38.51)	38.18 (0.64) (37.34 - 39.61)	-0.12 (0.76) (-1.29 - 1.16)	-1.97, 1.73	0.881	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.24 (0.43) (7.60 - 8.93)	8.60 (0.43) (7.09 - 10.30)	-0.37 (0.61) (-1.69 - 1.84)	-1.85, 1.12	0.570	5.41, 10.36 (5.43 - 9.86)
Protein	41.55 (0.50) (41.02 - 42.28)	41.15 (0.50) (39.72 - 42.14)	0.40 (0.71) (-0.56 - 2.56)	-1.34, 2.15	0.594	35.06, 43.58 (35.11 - 42.16)
Total Fat	15.75 (0.39) (14.71 - 16.63)	15.92 (0.39) (15.14 - 16.88)	-0.17 (0.47) (-1.26 - 0.87)	-1.33, 0.99	0.729	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	15.91 (0.71) (14.97 - 16.96)	14.90 (0.71) (12.85 - 17.01)	1.01 (0.72) (-0.86 - 2.24)	-0.75, 2.78	0.209	9.99, 22.21 (11.74 - 22.13)

Table F-19. Summary of Site INSH Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	16.38 (0.57) (15.58 - 17.62)	16.78 (0.57) (15.38 - 18.40)	-0.41 (0.81) (-2.59 - 2.23)	-2.38, 1.56	0.632	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.79 (0.020) (1.78 - 1.82)	1.78 (0.020) (1.73 - 1.83)	0.014 (0.019) (-0.047 - 0.057)	-0.032, 0.061	0.476	1.54, 1.88 (1.58 - 1.84)
Arginine	3.19 (0.054) (3.13 - 3.29)	3.21 (0.054) (3.11 - 3.31)	-0.021 (0.072) (-0.16 - 0.18)	-0.20, 0.15	0.776	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.79 (0.056) (4.75 - 4.84)	4.79 (0.056) (4.67 - 4.89)	-0.0033 (0.063) (-0.14 - 0.14)	-0.16, 0.15	0.959	4.04, 5.07 (4.06 - 4.89)
Cystine	0.56 (0.0084) (0.55 - 0.58)	0.58 (0.0084) (0.57 - 0.62)	-0.025 (0.012) (-0.072 - 0.010)	-0.054, 0.0044	0.083	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.74 (0.12) (7.69 - 7.80)	7.76 (0.12) (7.54 - 7.97)	-0.022 (0.13) (-0.27 - 0.25)	-0.34, 0.30	0.874	6.28, 8.18 (6.40 - 7.94)

Table F-19. Summary of Site INSH Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.81 (0.019) (1.80 - 1.82)	1.80 (0.019) (1.74 - 1.84)	0.014 (0.020) (-0.036 - 0.079)	-0.035, 0.064	0.506	1.52, 1.90 (1.54 - 1.85)
Histidine	1.08 (0.013) (1.06 - 1.09)	1.09 (0.013) (1.06 - 1.12)	-0.0072 (0.013) (-0.038 - 0.038)	-0.040, 0.025	0.603	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.93 (0.025) (1.92 - 1.95)	1.90 (0.025) (1.86 - 1.96)	0.036 (0.028) (-0.038 - 0.075)	-0.033, 0.10	0.247	1.62, 2.03 (1.60 - 2.00)
Leucine	3.19 (0.041) (3.17 - 3.21)	3.19 (0.041) (3.10 - 3.26)	0.0030 (0.045) (-0.088 - 0.11)	-0.11, 0.11	0.948	2.71, 3.38 (2.77 - 3.29)
Lysine	2.69 (0.026) (2.67 - 2.70)	2.69 (0.026) (2.63 - 2.75)	-0.0058 (0.025) (-0.070 - 0.064)	-0.066, 0.055	0.823	2.33, 2.81 (2.36 - 2.74)
Methionine	0.55 (0.0069) (0.54 - 0.57)	0.57 (0.0069) (0.55 - 0.59)	-0.016 (0.0097) (-0.054 - 0.0071)	-0.039, 0.0081	0.157	0.51, 0.59 (0.51 - 0.60)

Table F-19. Summary of Site INSH Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	Difference (Test minus Control) 95% Confidence Interval	Difference (Test minus Control) Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.18 (0.031) (2.13 - 2.22)	2.18 (0.031) (2.12 - 2.24)	0.0020 (0.034) (-0.063 - 0.098)	-0.081, 0.085	0.956	1.81, 2.33 (1.81 - 2.25)
Proline	1.99 (0.038) (1.87 - 2.11)	2.02 (0.038) (1.98 - 2.07)	-0.038 (0.053) (-0.20 - 0.13)	-0.17, 0.092	0.499	1.70, 2.13 (1.69 - 2.09)
Serine	2.16 (0.037) (2.13 - 2.18)	2.22 (0.037) (2.14 - 2.34)	-0.061 (0.035) (-0.16 - 0.032)	-0.15, 0.024	0.129	1.86, 2.33 (1.90 - 2.30)
Threonine	1.62 (0.012) (1.60 - 1.64)	1.59 (0.012) (1.56 - 1.63)	0.032 (0.017) (-0.00038 - 0.064)	-0.0085, 0.072	0.101	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.46 (0.014) (0.44 - 0.48)	0.44 (0.014) (0.38 - 0.47)	0.015 (0.020) (-0.023 - 0.10)	-0.034, 0.064	0.478	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.50 (0.026) (1.47 - 1.55)	1.51 (0.026) (1.42 - 1.56)	-0.015 (0.037) (-0.095 - 0.13)	-0.11, 0.077	0.707	1.28, 1.57 (1.28 - 1.55)

Table F-19. Summary of Site INSH Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	2.04 (0.025) (2.02 - 2.06)	1.99 (0.025) (1.96 - 2.07)	0.044 (0.028) (-0.050 - 0.095)	-0.026, 0.11	0.174	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	10.99 (0.075) (10.78 - 11.21)	11.19 (0.075) (11.16 - 11.22)	-0.19 (0.11) (-0.44 - 0.050)	-0.45, 0.067	0.120	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	4.62 (0.057) (4.45 - 4.72)	4.57 (0.057) (4.45 - 4.65)	0.045 (0.080) (-0.20 - 0.25)	-0.15, 0.24	0.593	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	22.26 (0.13) (22.07 - 22.50)	21.25 (0.13) (21.07 - 21.54)	1.01 (0.18) (0.90 - 1.19)	0.57, 1.45	0.001	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	52.72 (0.14) (52.33 - 53.25)	53.25 (0.14) (53.21 - 53.27)	-0.52 (0.19) (-0.92 - 0.037)	-0.99, -0.054	0.034	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	8.70 (0.098) (8.54 - 8.87)	8.98 (0.098) (8.83 - 9.18)	-0.28 (0.14) (-0.64 - -0.0039)	-0.62, 0.060	0.090	5.60, 11.61 (5.89 - 10.16)

Table F-19. Summary of Site INSH Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.31 (0.0096) (0.27 - 0.33)	0.33 (0.0096) (0.31 - 0.34)	-0.016 (0.013) (-0.066 - 0.0050)	-0.048, 0.016	0.269	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.14 (0.016) (0.081 - 0.16)	0.15 (0.016) (0.15 - 0.16)	-0.018 (0.014) (-0.066 - 0.0011)	-0.051, 0.016	0.243	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.26 (0.019) (0.22 - 0.31)	0.29 (0.019) (0.21 - 0.32)	-0.029 (0.024) (-0.091 - 0.022)	-0.088, 0.030	0.276	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	1.32 (0.053) (1.28 - 1.37)	1.35 (0.053) (1.20 - 1.47)	-0.024 (0.075) (-0.19 - 0.15)	-0.21, 0.16	0.765	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-20. Summary of Site INSH Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	1.58 (0.22) (1.10 - 1.97)	2.17 (0.22) (1.63 - 3.05)	-0.60 (0.31) (-1.40 - 0.30)	-1.35, 0.16	0.103	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	0.99 (0.042) (0.90 - 1.08)	0.94 (0.042) (0.87 - 1.03)	0.048 (0.060) (-0.13 - 0.21)	-0.098, 0.19	0.453	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.66 (0.034) (0.58 - 0.75)	0.68 (0.034) (0.62 - 0.74)	-0.024 (0.048) (-0.12 - 0.13)	-0.14, 0.094	0.638	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.12 (0.078) (3.91 - 4.29)	4.05 (0.078) (3.78 - 4.15)	0.069 (0.11) (-0.21 - 0.28)	-0.20, 0.33	0.546	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	30.60 (4.05) (28.25 - 32.72)	30.29 (4.05) (20.34 - 41.47)	0.31 (5.69) (-8.75 - 9.85)	-13.62, 14.23	0.958	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	981.19 (40.89) (926.76 - 1105.15)	1055.58 (40.89) (910.56 - 1132.28)	-74.39 (45.24) (-187.49 - 44.95)	-185.08, 36.30	0.151	0, 1756.99 (138.15 - 1548.98)

Table F-20. Summary of Site INSH Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	728.13 (30.47) (674.24 - 809.72)	773.66 (30.47) (675.92 - 836.15)	-45.53 (32.81) (-125.71 - 37.81)	-125.82, 34.76	0.214	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	106.91 (6.85) (83.78 - 131.31)	108.31 (6.85) (102.05 - 116.49)	-1.40 (9.69) (-22.23 - 22.61)	-25.11, 22.31	0.889	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-21. Summary of Site INSH Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.95 (0.21) (4.55 - 5.28)	5.50 (0.21) (4.98 - 5.93)	-0.55 (0.27) (-1.04 - 0.11)	-1.20, 0.11	0.086	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	63.40 (0.91) (62.62 - 64.84)	63.49 (0.91) (62.18 - 64.82)	-0.086 (1.20) (-1.80 - 2.66)	-3.02, 2.84	0.944	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	68.85 (0.66) (68.00 - 69.50)	68.38 (0.66) (67.30 - 69.30)	0.48 (0.92) (-0.40 - 1.40)	-1.78, 2.73	0.624	65.61, 80.67 (64.50 - 79.80)
Protein	23.23 (0.65) (22.52 - 23.57)	22.59 (0.65) (20.98 - 24.01)	0.64 (0.83) (-1.49 - 2.53)	-1.40, 2.67	0.472	13.77, 26.51 (16.56 - 27.76)
Total Fat	8.45 (0.52) (8.16 - 8.59)	8.41 (0.52) (7.59 - 9.33)	0.039 (0.63) (-0.83 - 1.00)	-1.50, 1.58	0.952	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	25.96 (1.24) (24.43 - 27.31)	27.36 (1.24) (24.68 - 29.02)	-1.40 (1.76) (-4.54 - 0.90)	-5.70, 2.90	0.455	23.12, 38.15 (22.60 - 41.29)

Table F-21. Summary of Site INSH Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	28.21 (1.41) (26.56 - 30.13)	30.37 (1.41) (26.89 - 32.60)	-2.16 (1.78) (-4.37 - 1.04)	-6.51, 2.19	0.270	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-22. Summary of Site KSLA Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	5.63 (0.092) (5.25 - 5.85)	5.55 (0.092) (5.33 - 5.71)	0.082 (0.13) (-0.41 - 0.38)	-0.24, 0.40	0.553	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	37.84 (0.65) (36.54 - 38.71)	38.65 (0.65) (37.51 - 40.40)	-0.81 (0.90) (-2.05 - 0.88)	-3.01, 1.40	0.406	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	6.80 (0.35) (5.72 - 8.32)	6.86 (0.35) (6.44 - 7.33)	-0.055 (0.50) (-0.72 - 1.62)	-1.28, 1.17	0.916	5.41, 10.36 (5.43 - 9.86)
Protein	39.25 (0.33) (38.90 - 39.92)	38.62 (0.33) (37.73 - 39.23)	0.62 (0.35) (0.013 - 1.20)	-0.23, 1.48	0.124	35.06, 43.58 (35.11 - 42.16)
Total Fat	17.31 (0.41) (16.57 - 17.89)	17.18 (0.41) (16.25 - 17.81)	0.13 (0.58) (-1.16 - 0.95)	-1.28, 1.53	0.834	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	14.62 (0.84) (12.94 - 15.27)	14.30 (0.84) (12.41 - 17.85)	0.32 (1.19) (-2.58 - 2.81)	-2.59, 3.23	0.796	9.99, 22.21 (11.74 - 22.13)

Table F-22. Summary of Site KSLA Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	15.22 (0.58) (13.68 - 17.02)	16.08 (0.58) (15.28 - 16.62)	-0.86 (0.68) (-1.60 - 0.62)	-2.53, 0.82	0.256	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.70 (0.010) (1.69 - 1.72)	1.67 (0.010) (1.65 - 1.68)	0.037 (0.013) (0.021 - 0.051)	0.0045, 0.069	0.031	1.54, 1.88 (1.58 - 1.84)
Arginine	3.05 (0.025) (3.03 - 3.08)	2.90 (0.025) (2.85 - 2.98)	0.15 (0.028) (0.074 - 0.23)	0.083, 0.22	0.001	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.56 (0.031) (4.51 - 4.62)	4.45 (0.031) (4.36 - 4.49)	0.10 (0.039) (0.030 - 0.18)	0.0079, 0.20	0.037	4.04, 5.07 (4.06 - 4.89)
Cystine	0.62 (0.0072) (0.60 - 0.63)	0.63 (0.0072) (0.61 - 0.65)	-0.016 (0.010) (-0.053 - 0.014)	-0.041, 0.0090	0.169	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.24 (0.057) (7.19 - 7.36)	7.03 (0.057) (6.86 - 7.10)	0.21 (0.066) (0.10 - 0.34)	0.046, 0.37	0.020	6.28, 8.18 (6.40 - 7.94)

Table F-22. Summary of Site KSLA Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.72 (0.010) (1.70 - 1.75)	1.69 (0.010) (1.66 - 1.70)	0.031 (0.011) (0.013 - 0.052)	0.0032, 0.059	0.034	1.52, 1.90 (1.54 - 1.85)
Histidine	1.04 (0.0082) (1.03 - 1.06)	1.01 (0.0082) (1.00 - 1.03)	0.031 (0.010) (0.013 - 0.044)	0.0064, 0.055	0.021	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.80 (0.019) (1.78 - 1.84)	1.77 (0.019) (1.70 - 1.81)	0.029 (0.026) (-0.023 - 0.10)	-0.035, 0.092	0.316	1.62, 2.03 (1.60 - 2.00)
Leucine	3.02 (0.020) (3.00 - 3.05)	2.96 (0.020) (2.90 - 2.99)	0.063 (0.025) (0.017 - 0.12)	0.0023, 0.12	0.043	2.71, 3.38 (2.77 - 3.29)
Lysine	2.57 (0.016) (2.55 - 2.61)	2.53 (0.016) (2.49 - 2.56)	0.036 (0.016) (0.015 - 0.055)	-0.0029, 0.075	0.063	2.33, 2.81 (2.36 - 2.74)
Methionine	0.57 (0.0058) (0.55 - 0.59)	0.57 (0.0058) (0.56 - 0.57)	0.0048 (0.0081) (-0.012 - 0.031)	-0.015, 0.025	0.573	0.51, 0.59 (0.51 - 0.60)

Table F-22. Summary of Site KSLA Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.04 (0.018) (1.98 - 2.07)	1.98 (0.018) (1.95 - 2.02)	0.055 (0.023) (0.027 - 0.097)	0.00024, 0.11	0.049	1.81, 2.33 (1.81 - 2.25)
Proline	1.91 (0.028) (1.90 - 1.93)	1.93 (0.028) (1.89 - 1.97)	-0.017 (0.038) (-0.074 - 0.039)	-0.11, 0.076	0.666	1.70, 2.13 (1.69 - 2.09)
Serine	2.10 (0.019) (2.06 - 2.13)	2.04 (0.019) (2.01 - 2.08)	0.053 (0.025) (-0.027 - 0.091)	-0.0086, 0.11	0.080	1.86, 2.33 (1.90 - 2.30)
Threonine	1.56 (0.012) (1.55 - 1.57)	1.55 (0.012) (1.51 - 1.58)	0.015 (0.017) (-0.029 - 0.063)	-0.027, 0.057	0.410	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.44 (0.0095) (0.43 - 0.45)	0.42 (0.0095) (0.40 - 0.44)	0.019 (0.012) (-0.0065 - 0.038)	-0.0096, 0.048	0.154	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.45 (0.016) (1.41 - 1.48)	1.40 (0.016) (1.37 - 1.44)	0.048 (0.018) (0.023 - 0.11)	0.0044, 0.091	0.035	1.28, 1.57 (1.28 - 1.55)

Table F-22. Summary of Site KSLA Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	1.91 (0.020) (1.88 - 1.96)	1.87 (0.020) (1.78 - 1.91)	0.045 (0.025) (-0.013 - 0.11)	-0.018, 0.11	0.129	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.89 (0.033) (11.84 - 11.94)	12.12 (0.033) (12.04 - 12.23)	-0.23 (0.046) (-0.39 - -0.13)	-0.34, -0.11	0.002	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	3.97 (0.023) (3.92 - 4.03)	3.92 (0.023) (3.87 - 4.01)	0.053 (0.028) (0.022 - 0.091)	-0.014, 0.12	0.102	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	19.70 (0.11) (19.60 - 19.81)	19.71 (0.11) (19.45 - 19.99)	-0.0098 (0.10) (-0.20 - 0.15)	-0.27, 0.25	0.928	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	54.62 (0.14) (54.38 - 54.90)	54.37 (0.14) (53.90 - 54.70)	0.25 (0.16) (-0.038 - 0.48)	-0.16, 0.65	0.186	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	9.08 (0.058) (8.96 - 9.19)	9.23 (0.058) (9.03 - 9.41)	-0.15 (0.082) (-0.45 - 0.033)	-0.35, 0.049	0.113	5.60, 11.61 (5.89 - 10.16)

Table F-22. Summary of Site KSLA Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.30 (0.0084) (0.29 - 0.30)	0.26 (0.0084) (0.25 - 0.30)	0.032 (0.0078) (0.0020 - 0.049)	0.013, 0.051	0.006	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.16 (0.0030) (0.15 - 0.16)	0.15 (0.0030) (0.15 - 0.16)	0.0054 (0.0042) (-0.0062 - 0.011)	-0.0047, 0.016	0.239	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.29 (0.018) (0.28 - 0.30)	0.23 (0.018) (0.20 - 0.30)	0.052 (0.016) (-0.0048 - 0.079)	0.013, 0.092	0.017	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	1.33 (0.092) (1.17 - 1.45)	1.23 (0.092) (1.01 - 1.37)	0.094 (0.10) (-0.084 - 0.23)	-0.16, 0.34	0.392	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-23. Summary of Site KSLA Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	2.17 (0.32) (1.22 - 2.81)	1.28 (0.32) (0.58 - 2.39)	0.88 (0.46) (-1.17 - 1.63)	-0.23, 2.00	0.101	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	1.72 (0.069) (1.63 - 1.81)	1.61 (0.069) (1.39 - 1.77)	0.11 (0.097) (-0.034 - 0.42)	-0.12, 0.35	0.280	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.87 (0.011) (0.86 - 0.88)	0.82 (0.011) (0.80 - 0.85)	0.043 (0.012) (0.020 - 0.063)	0.013, 0.073	0.011	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.33 (0.065) (4.27 - 4.42)	4.21 (0.065) (4.16 - 4.25)	0.12 (0.092) (0.064 - 0.26)	-0.10, 0.35	0.236	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	34.65 (4.00) (31.85 - 39.35)	36.99 (4.00) (26.29 - 54.88)	-2.34 (5.66) (-23.03 - 6.80)	-16.20, 11.52	0.693	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	1311.42 (35.73) (1232.55 - 1393.21)	1341.83 (35.73) (1268.27 - 1442.92)	-30.41 (48.92) (-148.91 - 98.29)	-150.10, 89.29	0.557	0, 1756.99 (138.15 - 1548.98)

Table F-23. Summary of Site KSLA Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	1017.66 (44.06) (887.87 - 1093.13)	1064.05 (44.06) (1002.79 - 1175.72)	-46.39 (59.96) (-159.29 - 66.50)	-193.11, 100.33	0.468	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	98.83 (10.16) (75.71 - 125.39)	101.86 (10.16) (92.22 - 117.90)	-3.03 (12.66) (-28.68 - 23.31)	-34.01, 27.95	0.818	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-24. Summary of Site ILCY Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	7.90 (0.30) (6.93 - 8.75)	7.32 (0.30) (7.07 - 8.04)	0.58 (0.42) (-0.13 - 1.34)	-0.45, 1.62	0.216	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	68.63 (1.06) (66.67 - 70.88)	67.21 (1.06) (65.25 - 70.63)	1.42 (1.49) (-3.97 - 4.90)	-2.23, 5.08	0.377	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	75.45 (0.59) (73.90 - 76.90)	73.90 (0.59) (73.10 - 75.00)	1.55 (0.73) (0.10 - 3.80)	-0.24, 3.34	0.078	65.61, 80.67 (64.50 - 79.80)
Protein	19.75 (0.61) (18.45 - 21.17)	19.41 (0.61) (17.88 - 20.50)	0.33 (0.86) (-2.05 - 3.29)	-1.78, 2.44	0.713	13.77, 26.51 (16.56 - 27.76)
Total Fat	3.74 (0.42) (3.41 - 4.03)	6.14 (0.42) (4.57 - 7.18)	-2.40 (0.42) (-3.15 - -0.86)	-3.42, -1.38	0.001	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	33.37 (1.43) (30.74 - 38.10)	29.10 (1.43) (26.28 - 32.84)	4.26 (2.03) (-0.081 - 11.81)	-0.70, 9.22	0.080	23.12, 38.15 (22.60 - 41.29)

Table F-24. Summary of Site ILCY Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	38.88 (1.58) (33.84 - 41.65)	34.35 (1.58) (32.51 - 38.64)	4.53 (2.18) (1.33 - 7.93)	-0.80, 9.87	0.082	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-25. Summary of Site NEYO Soybean Seed Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	4.98 (0.16) (4.54 - 5.30)	4.94 (0.16) (4.69 - 5.21)	0.045 (0.19) (-0.27 - 0.25)	-0.42, 0.51	0.818	4.43, 5.89 (4.43 - 6.14)
Carbohydrates	38.54 (0.60) (37.56 - 40.03)	39.13 (0.60) (37.69 - 40.86)	-0.60 (0.85) (-1.37 - 1.05)	-2.68, 1.49	0.509	32.36, 41.63 (33.43 - 40.39)
Moisture (% fwt)	8.46 (0.20) (7.98 - 8.94)	7.88 (0.20) (7.74 - 8.07)	0.58 (0.25) (-0.090 - 1.17)	-0.039, 1.20	0.061	5.41, 10.36 (5.43 - 9.86)
Protein	41.05 (0.14) (40.85 - 41.36)	40.14 (0.14) (40.03 - 40.21)	0.91 (0.15) (0.74 - 1.17)	0.54, 1.28	<0.001	35.06, 43.58 (35.11 - 42.16)
Total Fat	15.46 (0.55) (14.44 - 16.47)	15.80 (0.55) (14.09 - 16.95)	-0.34 (0.77) (-2.32 - 0.53)	-2.22, 1.55	0.677	13.15, 23.90 (15.71 - 22.65)
Fiber (% dwt)						
Acid Detergent Fiber	14.94 (0.47) (14.11 - 15.87)	15.41 (0.47) (14.25 - 16.05)	-0.48 (0.67) (-1.56 - 1.62)	-2.11, 1.16	0.503	9.99, 22.21 (11.74 - 22.13)

Table F-25. Summary of Site NEYO Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	16.28 (0.54) (14.95 - 17.17)	17.20 (0.54) (15.45 - 18.10)	-0.93 (0.76) (-2.65 - 1.72)	-2.79, 0.94	0.269	11.03, 23.27 (12.18 - 22.88)
Amino Acid (% dwt)						
Alanine	1.78 (0.013) (1.76 - 1.80)	1.73 (0.013) (1.70 - 1.74)	0.052 (0.013) (0.042 - 0.063)	0.020, 0.084	0.007	1.54, 1.88 (1.58 - 1.84)
Arginine	3.19 (0.035) (3.16 - 3.24)	3.02 (0.035) (2.93 - 3.12)	0.17 (0.026) (0.095 - 0.23)	0.10, 0.23	<0.001	2.51, 3.33 (2.57 - 3.24)
Aspartic Acid	4.77 (0.025) (4.74 - 4.81)	4.64 (0.025) (4.57 - 4.67)	0.13 (0.031) (0.082 - 0.17)	0.053, 0.20	0.005	4.04, 5.07 (4.06 - 4.89)
Cystine	0.60 (0.0069) (0.58 - 0.62)	0.62 (0.0069) (0.61 - 0.63)	-0.023 (0.0097) (-0.051 - 0.0037)	-0.046, 0.0013	0.059	0.51, 0.67 (0.54 - 0.69)
Glutamic Acid	7.68 (0.056) (7.60 - 7.76)	7.41 (0.056) (7.28 - 7.48)	0.27 (0.068) (0.22 - 0.32)	0.11, 0.44	0.007	6.28, 8.18 (6.40 - 7.94)

Table F-25. Summary of Site NEYO Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Glycine	1.79 (0.0080) (1.78 - 1.80)	1.74 (0.0080) (1.71 - 1.76)	0.052 (0.0096) (0.033 - 0.081)	0.028, 0.076	0.001	1.52, 1.90 (1.54 - 1.85)
Histidine	1.09 (0.0059) (1.08 - 1.09)	1.06 (0.0059) (1.04 - 1.07)	0.025 (0.0043) (0.017 - 0.035)	0.014, 0.035	0.001	0.91, 1.17 (0.93 - 1.16)
Isoleucine	1.92 (0.013) (1.90 - 1.93)	1.85 (0.013) (1.82 - 1.88)	0.066 (0.018) (0.020 - 0.096)	0.023, 0.11	0.009	1.62, 2.03 (1.60 - 2.00)
Leucine	3.18 (0.016) (3.17 - 3.21)	3.09 (0.016) (3.05 - 3.11)	0.099 (0.020) (0.084 - 0.13)	0.050, 0.15	0.002	2.71, 3.38 (2.77 - 3.29)
Lysine	2.68 (0.011) (2.66 - 2.68)	2.62 (0.011) (2.59 - 2.65)	0.058 (0.013) (0.034 - 0.074)	0.025, 0.090	0.004	2.33, 2.81 (2.36 - 2.74)
Methionine	0.57 (0.0048) (0.55 - 0.58)	0.57 (0.0048) (0.56 - 0.58)	0.0020 (0.0068) (-0.018 - 0.018)	-0.015, 0.019	0.783	0.51, 0.59 (0.51 - 0.60)

Table F-25. Summary of Site NEYO Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Phenylalanine	2.17 (0.018) (2.15 - 2.19)	2.08 (0.018) (2.03 - 2.13)	0.095 (0.020) (0.064 - 0.15)	0.046, 0.14	0.003	1.81, 2.33 (1.81 - 2.25)
Proline	2.02 (0.014) (2.00 - 2.04)	1.97 (0.014) (1.95 - 1.99)	0.051 (0.020) (0.029 - 0.091)	0.0011, 0.10	0.046	1.70, 2.13 (1.69 - 2.09)
Serine	2.19 (0.027) (2.17 - 2.24)	2.12 (0.027) (2.10 - 2.13)	0.074 (0.029) (0.052 - 0.11)	0.0023, 0.14	0.044	1.86, 2.33 (1.90 - 2.30)
Threonine	1.55 (0.016) (1.52 - 1.59)	1.58 (0.016) (1.55 - 1.63)	-0.028 (0.023) (-0.089 - 0.040)	-0.084, 0.028	0.261	1.40, 1.69 (1.36 - 1.68)
Tryptophan	0.44 (0.0084) (0.41 - 0.47)	0.45 (0.0084) (0.44 - 0.47)	-0.012 (0.012) (-0.030 - 0.0028)	-0.041, 0.016	0.324	0.36, 0.50 (0.38 - 0.48)
Tyrosine	1.47 (0.032) (1.41 - 1.54)	1.45 (0.032) (1.33 - 1.51)	0.028 (0.017) (-0.0033 - 0.078)	-0.013, 0.070	0.148	1.28, 1.57 (1.28 - 1.55)

Table F-25. Summary of Site NEYO Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dwt)						
Valine	2.03 (0.017) (2.00 - 2.05)	1.95 (0.017) (1.91 - 1.99)	0.083 (0.024) (0.0091 - 0.13)	0.025, 0.14	0.013	1.71, 2.13 (1.69 - 2.09)
Fatty Acid (% Total FA)						
16:0 Palmitic	11.63 (0.052) (11.59 - 11.71)	11.89 (0.052) (11.83 - 12.00)	-0.26 (0.073) (-0.41 - -0.11)	-0.44, -0.079	0.012	7.76, 13.14 (9.00 - 12.03)
18:0 Stearic	4.00 (0.033) (3.92 - 4.10)	4.02 (0.033) (3.94 - 4.07)	-0.021 (0.033) (-0.12 - 0.032)	-0.10, 0.060	0.549	3.06, 5.10 (3.49 - 4.97)
18:1 Oleic	19.45 (0.11) (19.38 - 19.60)	19.13 (0.11) (18.73 - 19.38)	0.32 (0.15) (0.0019 - 0.68)	-0.056, 0.70	0.082	17.37, 26.86 (18.93 - 25.33)
18:2 Linoleic	54.44 (0.14) (54.10 - 54.61)	54.47 (0.14) (54.29 - 54.81)	-0.029 (0.20) (-0.35 - 0.27)	-0.52, 0.46	0.890	50.14, 57.81 (51.57 - 56.25)
18:3 Linolenic	9.80 (0.091) (9.76 - 9.85)	9.82 (0.091) (9.59 - 9.99)	-0.017 (0.12) (-0.14 - 0.20)	-0.30, 0.27	0.889	5.60, 11.61 (5.89 - 10.16)

Table F-25. Summary of Site NEYO Soybean Seed Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% Total FA)						
20:0 Arachidic	0.30 (0.0029) (0.29 - 0.31)	0.30 (0.0029) (0.29 - 0.30)	0.00053 (0.0039) (-0.0097 - 0.0091)	-0.0089, 0.010	0.894	0.22, 0.39 (0.23 - 0.38)
20:1 Eicosenoic	0.095 (0.016) (0.075 - 0.14)	0.095 (0.016) (0.073 - 0.15)	0.00010 (0.023) (-0.066 - 0.071)	-0.057, 0.057	0.996	0.094, 0.23 (0.072 - 0.21)
22:0 Behenic	0.29 (0.0028) (0.29 - 0.30)	0.29 (0.0028) (0.28 - 0.30)	0.0028 (0.0040) (-0.00065 - 0.011)	-0.0069, 0.013	0.500	0.18, 0.43 (0.16 - 0.37)
Vitamin (mg/100g dwt)						
Vitamin E	0.96 (0.045) (0.94 - 0.99)	1.13 (0.045) (1.02 - 1.30)	-0.17 (0.059) (-0.31 - -0.065)	-0.31, -0.027	0.027	0.10, 2.85 (0.86 - 2.73)

[1]dwt = dry weight; fwt = fresh weight; FA = fatty acid.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-26. Summary of Site NEYO Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Antinutrient						
Lectin (H.U./mg dwt)	2.18 (0.27) (1.31 - 2.59)	1.72 (0.27) (1.25 - 2.27)	0.45 (0.24) (0.066 - 0.97)	-0.12, 1.03	0.103	0, 6.11 (0.60 - 6.99)
Phytic Acid (% dwt)	0.99 (0.064) (0.88 - 1.12)	0.97 (0.064) (0.86 - 1.08)	0.013 (0.073) (-0.027 - 0.043)	-0.17, 0.19	0.863	0.50, 1.92 (0.66 - 1.74)
Raffinose (% dwt)	0.66 (0.011) (0.65 - 0.66)	0.71 (0.011) (0.69 - 0.74)	-0.046 (0.015) (-0.072 - -0.028)	-0.084, -0.0087	0.023	0.39, 1.01 (0.45 - 0.93)
Stachyose (% dwt)	4.27 (0.030) (4.24 - 4.29)	4.11 (0.030) (4.05 - 4.16)	0.16 (0.042) (0.11 - 0.21)	0.057, 0.26	0.009	2.45, 5.34 (2.57 - 4.68)
Trypsin Inhibitor (TIU/mg dwt)	33.52 (2.48) (29.21 - 40.86)	33.08 (2.48) (29.37 - 36.43)	0.44 (3.44) (-7.22 - 11.49)	-7.98, 8.87	0.901	20.97, 50.01 (24.22 - 51.78)
Isoflavone (µg/g dwt)						
Daidzein	1425.70 (61.46) (1378.10 - 1471.56)	1262.35 (61.46) (1076.47 - 1533.78)	163.36 (86.92) (-121.04 - 363.95)	-49.34, 376.05	0.109	0, 1756.99 (138.15 - 1548.98)

Table F-26. Summary of Site NEYO Soybean Seed Anti-nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Isoflavone (µg/g dwt)						
Genistein	1050.47 (50.27) (1006.30 - 1085.77)	898.64 (50.27) (761.46 - 1087.78)	151.83 (71.10) (-81.48 - 324.31)	-22.14, 325.79	0.076	87.22, 1792.07 (335.67 - 1409.07)
Glycitein	99.73 (9.66) (89.15 - 107.95)	99.40 (9.66) (77.28 - 129.45)	0.33 (13.66) (-31.42 - 30.67)	-33.10, 33.75	0.981	8.13, 299.67 (66.83 - 280.71)

[1]dwt = dry weight; H.U. = Hemagglutinating Units; TIU = Trypsin Inhibitor Units.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

Table F-27. Summary of Site NEYO Soybean Forage Nutrients for MON 87712 vs. Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dwt)						
Ash	6.76 (0.35) (6.14 - 7.33)	7.83 (0.35) (6.56 - 8.74)	-1.07 (0.50) (-2.60 - 0.77)	-2.29, 0.15	0.074	4.29, 8.65 (4.82 - 8.98)
Carbohydrates	66.98 (0.83) (64.83 - 68.67)	65.75 (0.83) (63.01 - 67.97)	1.22 (1.17) (-2.36 - 5.67)	-1.65, 4.10	0.337	55.73, 77.45 (54.40 - 72.96)
Moisture (% fwt)	75.38 (0.33) (74.80 - 76.40)	74.73 (0.33) (74.40 - 75.40)	0.65 (0.37) (-0.30 - 1.70)	-0.26, 1.56	0.132	65.61, 80.67 (64.50 - 79.80)
Protein	21.01 (0.48) (20.44 - 21.53)	20.57 (0.48) (18.71 - 22.24)	0.44 (0.68) (-1.79 - 2.40)	-1.23, 2.11	0.542	13.77, 26.51 (16.56 - 27.76)
Total Fat	5.37 (0.29) (4.84 - 6.53)	5.84 (0.29) (5.57 - 6.21)	-0.47 (0.41) (-1.37 - 0.95)	-1.48, 0.54	0.298	0.54, 13.11 (2.73 - 12.11)
Fiber (% dwt)						
Acid Detergent Fiber	33.04 (1.31) (30.84 - 35.04)	31.24 (1.31) (27.71 - 33.66)	1.80 (1.85) (-2.82 - 7.33)	-2.72, 6.32	0.367	23.12, 38.15 (22.60 - 41.29)

Table F-27. Summary of Site NEYO Soybean Forage Nutrients for MON 87712 vs. Conventional Control (continued)

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dwt)						
Neutral Detergent Fiber	36.33 (1.71) (34.14 - 40.76)	35.65 (1.71) (32.73 - 41.41)	0.67 (2.42) (-6.68 - 8.04)	-5.26, 6.60	0.790	24.96, 43.33 (25.78 - 44.41)

[1]dwt = dry weight; fwt = fresh weight.
[2]Test refers to MON 87712.
[3]Mean (S.E.) = least-square mean (standard error).
[4]Control refers to the non-biotechnology derived, conventional control, A3525.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial substances. Negative limits set to zero.

References for Appendix F

AACC. 1998. Approved methods of the American Association of Cereal Chemists. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

AOAC. 2005a. AOAC official method 982.30: Protein efficiency ratio. Official Methods of Analysis of AOAC International, Association of Analytical Communities International, Gaithersburg, Maryland.

AOAC. 2005b. AOAC official method 923.03: Ash of flour. Official Methods of Analysis of AOAC International, Association of Analytical Communities International, Gaithersburg, Maryland.

AOAC. 2005c. AOAC official method 983.23: Fat in foods. Official Methods of Analysis of AOAC International, Association of Analytical Communities International, Gaithersburg, Maryland.

AOAC. 2005d. AOAC official method 955.04: Nitrogen (total) in fertilizers. Official Methods of Analysis of AOAC International, Association of Analytical Communities International, Gaithersburg, Maryland.

AOAC. 2007a. AOAC official method 960.39 and 948.22. Official Methods of Analysis of AOAC International, Association of Analytical Communities International, Gaithersburg, Maryland.

AOAC. 2007b. AOAC official method 926.08 and 925.09. Official Methods of Analysis of AOAC International, Association of Analytical Communities International, Gaithersburg, Maryland.

AOCS. 1997a. Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th Edition. Method Ce 2-66, American Oil Chemists' Society, Champaign, Illinois.

Brobst, K.H. 1972. Gas-liquid chromatography of trimethylsilyl derivatives. Pages 3-8 in Methods in Carbohydrate Chemistry. Volume 6. Academic Press, New York, New York.

Cort, W., T.S. Vicente, E.H. Waysek and B.D. Williams. 1983. Vitamin E content of feedstuffs determined by high-performance liquid chromatographic fluorescence. Journal of Agriculture and Food Chemistry 31:1330-1333.

Goering, H.K. and P.J. Van Soest. 1970. Forage and fiber analyses (apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379. U.S. Department of Agriculture, Agricultural Research Service, Washington, D.C.

Kakade, M.L., J.J. Rackis, J.E. McGhee and G. Puski. 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Pages 376-382 in Cereal Chemistry. Volume 51. American Association of Cereal Chemists, St. Paul, Minnesota.

Klurfeld, D.M. and D. Kritchevsky. 1987. Isolation and quantitation of lectins from vegetable oils. Lipids 22:667-668.

Lehrfeld, J. 1989. High-performance liquid chromatography analysis of phytic acid on a pH-stable macroporous polymer column. Cereal Chemistry 66:510-515.

Lehrfeld, J. 1994. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: Problems and solutions. Journal of Agricultural and Food Chemistry 42:2726-2731.

Liener, I.E. 1955. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. Archives of Biochemistry and Biophysics 54:223-231.

Mason, B.S. and H.T. Slover. 1971. A gas chromatographic method for the determination of sugars in foods. Journal of Agricultural and Food Chemistry 19:551-554.

McMurray, C.H., W.J. Blanchflower and D.A. Rice. 1980. Influence of extraction techniques on determination of □Tocopherol in animal feedstuffs. Journal of the Association of Official Analytical Chemists 63:1258-1261.

Merrill, A.L. and B.K. Watt. 1973. Energy value of foods... Basis and derivation. Agricultural Handbook No. 74. US Department of Agriculture, Agricultural Research Service, Washington, D.C.

OECD. 2001. Consensus document on compositional considerations for new varieties of soybean: Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2001)15. Series on the Safety of Novel Foods and Feeds No.2. Organisation for Economic Co-operation and Development, Paris, France.

Pettersson, H. and K.-H. Kiessling. 1984. Liquid chromatographic determination of the plant estrogens coumestrol and isoflavones in animal feed. Journal of Association of Official Analytical Chemists 67:503-506.

Seo, A. and C.V. Morr. 1984. Improved high performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. Journal of Agricultural and Food Chemistry 32:530-533.

Speek, A.J., J. de Schrijver and W.H.P. Schreurs. 1985. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric detection. Journal of Food Science 50:121-124.

Appendix G: Materials, Methods, and Individual-Site Results for Seed Dormancy and Germination Assessment of MON 87712

G.1. Materials

Seed dormancy and germination characteristics were assessed on seed from MON 87712, a conventional control A3525, and commercial reference varieties produced at the Stark County, Illinois; Macon County, Missouri; and Butler County, Missouri sites in 2009 field trials (Appendix G). The seed from MON 87712, the conventional control A3525, and the commercial reference varieties were harvested from four replications at each of the three field sites and pooled to produce one seed lot of MON 87712, the conventional control A3525, and each commercial reference variety from each field site for dormancy and germination testing (Table G-1).

G.2. Characterization of the Materials

For the MON 87712, conventional control A3525, and commercial reference variety starting seed, the presence or absence of MON 87712 was verified by event-specific polymerase chain reaction analyses.

G.3. Germination Testing Facility and Experimental Methods

Seed dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was qualified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts, a seed trade association (AOSA, 2000; 2006; 2007).

Seed lots of MON 87712, the conventional control A3525, and four commercial reference varieties were produced from each of three sites and tested under six different temperature regimes. Six germination chambers were maintained under dark conditions with one the of the following 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 assessment. For each seed lot, four replicated paper germination towels were prepared per facility SOPs for each temperature regime. Wax coated paper was placed on a large tray followed by a water-moistened germination towel. A target of 100 seeds per seed lot were placed on the germination towel (*i.e.,* one seed lot per towel) using a vacuum planting system. A second water-moistened germination towel was placed on top of the seed. The towels were then rolled up and secured with a rubber band. All rolled germination towels were placed into appropriately labeled buckets that were then covered with ventilated plastic bags attached with rubber bands. The buckets were arranged in the germination chambers in a split-plot design. For each split-plot design, the whole-plot was the seed production location and the sub-plot was the seed substance (*i.e.,* test, control, or reference substance).

A description of each germination characteristic evaluated and the timing of evaluations are presented in Table VII-1. 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 evaluated periodically during the study for normal germinated, abnormal germinated, hard, dead, and firm-swollen seed as defined by AOSA guidelines (AOSA, 2006; 2007). AOSA only provides guidelines (AOSA, 2007) for testing seed under optimal temperatures (20/30°C); however, additional temperature regimes were included to test a range of temperature conditions. Each rolled germination towel in the additional temperature regimes (*i.e.,* 10, 20, 30, 10/20, and 10/30°C) was evaluated periodically for germinated, viable hard, dead, and viable firm-swollen seed. Emergence and/or development of essential structures of seedlings that otherwise would be categorized as "normal germinated" under optimal temperature conditions may not be so at non-optimal temperatures. Therefore, for the additional temperature regimes, no distinction was made between normal and abnormal germinated seed.

G.4. Statistical Analysis

An analysis of variance was conducted using SAS® (2008) according to a split-plot design with four replications. MON 87712 was compared to the conventional soybean control for germination characteristics of seed produced within each site (*i.e.,* individual-site analyses) and a combined-site analysis in which the data were pooled across seed production sites. The seed germination characteristics analyzed included percent germinated (categorized as percent normal germinated and percent abnormal germinated for the AOSA temperature regime), percent viable hard seed, percent dead, and percent viable firm-swollen seed. The level of statistical significance was predetermined to be 5% (α=0.05). MON 87712 was not statistically compared to the reference substances, nor were comparisons made across temperature regimes. The minimum and maximum mean values (reference range) were determined from the reference substances across the seed production sites. The following is a summary of the results from the individual-site analysis. Results from the combined-site analysis are presented in Table VII-2.

G.5. Individual-Site Seed Dormancy and Germination Analysis

In the individual-site analysis, four statistically significant differences were detected between MON 87712 and the conventional control A3525 (Table G-2). Four statistically significant differences were detected between MON 87712 and the conventional control A3525 for seed produced at the ILWY and MOAN site. At 10/20°C, MON 87712 had higher percent germinated seed than the conventional control A3525 at 10/20°C (100.0% vs. 99.3%) and lower percent dead seed than the conventional control A3525 (0.0% vs 0.8%) at the ILWY site. At 20/30°C, MON 87712 had higher percent normal germinated seed than the conventional control A3525 (99.8% vs. 97.8%) and lower percent abnormal germinated seed than the conventional control A3525 (0.0% vs. 2.0%) at the MOAN site.

® SAS is a registered trademark of SAS Institute, Inc.

Statistical differences detected between MON 87712 and the conventional control A3525 for germination characteristics in the individual-site analysis were not consistently detected across temperature regimes or seed production sites. Furthermore, the differences were not detected in the combined site analysis indicating that there is no increased weed potential for MON 87712 compared to conventional soybean.

Table G-1. Starting Seed of MON 87712, Conventional Control and Commercial Reference Varieties Used in Dormancy Assessment

Site[1]	Material Type	Material Name[2]	Phenotype	Sample ID
MOAN	Control	A3525	Conventional	11262199
MOAN	Test	MON 87712	Soybean Intrinsic Yield	11262197
MOAN	Reference	SB3369R	Glyphosate-Tolerant[3]	11262200
MOAN	Reference	Pioneer 93M14	Conventional	11262201
MOAN	Reference	NutriPride 8339	Glyphosate-Tolerant[3]	11262211
MOAN	Reference	Pioneer 93M62	Conventional	11262212
MOFI	Control	A3525	Conventional	11262057
MOFI	Test	MON 87712	Soybean Intrinsic Yield	11262055
MOFI	Reference	Stine 3300-0	Conventional	11262058
MOFI	Reference	SB3888R	Glyphosate-Tolerant[3]	11262059
MOFI	Reference	NK S30-D4	Glyphosate-Tolerant[3]	11262061
MOFI	Reference	LG-C3540	Conventional	11262063
ILWY	Control	A3525	Conventional	11262070
ILWY	Test	MON 87712	Soybean Intrinsic Yield	11262065
ILWY	Reference	SB3369R	Glyphosate-Tolerant[3]	11262072
ILWY	Reference	Pioneer 93M11	Glyphosate-Tolerant[3]	11262075
ILWY	Reference	Stine 3300-0	Conventional	11262077
ILWY	Reference	SB3819	Conventional	11262081

[1] Site = Site where seed lot was produced; ILWY = Stark County, IL; MOAN = Macon County, MO; and MOFI = Butler County, MO

[2] Material Name = Test material name is a Monsanto Regulatory ID. Control and reference material names are commercial names.

[3] Glyphosate - tolerant = Commercially-available Roundup Ready soybean.

Table G-2. Dormancy and Germination Characteristics of MON 87712 and the Conventional Control A3525 Seed Produced at each of the Three Field Sites

		ILWY[2]		MOAN[2]		MOFI[2]	
		Mean % (S.E.)[3]		Mean % (S.E.)[3]		Mean % (S.E.)[3]	
Temperature Regime	Germination Category[1]	MON 87712	Control	MON 87712	Control	MON 87712	Control
10 °C	Germinated	99.8 (0.3)	99.5 (0.3)	100.0 (0.0)	99.8 (0.3)	99.5 (0.3)	99.5 (0.3)
	Viable Hard †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	0.3 (0.3)	0.5 (0.3)	0.0 (0.0)	0.3 (0.3)	0.5 (0.3)	0.5 (0.3)
	Viable Firm Swollen	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20 °C	Germinated	99.8 (0.3)	100.0 (0.0)	99.8 (0.3)	99.3 (0.5)	98.8 (0.5)	98.8 (0.3)
	Viable Hard †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	0.3 (0.3)	0.0 (0.0)	0.3 (0.3)	0.8 (0.5)	1.3 (0.5)	1.3 (0.3)
	Viable Firm Swollen †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
30 °C	Germinated	100.0 (0.0)	100.0 (0.0)	99.0 (0.4)	99.5 (0.3)	99.0 (0.7)	99.0 (0.4)
	Viable Hard †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	0.0 (0.0)	0.0 (0.0)	1.0 (0.4)	0.5 (0.3)	1.0 (0.7)	1.0 (0.4)
	Viable Firm Swollen †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table G-2. Dormancy and Germination Characteristics of MON 87712 and the Conventional Control A3525 Seed Produced at each of the Three Field Sites (continued)

		ILWY[2]		MOAN[2]		MOFI[2]	
		Mean % (S.E.)[3]		Mean % (S.E.)[3]		Mean % (S.E.)[3]	
Temperature Regime	Germination Category[1]	MON 87712	Control	MON 87712	Control	MON 87712	Control
10/20 °C	Germinated	100.0 (0.0)*	99.3 (0.3)	99.8 (0.3)	100.0 (0.0)	99.8 (0.3)	99.3 (0.5)
	Viable Hard †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	0.0 (0.0)*	0.8 (0.3)	0.3 (0.3)	0.0 (0.0)	0.3 (0.3)	0.8 (0.5)
	Viable Firm Swollen †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10/30 °C	Germinated	99.8 (0.3)	100.0 (0.0)	100.0 (0.0)	99.5 (0.3)	98.8 (0.5)	99.0 (0.6)
	Viable Hard †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)	0.5 (0.3)	1.3 (0.5)	1.0 (0.6)
	Viable Firm Swollen †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20/30 °C	Normal Germinated	96.5 (1.0)	97.8 (1.0)	99.8 (0.3)*	97.8 (1.1)	94.0 (0.8)	94.3 (1.3)
(AOSA)	Abnormal Germinated	2.5 (0.9)	2.0 (0.8)	0.0 (0.0)*	2.0 (0.9)	5.3 (0.8)	5.0 (1.2)
	Viable Hard †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	1.0 (0.4)	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0.8 (0.5)	0.8 (0.5)
	Viable Firm Swollen †	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Note: The experimental design was a split-plot with four replications.
* Indicates a statistically significant difference (α=0.05) between MON 87712 and the conventional control A3525.
† No statistical comparisons could be made due to lack of variability in the data.
[1] Dormancy and germination characteristics were analyzed using an analysis of variance (ANOVA) model.
[2] Site codes are as follows: ILWY = Stark County, IL; MOAN = Macon County, MO; and MOFI = Butler County, MO
[3] Mean based on n = 4. S.E. = Standard Error. In some instances, the total percentage of both MON 87712 and the conventional control A3525 did not equal exactly 100% due to numerical rounding of the means.

References for Appendix G

AOSA. 2000. Tetrazolium testing handbook. Contribution no. 29. Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2006. Seedling evaluation handbook. Contribution no. 35. Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2007. Tetrazolium testing handbook - Family: Brassicaceae I. Association of Official Seed Analysts, Lincoln, Nebraska.

Appendix H: Materials, Methods, and Individual-Site Results from Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 87712 under Field Conditions

H.1. Materials

The soybean materials for the phenotypic and environmental assessment in the field included MON 87712, the conventional soybean control A3525, and 18 commercial reference varieties. The list of the soybean materials planted at each of 19 field sites is presented in Table H-1.

H.2. Characterization of the Materials

The identities of MON 87712 and the conventional control A3525 seed were verified by event-specific polymerase chain reaction analyses.

H.3. Field Sites and Plot Design

Data were collected from field sites conducted in 2009 at 19 sites within the U.S. soybean production regions (Table VII-3). These 19 sites provided a range of environmental and agronomic conditions representative of major U.S. soybean-growing regions. The field cooperators at each site were familiar with the growth, production, and evaluation of soybean characteristics.

The experiment was established at each of the 19 sites in a randomized complete block design with four replications. Each plot at the ARNE, ILSE, MOCB, and MOSL sites consisted of twelve rows spaced approximately 30 inches apart and approximately 30 feet in length. Rows # 2 and 3 were designated for the collection of phenotypic data. Rows # 4 and 5 were designated for the collection of abiotic stress response, disease damage, and arthropod-related damage data. Rows # 7 and 9 were designated for the collection of arthropod samples. Rows # 1, 6, 8, and 10-12 were used as buffer rows. Each plot was surrounded by approximately 5-15 feet of a commercial soybean variety by planting border rows in the alleyways between the blocks and around the entire perimeter of the plot area. The purpose of the planted borders was to create a continuous soybean stand across the plot area to ensure collection of more robust arthropod abundance data within the test area.

Each plot at the IAJA, IARL, ILCY, ILMS, ILWY, INRC, INSH, KSLA, MOAN, MOFI, MOKI, MOWR, NEYO, PAGR, and PAHM sites consisted of four 20 feet long rows spaced approximately 30 inches apart. Rows # 2 and 3 were designated for the collection of phenotypic, abiotic stress response, disease damage, and arthropod-related damage data. Rows # 1 and 4 were used as buffer rows. The entire plot area was surrounded by a border of a commercial soybean variety approximately 10 feet (four rows) in width.

H.4. Planting and Field Operations

Field and planting information are listed in Table H-2. Agronomic practices used to prepare and maintain each study site were characteristic of those used in each respective

geographic region. All maintenance operations were performed uniformly over the entire trial area.

Table H-1. Starting Seed for Phenotypic, Agronomic, and Environmental Interaction Assessment

Material	Material Type[1]	Phenotype[2]	Monsanto Lot Number	Sites[3]
MON 87712	T	SIY	11223539	All
A3525	C	Conventional	11223542	All
SB3369R	R	GT	11226924	ILWY, MOAN, MOSL, NEYO
SB3579R	R	GT	11226925	ILCY, MOCB, MOKI
SB3888R	R	GT	11226926	ARNE, INRC, KSLA
NuPride 8339	R	GT	11226939	ILCY, ILMS, MOAN, MOKI
NC+ 2A95	R	GT	11226838	IARL, ILMS, KSLA, MOCB, PAHM
Pioneer 93M43	R	GT	11226839	IAJA, ILSE, INSH, MOWR, PAHM
Pioneer 93M11	R	GT	11226840	ARNE, ILWY, MOWR, NEYO
NK S28-B4	R	GT	11226842	IARL, INRC, INSH, MOSL, PAGR
NK S30-D4	R	GT	11226843	IAJA, ILSE, MOLP, MOFI, PAGR
Midland 363	R	Conventional	11226698	IAJA, INRC, KSLA, MOCB
Stine 3300-0	R	Conventional	10001134	ILCY, ILWY, MOCB, MOFI, NEYO
Pioneer 9382	R	Conventional	11226581	IAJA, ILSE, MOWR, PAHM
Pioneer 93M62	R	Conventional	11226582	ILMS, MOAN, MOWR, PAGR
SB3819	R	Conventional	11226928	ILWY, INSH, MOKI, NEYO
LG 3211	R	Conventional	11226860	IARL, ILCY, INSH, MOSL, PAHM
LG C3540	R	Conventional	11226858	IARL, INRC, MOFI, PAGR
Pioneer 93M14	R	Conventional	11226720	ARNE, ILMS, MOAN, MOSL
NuPride 3202	R	Conventional	11226938	ARNE, ILSE, KSLA, MOKI

Note: The study also included two additional experimental materials that were outside of the scope of the objectives of this evaluation of MON 87712.

[1] T = Test, C = Control, and R = reference

[2] Phenotypic abbreviations: SIY = soybean intrinsic yield, GT = glyphosate-tolerant, Conventional = conventional commercial

[3] Site codes are as follows: ARNE = Jackson County, AR; IAJA = Guthrie County, IA; IARL = Jefferson County, IA; ILCY = Clinton County, IL; ILMS = Effingham County, IL; ILSE = Champaign County, IL; ILWY = Stark County, IL; INRC = Parke County, IN; INSH = Boone County, IN; KSLA = Pawnee County, KS; MOAN = Macon County, MO; MOCB = Shelby County, MO; MOFI = Butler County, MO; MOKI = Callaway County, MO; MOSL = St. Louis County, MO; MOWR = Lincoln County, MO; NEYO = York County, NE; PAGR = Berks County, PA, site 1; PAHM = Berks County, PA, site 2.

Table H-2. Field and Planting Information

Site[1]	Planting Date[2]	Planting rate (seeds/ft)	Planting depth (in)	Plot size (ft)[3]	Rows/ plot	Soil series, organic matter, pH	Cropping History	
							2007	2008
ARNE	6-9-09	9	1.0	30 × 30	12	Bosket sandy loam; 1.0%; 6.0	Soybean	Corn
IAJA	6-5-09	9	1.5	10 × 20	4	Clarion loam; 2.5%; 5.6	Soybean	Corn
IARL	6-5-09	9	1.0	10 × 20	4	Mahaska silty clay loam; 3.54%; 7.01	Corn / Wheat	Sorghum / Soybean
ILCY	6-30-09	9	1.4	10 × 20	4	Hoyleton-Darmstadt complex / silt loam; 2.6%; 7.3	Soybean	Milo
ILMS	6-30-09	9	1.0	10 × 20	4	Bluford silt loam; 2.0%; 6.3	Corn	Soybean
ILSE	6-24-09	9	1.5	30 × 30	12	Flaragan silt loam; 5.0%; 6.5	Sweet corn	Corn
ILWY	6-6-09	9	2.0	10 × 20	4	Plano silt loam; 3.4%; 6.3	Soybean	Corn
INRC	6-25-09	9	1.0	10 × 20	4	Reesville silt clay loam; 1.4%; 7.0	Soybean	Wheat / Fallow
INSH	6-8-09	9	1.5	10 × 20	4	Crosby silt loam; 2.1%; 5.8	Corn	Corn
KSLA	6-8-09	9	2.0	10 × 20	4	Silt loam; 2.6%; 7.6	Sunflower / Sorghum / Fallow / Corn	Sunflower
MOAN	6-29-09	9	1.0	10 × 20	4	Mexico silt loam; 2.1%; 7.2	Soybean	Wheat
MOCB	6-25-09	9	1.0	30 × 30	12	Putnam silt loam; 2.5%; 6.8	Soybean	Corn
MOFI	6-12-09	9	0.5	10 × 20	4	Amagon silt loam; 1.8%; 5.0	Soybean	Rice
MOKI	6-25-09	9	1.25	10 × 20	4	Mexico silt loam; 3.1%; 6.4	Soybean	Corn
MOSL	6-5-09	9	1.0	30 × 30	12	Peers silty clay; 2.9%; 7.5	Soybean	Corn
MOWR	6-6-09	9	1.0	10 × 20	4	Hatton silt loam; 2.9%; 6.5	Soybean	Fallow
NEYO	6-5-09	9	1.0	10 × 20	4	Hastings silt loam; 3.0%; 6.2	Soybean	Sorghum
PAGR	6-8-09	9	1.5	10 × 20	4	Bedington-Berks complex clay loam; 1.4%; 5.2	Corn	Corn
PAHM	6-25-09	9	1.5	10 × 20	4	Philo / Atkins loam; 1.2%; 6.4	Soybean	Corn

[1] Site codes are as follows: ARNE = Jackson County, AR; IAJA = Guthrie County, IA; IARL = Jefferson County, IA; ILCY = Clinton County, IL; ILMS = Effingham County, IL; ILSE = Champaign County, IL; ILWY = Stark County, IL; INRC = Parke County, IN; INSH = Boone County, IN; KSLA = Pawnee County, KS; MOAN = Macon County, MO; MOCB = Shelby County, MO; MOFI = Butler County, MO; MOKI = Callaway County, MO; MOSL = St. Louis County, MO; MOWR = Lincoln County, MO; NEYO = York County, NE; PAGR = Berks County, PA, site 1; PAHM = Berks County, PA, site 2.

[2] Month-day-year.

[3] Width × length. Sites with arthropod collection (ARNE, ILSE, MOCB, and MOSL) had larger plot areas.

H.5. Phenotypic Observations

The description of characteristics measured and the designated developmental stages when observations occurred are listed in Table VII-1.

H.6. Environmental Observations

Environmental interactions (*i.e.,* interactions between the crop plants and their receiving environment) were used to characterize MON 87712 by evaluating plant response to abiotic stressors, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance in the plots using the methods described in G.7 ad G.8.

H.7. Abiotic Stress Response, Disease Damage, and Arthropod-Related Damage

MON 87712 and the conventional control A3525 were evaluated at all 19 sites for differences in plant response to abiotic stressors, disease damage, and arthropod-related damage. Three abiotic stressors, three diseases, and three arthropod pests were evaluated four times during the growing season at the following intervals:

Observation 1: V2 – V5 growth stage

Observation 2: R1 – R2 growth stage

Observation 3: R3 – R5 growth stage

Observation 4: R6 – R8 growth stage

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

Abiotic stressors and disease damage observations were collected from each plot using a continuous 0 – 9 scale of increasing severity. Data were collected numerically and then placed into one of the following categories for reporting purposes:

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

Arthropod-related damage was assessed from each plot on the upper four nodes of 10 non-systematically selected plants using the arthropod-specific 0 – 5 rating scales of increasing severity listed below.

Defoliating arthropods (*e.g.*, corn earworm, bean leaf beetle, Japanese beetle, soybean looper)	
Rating	**Severity of plant damage**
0	None
1	1 – 20 % defoliation
2	21 – 40% defoliation
3	41 – 60% defoliation
4	61 – 80% defoliation
5	> 80% defoliation

Pod feeding arthropods (*e.g.*, corn earworm, bean leaf beetle, stink bug, Lygus bug on reproductive plant parts)	
Rating	**Severity of plant damage**
0	None
1	1 – 20 % damaged pods
2	21 – 40% damaged pods
3	41 – 60% damaged pods
4	61 – 80% damaged pods
5	> 80% damaged pods

Leafhoppers (*e.g.*, potato leafhopper)	
Rating	**Severity of plant damage**
0	None
1	1 – 50% of foliage with leaf yellowing; no leaf puckering or leaf margin necrosis
2	1 – 50% of foliage with leaf yellowing, leaf puckering and/or leaf margin necrosis
3	> 50% of foliage with leaf yellowing; no leaf puckering or leaf margin necrosis
4	> 50% of foliage with leaf yellowing, leaf puckering, and/or leaf margin necrosis
5	> 50% of foliage with necrotic leaves (leaves dead due to leafhopper damage)

Aphids (*e.g.*, soybean aphid)	
Rating	**Severity of plant damage**
0	None
1	1 – 100 aphids per plant; no leaf puckering
2	101 – 250 aphids per plant; no leaf puckering
3	≥ 250 aphids per plant with leaf puckering
4	≥ 250 aphids per plant with leaf puckering and leaf yellowing and/or necrosis
5	≥ 250 aphids per plant with plant stunting

H.8. Arthropod Abundance

Pest and beneficial arthropods were collected at the ARNE, ILSE, and MOCB sites four times during the growing season at the following intervals:

Collection 1: R1 – R2 growth stage

Collection 2: Approximately two weeks after collection 1

Collection 3: Approximately two weeks after collection 2

Collection 4: Approximately two weeks after collection 3

Arthropods were collected using a vertical beat sheet sampling method (Drees and Rice, 1985). The beat sheet was approximately 36×36 inch sheet constructed of a stiff material with a collecting trough at the bottom. The sheet was placed between row 7 and row 8 and the collecting trough was positioned near the base of the plants in row 7. Plants were shaken vigorously along the length of the beat sheet to dislodge arthropods from the plants. Another sub-sample was collected from the same row, approximately 8 ft from the first sub-sample. Two sub-samples were collected from row 9 in the same manner. A total of four sub-samples were collected in this way from each plot. The four sub-samples were combined into one pre-labeled container and placed on freezer packs. The samples were then sent to Monsanto Company, St. Louis, MO for arthropod identification and enumeration.

A maximum of six pest and six beneficial arthropods were enumerated for each collection. Three preselected pest and beneficial arthropods (or arthropod groups), namely bean leaf beetle, green clover worm, and stink bugs for the pests, and Araneae (spiders), *Nabis* sp., and *Orius* sp. for the beneficial arthropods, were enumerated at all sites for each collection time. Additionally, for each individual collection (*e.g.,* Collection 1, ARNE site), four non-systematically selected samples were examined to determine presence and relative abundance of up to three additional pest and beneficial arthropods to be enumerated for that particular collection and site. Thus, the suite of pest and beneficial arthropods assessed often varied between collections from a site and between sites due to differences in temporal activity and geographical distribution of arthropod taxa.

H.9. Environmental Interactions Evaluation Criteria

For the assessments of abiotic stress response and disease damage, MON 87712 and the conventional control A3525 were considered different in susceptibility or tolerance to an abiotic stressor or disease on a particular observation date at a site if the range of injury severity to MON 87712 did not overlap with the range of injury severity to the conventional control A3525 across all four replications. These data are categorical and were not subjected to statistical analysis. For each observation at a site, the range of injury severity across the commercial reference varieties provided data that are representative of commercial soybean varieties. Arthropod-related damage and arthropod abundance were quantitatively evaluated and subjected to statistical analysis, as appropriate.

H.10. Data Assessment

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

H.11. Statistical Analysis

The data from each experiment were analyzed separately according to a randomized complete block design using SAS® (2008). MON 87712 was compared to the conventional control A3525 within each site (individual-site analyses) and in a combined-site analysis, in which the data were pooled across sites, for early stand count, seedling vigor, days to 50% flowering, days to 50% end of flowering, days to 50% senescence, days to physiological maturity, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, and yield. Growth stage, flower color, abiotic stress response, and disease damage data were categorical and not statistically analyzed. Arthropod-related damage and pest and beneficial arthropod abundance data were statistically analyzed only within individual observations/collections and sites due to the variation in temporal activity and geographical distribution of the taxa.

No statistical comparisons were made between MON 87712 and commercial reference varieties. The reference range for each measured phenotypic characteristic was determined from the minimum and maximum mean values from the 18 commercial reference varieties planted among the sites. The reference range for the abundance and damage of each arthropod evaluated from a given collection/observation and site was determined from the minimum and maximum mean abundance or damage values collected from the reference varieties at the site. Data excluded from the study and the reasons for their exclusions are listed in Table H-3.

Table H-3. Data Missing or Excluded from Analysis

Site[1]	Material name	Material type	Plots	Characteristics	Reason for exclusion
All	All	All	All	Animal damage	Animal damage is a non-uniform environmental stressor.
All	All	All	All	Herbicide injury	Herbicide injury is a non-uniform environmental stressor.
ILCY	All	All	All	Brown stem rot, observation 4	Assessment of brown stem rot was not performed at all plots.
ILCY	All	All	All	Plant height	Data were not collected.
IAJA	All	All	All	Growth stage Monitoring	Growth stage monitoring data collected at senescence indicated the observations were made after harvest which could not be possible. Therefore, growth stage monitoring data collected at senescence were excluded.
IAJA	All	All	All	Arthropod damage	Data were not collected.
IAJA	All	All	All	Disease and abiotic stressors, observations 2 & 3	Data were not collected.
IAJA	MON 87712 A3525 Pioneer 93B82	Test Control Reference	209, 305; 303; 208	Final stand count	Data were not collected.

Table H-3. Data Missing or Excluded from Analysis (continued)

Site[1]	Material name	Material type	Plots	Characteristics	Reason for exclusion
IAJE	All	All	All	All	Site was dropped due to plant damage from frost.
IARL	MON 87712	Test	405	Flower color	Data was not collected.
IARL	All	All	All	Disease and abiotic stressors, observation 2	Inconsistent use of stressors.
IARL	NC+ 2A95	Reference	408	Drought, observation 4	Data were not collected.
ILMS	Pioneer 93M14	Reference	103, 207, 301, 404	All	The wrong number of seed was counted and placed into the planting envelopes. As a result, excessive number of seed was planted.
ILSE	All	All	All	Plant height	Data were not collected.
ILSE	MON 87712	Test	408	Days to 50% end of flowering	Original data in paper notebook was crossed out but no explanation was provided. As a result, the electronic data could not be verified.
ILSE	MON 87712	Test	408	Days to 50% end of senescence	Original data in paper notebook was crossed out but no explanation was provided. As a result, the electronic data could not be verified.

Table H-3. Data Missing or Excluded from Analysis (continued)

Site[1]	Material name	Material type	Plots	Characteristics	Reason for exclusion
ILSE	All	All	All	Disease and abiotic stressors, observation 2, 3, and 4	Data were not collected.
ILSE	All	All	All	Growth stage Monitoring after R4	Data were not collected.
ILSE	All	All	All	Arthropod damage	Incorrect rating scale was used.
INSH	LG 3211	Reference	206	Yield	Only one row of the plot was harvested due to equipment malfunction.
INSH	All	All	All	Disease and abiotic stressors, observation 2	Data were not collected.
INRC	All	All	All	Arthropod damage	Data were not collected.
INRC	All	All	All	Seedling vigor	Data were not collected.
INRC	All	All	All	Disease and abiotic stressors, observation 2	Data were not collected.
KSLA	All	All	All	Days to 50% end of flowering	Data were not collected.

Table H-3. Data Missing or Excluded from Analysis (continued)

Site[1]	Material name	Material type	Plots	Characteristics	Reason for exclusion
KSLA	All	All	All	Eyespot	Eyespot is a corn disease and inappropriate to be evaluated as a soybean disease stressor.
KSLA	All	All	All	Frost damage	Assessment of frost damage was not performed on all plots.
MOAN	Pioneer 93M14	Reference	110, 205, 309, 402	All	The wrong number of seed was counted and placed into the planting envelopes. As a result, excessive number of seed was planted.
MOAN	MON 87712	Test	407	Days to physiological maturity	Data was not collected.
MOCB	Stine 3300-0	Reference	401	Days to 50% end of flowering	Data was not collected.
MOCB	SB3579R	Reference	110	Days to physiological maturity	Data was not collected.
MOLP	All	All	All	All	Site was dropped due to poor plant stand from flood.

Table H-3. Data Missing or Excluded from Analysis (continued)

Site[1]	Material name	Material type	Plots	Characteristics	Reason for exclusion
MOKI	A3525	Control	108	Early stand count	Data was recorded on a date when the vast majority of the seedlings had not yet emerged.
MOKI	All	All	All	Seedling vigor	Data were not collected.
MOKI	All	All	All	Disease and abiotic stressors, observations 1 & 2	Data were not collected.
MOSL	SB3369R	Reference	304	Sudden death, observation 4	Data were not collected.
MOSL	All	All	All	Arthropod abundance	Data were not collected
MOSL	All	All	All	Biomass and photosynthetic data	Data were of poor quality due to the lack of available sites.
MOWR	Pioneer 93M62	Reference	406	Arthropod stressors, observation 2	Data from two of the three arthropod stressors were not collected.
MOWR	Pioneer 93M43	Reference	110	Disease and abiotic stressors, observation 3	Data were not collected.

Table H-3. Data Missing or Excluded from Analysis (continued)

Site[1]	Material name	Material type	Plots	Characteristics	Reason for exclusion
PAGR	LG 3211	Reference	101	Alternaria, observation 1	Data were not collected.
PAGR	MON 87712	Test	102, 203, 304, 404;	Plant height	Data were not collected.
	MON 87712(-)	Control	302, 401;		
	A3525	Control	204, 306;		
	A3525(-)	Control	205, 305, 405;		
	Pioneer 93M62	Reference	101, 206, 308, 409		
PAHM	LG C3540	Reference	409	Bacterial blight, observation 2	Data were not collected

[1] Site codes are as follows: ARNE = Jackson County, AR; IAJA = Guthrie County, IA; IAJE = Greene County, IA; IARL = Jefferson County, IA; ILCY = Clinton County, IL; ILMS = Effingham County, IL; ILSE = Champaign County, IL; ILWY = Stark County, IL; INRC = Parke County, IN; INSH = Boone County, IN; KSLA = Pawnee County, KS; MOAN = Macon County, MO; MOCB = Shelby County, MO; MOFI = Butler County, MO; MOLP = Adair County, MO; MOKI = Callaway County, MO; MOSL = St. Louis County, MO; MOWR = Lincoln County, MO; NEYO = York County, NE; PAHM = Berks County, PA, site 1; PAGR = Berks County, PA, site 2.

H.12. Individual Field Site Plant Growth and Development Results and Discussion

In the individual-site analysis, a total of 67 statistically significant differences ($\alpha = 0.05$) were detected out of a total of 211 comparisons made between MON 87712 and the conventional control A3525 (Table H-4). These differences were distributed among eleven of the 14 phenotypic characteristics. No statistical comparisons could be made in 30 instances where p-values could not be generated due to lack of variability. The nineteen flower color comparisons were categorical and were not statistically analyzed; however, at each site, all plants of MON 87712 and the conventional control A3525 had purple flowers as expected (Table H-4). MON 87712 and the conventional control A3525 were within the same range of plant growth stage observations among the sites (Table H-5).

Differences in stand count were observed between MON 87712 and the conventional control A3525 at the individual sites. Early stand count was higher for MON 87712 than the conventional control A3525 at the IAJA site (321.3 vs. 303.3 plants/plot), the KSLA site (312.8 vs. 293.8 plants/plot), the MOFI site (289.3 vs. 266.5 plants/plot), and the MOWR site (354.0 vs. 343.3 plants/plot), and lower than the control at the IARL site (333.3 vs. 349.0 plants/plot). Final stand count was higher for MON 87712 than the conventional control A3525 at the IAJA site (310.0 vs. 249.0 plants/plot), the ILSE site (260.3 vs. 232.3 plants/plot), the INSH site (327.5 vs. 305.8 plants/plot), the KSLA site (300.5 vs. 277.8 plants/plot), the MOWR site (347.8 vs. 338.3 plants/plot), and the NEYO site (343.5 vs. 307.8 plants/plot).

Statistically significant differences in flowering time were observed between MON 87712 and the conventional control A3525 at individual sites. Plants of MON 87712 flowered earlier than the conventional control A3525 at the IAJA site (204.3 vs. 207.3 days after 1 Jan. 2009) and the PAGR site (209.3 vs. 210.5 days after 1 Jan. 2009), and later than the conventional control A3525 at the ARNE site (193.0 vs. 190.8 days after 1 Jan. 2009), the ILWY site (206.3 vs. 205.3 days after 1 Jan. 2009), the INRC site (218.0 vs. 216.5 days after 1 Jan. 2009), and the NEYO site (206.3 vs. 205.0 days after 1 Jan. 2009). Plants of MON 87712 ended flowering earlier than the conventional control A3525 at the INRC site (254.0 vs. 255.0 days after 1 Jan. 2009) and the MOFI site (221.5 vs. 223.3 days after 1 Jan. 2009), and later than the conventional control A3525 at the ILCY site (234.0 vs. 232.8 days after 1 Jan. 2009), the MOKI site (243.0 vs. 239.3 days after 1 Jan. 2009), and the PAGR site (226.8 vs. 225.5 days after 1 Jan. 2009).

Plants of MON 87712 reached 50% senescence significantly later than the conventional control A3525 at all but three sites (ILWY, INRC, and INSH). Plants of MON 87712 reached physiological maturity significantly later than the conventional control A3525 at 16 sites. MON 87712 had significantly more lodging than the conventional control A3525 at the KSLA site (3.0 vs. 1.8 ranting).

Pod shattering was significantly lower for MON 87712 than the conventional control A3525 at the NEYO site (1.0 vs. 1.8 rating).

Grain moisture was significantly higher for MON 87712 than the conventional control A3525 at the IARL site (13.0 vs. 12.0%) and the PAGR site (16.0 vs. 15.7%), and significantly lower than the conventional control A3525 at the MOFI site (11.6 vs. 11.9%).

The weight of 100 seeds was significantly higher for MON 87712 than the conventional control A3525 at the INRC site (14.7 vs. 13.7 g) and the MOKI site (20.5 vs. 17.5 g).

Statistically significant differences in yield were observed between MON 87712 and the conventional control A3525 at the individual sites. Yield was higher for MON 87712 than the conventional control A3525 at the INSH site (54.5 vs. 47.9 bu/ac), the KSLA site (68.9 vs. 60.0 bu/ac), the MOWR site (65.1 vs. 58.3 bu/ac), the NEYO site (51.7 vs. 46.9 bu/ac), the PAGR site (69.3 vs. 61.4 bu/ac), and the PAHM site (52.4 vs. 48.2 bu/ac).

Considering that the statistical differences detected in the individual-site analyses for days to 50% flowering, days to 50% end of flowering, lodging, pod shattering, grain moisture, and 100 seed weight were not detected in the combined-site analysis, this suggests these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of increased weediness potential of MON 87712 compared to the conventional control A3525. While statistical differences were detected for early stand count at five sites, days to 50% senescence at sixteen sites, days to physiological maturity at sixteen sites, final stand count at six sites, and yield at six sites, and statistical differences were detected for these phenotypic characteristics in the combined-site analysis, the assessed phenotypic values of MON 87712 were within their respective variation of the study references.

H.13 Individual Field Site Environmental Interaction Results and Discussion

In an individual site assessment, no statistically significant differences ($\alpha = 0.05$) were observed between MON 87712 and the conventional control A3525 for any of the 186 comparisons for the assessed abiotic stressors (Table H-6) or for any of the 198 comparisons for the assessed diseases (Table H-7).

In an assessment of arthropod-related damage, no statistically significant differences were detected ($\alpha = 0.05$) between MON 87712 and the conventional control A3525 for 129 out of 137 comparisons for the assessed arthropods (Table H-8). Statistical comparisons could not be made between MON 87712 and the conventional control A3525 for 55 additional arthropod-related damage comparisons for which p-values could not be generated due to lack of variability in the data. A total of eight statistically significant differences involving three taxa were detected between MON 87712 and the conventional control A3525 out of the 137 comparisons. The bean leaf beetle damage was higher in MON 87712 than the conventional control A3525 in Observation 1 at the ILCY site (0.53 vs. 0.23 rating) but lower in Observation 2 at the MOAN site (0.65 vs. 0.93 rating). MON 87712 had more damage than the conventional control A3525 from green cloverworms in Observation 4 from the MOWR site (0.60 vs. 0.48 rating). MON 87712 had more damage than the conventional control A3525 from Japanese

beetles in Observation 1 at the ILMS site (0.50 vs. 0.30 rating). MON 87712 had more damage than the conventional control A3525 from leafhoppers in Observation 1at the PAHM site (0.55 vs. 0.15 rating). MON 87712 had less damage than the conventional control A3525 from soybean loopers in Observation 2 at the MOAN site (0.55 vs. 0.90 rating) and Observation 3 at the MOFI site (0.45 vs. 0.85 rating). MON 87712 had less damage than the conventional control A3525 from velvetbean caterpillars in Observation 3 at the MOFT site (0.45 vs. 0.85 rating). The mean damage ratings for these detected differences were outside their respective reference range. However, the differences detected were small in magnitude and these differences were not consistent across observations or sites. Thus, these detected differences in arthropod-related damage were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of an adverse environmental impact of MON 87712 compared to the conventional control A3525.

In an assessment of pest and beneficial arthropod abundance, no statistically significant differences were detected ($\alpha = 0.05$) between MON 87712 and the conventional control A3525 for 106 out of 115 comparisons, including 59 pest arthropod comparisons (Table H-9) and 56 beneficial arthropod comparisons (Table H-10). Statistical comparisons could not be made between MON 87712 and the conventional control A3525 for eight additional comparisons, including six pest arthropod comparisons and two beneficial arthropod comparisons, for which p-values could not be generated due to lack of variability in the data. A total of nine statistically significant differences were detected between MON 87712 and the conventional control A3525 for arthropod abundance, including five differences for pest arthropods and four differences for beneficial arthropods.

The five differences detected out of the 59 pest arthropod comparisons involved aphids, bean leaf beetles, green cloverworms, and stink bugs (Table H-8). MON 87712 had higher aphid abundance than the conventional control A3525 in Collection 4 at the MOCB site (37.5 vs. 19.3 per plot). MON 87712 had higher bean leaf beetle abundance than the conventional control A3525 in Collection 2 at the ILSE site (1.3 vs. 0.3 per plot). MON 87712 had lower green cloverworm abundance than the conventional control A3525 in Collection 3 at the MOCB site (0.8 vs. 5.5 per plot). The abundance of stink bug was lower in MON 87712 then the conventional control A3525 in Collection 3 at the ARNE site (0.3 vs. 2.0 per plot) but higher in Collection 3 at the MOCB site (0.8 vs. 0.0 per plot). The mean abundance values for aphids in Collection 4 at the MOCB site, green cloverworms in Collection 3 at the MOCB, and stink bugs in Collection 3 at the ARNE site were within their respective reference range. The mean abundance values for bean leaf beetle in Collection 2 at the ILSE site and stink bug in Collection 3 at the MOCB site were outside their respective reference range. However, the differences detected for these taxa were not consistently detected across collections or sites. Thus, the detected differences in pest arthropod abundance were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of an adverse environmental impact of MON 87712 compared to conventional soybean.

The four differences detected out of the 56 beneficial arthropod comparisons involved big-eyed bugs, ladybird beetles, micro-parasitic hymenoptera, and *Orius* spp. (Table H-

9). MON 87712 had higher big-eyed bug abundance than the conventional control A3525 in Collection 1 at the ARNE site (4.3 vs. 1.8 per plot). MON 87712 had higher ladybird beetle abundance than the conventional control A3525 in Collection 4 at the ILSE site (8.3 vs. 3.5 per plot). MON 87712 had higher micro-parasitic hymenoptera abundance than the conventional control A3525 in Collection 4 at the MOCB site (2.3 vs. 0.8 per plot). MON 87712 had lower *Orius* spp. abundance than the conventional control A3525 in Collection 1 at the ILSE site (0.0 vs. 1.0 per plot). The mean abundance values for micro-parasitic hymenoptera in Collection 4 at the MOCB site and *Orius* spp. in Collection 1 at the ILSE site were within their respective reference range. The mean abundance value for big-eyed bugs in Collection 1 at the ARNE site and ladybird beetles in Collection 4 at the ILSE site were outside their respective reference range. However, the differences detected for these taxa were not consistently detected across collections or sites. Thus, the detected differences in beneficial arthropod abundance were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of adverse environmental impact of MON 87712 compared to conventional soybean.

Table H-4. Individual-Site Phenotypic Comparison of MON 87712 to Conventional Control A3525

	Phenotypic Characteristic (units)					
	Early stand count (# of plants/plot)		Seedling vigor (1-9 scale)		Days after 1 Jan 2009 to 50% flowering[3]	
Site[1]	MON 87712 (SE)[2]	Control (SE)	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)
ARNE	301.5 (2.7)	299.0 (7.4)	4.8 (0.6)	4.5 (0.7)	193.0 (0.0)*	190.8 (0.5)
IAJA	321.3 (4.4)*	303.3 (16.4)	2.0 (0.0)	2.3 (0.3)	204.3 (0.5)*	207.3 (0.3)
IARL	333.3 (4.9)*	349.0 (2.5)	1.3 (0.3)	1.3 (0.3)	205.0 (0.0)	204.5 (0.3)
ILCY	325.5 (9.6)	319.5 (6.9)	1.0 (0.0)	1.3 (0.3)	214.8 (0.3)	215.8 (0.5)
ILMS	310.3 (2.1)	320.3 (6.9)	2.5 (0.3)	2.5 (0.3)	218.5 (0.9)	219.3 (0.6)
ILSE	234.8 (17.7)	224.3 (9.6)	7.3 (0.5)	7.3 (0.3)	218.3 (0.3)	218.0 (0.4)
ILWY	336.8 (9.6)	347.3 (3.1)	2.0 (0.0)†	2.0 (0.0)	206.3 (0.3)*	205.3 (0.3)
INRC	273.3 (11.4)	269.5 (10.9)	—	—	218.0 (0.0)*	216.5 (0.5)
INSH	321.5 (9.1)	315.0 (6.8)	5.3 (0.3)	5.5 (0.5)	205.8 (0.6)	205.8 (0.6)
KSLA	312.8 (3.8)*	293.8 (10.8)	3.0 (0.0)	3.3 (0.3)	201.0 (0.0)	200.8 (0.3)
MOAN	330.8 (1.1)	322.3 (5.5)	3.0 (0.0)	3.0 (0.0)	219.3 (0.3)	219.5 (0.3)
MOCB	308.0 (6.7)	278.2 (33.7)	2.5 (0.3)	3.5 (0.5)	216.8 (0.3)	217.5 (0.3)
MOFI	289.3 (2.8)*	266.5 (7.6)	3.0 (0.0)	3.0 (0.4)	201.0 (0.4)	200.3 (0.6)
MOKI	153.5 (12.2)	139.3 (28.6)	—	—	217.5 (0.5)	217.0 (0.7)
MOSL	349.5 (2.7)	351.0 (3.2)	1.0 (0.0)†	1.0 (0.0)	196.0 (0.4)	195.8 (0.3)
MOWR	354.0 (0.9)*	343.3 (1.4)	1.0 (0.0)†	1.0 (0.0)	199.3 (0.3)	198.5 (0.5)
NEYO	338.3 (4.6)	314.5 (8.4)	3.0 (0.0)†	3.0 (0.0)	206.3 (0.3)*	205.0 (0.0)
PAGR	244.5 (5.7)	238.8 (14.2)	4.0 (0.6)	4.3 (0.5)	209.3 (0.3)*	210.5 (0.3)
PAHM	312.7 (7.4)	309.5 (4.4)	2.3 (0.5)	2.8 (0.3)	220.0 (0.4)	220.5 (0.3)

Table H-4. Individual-Site Phenotypic Comparison of MON 87712 to Conventional Control A3525 (continued)

	Phenotypic Characteristic (units)					
	Days after 1 Jan 2009 to 50% end of flowering[4]		Days after 1 Jan 2009 to 50% senescence[5]		Days after 1 Jan 2009 to physiological maturity[6]	
Site[1]	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)
ARNE	222.0 (0.0)	220.3 (0.5)	256.3 (0.3)*	253.0 (0.0)	268.0 (0.0)*	263.8 (0.5)
IAJA	230.8 (0.3)	231.5 (0.5)	266.8 (0.3)*	264.8 (0.3)	287.3 (0.5)	285.8 (0.3)
IARL	225.8 (0.5)	226.0 (0.0)	268.3 (0.3)*	264.8 (0.3)	290.5 (0.3)*	281.5 (0.3)
ILCY	234.0 (0.6)*	232.8 (0.3)	267.0 (0.0)*	264.5 (0.7)	285.0 (0.4)*	280.5 (0.3)
ILMS	237.0 (0.4)	237.5 (0.5)	269.3 (0.3)*	268.0 (0.4)	282.8 (0.5)*	277.5 (0.3)
ILSE	241.0 (1.0)	239.8 (0.9)	280.3 (0.7)*	275.0 (0.7)	284.8 (0.3)*	280.8 (0.3)
ILWY	225.5 (0.5)	226.5 (0.5)	269.8 (1.0)	268.5 (0.9)	279.0 (0.0)†	279.0 (0.0)
INRC	254.0 (0.0)*	255.0 (0.0)	272.5 (0.3)	272.0 (0.7)	283.0 (0.0)†	283.0 (0.0)
INSH	228.0 (0.0)	228.5 (0.3)	265.3 (0.9)	264.0 (1.0)	274.3 (1.0)*	271.0 (1.0)
KSLA	—	—	265.0 (0.0)*	259.5 (0.3)	273.0 (0.0)*	266.5 (0.3)
MOAN	244.3 (0.3)	243.3 (1.1)	275.0 (0.7)*	271.3 (0.8)	290.7 (0.3)*	287.0 (0.4)
MOCB	240.5 (1.2)	240.0 (1.4)	277.0 (0.0)*	271.8 (1.4)	291.8 (0.3)*	287.0 (1.5)
MOFI	221.5 (0.3)*	223.3 (0.3)	255.8 (0.3)*	252.0 (0.4)	283.8 (0.3)*	282.0 (0.0)
MOKI	243.0 (0.0)*	239.3 (0.6)	276.8 (0.3)*	273.3 (0.3)	276.8 (0.3)*	273.0 (0.0)
MOSL	219.0 (0.0)	218.0 (0.0)	259.5 (0.5)*	255.8 (0.5)	267.3 (0.5)*	263.0 (0.4)
MOWR	219.5 (0.3)	219.8 (0.3)	260.0 (0.0)*	257.0 (1.0)	268.0 (0.0)*	262.8 (0.3)
NEYO	224.5 (0.3)	224.8 (0.3)	267.0 (0.0)*	264.8 (0.3)	278.0 (0.0)*	277.3 (0.3)
PAGR	226.8 (0.5)*	225.5(0.3)	266.0 (0.0)*	264.8(0.3)	280.5 (0.3)*	278.5 (0.3)
PAHM	235.8 (0.3)	235.5 (0.3)	272.8 (0.3)*	270.5 (0.7)	294.0 (0.6)*	291.5 (0.5)

Table H-4. Individual-Site Phenotypic Comparison of MON 87712 to Conventional Control A3525 (continued)

	Phenotypic Characteristic (units)					
	Flower color[7]		Plant height (in)		Lodging (1-9 scale)	
Site[1]	MON 87712	Control	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)
ARNE	purple	purple	38.3 (0.7)	37.1 (0.7)	4.8 (0.3)	4.0 (0.0)
IAJA	purple	purple	41.9 (0.5)	40.9 (0.2)	1.0 (0.0)†	1.0 (0.0)
IARL	purple	purple	36.0 (0.6)	35.9 (1.1)	4.5 (0.3)	3.8 (0.5)
ILCY	purple	purple	—	—	1.0 (0.0)†	1.0 (0.0)
ILMS	purple	purple	21.0 (0.7)	21.0 (0.5)	1.0 (0.0)†	1.0 (0.0)
ILSE	purple	purple	—	—	3.8 (0.3)	3.8 (0.3)
ILWY	purple	purple	35.7 (0.5)	35.1 (1.8)	3.3 (0.3)	3.3 (0.3)
INRC	purple	purple	24.3 (0.6)	24.1 (0.7)	1.0 (0.0)†	1.0 (0.0)
INSH	purple	purple	27.1 (0.8)	26.4 (0.4)	1.0 (0.0)†	1.0 (0.0)
KSLA	purple	purple	41.2 (1.2)	38.9 (1.1)	3.0 (0.0)*	1.8 (0.3)
MOAN	purple	purple	26.7 (0.5)	26.5 (0.4)	1.0 (0.0)	1.0 (0.0)
MOCB	purple	purple	26.0 (0.4)	26.0 (0.9)	4.0 (0.4)	3.3 (0.3)
MOFI	purple	purple	25.8 (0.5)	22.8 (1.7)	1.0 (0.0)†	1.0 (0.0)
MOKI	purple	purple	25.2 (0.6)	26.7 (0.7)	1.0 (0.0)†	1.0 (0.0)
MOSL	purple	purple	34.3 (0.8)	32.8 (0.7)	1.0 (0.0)†	1.0 (0.0)
MOWR	purple	purple	31.8 (0.6)	30.1 (0.9)	1.0 (0.0)†	1.0 (0.0)
NEYO	purple	purple	36.0 (0.5)	35.8 (0.4)	1.0 (0.0)†	1.0 (0.0)
PAGR	purple	purple	—	36.7 (1.9)	4.3 (0.5)	4.0 (0.4)
PAHM	purple	purple	35.1 (0.5)	34.5 (0.1)	3.3 (1.0)	4.0 (0.7)

Table H-4. Individual-Site Phenotypic Comparison of MON 87712 to Conventional Control A3525 (continued)

	Phenotypic Characteristic (units)					
	Pod Shattering (1-9 scale)		Final stand count (# of plants/plot)		Grain moisture (%)	
Site[1]	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)
ARNE	1.0 (0.0)†	1.0 (0.0)	293.5 (4.4)	284.3 (5.0)	12.3 (0.1)	12.6 (0.1)
IAJA	1.0 (0.0)†	1.0 (0.0)	310.0 (7.0)*	249.0 (10.1)	13.1 (0.4)	12.6 (0.1)
IARL	1.0 (0.0)†	1.0 (0.0)	316.0 (7.3)	318.5 (8.1)	13.0 (0.5)*	12.0 (0.1)
ILCY	1.0 (0.0)†	1.0 (0.0)	287.0 (22.8)	294.5 (8.7)	10.1 (0.1)	10.2 (0.1)
ILMS	1.0 (0.0)†	1.0 (0.0)	284.3 (7.1)	276.8 (7.6)	13.4 (0.1)	13.4 (0.0)
ILSE	1.0 (0.0)	1.3 (0.3)	260.3 (9.9)*	232.3 (9.1)	13.7 (0.1)	13.8 (0.2)
ILWY	1.0 (0.0)†	1.0 (0.0)	317.0 (3.9)	320.5 (5.6)	12.8 (0.0)	12.7 (0.1)
INRC	1.0 (0.0)†	1.0 (0.0)	273.3 (11.4)	269.5 (10.9)	11.7 (0.2)	12.1 (0.2)
INSH	1.0 (0.0)†	1.0 (0.0)	327.5 (3.6)*	305.8 (5.8)	12.8 (0.1)	13.1 (0.1)
KSLA	2.5 (0.3)	4.0 (0.8)	300.5 (3.3)*	277.8 (12.4)	11.7 (0.2)	12.3 (0.4)
MOAN	1.0 (0.0)†	1.0 (0.0)	314.5 (4.7)	314.0 (9.0)	11.2 (0.1)	16.8 (0.1)
MOCB	1.0 (0.0)†	1.0 (0.0)	278.7 (4.7)	260.0 (28.7)	14.2 (0.3)	14.0 (0.5)
MOFI	1.0 (0.0)†	1.0 (0.0)	274.5 (2.0)	259.3 (7.6)	11.6 (0.1)*	11.9 (0.1)
MOKI	1.0 (0.0)†	1.0 (0.0)	188.8 (12.1)	206.8 (29.9)	11.9 (0.3)	12.5 (0.4)
MOSL	1.0 (0.0)†	1.0 (0.0)	337.5 (2.7)	336.8 (2.5)	16.7 (0.4)	17.1 (0.4)
MOWR	1.0 (0.0)†	1.0 (0.0)	347.8 (1.4)*	338.3(0.8)	13.1 (0.5)	12.7 (0.1)
NEYO	1.0 (0.0)*	1.8 (0.3)	343.5 (1.4)*	307.8 (6.4)	11.1 (0.0)	11.1 (0.5)
PAGR	1.0 (0.0)	1.5 (0.5)	266.3 (10.2)	261.8 (6.6)	16.0 (0.3)*	15.7 (0.3)
PAHM	2.5 (0.3)	2.8 (0.5)	325.3(2.3)	319.3 (0.0)	14.9 (0.5)	14.8 (0.4)

Table H-4. Individual-Site Phenotypic Comparison of MON 87712 to Conventional Control A3525 (continued)

	Phenotypic Characteristic (units)			
	100 seed weight (g)		Yield (bu/ac)	
Site[1]	MON 87712 (SE)	Control (SE)	MON 87712 (SE)	Control (SE)
ARNE	16.4 (0.2)	16.1 (0.3)	65.0 (1.1)	62.6 (1.5)
IAJA	15.9 (0.2)	16.0 (0.2)	64.1 (3.2)	61.7 (0.3)
IARL	16.0 (0.4)	15.0 (0.0)	48.3 (2.1)	45.8 (2.2)
ILCY	15.8 (0.2)	15.5 (0.5)	66.0 (1.4)	58.4 (4.0)
ILMS	14.0 (0.1)	13.2 (0.3)	45.6 (2.2)	40.9 (3.6)
ILSE	15.3 (0.1)	15.6 (0.5)	33.7 (2.1)	29.9 (2.9)
ILWY	15.8 (0.3)	15.8 (0.2)	33.0 (1.9)	34.6 (2.0)
INRC	14.7 (0.3)*	13.7 (0.5)	37.2 (2.9)	33.1 (1.3)
INSH	15.7 (0.2)	15.8 (0.2)	54.5 (1.5)*	47.9 (1.7)
KSLA	17.5 (0.3)	18.3 (0.3)	68.9 (2.9)*	60.0 (2.3)
MOAN	14.0 (0.7)	13.5 (0.3)	48.3 (0.9)	47.7(1.5)
MOCB	14.8 (0.2)	14.5 (0.1)	54.3 (1.4)	50.7 (3.5)
MOFI	17.0 (0.2)	16.6 (0.3)	44.1(1.6)	43.7 (2.9)
MOKI	20.5 (1.0)*	17.5 (0.7)	42.3 (1.5)	46.9 (2.6)
MOSL	19.5 (0.3)	19.0 (0.0)	55.2 (1.8)	52.5 (1.3)
MOWR	17.9 (0.5)	17.3 (0.1)	65.1 (1.7)*	58.3 (0.9)
NEYO	15.3 (0.3)	14.8 (0.3)	51.7 (3.3)*	46.9 (0.2)
PAGR	17.7 (0.1)	16.8 (0.3)	69.3 (1.6)*	61.4 (0.8)
PAHM	16.3 (0.1)	17.0 (0.2)	52.4 (2.2)*	48.2 (1.6)

Note: The experimental design was a randomized complete block with four replications.

* Statistically significant differences were detected (α=0.05) between MON 87712 and the conventional control A3525.

† No statistical comparisons were made due to lack of variability in the data.

[1] Site codes are as follows: ARNE = Jackson County, AR; IAJA = Guthrie County, IA; IARL = Jefferson County, IA; ILCY = Clinton County, IL; ILMS = Effingham County, IL; ILSE = Champaign County, IL; ILWY = Stark County, IL; INRC = Parke County, IN; INSH = Boone County, IN; KSLA = Pawnee County, KS; MOAN = Macon County, MO; MOCB = Shelby County, MO; MOFI = Butler County, MO; MOKI = Callaway County, MO; MOSL = St. Louis County, MO; MOWR = Lincoln County, MO; NEYO = York County, NE; PAGR = Berks County, PA, site 1; PAHM = Berks County, PA, site 2.

Table H-4. Individual-Site Phenotypic Comparison of MON 87712 to Conventional Control (continued)

[2] Mean based on n = 4 for MON 87712 and the conventional control A3525 for all data characteristics except as follows. For Early Stand County n = 3 for the conventional control A3525 at the MOKI site. For Days to 50% end of flowering n = 3 for MON 87712 at the ILSE site. For Days to 50% senescence n = 3 for the conventional control A3525 at the ILSE site. For Days to physiological maturity n = 3 for MON 87712 at the MOAN site. For Plant height n = 2 for the conventional control A3525 at the PAGR site. For Final stand count n = 3 for the conventional control A3525 at the IAJA site. SE = Standard error.
[3] Calendar day number (days after 1 Jan 2009) when 50% of the plants in each plot were flowering.
[4] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot have stopped flowering.
[5] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot reached 50% senescence.
[6] Calendar day number (days after 1 Jan 2009) when 50% of the marked plants in each plot reached physiological maturity.
[7] Flower color data were categorical and were not statistically analyzed.
— Dashes indicate information not collected.

Table H-5. Growth Stage Monitoring of MON 87712, Conventional Control A3525, and the Commercial Reference Varieties

		Assessment Date and Range of Growth Stages Observed[1]								
Site[2]	Substance	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9
ARNE		6/29/2009	7/13/2009	7/21/2009	8/3/2009	8/26/2009	9/11/2009	—	—	—
	MON 87712	V2	R2	R2	R4	R6	R6	—	—	—
	Control	V2	R2	R2	R4	R6	R6	—	—	—
	References	V2	R2	—	—	—	—	—	—	—
IAJA		7/9/2009	7/16/2009	8/19/2009	8/27/2009	—	—	—	—	—
	MON 87712	V2	V5	R2	R3	—	—	—	—	—
	Control	V2	V5	R2	R3	—	—	—	—	—
	References	V2	V5	R2	R3	—	—	—	—	—
IARL		6/30/2009	7/2/2009	7/14/2009	7/27/2009	8/4/2009	8/17/2009	9/7/2009	9/14/2009	10/5/2009
	MON 87712	V2	V2	V6-V7	R2	R3	R5	R6	R6	R7
	Control	V2	V2	V5-V6	R2	R3-R4	R5	R6	R6	R7
	References	V2	V2	V5-V8	R2	R3-R4	R5	—	—	—
ILCY		7/22/2009	7/29/2009	8/10/2009	8/14/2009	8/22/2009	—	—	—	—
	MON 87712	V2	V4	V5-V6	V9	R3	—	—	—	—
	Control	V2	V4	V5-V6	V9	R3	—	—	—	—
	References	V2	—	V5-V6	—	R3	—	—	—	—
ILMS		7/24/2009	7/27/2009	8/20/2009	9/8/2009	9/29/2009	—	—	—	—
	MON 87712	V2	V3	R2	R4-R5	R6	—	—	—	—
	Control	V2	V3	R2	R4-R5	R6	—	—	—	—
	References	V2	—	R2	R4-R5	—	—	—	—	—

Table H-5. Growth Stage Monitoring of MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Assessment Date and Range of Growth Stages Observed[1]								
Site[2]	Material	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9
ILSE		7/17/2009	8/3/2009	8/12/2009	9/25/2009	—	—	—	—	—
	MON 87712	V2	V5	R2	R4	—	—	—	—	—
	Control	V2	V5	R2	R4	—	—	—	—	—
	References	V2	—	R2	R4	—	—	—	—	—
ILWY		7/10/2009	7/27/2009	8/17/2009	9/7/2009	9/28/2009	—	—	—	—
	MON 87712	V2	R1	R4	R6	R7	—	—	—	—
	Control	V2	R1	R4	R6	R7	—	—	—	—
	References	V2	—	—	—	—	—	—	—	—
INRC		7/18/2009	7/28/2009	8/22/2009	10/2/2009	—	—	—	—	—
	MON 87712	V1	V2	R2	R6	—	—	—	—	—
	Control	V1	V2	R2	R6	—	—	—	—	—
	References	V1	V2	R2	R6	—	—	—	—	—
INSH		7/1/2009	7/7/2009	7/15/2009	7/29/2009	8/12/2009	8/28/2009	9/28/2009	—	—
	MON 87712	V2	V2	V5	R1-R2	R3	R4	R7	—	—
	Control	V2	V2	V5	R1-R2	R3	R4	R7	—	—
	References	V2	V2	V5	R1-R2	R3	R4	R7	—	—
KSLA		7/2/2009	7/17/2009	7/22/2009	7/31/2009	8/14/2009	8/31/2009	9/11/2009	—	—
	MON 87712	V3	R1	R2	R2	R4	R5	R6	—	—
	Control	V3	R1	R2	R2	R4	R5-R6	R6	—	—
	References	V3	R1	R2	R2	R4	R5-R6	R6	—	—

Table H-5. Growth Stage Monitoring of MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Assessment Date and Range of Growth Stages Observed[1]								
Site[2]	Material	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9
MOAN		7/22/2009	8/10/2009	8/27/2009	9/25/2009	10/13/2009	—	—	—	—
	MON 87712	V2	R1	R5	R6	R6-R7	—	—	—	—
	Control	V2	R1	R5	R6	R7	—	—	—	—
	References	V2	R1	—	—	—	—	—	—	—
MOCB		7/20/2009	8/4/2009	8/25/2009	9/24/2009	10/13/2009	—	—	—	—
	MON 87712	V2-V3	V6-V7	R4-R5	R6	R7	—	—	—	—
	Control	V2-V3	V6	R4-R5	R6	R7-8	—	—	—	—
	References	V2-V3	—	—	—	—	—	—	—	—
MOFI		7/1/2009	7/7/2009	7/22/2009	8/12/2009	9/2/2009	—	—	—	—
	MON 87712	V2	V3	V6-V7	R3	R6	—	—	—	—
	Control	V2	V3	V6	R3	R6	—	—	—	—
	References	V2	V3	V5-V7	R3	R6	—	—	—	—
MOKI		7/20/2009	8/1/2009	8/10/2009	8/20/2009	9/24/2009	—	—	—	—
	MON 87712	V1	V4-V5	V5	R4	R6	—	—	—	—
	Control	V1	V4-V5	V5	R4	R6	—	—	—	—
	References	V1	V4	V5	R4	R6	—	—	—	—
MOSL		6/28/2009	7/15/2009	8/1/2009	8/21/2009	9/13/2009	—	—	—	—
	MON 87712	V2	V7-V8	R3	R5	R6	—	—	—	—
	Control	V2	V7-V8	R3	R5	R6	—	—	—	—
	References	V2	V7-V8	—	—	—	—	—	—	—

Table H-5. Growth Stage Monitoring of MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Assessment Date and Range of Growth Stages Observed[1]								
Site[2]	Material	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9
MOWR		6/28/2009	7/13/2009	7/23/2009	8/1/2009	8/20/2009	9/13/2009	—	—	—
	MON 87712	V2	V5-V6	R2	R3	R5	R6	—	—	—
	Control	V2	V5	R2	R3	R5	R6	—	—	—
	References	V2	V5	R2	—	—	R6	—	—	—
NEYO		7/2/2009	7/10/2009	7/22/2009	8/3/2009	8/11/2009	9/1/2009	9/28/2009	—	—
	MON 87712	V3	V5	V7	R2	R3-R4	R5	R7	—	—
	Control	V3	V5	V7	R2	R3-R4	R5	R7	—	—
	References	V3	V5	—	R2	—	R5	—	—	—
PAGR		7/2/2009	7/22/2009	8/11/2009	8/31/2009	9/21/2009	10/6/2009	—	—	—
	MON 87712	V2	V6-V7	R3-R4	R6	R6	R7	—	—	—
	Control	V2	V6	R3-R4	R6	R6	R7-R8	—	—	—
	References	V2	V6	R4	R6	R6	R7-R8	—	—	—
PAHM		7/22/2009	8/12/2009	9/3/2009	9/23/2009	—	—	—	—	—
	MON 87712	V2-V3	R1	R5	R6	—	—	—	—	—
	Control	V2	R1	R5	R6	—	—	—	—	—
	References	—	R2	R5	R6	—	—	—	—	—

Obs. = Observation number

[1] Month-day-year.

[2] Site codes are as follows: ARNE = Jackson County, AR; IAJA = Guthrie County, IA; IA; IARL = Jefferson County, IA; ILCY = Clinton County, IL; ILMS = Effingham County, IL; ILSE = Champaign County, IL; ILWY = Stark County, IL; INRC = Parke County, IN; INSH = Boone County, IN; KSLA = Pawnee County, KS; MOAN = Macon County, MO; MOCB = Shelby County, MO; MOFI = Butler County, MO; MOKI = Callaway County, MO; MOSL = St. Louis County, MO; MOWR = Lincoln County, MO; NEYO = York County, NE; PAGR = Berks County, PA, site 1; PAHM = Berks County, PA, site 2.

— Dashes indicate information not provided.

Table H-6. Abiotic Stress Response Evaluations of MON 87712 and Conventional Control A3525 Using an Observational Severity Scale

Abiotic Stressor	Number of observations across all sites	Number of observations where no differences were observed between MON 87712 and the Conventional Control A3525
Total	186	186
Cold	6	6
Compaction[1]	12	12
Drought[2]	45	45
Flood[3]	41	41
Frost damage	5	5
Hail damage	14	14
Mineral toxicity	2	2
Nutrient deficiency	29	29
Wind damage	32	32

No differences were observed between MON 87712 and the conventional control A3525 during any observation for damage caused by any of the assessed abiotic stressors. Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with four replications. All observations were made during the following four crop developmental stages: V2 –V5; R1 - R2; R3 - R5; and R6 - R8.

[1]Includes soil compaction.

[2]Includes heat.

[3]Includes wet soil, excess moisture.

Table H-7. Disease Damage Evaluations of MON 87712 and Conventional Control A3525 Using an Observational Severity Scale

Disease stressor	Number of observations across all sites	Number observations where no differences were observed between MON 87712 and the Conventional Control A3525
Total	198	198
Anthracnose	6	6
Aster yellow phytoplasma	1	1
Bacterial blight[1]	19	19
Bacterial leaf spot	3	3
Brown stem rot	6	6
Cercospora[2]	7	7
Charcoal rot	3	3
Downy mildew	25	25
Frogeye leaf spot	17	17
Fusarium	1	1
Leaf spots (*Septoria* and *Alternaria*)[3]	18	18
Phytophtora[4]	13	13
Powdery mildew	16	16
Pythium	3	3
Rhizoctonia	1	1
Seedling blight	1	1
Septoria brown spot[5]	24	24
Southern blight	1	1
Soybean mosaic virus	3	3
Soybean rust	6	6
Stem canker	3	3
Sudden death	15	15
White mold	6	6

No differences were observed between MON 87712 and the conventional control A3525 during any observation for damage caused by any of the assessed disease stressors. Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with four replications. All observations were made during the following four crop developmental stages: V3 – V5; R1 - R2; R3 - R5; and R6 - R8.

[1] Includes *Pseudomonas*.

[2] Includes *Cercospora* leaf blight and *Cercospora* leaf spot.

[3] Includes *Alternaria* black spot and *Alternaria* leaf spot.

[4] Includes root rot.

[5] Includes brown spot.

Table H-8. Arthropod-Related Damage Evaluations of MON 87712 and Conventional Control A3525 Using an Observational Severity Scale

Arthropod	Number of observations across sites[1]	Number of observations where no differences were detected between MON 87712 and the conventional control A3525[2]	Statistically Significant Differences[4]				
					Arthropod Damage Rating (0-5 scale)		
			Site[3]	Observation Number	MON 87712 Mean (S.E.)	Control Mean (S.E.)	Reference Range
Aphids[5] (Aphididae)	20	20	–	–	–	–	–
Armyworms[6] (*Spodoptera* spp.)	10	10	–	–	–	–	–
Bean leaf beetles (*Cerotoma trifurcata*)	35	33	ILCY	1	0.53(0.05)	0.23(0.06)	0.33-0.40
			MOAN	2	0.65(0.12)	0.93(0.09)	0.75-0.92
Blister beetles (Meloidae)	5	5	–	–	–	–	–
Cabbage loopers (*Trichoplusia ni*)	1	1	–	–	–	–	–
Grasshoppers (Acrididae)	29	29	–	–	–	–	–
Green cloverworms (*Plathypena scabra*)	16	15	MOWR	4	0.60(0.04)	0.48 (0.05)	0.48-0.55
Japanese beetles (*Popillia japonica*)	21	20	ILMS	1	0.50(0.04)	0.30(0.04)	0.33-0.40
Leafhoppers[7] (Cicadellidae)	6	5	PAHM	1	0.55(0.12)	0.15(0.06)	0.30-0.50
Silver spotted skippers (*Epargyreus clarus*)	1	1	–	–	–	–	–

Table H-8. Arthropod-Related Damage Evaluations of MON 87712 and Conventional Control A3525 Using an Observational Severity Scale (continued)

Arthropod	Number of observations across sites[1]	Number of observations where no differences were detected between MON 87712 and the conventional control A3525[2]	Statistically Significant Differences[4]				
					Arthropod Damage Rating (0-5 scale)		
			Site[3]	Observation Number	MON 87712 Mean (S.E.)	Control Mean (S.E.)	Reference Range
Soybean loopers (*Pseudoplusia includens*)	11	9	MOAN	2	0.55(0.10)	0.90(0.09)	0.70-0.86
			MOFI	3	0.45(0.05)	0.85(0.05)	0.80-0.93
Stink bugs (Pentatomidae)	22	22	–	–	–	–	–
Three-cornered alfalfa hoppers (*Spissistilus*	4	4	–	–	–	–	–
Thrips (Thripidae)	2	2	–	–	–	–	–
Velvetbean caterpillars (*Anticarsia gemmatalis*)	4	3	MOFI	3	0.45(0.05)	0.85(0.05)	0.80-0.93

Note: The experimental design was a randomized complete block with four replications. Observations were conducted at four crop developmental stages: Observation 1 = V3-V5, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8.

[1] A total of 192 arthropod damage observations were made across sites. Lack of variability in the data precluded statistical comparisons for 55 of the observations. Statistical comparisons could be made between MON 87712 and conventional control A3525 for 137 of the observations.

[2] No statistically significant differences were detected (α=0.05) or numerical differences between MON 87712 and the conventional control A3525 where p-values could not be generated due to lack of variability in the data.

[3] Site codes are as follows: ILCY = Clinton County, IL; ILMS = Effingham County, IL; MOAN = Macon County, MO; MOFI = Butler County, MO; MOWR = Lincoln County, MO; PAHM = Berks County, PA site 2.

[4] Means, standard errors (S.E.), and reference ranges are reported for a statistically significant difference that was detected (α=0.05) between MON 87712 and the conventional control A3525. Reference range = minimum and maximum mean values among the commercial reference varieties.

[5] Aphid includes soybean aphid.

[6] Armyworm includes fall armyworm and beet armyworm.

[7] Leafhopper includes potato leafhopper.

Dash (−) indicates that there were no statistically significant differences were detected (α=0.05) between MON 87712 and the conventional control A3525.

Table H-9. Abundance of Pest Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties

		Pest Arthropod[1]								
		Aphids (Aphididae)			Bean leaf beetles (*Cerotoma trifurcata)*			Corn earworms (*Heliothis zea*)		
Coll.	Site[3]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	–	–	–	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0	–	–	–
	ILSE	0.8 (0.5)	0.0 (0.0)	0.5 - 2.0	1.5 (1.0)	1.8 (0.3)	0.8 - 2.5	–	–	–
	MOCB	–	–	–	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0	–	–	–
2	ARNE	–	–	–	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0	0.8 (0.3)	0.5 (0.3)	0.3 - 0.8
	ILSE	2.3 (0.9)	2.8 (2.1)	1.3 - 4.0	1.3 (0.3)*	0.3 (0.3)	0.5 - 1.0	–	–	–
	MOCB	–	–	–	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	–	–	–
3	ARNE	–	–	–	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0	–	–	–
	ILSE	0.5 (0.5)	1.0 (0.6)	0.5 - 2.0	10.3 (1.9)	12.8 (3.6)	5.8 - 10.0	–	–	–
	MOCB	61.5 (56.2)	31.8 (27.9)	16.3 - 61.8	0.0 (0.0)	0.0 (0.0)	0.0 - 0.3	–	–	–
4	ARNE	–	–	–	0.0 (0.0)	0.0 (0.0)	0.0 - 0.3	–	–	–
	ILSE	1.5 (0.6)	1.0 (0.6)	0.0 - 1.8	35.8 (5.1)	25.5 (4.3)	17.0 - 30.8	–	–	–
	MOCB	37.5 (12.0)*	19.3 (7.1)	18.3 - 42.5	0.8 (0.3)	0.5 (0.3)	0.5 - 1.3	–	–	–

Table H-9. Abundance of Pest Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Pest Arthropod[1]								
		Green cloverworms (*Plathypena scabra*)			Garden flea hoppers (*Halticus bractacus*)			Leafhoppers[2] (Cicadellidae)		
Coll.	Site[3]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	1.8 (0.5)	4.3 (2.3)	1.5 - 2.0	–	–	–	–	–	–
	ILSE	7.3 (2.8)	13.5 (3.7)	9.0 - 12.8	–	–	–	–	–	–
	MOCB	4.0 (1.7)	3.0 (1.2)	1.3 - 3.0	1.0 (0.7)	1.0 (0.7)	0.8 - 1.8	1.0 (0.7)	0.0 (0.0)	0.3 - 2.3
2	ARNE	1.5 (0.9)	4.5 (2.1)	2.3 - 3.0	–	–	–	–	–	–
	ILSE	10.0 (2.0)	11.0 (2.9)	9.0 - 15.5	–	–	–	–	–	–
	MOCB	3.8 (1.1)	2.0 (0.6)	1.3 - 3.0	0.3 (0.3)	0.3 (0.3)	0.0 - 1.3	–	–	–
3	ARNE	2.8 (0.5)	4.3 (1.9)	2.0 - 3.5	–	–	–	–	–	–
	ILSE	0.0 (0.0)	0.5 (0.5)	0.0 - 0.3	0.0 (0.0)	0.3 (0.3)	0.0 - 0.5	–	–	–
	MOCB	0.8 (0.5)*	5.5 (2.6)	0.8 - 4.8	2.8 (1.3)	1.5 (1.2)	0.8 - 2.8	–	–	–
4	ARNE	3.5 (1.6)	4.8 (1.8)	0.8 - 4.0	–	–	–	–	–	–
	ILSE	0.0 (0.0)†	0.0 (0.0)	0.0 - 0.0	–	–	–	–	–	–
	MOCB	0.3 (0.3)	0.0 (0.0)	0.0 - 0.0	8.0 (4.3)	4.3 (2.3)	1.5 - 4.5	–	–	–

Table H-9. Abundance of Pest Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Pest Arthropod[1]								
		Soybean loopers (*Pseudoplusia includens*)			Stink bugs (Pentatomidae)			Three-cornered alfalfa hoppers (*Spissistilus festinus*)		
Coll.	Site[3]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	–	–	–	0.8 (0.5)	0.5 (0.5)	0.0 - 2.5	3.5 (0.9)	6.3 (3.4)	0.0 - 3.3
	ILSE	0.0 (0.0)	0.0 (0.0)	0.0 - 0.5	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	–	–	–
	MOCB	–	–	–	0.0 (0.0)†	0.0 (0.0)	0.0 - 0.0	–	–	–
2	ARNE	–	–	–	1.8 (0.8)	1.8 (0.6)	0.5 - 3.5	0.3 (0.3)	1.8 (1.2)	0.3 - 3.0
	ILSE	–	–	–	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	–	–	–
	MOCB	–	–	–	0.0 (0.0)	0.0 (0.0)	0.0 - 0.0	–	–	–
3	ARNE	–	–	–	0.3 (0.3)*	2.0 (0.4)	0.3 - 2.3	0.3 (0.3)	0.8 (0.3)	0.5 - 1.5
	ILSE	–	–	–	1.3 (0.3)	0.5 (0.3)	0.0 - 1.5	–	–	–
	MOCB	–	–	–	0.8 (0.5)*	0.0 (0.0)	0.0 - 0.5	–	–	–
4	ARNE	–	–	–	1.0 (1.0)	2.5 (1.0)	1.0 - 5.5	3.5 (1.2)	4.8 (2.9)	1.0 - 2.5
	ILSE	–	–	–	0.8 (0.5)	1.3 (0.8)	0.3 - 0.8	–	–	–
	MOCB	–	–	–	0.3 (0.3)	0.0 (0.0)	0.0 - 1.0	–	–	–

Table H-9. Abundance of Pest Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Pest Arthropod[1]								
		Tarnished plant bugs (*Lygus lineolaris*)			Yellow-striped armyworms (*Spodoptera ornithogalli*)			Yellow woollybear caterpillars (*Spilosoma virginica*)		
Coll.	Site[3]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	0.3 (0.3)	0.0 (0.0)	0.3 - 0.5	–	–	–	–	–	–
	ILSE	0.0 (0.0)	0.5 (0.5)	0.0 - 0.8	–	–	–	–	–	–
	MOCB	–	–	–	–	–	–	–	–	–
2	ARNE	–	–	–	1.0 (0.4)	0.3 (0.3)	0.0 - 1.8	–	–	–
	ILSE	0.3 (0.3)	0.5 (0.3)	0.3 - 0.3	–	–	–	0.3 (0.3)	0.0 (0.0)	0.0 - 0.3
	MOCB	–	–	–	–	–	–	–	–	–
3	ARNE	–	–	–	0.3 (0.3)	0.5 (0.5)	0.0 - 0.8	–	–	–
	ILSE	1.0 (0.4)	2.3 (1.9)	0.3 - 1.0	–	–	–	–	–	–
	MOCB	–	–	–	–	–	–	–	–	–
4	ARNE	–	–	–	0.5 (0.5)	0.5 (0.5)	0.0 - 0.3	0.3 (0.3)	0.0 (0.0)	0.0 - 0.3
	ILSE	2.3 (0.5)	7.8 (2.8)	1.5 - 3.5	–	–	–	–	–	–
	MOCB	1.0 (0.7)	0.3 (0.3)	0.0 - 0.8	–	–	–	–	–	–

Note: The experimental design was a randomized complete block with four replications. Arthropod collection 1 was made at R1 - R2 growth stage and the three subsequent collections at approximately two week intervals thereafter. Numbers represent sample means with standard error (SE) in parentheses.

[*] Indicates a statistically significant difference (α=0.05) between MON 87712 and the conventional control A3525.

[†] No p-values were generated due to lack of variability in the data.

[1] MON 87712 and conventional control A3525 values represent mean number of arthropods from four replications. A dash (−) indicates arthropod not evaluated.

[2] Leafhopper includes potato leafhopper.

[3] Site codes are as follows: ARNE = Jackson County, AR; ILSE = Champaign County, IL; MOCB = Shelby County, MO.

Table H-10. Abundance of Beneficial Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties

		Beneficial Arthropod[1]								
		Spiders (Araneae)			Big-eyed bugs (*Geocoris* spp.)			Carabid beetles (Carabidae)		
Coll.	Site[2]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	1.5 (0.6)	2.8 (1.3)	0.5 - 3.0	4.3 (0.9)*	1.8 (1.0)	0.8 - 3.8	–	–	–
	ILSE	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	–	–	–	–	–	–
	MOCB	0.8 (0.5)	0.3 (0.3)	0.0 - 0.3	–	–	–	–	–	–
2	ARNE	2.8 (1.0)	2.5 (0.6)	1.8 - 5.8	4.0 (1.5)	2.5 (1.0)	0.8 - 7.0	–	–	–
	ILSE	0.5 (0.5)	1.3 (0.5)	0.3 - 1.5	–	–	–	–	–	–
	MOCB	1.3 (0.5)	0.3 (0.3)	0.3 - 1.0	–	–	–	–	–	–
3	ARNE	2.8 (0.9)	1.0 (0.4)	0.5 - 2.0	1.0 (0.6)	1.0 (0.6)	0.3 - 1.0	–	–	–
	ILSE	1.8 (0.3)	0.5 (0.3)	0.5 - 1.3	–	–	–	0.5 (0.3)	0.0 (0.0)	0.0 - 0.8
	MOCB	1.3 (1.3)	0.8 (0.3)	1.8 - 2.8	–	–	–	–	–	–
4	ARNE	1.8 (0.9)	1.3 (0.3)	0.8 - 2.5	0.3 (0.3)	1.8 (1.4)	0.3 - 1.8	–	–	–
	ILSE	2.0 (0.7)	0.8 (0.5)	0.8 - 1.5	–	–	–	–	–	–
	MOCB	2.8 (0.8)	1.3 (0.6)	0.5 - 2.0	–	–	–	–	–	–

Table H-10. Abundance of Beneficial Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Beneficial Arthropod[1]								
		Lacewings (Chrysopidae)			Ladybird beetles (Coccinellidae)			Parasitic Wasps (Micro-parasitic hymenoptera)		
Coll.	Site[2]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	–	–	–	–	–	–	–	–	–
	ILSE	0.0 (0.0)	0.0 (0.0)	0.0 - 0.5	0.0 (0.0)	0.0 (0.0)	0.0 - 0.5	–	–	–
	MOCB	–	–	–	1.0 (0.4)	0.5 (0.3)	0.3 - 1.3	–	–	–
2	ARNE	–	–	–	–	–	–	0.3 (0.3)	0.0 (0.0)	0.0 - 0.0
	ILSE	1.0 (0.4)	0.3 (0.3)	0.0 - 1.3	0.8 (0.3)	0.0 (0.0)	0.5 - 1.0	–	–	–
	MOCB	–	–	–	1.3 (0.5)	0.5 (0.3)	0.0 - 2.0	–	–	–
3	ARNE	–	–	–	–	–	–	–	–	–
	ILSE	0.3 (0.3)	0.3 (0.3)	0.0 - 0.8	4.0 (1.2)	3.5 (1.0)	3.8 - 8.8	–	–	–
	MOCB	0.3 (0.3)	0.8 (0.5)	0.5 - 1.0	1.0 (0.6)	0.5 (0.3)	0.5 - 3.0	–	–	–
4	ARNE	–	–	–	–	–	–	–	–	–
	ILSE	0.5 (0.3)	0.0 (0.0)	0.0 - 0.3	8.3 (1.7)*	3.5 (1.0)	1.0 - 2.8	4.3 (1.4)	3.0 (1.3)	1.0 - 1.8
	MOCB	2.8 (0.8)	1.0 (0.7)	1.5 - 3.3	3.8 (0.6)	2.8 (0.9)	2.5 - 5.3	2.3 (0.5)*	0.8 (0.5)	1.3 - 2.3

Table H-10. Abundance of Beneficial Arthropods in Samples Collected from MON 87712, Conventional Control A3525, and the Commercial Reference Varieties (continued)

		Beneficial Arthropod[1]					
		Damsel bugs (*Nabis* spp.)			*Orius* spp.		
Coll.	Site[2]	MON 87712 Mean (SE)	Control Mean (SE)	Reference range	MON 87712 Mean (SE)	Control Mean (SE)	Reference range
1	ARNE	0.3 (0.3)	0.3 (0.3)	0.0 - 1.3	0.0 (0.0)	0.8 (0.5)	0.0 - 1.0
	ILSE	0.0 (0.0)	0.0 (0.0)	0.0 - 0.3	0.0 (0.0)*	1.0 (0.4)	0.0 - 0.8
	MOCB	0.3 (0.3)	0.0 (0.0)	0.0 - 0.5	5.0 (2.6)	2.8 (1.0)	1.3 - 4.5
2	ARNE	2.3 (1.3)	2.5 (1.0)	0.5 - 4.3	0.0 (0.0)	0.0 (0.0)	0.0 - 0.0
	ILSE	0.0 (0.0)	0.0 (0.0)	0.0 - 1.3	2.8 (0.5)	1.3 (0.8)	1.0 - 2.5
	MOCB	0.5 (0.5)	0.0 (0.0)	0.0 - 0.5	0.3 (0.3)	0.0 (0.0)	0.5 - 2.3
3	ARNE	1.5 (0.6)	1.0 (1.0)	0.3 - 2.0	0.0 (0.0)†	0.0 (0.0)	0.0 - 0.0
	ILSE	0.3 (0.3)	0.8 (0.5)	0.5 - 1.5	17.3 (6.3)	6.3 (1.3)	4.3 - 11.5
	MOCB	0.8 (0.3)	0.5 (0.5)	0.0 - 0.5	0.8 (0.8)	3.5 (2.4)	6.0 - 11.5
4	ARNE	1.0 (0.6)	0.3 (0.3)	0.8 - 2.0	0.0 (0.0)†	0.0 (0.0)	0.0 - 0.0
	ILSE	0.8 (0.5)	0.5 (0.5)	0.5 - 2.0	19.5 (4.9)	16.3 (8.1)	4.5 - 11.0
	MOCB	1.0 (0.4)	0.0 (0.0)	0.5 - 1.3	14.5 (4.2)	16.3 (4.7)	7.5 - 13.0

* Indicates a statistically significant difference (α=0.05) between MON 87712 and the conventional control A3525.
Note: The experimental design was a randomized complete block with four replications. Arthropod collection 1 was made at R1 - R2 growth stage and the three subsequent collections at approximately two week intervals thereafter. Numbers represent sample means with standard error (SE) in parentheses.
† No p-values were generated due to lack of variability in the data.
[1] MON 87712 and conventional control A3525 values represent mean number of arthropods from four replications. A dash (−) indicates arthropod not evaluated.
[2] Site codes are as follows: ARNE = Jackson County, AR; ILSE = Champaign County, IL; MOCB = Shelby County, MO.

References for Appendix H

Drees, B.M. and M.E. Rice. 1985. The vertical beat sheet: A new device for sampling soybeans insects. Journal of Economic Entomology 78:1507-1510.

Appendix I: Materials and Methods for Pollen Morphology and Viability Assessment

I.1. Plant Production

MON 87712, a conventional soybean control A3525, and four commercial reference varieties were grown under similar agronomic conditions in a field trial in St. Louis County, MO (Table H-1; MOSL site). The trial was arranged in a randomized completed block design with four replications. Each plot consisted of twelve rows approximately 30 feet in length.

I.2. Flower Collection

When the soybean plants were flowering, approximately twenty whole flowers were collected from the first and fourth row of each plot. One flower from the bottom of the plant, two flowers from the middle, and one flower from the top were collected from each of five representative plants per plot. All flowers from a plot were placed in a single, clean container. The container was labeled with the plot number from which the sample originated, entry number, and entry name. The containers were kept on wet ice from collection until the pollen was prepared and stained.

I.3. Pollen Sample Preparation

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

I.4. Data Collection

Pollen characteristics were assessed by viewing samples with an Olympus BX51 light/fluorescence microscope equipped with an Olympus DP70 digital color camera. The microscope and camera were connected to a computer running Microsoft Windows 2000 Professional and installed with associated DP Controller c 1.2.1.108 and DP Manager V1.2.1.107 camera software and Image-Pro Plus v6.2.1.491 imaging software.

I.4.1. Pollen Viability

When exposed to the stain solution, viable pollen grains stained red to purple due to the presence of living cytoplasmic content. Nonviable pollen grains stained blue to green and may have appeared round to collapsed in shape, depending on the degree of hydration. For each pollen sample, the number of viable and nonviable pollen grains was counted from a minimum of 75 pollen grains from a random field of view under the

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

I.4.2. Pollen Diameter

Micrographs (400× resolution) of 10 selected pollen grains were taken and imported into the imaging software. The software was used to measure pollen grain diameter along two perpendicular axes for each selected pollen grain. Mean pollen diameter from each plot was calculated from 20 total measurements.

I.4.3. General Pollen Morphology

General pollen morphology was observed from digital images of MON 87712, the conventional control A3525, and commercial reference varieties that also were used for pollen diameter measurements.

I.5. Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS® (2008). The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). MON 87712 was compared to the conventional control for percent viable pollen and pollen diameter. No statistical comparisons were made between MON 87712 and the commercial reference varieties. A reference range for each measured characteristic was determined from the minimum and maximum mean values from among the references. General pollen morphology was qualitative; therefore, no statistical analysis was conducted on these observations.

References for Appendix I

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

Appendix J: Materials and Methods for Symbiont Assessment

J.1. Materials

The soybean materials for the symbiont interaction assessment included MON 87712, the conventional control A3525, and six commercial references varieties (Table J-1). Nodule, root tissue, and shoot tissue collected from MON 87712, the conventional control A3525, and the commercial reference varieties were evaluated.

Table J-1. Starting Seed of MON 87712, Conventional Control, and Commercial Reference Varieties Used in the Symbiont Assessment

T/C/R[1]	Starting Seed Name[2]	Phenotype	Orion Number
Test	MON 87712	High yield	11223539
Control	A3525	Conventional	11223542
Reference	A2553	Conventional	10000961
Reference	Midland 363	Conventional	11243106
Reference	Stewart SB3454	Conventional	11242910
Reference	Gateway 427	Conventional	11225759
Reference	Pioneer 93B15	Conventional	11242904
Reference	Hoegemeyer 333	Conventional	11242905

[1] T/C/R = test, control, and reference
[2] The test starting seed name is a Monsanto Regulatory identification; the conventional control A3525 and commercial reference varieties starting seed names are variety names.

J.2. Characterization of the Methods

The identities of MON 87712 and the conventional control A3525 seed were verified by event-specific polymerase chain reaction analyses.

J.3. Greenhouse Phase and Experimental Design

MON 87712, the conventional control A3525, and the commercial reference varieties starting seed were planted in 6-inch pots containing nitrogen-deficient potting medium (Sunshine® Mix #3 Basic/LB2) composed of primarily peat, vermiculite, and perlite. Plants from MON 87712, the conventional control A3525, and commercial reference varieties starting seed were grown in a greenhouse where actual temperatures ranged from approximately 18 to 35°C. Eight replicate pots were planted with three seeds per pot for each of MON 87712, the conventional control A3525, and commercial reference varieties. At planting, each seed was inoculated with approximately 1×10^7 cells of *Bradyrhizobium japonicum* (VAULT® NP, Becker, Underwood, Ames, IA) in phosphate-buffered saline. Pots were arranged in eight replicated blocks for the 6-week sampling period using a randomized complete block design.

The starting seeds for replicates 1, 2, and 3 were planted on July 27, 2010, replicates 4, 5, and 6 were planted on July 28, 2010, and replicates 7, 8, and 9 were planted on July 29, 2010. In all cases, replicate pots had a minimum of one plant emerge within one week. A solution of nitrogen-free nutrient solution (approximately 250 ml) was added weekly after plant emergence.

J.4. Plant Harvesting/Data Collection

Six weeks after emergence, plants were excised at the surface of the potting medium and shoot and root plus nodule material were removed from pots. The shoot material was cut into smaller pieces and placed in labeled bags. The plant roots with nodules were separated from the potting medium by washing with water. Excess moisture was removed using absorbent paper towels and the roots plus nodules were placed in labeled bags. The same day that plants were harvested, nodules were removed by hand from the roots of each plant, enumerated, and weighed to determine the fresh weight (fwt) of the nodules.

The remaining root and shoot fresh weight were determined for each plant. Nodules as well as root and shoot material were placed in a drying oven on the same day as collected. The plant material was dried for at least 72 hours at approximately 65°C to determine dry weight (dwt). The shoot tissue was ground using a Harbil 5G high-speed paint shaker prior to total nitrogen analysis. Shoot total nitrogen was determined by combustion using a nitrogen analyzer (Rapid N Cube, Elementar Americas, Inc).

J.5. Statistical Analysis

The data consisted of six measurements taken at the six week sampling period: nodule number, nodule dwt (g), shoot dwt (g), root dwt (g), shoot percent total nitrogen, and shoot total nitrogen (g). An analysis of variance was conducted using a randomized complete block design with eight replications. Data were analyzed using SAS® (2008). The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$). No statistical comparisons were made between MON 87712 and the commercial reference varieties. Instead, a reference range for each measured characteristic was determined from the minimum and maximum mean values from among the six commercial reference varieties.

References for Appendix J

SAS. 2008. SAS/STAT software version 9.2. SAS Institute, Inc., Cary, North Carolina..

Appendix K: Materials and Methods for Volunteer Potential Assessment

K.1. Materials

The soybean materials for the volunteer potential assessment in the field included MON 87712, the conventional control A3525, and commercial reference varieties. The list of the soybean materials planted at each of four field sites is presented in Table K-1.

The viability of the starting seed material was determined in the laboratory (BioDiagnostics Inc.) by conducting warm germination testing of each seed lot. The viability of MON 87712 and the conventional control A3525 from all sites was ≥90%. The viability of the commercial reference varieties from among the sites was ≥80% with two exceptions: two commercial reference varieties harvested at the Jackson County, MO (ARNE) site had germination rates of 42 and 31% respectively. Based upon the viability data, it was determined that the seed were acceptable for evaluating volunteer potential.

Table K-1. Starting Seed for Volunteer Potential Assessment

Substance	Substance Type	Phenotype/Genotype	Site[1]	Orion ID #			
				Rep 1	Rep 2	Rep 3	Rep 4
MON 87712	Test	Soybean Intrinsic Yield	ARAU	11259962-002	11259970-002	11259979-002	11259987-002
MON 87712	Test	Soybean Intrinsic Yield	ARNE	11260951-002	11260927-002	11260953-002	11260942-002
MON 87712	Test	Soybean Intrinsic Yield	ILWY	11259999-002	11260022-002	11260014-002	11260007-002
MON 87712	Test	Soybean Intrinsic Yield	MOAN	11259953-002	11259931-002	11259939-002	11259945-002
A3525	Control	Conventional	ARAU	11259967-002	11259974-002	11259990-002	11259983-002
A3525	Control	Conventional	ARNE	11260932-002	11260949-002	11260947-002	11260940-002
A3525	Control	Conventional	ILWY	11260003-002	11260010-002	11260018-002	11259995-002
A3525	Control	Conventional	MOAN	11259958-002	11259943-002	11259934-002	11259950-002
SB3888R	Reference	Conventional	ARAU	11259986-002	11259978-002	11259971-002	11259963-002
NK S30-D4	Reference	Conventional	ARAU	11259984-002	11259977-002	11259968-002	11259961-002
LG-C3540	Reference	Conventional	ARAU	11259982-002	11259966-002	11259975-002	11259991-002
Stine 3300-0	Reference	Conventional	ARAU	11259989-002	11259980-002	11259973-002	11259964-002
NuPride 3202	Reference	Conventional	ARNE	11260946-002	11260956-002	11260931-002	11260939-002
Pioneer 93M14	Reference	Conventional	ARNE	11260944-002	11260954-002	11260929-002	11260937-002
Pioneer 93M11	Reference	Conventional	ARNE	11260933-002	11260938-002	11260948-002	11260955-002
SB3888R	Reference	Conventional	ARNE	11260935-002	11260930-002	11260945-002	11260952-002
Stine 3300-0	Reference	Conventional	ILWY	11260011-002	11260002-002	11259994-002	11260019-002
Pioneer 93M11	Reference	Conventional	ILWY	11260006-002	11260015-002	11259998-002	11260023-002
SB3819	Reference	Conventional	ILWY	11260009-002	11260000-002	11259992-002	11260017-002
SB3369R	Reference	Conventional	ILWY	11260005-002	11260012-002	11260021-002	11259996-002
SB3369R	Reference	Conventional	MOAN	11259936-002	11259944-002	11259952-002	11259925-002
NutriPride 8339	Reference	Conventional	MOAN	11259937-002	11259946-002	11259955-002	11259929-002
Pioneer 93M62	Reference	Conventional	MOAN	11259942-002	11259951-002	11259935-002	11259959-002
Pioneer 93M14	Reference	Conventional	MOAN	11259948-002	11259940-002	11259933-002	11259957-002

[1] ARAU = Woodruff County, AR; ARNE = Jackson County, AR; ILWY = Stark County, IL; MOAN = Shelby County, MO.

K.2. Characterization of the Materials

The identities of MON 87712 and the conventional control A3525 were verified by event-specific polymerase chain reaction analyses.

K.3. Field Sites and Plot Designs

Data were collected from field sites conducted in 2009-2010 growing season at four sites within the U.S. soybean production regions. These four sites provided a range of environmental and agronomic conditions representative of major U.S. soybean-growing regions. The Principle Investigator at each site was familiar with soybean production and the identification of soybean volunteers.

At each site, the study area used for volunteer potential assessment had not been used for soybean production the previous growing season. Planting, soil, and cropping history information for each site are presented in Table K-2. The experiment was established at each of four locations in a randomized complete block design with four replications. Each plot was 5-8 ft wide by 20 ft long. To avoid mixing of the materials between adjacent plots during seed incorporation, a confined seeding area was established within each plot that consisted of the center area of the plot located approximately 1 ft from each plot edge. Consequently, the actual seeded area of each plot was 3-6 ft wide by 18 ft long. Alleyways between experimental blocks were 5-10 ft wide.

Table K-2. Seeding Information, Soil Description, and Cropping History Information for Volunteer Potential Assessment

Site[1]	Seeding Date[2]	Target Seeding Rate (seed/plot)	Approximate Seed Incorporation Depth (in)	Plot Size (ft)	Actual Seeding Area (ft)	Reps	Soil Series Description; Organic Matter (%); and pH	Previous Cropping History	
								2007	2008
ARAU	11/23/2009	400	1-3	8 x 20	6 x 18	4	Wiville fine sandy loam; 1.25 %; 6.2	Fallow	Fallow
ARNE	11/23/2009	400	1-3	8 x 20	6 x 18	4	Bosket fine sandy loam; 2.0 %; 5.8	Soybeans	Fallow
ILWY	11/23/2009	400	1-3	5 x 20	3 x 18	4	Plano silt loam; 3.6 %; 6.1	Corn	Fallow
MOAN	12/02/2009	400	0.5-2	5 x 20	3 x 18	4	Mexico silt loam; 2.7 %; 6.0	Corn	Fallow

[1]ARAU = Woodruff County, AR; ARNE = Jackson County, AR; ILWY = Stark County, IL; MOAN = Shelby County, MO.
[2] month/day/year.

K.4. Planting and Field Operations

Each plot was hand-seeded by uniformly scattering approximately 400 seed on the soil surface within the confined seeding area. Seed were then mechanically incorporated to a maximum depth of approximately depth of approximately 0.5-3 inches to avoid surface predation.

Agronomic practices used to prepare and maintain each study site were characteristic of each respective region. No irrigation was applied to the study areas, and no plot management was required after the seed were scattered in the plots.

K.5. Data Collection

The plots at each site were monitored for volunteer plants starting approximately two weeks after planting. Counts occurred approximately every two weeks thereafter until environmental conditions were no longer conducive for germination and emergence. Monitoring resumed in the spring of 2010, when environmental conditions became favorable for soybean germination and emergence. Counts continued approximately every two weeks until mid-June 2010. A total of seven germination and emergence observations were conducted at each site throughout the study.

K.6. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all stages of data collection, summarization, and analysis. Study personnel ensured that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated by the Lead Scientist for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues during the study that would impact the study objectives were noted by the Lead Scientist.

K.7. Statistical Analysis

There were few volunteers observed in the study. Due to insufficient number of volunteer plants and lack of variability, statistical analysis of the data could not be performed.

K.8. Individual-Site Volunteer Potential Results and Discussion

The environmental conditions after planting were not favorable for germination and emergence at any of the four sites, therefore no volunteers were observed in fall of 2009. Monitoring resumed in the spring of 2010, when environmental conditions became favorable for germination and emergence of soybean volunteers. No volunteer plants were observed at the ARAU and ARNE sites for MON 87712, the conventional control A3525 or commercial reference varieties. A small number of volunteer plants were observed at the ILWY and MOAN sites (Table VII-9). The small number of volunteer observed for MON 87712 and the conventional control A3525 where within the range of

the number of volunteers observed for the commercial reference varieties. However, no statistical analysis was performed due to insufficient number of volunteers observed and lack of variability

Appendix L: Materials and Methods for Persistence Outside of Cultivation Assessment

L.1. Materials

The soybean materials for the persistence outside of cultivation assessment in the field included MON 87712, the conventional control A3525, and commercial reference varieties. The list of the soybean materials planted at each of four field sites is presented in Table L-1.

Table L-1. Starting Seed for Persistence Outside of Cultivation Assessment

Material Name[1]	Material Type	Phenotype	Monsanto Lot Number	Sites[2]
MON 87712	Test	High yield	11223539	All
A3525	Control	Conventional	11223542	All
Garst 3585N	Reference	Conventional	10001119	INSH, NEYO
Pioneer 93M62	Reference	Conventional	11226582	INSH, NEYO
Stewart SB3579R	Reference	Glyphosate-tolerant	11226925	INSH, NEYO
Pioneer 93M11	Reference	Glyphosate-tolerant	11226840	INSH, NEYO
Pioneer 93B82	Reference	Conventional	11226581	MOCB
Trisoy 3535CN	Reference	Conventional	10001811	MOCB
NuPride 8339	Reference	Glyphosate-tolerant	11226939	MOCB
NK S30-D4	Reference	Glyphosate-tolerant	11226843	MOCB
Midland/Phillips 363	Reference	Conventional	11226698	MOFI
Stewart SB3454	Reference	Conventional	10001130	MOFI
Stewart SB3993R	Reference	Glyphosate-tolerant	11226927	MOFI
Pioneer 93M43	Reference	Glyphosate-tolerant	11226839	MOFI

[1] The use of the word "material" is synonymous with the use of the word "substance" in the statistical report (Appendix A).
[2] Sites: INSH = Boone County, IN; MOCB = Shelby County, MO; MOFI = Butler County, MO; and NEYO = York County, NE

L.2. Characterization of the Materials

Identities of MON 87712 and the conventional control A3525 seed were verified by event-specific polymerase chain reaction analyses.

L.3. Field Sites and Plot Design

Data were collected from field sites conducted in 2009 at four sites within the U.S. soybean production regions. The study sites were located within major soybean growing regions of the U.S. where seed or grain may be incidentally returned to the environment during harvest, handling, or transport.

The experiment was established in 2009 at four sites in a randomized complete block design with three replications. Each plot was approximately 100 ft^2 in size, consisting of four rows, approximately 10 ft in length, with an inter-row spacing of approximately 30 inches.

Due to strong soybean plant stands in all plots at the MOCB site, the experiment was continued for a second season. The site was established in January, 2010 and continued through July, 2010. Each entry of every replication was established in exactly the same plot area in which that entry had previously been located during 2009. An exception was that the three plots which were planted with a material dropped from development were left unused after the plant and seed material from these plots was removed and destroyed. The seed for each plot was hand scattered over the existing vegetation in three ten foot rows. Each row was marked with a colored flag to facilitate locating new plants in the spring of 2010. Each plot was then covered with a wire mesh with 0.5 inch openings in order to prevent surface predation of the seeds by animals. The mesh was secured on the edges of all plots. An animal exclusion electric fence was constructed around the perimeter of the study area. A 15 foot isolation border was established around the outside of the fenced study area. This isolation border was the same area as the original 15 foot isolation border.

L.4. Planting and Field Operations

One hundred seeds were planted in each row and the planting density at all sites was 10 seeds/ft resulting in 400 seeds/plot being planted at all sites.

Within each study area, the study materials were grown under similar conditions. The study areas consisted of unmanaged areas and no plot preparation was performed other than mowing the area at approximately 3-6 inches. No fertilizers, tillage, or herbicides were applied to the study area prior to or after planting. Planting information and a soil description of each study site are provided in Tables L-2 and L-3.

The INSH study area was well-drained unused pasture. The ground cover was mostly perennial grass with some broadleaf species and was estimated to be approximately 100% cover. The MOCB study area was on the perimeter of a cultivated field and had not been in agricultural use during the previous two years. The ground cover was estimated to be approximately 90% and consisted of fescue grass and clover. The MOFI study area was

a frequently mowed area near the farm house that was 100% covered in annual grasses and broadleaf annuals. The area had not been in agricultural production for at least ten years. The NEYO study area was a well drained level field margin adjacent to a field under cultivation and contained mostly perennial brome grass. The ground cover was estimated to be approximately 100%. None of the study sites had been used for crop production for at least the previous two years. Weather conditions at the sites during 2009 and 2010 were typical for their respective regions and there were no unusual weather events.

Table L-2. Seeding Information, Soil Description, and Cropping History Information for Persistence Outside of Cultivation Assessment

Site[1]	Planting Date (mm/dd/yyyy)	Planting Rate (seeds/plot)	Planting Depth (in)	Plot Size (ft)	Reps	Soil Series; Organic Matter; pH
INSH	06/05/2009	400	1.5	10 × 10	3	Crosby silt loam; 2.5%; 6.0
MOCB	06/23/2009	400	1.0	10 × 10	3	Mexico silt loam; 2.7%; 7.2
MOFI	06/12/2009	400	0.5	10 × 10	3	Wiville loamy fine sand; 1.3%; 6.6
NEYO	06/03/2009	400	0.75	10 × 10	3	Hastings silt loam; 3.0%; 6.5
MOCB	01/25/2010	varies by plot	surface	10 × 10	3	Mexico silt loam; 2.7%; 7.2

[1] Sites: INSH = Boone County, IN; MOCB = Shelby County, MO; MOFI = Butler County, MO; and NEYO = York County, NE

Table L-3. Study Area Planting Information and Soil Description for 2010 Extended Season at MOCB Site

Site[1]	Planting Date (mm/dd/yyyy)	Planting Rate (seeds/plot)	Planting Depth (in)	Plot Size (ft)	Reps	Soil Series; Organic Matter; pH
MOCB	01/25/2010	varies by plot	surface	10 × 10	3	Mexico silt loam; 2.7%; 7.2

[1] Sites: MOCB = Shelby County, MO

L.5. Data Collection

The characteristics evaluated and the timing of their evaluation are presented in Table L-4. Replacement values were calculated and used to evaluate the ability of the study materials to persist outside of cultivation. The replacement value is the ratio of the number of seeds produced to the number of seeds sown. A replacement value greater than one means that more seeds were produced than were sown and indicates a population that is increasing. A replacement value of less than one means that fewer seeds were produced than sown. This is indicative of a population that will not replace itself and will not persist.

Table L-4. Data Characteristics Evaluated at Each Site for Persistence Outside of Cultivation Assessment

Characteristic	Timing of Evaluation	Evaluation Description
Stand count	Approximately every 14 days	Number of emerged plants per plot
Growth stage monitoring	Approximately every 14 days	Average plant growth stage of emerged soybean plants using guidelines outlined in *Soybean Growth and Development* (Pedersen, 2004).
Vigor monitoring	Approximately every 14 days up to R1 growth stage	Vigor rating on a 1 – 9, where 1 = excellent vigor and 9 = poor vigor
Number of plants producing pods	R8 growth stage	Total number of plants in a plot which produced pods
Number of seed produced	R8 growth stage	Total number of seeds produced in a plot
Seed weight	R8 growth stage	Total weight (g) of all seeds produced in a plot

L.6. Data Assessment

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

L.7. Statistical Analysis

Analysis of variance was conducted according to a randomized complete block design using SAS® (2008). The level of significance was $\alpha = 0.05$. For each statistically analyzed characteristic, MON 87712 was compared to the conventional control A3525 at each site. There was no intention to analyze the data across all sites since the sites were selected for their varying attributes. The reference range was determined for each characteristic from the minimum and maximum mean values of the references.

L.8. Individual-Site Persistence Outside of Cultivation Results and Discussion

Plant growth stage data were categorical and not statistically analyzed. However, there were no instances at any of the sites where the range of growth stages of MON 87712 and the conventional control A3525 did not overlap (Table L-4).

No statistically significant differences were observed between MON 87712 and the conventional control A3525 at the MOFI, MOCB, and NEYO sites for stand count at any data collection time during the 2009 growing season. At the INSH site, MON 87712 had a greater stand count than the conventional control A3525 at the second observation (82.0 vs. 28.3 plants/plot). Although the mean values for stand count for MON 87712 and the conventional control A3525 at the INSH site were outside the reference range (29-81 plants/plot), the difference was not observed in the subsequent observation (1.3 vs. 3.3 plants/plot) at the INSH site, at any of the six other observation times at the INSH site, or at any of the observations at the other sites (Table L-5).

No statistically significant differences were observed between MON 87712 and the conventional control A3525 at the INSH, MOFI, and NEYO sites for plant vigor at any data collection time during the 2009 growing season (Table L-6). At the MOCB site, MON 87712 had slightly decreased plant vigor than the conventional control A3525 at the sixth observation (5.0 vs. 4.7 rating). The mean value for plant vigor for MON 87712 at the MOCB site was within the range of the references (4.0-5.7 rating) and the difference was not observed at the other observation times at the MOCB site or at any observations at the other sites (Table VII-8).

® SAS is a registered trademark of the SAS Institute.

No statistically significant differences were observed between MON 87712 and the conventional control A3525 at the end of the 2009 season for number of plants producing pods per plot, number of seeds produced per plot, or weight of seeds produced per plot (Table VII-8).

At the INSH, MOFI, and NEYO sites, MON 87712 and the conventional control A3525 produced no seed per plot (Table VII-8). This resulted in replacement values of zero at these sites. Therefore, the experiment was completed at these sites upon harvest in 2009. The replacement values of 2.72 for MON 87712 and 2.63 for the conventional control A3525 at the MOCB site in 2009 indicated that the plots produced more seed than was planted. However, no plants emerged in any of the plots during the second season at the MOCB site and thus, the replacement value was zero. This indicates that the populations were all in decline, and did not increase over time in the unmanaged conditions.

Table L-5. Growth Stage Monitoring of MON 87712, the Conventional Control A3525, and the Commercial Reference Varieties in 2009

		Assessment Date and Range of Growth Stages Observed[2]									
Site[1]	Material	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9	Obs. 10
INSH		6-24-09	7-8-09	7-21-09	8-12-09	8-24-09	9-11-09	9-28-09	10-14-09	–	–
	MON 87712	V1	V2	V3	V2	V1-R3	R3	R5-R6	R7	–	–
	A3525	V1	V2	V3	V2	R1-R3	R3-R4	R6	R7	–	–
	References	V1	V2	V3	V2	V2-R3	V1-R3	R6	R7	–	–
MOFI		6-26-09	7-10-09	7-27-09	8-8-09	8-21-09	9-4-09	9-19-09	10-5-09	10-21-09	11-5-09
	MON 87712	V1	V2-V3	V2-V4	R1	R3-R4	R5	R7	R7-R8	R8	R8
	A3525	V1	V2	V2-V3	R1	R3	R5	R7	R8	R8	R8
	References	V1	V2-V3	V2-V4	R1-R2	R3-R4	R5-R6	R7-R8	R7-R8	R8	R8
MOCB		7-7-09	7-21-09	8-4-09	8-19-09	8-31-09	9-16-09	9-28-09	10-13-09	10-29-09	–
	MON 87712	VC	V2	V4	R3-R4	R5	R5	R6	R7	R8	–
	A3525	VC	V2	V4	R4	R5	R5	R6	R7	R8	–
	References	VC	V2	V4-R1	R4	R5	R5	R6-R7	R7	R8	–
NEYO		6-18-09	7-1-09	7-17-09	–	–	–	–	–	–	–
	MON 87712	VC	V2	V3	–	–	–	–	–	–	–
	A3525	VC	V2	V3	–	–	–	–	–	–	–
	References	VC	V2	V3	–	–	–	–	–	–	–

Obs. = Observation number

— Dashes indicate information not provided due to no plants remaining in the field.

[1] Sites: INSH = Boone County, IN; MOCB = Shelby County, MO; MOFI = Butler County, MO; and NEYO = York County, NE

[2] Month-day-year.

Table L-6. Stand Counts of MON 87712, the Conventional Control A3525, and the Commercial Reference Varieties in 2009

Site[1]	Material	Means and Ranges of Stand Counts Observed									
		Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9	Obs. 10
INSH											
	MON 87712	124.0	82.0*	1.3	4.7	0.3	0.7	0.7	0.7	–	–
	A3525	99.0	28.3	3.3	3.7	1.0	0.7	0.3	0.3	–	–
	References	89-140	29-81	2-9	2-9	0-2	0-1	0-1	0-1	–	–
MOFI											
	MON 87712	187.3	121.0	92.7	67.3	62.7	62.3	14.0	12.7	11.3	10.7
	A3525	170.3	97.0	45.3	36.0	27.3	22.7	7.0	6.0	5.3	5.3
	References	151-243	93-207	50-179	45-118	36-84	32-70	10-23	10-20	9-20	8-21
MOCB											
	MON 87712	330.0	342.3	328.3	321.3	327.0	312.3	296.3	278.3	246.7	–
	A3525	341.3	345.7	340.3	335.3	332.7	321.0	295.7	285.3	266.3	–
	References	221-331	250-344	241-345	261-323	253-342	249-339	219-339	211-330	216-335	–
NEYO											
	MON 87712	193.7	110.3	11.0	–	–	–	–	–	–	–
	A3525	188.0	103.3	51.3	–	–	–	–	–	–	–
	References	140-200	44-139	3-77	–	–	–	–	–	–	–

Note: The experimental design at each site was a randomized complete block with three replications. Values for MON 87712 and the conventional control A3525 are means. References are a range of the minimum and maximum mean values of the four commercial reference varieties at each site.

— Dashes indicate information not provided due to no plants remaining in the field.

* Indicates a statistically significant difference between MON 87712 and the control ($\alpha = 0.05$).

[1] Sites: INSH = Boone County, IN; MOCB = Shelby County, MO; MOFI = Butler County, MO; and NEYO = York County, NE

Table L-7. Plant Vigor Monitoring of MON 87712, the Conventional Control A3525, and the Commercial Reference Varieties in 2009

		Means and Ranges of Vigor Ratings Observed[2]									
Site[1]	Material	Obs. 1	Obs. 2	Obs. 3	Obs. 4	Obs. 5	Obs. 6	Obs. 7	Obs. 8	Obs. 9	Obs. 10
INSH											
	MON 87712	2.0	2.0	9.0	9.0†	3.0	–	–	–	–	–
	A3525	2.0	2.0	8.0	9.0	3.0	–	–	–	–	–
	References	1.3-2.0	2.0-2.7	7.3-8.7	9.0-9.0	0.0-6.0	–	–	–	–	–
MOFI											
	MON 87712	5.0	6.7	7.7	8.7	–	–	–	–	–	–
	A3525	5.3	7.3	8.3	9.0	–	–	–	–	–	–
	References	3.7-5.7	4.7-7.0	5.7-7.7	8.0-9.0	–	–	–	–	–	–
MOCB											
	MON 87712	2.0	3.0	NA	2.0	4.7	5.0*	5.0	3.3	1.7	–
	A3525	2.7	3.0	NA	1.7	3.7	4.7	5.7	3.0	1.7	–
	References	2.0-4.0	3.0-4.0	NA	1.3-2.7	4.3-5.7	4.0-5.7	5.3-7.0	2.7-3.3	1.7-2.3	–
NEYO											
	MON 87712	5.0	8.0	8.7	–	–	–	–	–	–	–
	A3525	5.3	7.7	8.3	–	–	–	–	–	–	–
	References	5.0-5.7	7.0-9.0	7.7-9.0	–	–	–	–	–	–	–

Note: The experimental design at each site was a randomized complete block with three replications. Values for MON 87712 and the conventional control A3525 are means. References are a range of the minimum and maximum mean values of the four commercial reference varieties at each site.

[1] Sites: INSH = Boone County, IN; MOCB = Shelby County, MO; MOFI = Butler County, MO; and NEYO = York County, NE

[2] Vigor was rated on a 1-9 scale, where 1 = excellent vigor and 9 = poor vigor. Plant vigor was not required after the plants reached the R1 growth stage.

— Dashes indicate information not provided due to no plants remaining in the field or data was not required. NA = not available.

* Indicates a statistically significant difference between MON 87712 and the control ($\alpha = 0.05$).

† No statistical comparisons were made due to lack of variability in the data.

Table L-8. Phenotypic Comparison of MON 87712 to the Control within Each Site in 2009

	Number of plants producing pods per plot			
	Means (SE)		Reference Range[2]	
Site[1]	MON 87712	Control	Minimum	Maximum
INSH	0.7 (0.7)	0.3 (0.3)	0.0	1.0
MOFI	0.0 (0.0)	0.3 (0.3)	0.3	1.0
MOCB	136.0 (60.0)	140.7 (40.2)	124.3	201.7
NEYO	0.0 (0.0)†	0.0 (0.0)	0.0	0.0

Note: The experimental design at each site was a randomized complete block with three replications. The number of values used in the calculation of the means (n)=3. SE = standard error.
† No statistical comparisons were made due to lack of variability in the data.
[1] Sites: INSH = Boone County, IN; MOCB = Shelby County, MO; MOFI = Butler County, MO; and NEYO = York County, NE
[2] Reference range = Minimum and maximum mean values among the commercial reference varieties.

References for Appendix L

Pedersen, P. 2004. Soybean growth and development. Iowa State University, Ames, Iowa.

SAS. 2008. SAS/STAT software version 9.2. SAS Institute, Inc., Cary, North Carolina.

Appendix M: Petitioner's Environmental Report

Summary

Monsanto has developed the biotechnology derived soybean line MON 87712 that will be used in traditional breeding programs to produce varieties with increased yield opportunity compared to conventional, non-biotechnology-derived soybeans of the same genetic background. Crop yield is a variable and complex parameter that depends on genetic as well as environmental factors. In field trials using MON 87712, yield increases of 7 to 11 percent were measured compared to control soybean grown in the same trials. The yield increases are achieved using a gene from the plant *Arabadopsis thaliana* that produces a protein that impacts the plant's day/night processes. Research data indicate this change in day/night processes results in increased availability of carbon and nitrogen assimilates in the plant leading to higher yield.

This environmental report (ER), which has been prepared to support APHIS' obligations under the National Environmental Policy Act (NEPA), evaluates two alternatives: the "deregulation in whole" alternative and the no action alternative. Under the deregulation in whole alternative, MON 87712 would no longer be a regulated article under 7 CFR Part 340 and would be widely available for planting. Under the no action alternative MON 87712 would remain a regulated article.

Affected Environment

U.S. Soybean Production:

In 2009, soybean represented 59% of world oilseed production, and approximately 35% of those soybeans (valued at $33.6 billion) were produced in the U.S. on 77.5 million acres throughout the eastern half of the U.S., with a concentration in the Midwest. During 2009, the U.S. exported 40.9 million metric tons (1.50 billion bushels) of harvested soybean seed, which accounted for 44% of the world's soybean exports. Most of the soybean supply (85%) is crushed to produce meal and oil, which is used to supply either the feed industry for livestock use or the food industry for edible vegetable oil and soybean protein isolates. Soybean is also used on a limited basis as forage for animal feed.

Soybean Growth, Varieties, Yield and Land Use:

Every year growers can choose from among hundreds of soybean varieties with different characteristics such as maturity group and subgroup, disease resistance, insecticide seed treatment, and herbicide tolerance.

Soybean growth stages are important to growers because different growth stages are critical for certain agronomic practices (e.g., weed control, application of fungicide/insecticide), and certain other stages are critical for yield determination. The time of onset and the duration of the various growth stages in soybean are highly dependent on day length, which has led to different varieties being developed for different latitudes. In the U.S., soybean varieties are classified into one of ten major

"maturity groups", which are designated by 10 north-south bands from North Dakota to Florida.

Soybean varieties are tested in performance trials by public and private institutions to assess yield and other desirable characteristics. Breeders have historically used desirable traits such as yield for crop domestication and improvement by retaining plants that exhibit desirable traits for future breeding. In recent years molecular breeding techniques have been used to accelerate this process. As a result of conventional breeding combined with improved agronomic practices, soybean yields have steadily increased over the years, at a rate of approximately 0.35 bushels/acre since 1924, to the current U.S. average yield of 44 bushels per acre. While acres planted to some crops such as soybeans have increased over the years, the total acre of crops planted to cropland is the same as it was 100 years ago. Some crops such as soybean have increased in acreage, and others, such as wheat, barley and sorghum have decreased in acreage.

Seed Production:

Because seeds from a given maturity group are produced within the general geographic boundaries of that maturity group, seed production occurs throughout the soybean growing area. While field operations and management practices for seed production are similar to those for commercial soybeans, extra care must be taken to ensure high purity and practices that ensure high germination rates of the harvested seed. Not all commercial seed is certified, but most commercial seed meets certification standards.

Organic Soybean Production:

In the 2008 Census of Agriculture, the most recent data source available, USDA reported that organic soybeans were harvested on 98,199 acres in 28 states, with a total production of 2.58 million bushels, representing 0.09% of the total U.S. soybean production for 2008. U.S. organic soybean production peaked in 2001, then declined as a result of competition from overseas. A 2006 study found that the price premiums organic farmers receive make up for higher production costs and lower yields compared to nonorganic soybeans.

Agricultural Practices:

Variety selection is critical to optimize yields and growers select varieties of the correct maturity group that yield well and are adapted to the length of the growing season in their geographical location. Tillage and no-till or con-till methods may be used for production of soybean. Soybean requires 16 essential elements for growth and development. The primary or major essential elements are nitrogen, potassium and phosphorous. Nitrogen is seldom applied to soybean fields because soybean is a member of the legume family and fixes its own nitrogen. Potassium and phosphorous are also seldom applied because sufficient amounts of these two elements remain in the soil from the previous season's application to well fertilized crops like corn and wheat which are typically rotated with soybean.

Within the U.S., 95% of the soybean-planted acreage has been in some form of a crop rotation system since 1991, with the majority being rotated to corn. Rotation has a

number of benefits, including increased yields, decreased fertilizer needs, and reductions in disease and weed losses.

Insect damage in soybeans usually does not reach levels that cause economic loss in the U.S.. Major diseases resulting in yield loss include, in order of economic importance, soybean cyst nematode (*Heterodera gylcines*), Phytophthora root and stem rot (*Phytophthora sojae*), seedling diseases, charcoal rot (*Macrophomina phaseolina*) sudden death syndrome (*Fusarium virguliforme*), and sclerotinia stem rot (*Sclerotinia sclerotiorum)*. Soybean cyst nematode causes by far the greatest soybean yield losses. Selecting resistant varieties and crop rotation are the primary tools growers have for disease control.

When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75%. Cultural (e.g. crop rotation, narrow row spacing and planting date) and mechanical weed control practices (tillage) can be important components of an effective weed management program. Nearly all soybean fields receive some type of herbicide treatment and approximately 90% of soybeans planted are genetically modified for herbicide tolerance.

Human and Animal Health:

Both humans and animals have consumed soybean in various forms for thousands of years. Soybean improved with new traits produced by biotechnology pose no unique risks relative to other soybean and have been extensively evaluated for their safety prior to introduction.

Animal and Plants:

The affected environment for growing soybean plants can generally be considered the agroecosystem (managed agricultural fields) plus some area extending beyond the intended plantings that might be affected by agricultural operations. Mammals and birds may seasonally consume seed, and invertebrates can feed on the plant during the entire growing season. Plants growing in this adjacent area can be affected by fertilizer runoff, water runoff and/or herbicide drift.

Physical Environment:

Surface water may be impacted from soybean production, primarily by runoff of soil particles from soybean fields, but also by herbicides or other pesticides that may make it to streams, rivers, lakes, wetlands and other water bodies either through runoff or drift. Groundwater impacts may be of potential concern in some areas where nitrogen levels are either approaching or have exceeded the maximum contaminant level; however, soybean generally does not require addition of nitrogen fertilizer.

Adjacent Agricultural Crops and Non-Agricultural Plants:

Soybean is primarily grown adjacent to other large acre crops such as corn, wheat, and alfalfa. Soybean is highly self-pollinating and exhibits a very low level of outcrossing.

Soybean does not have any related wild relatives in the U.S. with which it can hybridize. Soybean is not found outside of cultivation and is not invasive or weedy.

Environmental consequences

Potential environmental impacts of the no action and deregulation in whole alternatives are summarized below:

Attribute/Measure	No Action	Deregulation in Whole
Commercial Production and Use	No change to the affected environment. MON 87712 not available for wide scale production, other biotechnology-derived soybean would be available.	No change except for the potential for higher yields. Composition comparable to conventional control; widespread use of biotechnology-derived soybean.
Economic	No change to the affected environment. Higher yielding soybean varieties continue to be introduced, providing economic benefit.	Higher yielding soybean varieties more readily available than if developed using conventional breeding programs.
Land Use	No change to the affected environment. Soybean will continue to be grown on land devoted to crop production – acreage driven by soybean demand.	Not different from no action alternative. Soybean acres could potentially decrease due to higher soybean yields - acreage driven by soybean demand.
Seed Production	No change to the affected environment. Current production practices in place to handle specialty, organic and seed production for soybean would continue.	Not different from no action alternative – similar production practices would be used to produce MON 87712 seed.
Organic Soybean Production	No change to the affected environment. Biotechnology-derived soybean has been on the market for over 14 years and co-existence practices are established – these practices will continue.	Not different from no action alternative – MON 87712 will utilize similar production practices already established for commodity biotechnology-derived soybean.
Cropping Practices (tillage, irrigation)	No change to the affected environment. Growers will continue to use cropping	Not different from no action alternative - No change in cropping practices expected based on

Attribute/Measure	No Action	Deregulation in Whole
	practices and variety selection to maximize yield and return.	observations of MON 87712 during confined release field trials.
Insect & Disease Management	No change to the affected environment. Insect & disease management practices will continue to be driven by pest and disease pressure.	Not different from no action alternative - MON 87712 shows no change in insect & disease susceptibility.
Variety Development	No change to the affected environment. Current breeding practices and variety selection continue.	Not different from the no action alternative. Slight delay in physiological maturity will be compensated for during new variety development and selection process.
Weed Management	No change to the affected environment. Current weed management practices expected to continue.	Not different from no action alternative – MON 87712 will likely be bred with herbicide tolerant soybean and weed control practices used with herbicide tolerant soybean will be used.
Human Health and Worker Safety	No change to the affected environment. MON 87712 not available for wide scale production and soybean currently on the market are consumed.	Not different from no action alternative. Soybean produced by MON 87712 are compositionally comparable to conventional soybean and the BBX32 protein is safe for consumption, therefore, MON 87712 will not impact human health and worker safety.
Plant & Animal Communities; TES	No change to the affected environment. MON 87712 would be grown in isolation on small acreages. Higher yielding soybean will continue to be introduced through conventional breeding..	Not different from no action alternative. MON 87712 would potentially increase yield in soybean varieties. The increase in yield associated with MON 87712 is unlikely to impact plants, animals or TES.
Soil Microbes	No change to the affected environment. Higher yielding soybean will continue to be introduced.	Not different from no action alternative. Exposure to BBX32 protein not expected to change soil microbial populations.

Attribute/Measure	No Action	Deregulation in Whole
Non-crop & Non-Agricultural Areas	No change to the affected environment. High yielding soybean would continue to be introduced	Not different from no action alternative - MON 87712 has no change in invasive characteristics from conventional soybean.
Cumulative Impact Due to Breeding with Previously Deregulated Events Stacking	No change to the affected environment. MON 87712 not available for stacking with previously deregulated events under this alternative.	MON 87712 available for breeding with previously deregulated events and potentially events under review. The stability of MON 87712 over multiple generations has been demonstrated. No cumulative impacts are expected.

M.1. Introduction

This environmental report (ER) has been prepared for APHIS to facilitate the agency's compliance with the National Environmental Policy Act (NEPA), including compliance with the Council on Environmental Quality's (CEQ) regulations that implement NEPA.[8]

M.1.1. Background and Rationale

Monsanto Company has developed the biotechnology-derived soybean line MON 87712, which will be used in traditional breeding programs to produce commercial varieties with increased yield opportunity. The yield increase in MON 87712 is achieved using the *BBX32* gene from the plant *Arabidopsis thaliana* that produces a protein that interacts with one or more endogenous transcription factors to regulate the plant's day/night processes and results in increased availability of assimilates (products of plant metabolism) in MON 87712 compared to an appropriate comparator without this gene. Increased plant nutrient availability in MON 87712 is supported by the measurement of factors indicative of an extended period of photosynthetic activity in MON 87712 and evidence of changes in diurnal metabolism during the reproductive phase of the soybean plant, as well as by the significantly higher yield of MON 87712 when compared to control, as observed in multisite field studies in the U.S. Higher yielding soybeans offer the opportunity for benefits to growers and the soybean food and feed chain, and help meet global demand for soybean.

M.1.2. Purpose and Need for Action

M.1.2.1 Regulatory Authority

"Protecting American agriculture" is the basic charge of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. In 1986, the Federal Government's Office of Science and Technology Policy (OSTP) published a policy document known as the Coordinated Framework for the Regulation of Biotechnology. This document specifies three Federal agencies that are responsible for regulating agricultural biotechnology in the U.S.: USDA APHIS, the U.S. Department of Health and Human Services' Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). APHIS regulates biotechnology-derived organisms under the Plant Protection Act of 2000. FDA regulates food and feed derived from biotechnology-derived organisms under the authority of the Federal Food, Drug, and Cosmetic Act. The FDA policy statement concerning regulation of products derived from new plant varieties, including those biotechnology-derived, was published in the Federal Register on May 29, 1992.[9] Under this policy, FDA uses a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of food.

---

[8] Title 40 of the Code of Federal Regulations (40 CFR) Parts 159901508.
[9] 57 FR 22984- 23005

M.1.2.2. USDA Regulation of Biotechnology-derived Organisms

The APHIS Biotechnology Regulatory Service's (BRS) mission is to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of biotechnology-derived organisms. APHIS regulations,[10] which were promulgated pursuant to authority granted by the Plant Protection Act, as amended,[11] regulate the introduction (importation, interstate movement, or release into the environment) of certain biotechnology-derived organisms and products. A biotechnology-derived organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR 340.2) and is also considered a plant pest. A biotechnology-derived organism may also be regulated under Part 340 when APHIS has reason to believe that the biotechnology-derived organism may be a plant pest or APHIS does not have sufficient information to determine if the biotechnology-derived organism is unlikely to pose a plant pest risk.

Under 7 CFR 340.6 entitled "Petition for Determination of Nonregulated Status", A person may petition the agency to evaluate submitted data and determine that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, should no longer be regulated. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A biotechnology-derived organism is no longer subject to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk.

M.1.2.3. Petition for Determination of Nonregulated Status: MON 87712

Monsanto has submitted a petition to APHIS seeking a determination of non-regulated status for MON 87712 (petition; #11-SY-271U). As detailed in the petition, MON 87712 contains a gene, *BBX32*, encoding for the production of a transcriptional accessory protein that modulates existing diurnally regulated processes resulting in increased incorporation of nutrients into the plant. Carbon and nitrogen metabolism is a diurnally regulated process; altering a plant's response to the day/night cycle can thus affect carbon and nitrogen metabolism. Alteration of a plant's reproductive responses to day/night length is a known mechanism for increasing yield components (Kantolic and Slafer, 2005). The BBX32 activity shifts the diurnally regulated gene transcription, thereby influencing the plant's day/night cycle. This modulation of the diurnally regulated carbon and nitrogen metabolism leads to increased availability of assimilates and hence increased yield. Interstate movements and field trials of MON 87712 have been conducted under permits issued or notifications acknowledged by APHIS since 2006.

The petition includes information that has been collected from field trials, laboratory and greenhouse studies, and published literature to assess whether the increase in yield through production of the BBX32 protein and/or the gene insertion process has altered

[10] 7 CFR § 340
[11] Title IV Pub. L. 106-224, 114 Stat. 438, Title 7 of the U.S. Code (7 USC) § 7701-7772[font]

MON 87712 in any way that would make these plants more of a plant pest compared to conventional soybeans, or cause significant environmental impacts, including cumulative impacts.

M.1.2.4. APHIS Action

Under the authority of 7 CFR part 340, APHIS has the responsibility for the safe development and use of biotechnology-derived organisms under the provisions of the Plant Protection Act. APHIS must respond to petitioners that request a determination of the nonregulated status of biotechnology-derived organisms, including biotechnology-derived crop plants such as MON 87712. If a petition for nonregulated status is submitted, APHIS must make a determination if the biotechnology-derived organism is not likely to pose a plant pest risk.

MON 87712 has been field tested in the U.S. since 2006 in APHIS authorized trials. Associated notifications acknowledged by APHIS are listed in the petition in Appendix A. Field tests conducted under APHIS oversight allow for evaluation in agricultural settings under confinement measures designed to minimize the likelihood of persistence in the environment after completion of the field trial. Under confined field trial conditions, data are gathered on multiple parameters and used by applicants to evaluate agronomic characteristics and product performance. These data are also valuable to APHIS for assessing the potential for a biotechnology-derived plant to pose a plant pest risk.

As a Federal agency subject to compliance with the National Environmental Policy Act (NEPA),[12] APHIS must consider the potential environmental effects of its actions/decisions and reasonable alternatives to those actions, consistent with NEPA regulations[13] and the USDA and APHIS NEPA implementing regulations and procedures.[14] This environmental report (ER) evaluates the potential impacts that may result from deregulation of MON 87712 and has been prepared to support APHIS' compliance with NEPA.

M.1.2.5. Submissions to Other Regulatory Agencies

In accordance with FDA's consultation policy (discussed in Appendix M.1.2.1), Monsanto will submit a food and feed safety assessment and nutritional assessment summary for MON 87712. The EPA regulates plant-incorporated protectants (PIPs) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). MON 87712 is not a PIP and does not have pesticidal activity and therefore is not within the scope of EPA regulations for pesticides. In addition, the MON 87712 trait does not provide herbicide tolerance or otherwise impact herbicide use. No submissions to EPA are required.

---

[12] 42 USC §4321 *et seq.*
[13] 40 CFR Parts 1500-1508
[14] 7 CFR 1b and 7 CFR Part 372[font]

To support commercial introduction of MON 87712 in the U.S., regulatory submissions will be made to countries that will eventually commercialize or import significant quantities of soybean and/or soybean products from the U.S. These will include submissions to a number of foreign government regulatory authorities, including: Ministry of Agriculture, People's Republic of China; Japan's Ministry of Agriculture, Forestry, and Fisheries, Ministry of Environment, and the Ministry of Health, Labor, and Welfare; the Canadian Food Inspection Agency and Health Canada; the Intersectoral Commission for Biosafety of Genetically Modified Organisms, Mexico; the European Food Safety Authority, as well as to regulatory authorities in other soybean importing countries with functioning regulatory systems. As appropriate, notifications of importation will be made to importing countries that do not have a formal approval process.

M.2. Affected Environment

This section describes the environment in which soybeans are currently grown and utilized in the U.S.

M.2.1. Commercial Soybean Production and Use

Commercial soybean production and uses are discussed in Section VIII.B of the petition and summarized here; refer to the petition for more detail.

Soybean was a relatively minor crop used mostly for forage in the U.S. from the late 1700s to the 1920s and 1930s. When breeders developed cultivars with shatter-resistant seeds that allowed for use of mechanical harvesting equipment, acreage expanded rapidly, major investments were made in breeding in the 1930s, and today soybean is the world's most important oilseed crop.

Soybean is grown as a commercial crop in over 35 countries and is one of the most valued agricultural commodities because of its high protein and oil content. In 2009, soybean represented 59% of world oilseed production, and approximately 35% of those soybean were produced in the U.S. (ASA, 2008). In 2009, the U.S. exported 40.9 million metric tons (1.50 billion bushels) of soybean, which accounted for 44% of the world's export of whole soybean seeds (USDA FAS, 2011). Total U.S. soybean exports (including soybean meal and oil) were valued at $22.1 billion in fiscal year 2010 (USDA, 2011). USDA world projections through 2020 show world soybean production and exports increasing greatly, but with the U.S. share slightly declining due to greatly increased production in Brazil (USDA, 2011).

Currently, approximately 85% of the world's harvested soybean supply is crushed to produce soybean meal and oil (Soyatech, 2010), and the majority was used to supply the feed industry for livestock use or the food industry for edible vegetable oil and soybean protein isolates.

The U.S. soybean acreage in the past 10 years has varied from approximately 64.7 to 77.5 million acres. Average soybean yields have varied from 33.9 to 44.0 bushels per acre over this same time period. According to data from USDA NASS (2011), soybean was

planted on approximately 77.5 million acres in the U.S. in 2009, producing 3.36 billion bushels of soybean with a value of $32.1 billion (USDA NASS, 2011).

In the U.S., soybean production occurs throughout much of the eastern half of the U.S. and is concentrated in the Midwest (Table M-1). Table M-1 shows average state production by region in 2010.

Table M-1. 2010 Soybean Productivity by Region

Region	2010 U.S. Soybean Acreage[1]	2010 Average Yield (bushels per acre)	Range of Average State Yields (bushels per acre)
Midwest/Great Plains	64.2	43.9	32.5 – 52.5
Southeast	10.3	31.0	23.0 – 41.0
Eastern Coastal	2.1	34.0	26.0 – 48.0

[1]U.S. soybean acreage – million acres
Source USDA NASS (2011)

M.2.1.1. Soybean Growth Stages

Soybean growth stages are important to growers because different growth stages are critical for certain agronomic practices (e.g., weed control, application of fungicide/insecticide), and certain other stages are critical for yield determination (Koger 2011, Pederson 2004, McWilliams et al., 2004). Growth stages are designated by two characters, beginning with either "V" for vegetative or "R" for reproductive. Some of the vegetative stage designations are VE for emergence, and V1 through Vn for the appearance of the sets of three-part (trifoliate) leaves. There are eight designated reproduction stages, for example, R1 for the appearance of the first flower, R3 for beginning of pod development, R5 for beginning of seed development, and R8 for full maturity (Pederson, 2004; Heatherly and Elmore, 2004).

The time of onset and the duration of the various growth stages in soybean are highly dependent on photoperiod (hours of daylight and darkness) and temperature (Major et al., 1975), and therefore, for the same soybean plant grown at different latitudes, the onset and duration of the growth stages and the total time from planting to maturity would be different. Also, in contrast to most other temperate-season crops, soybean is a "short-day" plant, meaning that maturity is delayed by longer day length (Major et al., 1975). In soybeans, flowering is initiated only after the night is longer (and days grow shorter) than a critical length (Holshouser, 2010). Once flowering begins, temperature controls the duration of flowering time (Heatherly and Elmore, 2004).

M.2.1.2. Soybean Variety Development and Yield

Maturity Groups. Because soybean growth is so dependent on day/night length, different varieties are developed for different latitudes. In the U.S. ten geographically-designated "maturity groups" originally defined by Scott and Aldrich (1970) are widely used (Zhang et al., 2007). These maturity groups are mapped as bands extending from north to south, beginning with Group 00 at the far north and ending with Group VIII in the far south.[15] Groups II, III and IV, which extend from approximately the northern border of Iowa to the southern tip of Illinois, account for approximately 76% (24%, 36%, and 16%, respectively) of the soybean planted in the U.S. (T. Schlueter, personal communication, August 2008). Because day length delays maturity, a soybean cultivar suited to a southern maturity group would mature too late if planted too far north. Conversely, a northern cultivar would mature too early if planted in the south (Heatherly and Elmore 2004).

Maturity groups are often designated by Arabic rather than Roman numerals, so the sequence is 00 to 8, and there are subdivisions within the major maturity groups. These are designated by a decimal value. For example, a variety with maturity group designation 2.9 would be at the southern end of Group II.

Variety Development. Crop domestication and improvement through breeding has been largely achieved through selection of genes that regulate the expression of desirable traits, such as those associated with higher yields or disease resistance. Once plants with the desired traits have been selected, a population of those plants with similar characteristics are classified as varieties. Historically breeders have developed desirable varieties by retaining for further breeding those plants that possess the desirable traits, as determined by visual inspection or by testing. In recent years breeders have used the more direct methods of molecular breeding techniques, such as marker assisted breeding, to accelerate the process of identifying breeding lines containing a desired set of positive traits. These techniques rely on inventories of genomic regions or genetic markers that have been positively associated with the desirable traits. Once the genetic markers associated with the desired traits have been identified, molecular breeders can quickly select the offspring inheriting the genes for further development and testing in the field (Voosen, 2009).

Hundreds of soybean varieties are tested each year in performance trials (variety trials) conducted by universities and private companies in all the major soybean growing states. The following information can typically be obtained from the results of variety trials: maturity group, disease resistance, yield, maturity date, percent lodging (plants fallen over on the ground), height, and herbicide resistance (Tylka et al., 2010). In different parts of the country and/or in other trials, additional characteristics may be identified, such as iron deficiency tolerance or protein or oil content (Pederson, 2008a).

---

[15] They were originally designated north to south as Groups I through VIII. Groups 0 and 00 were later added to the north.

M.2.1.3. Yield Increases in U.S. Soybean Production

As a result of new varieties developed through selective breeding, improved fertilizer and pesticide applications, and improved management practices, soybean yields have steadily increased over the past century (Ash et al., 2006). Figure M-1 shows the average annual soybean yield for the U.S. from the mid-1920s through 2010. As shown in the figure, from the mid-1920s to 2010, the average annual yield in the U.S. increased from approximately 11 to 44 bushels per acre (bu/acre), or a rate of 0.35 bu/acre, equivalent to a yield increase of approximately 400%.

There have been many factors that have led to this astounding increase in soybean productivity. Specific major management decisions that positively impact yield for growers in recent years include variety selection, management of soybean cyst nematode, early planting, narrow rows, elimination of weed competition, keeping insect pressure below economic thresholds, and crop rotation (Pederson, 2010). Some university agronomists believe there is still substantial yield potential that can be realized through implementation of good management decisions (Pederson, 2011; Pederson, 2010), although others believe a yield plateau has been reached (Moore, 2009). Record maximum yields for soybean are over 160 bu/acre for irrigated soybean and nearly 100 bu/acre for non-irrigated soybean (Alsager 2010). USDA projections through 2020 show an average annual rate of increased yields of 0.33 bu/acre for the period 2010 to 2020, which results in an average U.S. yield of 47.6 bu/acre in 2020/21 (USDA, 2011). While USDA projects increasing yields, the projected rate of increase is lower than the past rate. Current and future factors that negatively affect yield increases are the expansion of soybean production into northern and western parts of the country, where yields are typically lower than in the core Midwestern production acre, and a shift in some areas away from narrow rows to improve air circulation, which helps combat disease (USDA, 2010).

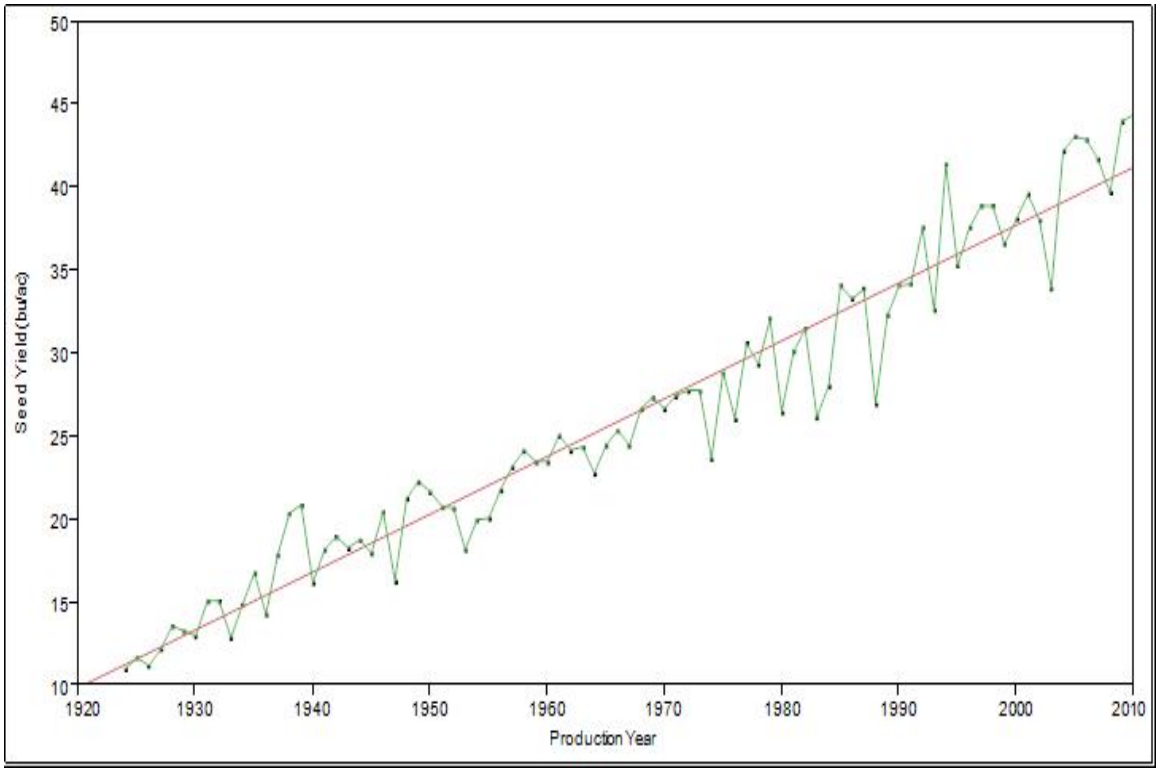


Figure M-1. Annual U.S. soybean yield 1924 – 2010.
Soybean yield rose at an annual average rate of 0.35 bu/A between 1924 – 2010. Linear regression analysis was conducted on data from the USDA National Agricultural Statistics Service (USDA-NASS, 2011, http://www.nass.usda.gov).

M.2.1.4. Regional and Temporal Yield Variations

As shown in Figure M-1, soybean yields can be highly variable from year to year. For example, between 1987 and 1988 (a drought year in the Midwest), average U.S. yields dropped over 20%, from 33.9 to 27.0 bu/acre; between 1993 and 1994, average yields increased 27%, from 32.6 to 41.4 bu/acre. Yields also vary geographically. For example, in 2010, the average yield in Nebraska (55 bu/acre) was 139% higher than the average yield in South Carolina (23 bu/acre). Within a state there is considerable local variability, as, for example, in eastern Nebraska, where the 2010 yields for Lancaster and Hamilton counties were 43.6 bu/acre and 64.2 bu/acre, respectively (USDA NASS, 2011). For another example, in two adjacent counties in Iowa, Hardin and Wright, in 2010 the average yield in Hardin County (55.3 bu/ac) was 33% greater than the 2010 yield in Wright County (41.7 bu/acre). In 2009, on the other hand, the average yield in Wright County (52 bu/acre) was 13% greater than the average yield in Hardin County (46 bu/acre). Within each county between 2009 and 2010, the average yield in Hardin increased by 20% and the average yield in Wright decreased by 20% (USDA NASS, 2011). Since these values represent averages over counties, variation from farm to farm and within a farm from year to year would be expected to be even greater. The current world record for yield is 160.6 bushels per acre in 2010, on a farm in Missouri, where the statewide average yield in 2010 was 42 bushels per acre (Alsager 2010, 2010). Thus,

even with the positive management decisions that were previously discussed, observed yield is highly variable, is dependent on many factors and likely rarely reaches the theoretical maximum yield potential.

Growers are accustomed to this regional and temporal variability in yield, influenced by variable environmental conditions and the genetics of the varieties they select for planting. However, growers are also accustomed to the steady improvements of average soybean yield observed over past decades and generally continue to pursue varieties that further increase productivity and profitability on their farm. Within this context, U.S. farmers will understand the value of the trait MON 87712 that offers the opportunity to increase the yield of their soybean production.

M.2.1.5. Crop Storage and Transportation

Once soybean are harvested from a field, they may be stored directly on farm, stored off the farm at another location, delivered to a grain elevator or directly to a soybean crusher. Grain elevators play an important role with their long term storage of the grain. Since processing facilities crush soybeans throughout the calendar year, soybeans used to supply these crush plants need to be stored year round.

A larger than normal crop can stress the storage and transportation system for the crop. Because of the very high variability in crop production, storage facilities are not always adequate. Soybeans and other grain must sometimes be stored in temporary structures or in other existing buildings if storage facilities are overloaded, and this may result in additional costs for constructing or renting temporary facilities and/or potential losses from exposure (Hellevang, 1998; Dorn, 2011). The same conditions can result when prices are low and growers want to hold on to their crops in the hopes of selling at higher prices (Maier and Wilcke, 1998). Soybeans can also compete with corn for available storage space; and while corn can be stored on the ground, soybeans rarely are (Hurburgh, 2005). University extension services provide practical guidelines for temporary storage of soybeans and other crops (Harner et al., 1998; Hellevang, 1998; Dorn, 2011; Maier and Wilke, 1998; Hurburgh, 2005).

Growers usually deliver their soybeans to the sale point using their own trucks. From the elevator or processing facility, the soybeans or oil and meal are shipped by rail, barge or truck. Approximately 24% of soybeans are transported by rail, although higher percentages of meal and soybean oil are transported by rail (STC, 2010). More than half of U.S. soybean exports are first shipped by barge on the Mississippi River (Ash et al., 2006). USDA reported in 2006 that recent record-large soybean harvests have tested the capacity of the U.S. bulk transportation system; however, no specifics were provided (Ash et al., 2006). Large crops can result in greater shipping competition and higher shipping costs, which translates to lower prices offered to growers (Ash et al., 2006).

M.2.1.6. Economics

In the short term, individual growers' decisions about whether to plant and which crop to plant are typically based on the relationship between operating costs and expected prices; i.e., on expected crop profitability (Ash et al., 2006). Managing input costs and managing the crop for yield are major components to the economics of producing a soybean crop. Growers' costs include both overhead costs and operating costs. Overhead costs are those that are not associated with a particular crop and/or that are present whether or not a crop is grown, such as the cost of land and the depreciation of equipment. Operating costs are those associated with growing a particular crop in a given year, such as seed and fertilizer. A producer's cost of growing a particular crop includes a proportional part of the overhead of his or her entire farming operation, plus all the operating costs associated with that crop.

Figure M-2 shows the average per acre net value of soybean production in the U.S. from 1975 to 2010, based on data compiled by the USDA Economic Research Service (ERS). The net value is the value of the soybeans produced less all costs of production, both the allocated overhead and the operating costs. For comparison, corn and wheat are also shown. USDA's data does not include crop subsidies. Overhead costs represent well over half the total costs (up to 69%), with the "opportunity cost of land (rental rate)" and the capital recovery cost of machinery and equipment representing the bulk of the overhead costs. The largest single operating cost is seed (USDA ERS, 2011). As the data show, farming is often not profitable when all costs, including land value costs, are included. For example, USDA reports that in 2004, 70 percent of soybean-producing farm operations were considered profitable, not considering government payments. The percent profitable rises to 76 when government payments are included (Ash et al, 2006).

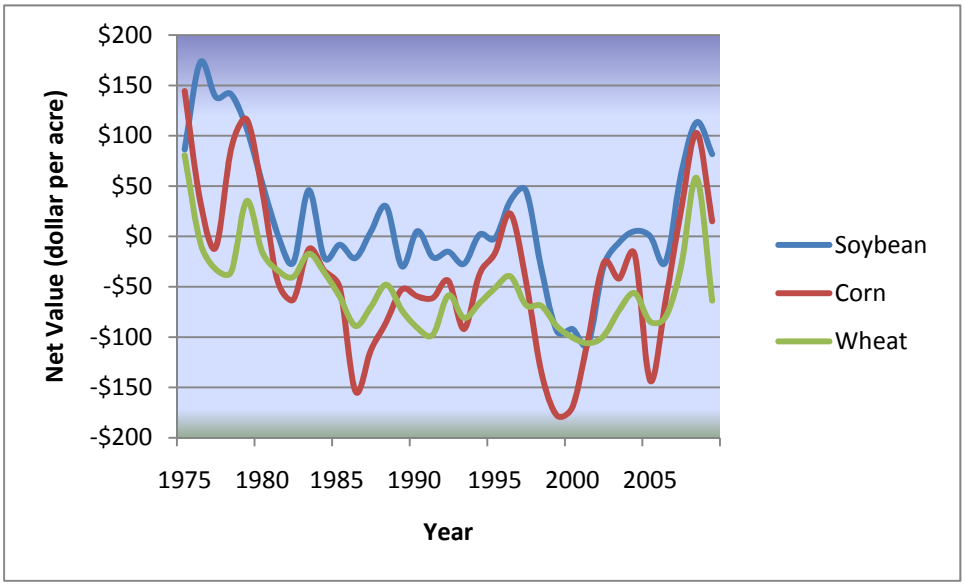


Figure M-2. U.S. Net Value of Soybean Production, Dollars Per Acre
Inflation Adjusted (to 2011) Values from 1975-2009, and does not include Government Subsidies
Sources: USDA ERS 2011, U.S. Bureau of Labor Statistics 2011

While an individual grower typically makes planting decisions based on the relationship between operating costs and expected prices (Ash et al., 2006), many factors influence both operating costs and expected prices. Government price supports can have a large effect on costs, and supply and demand governs prices. These are discussed in more detail in the next section.

M.2.1.7. Land Use

Land Used for Growing Soybeans. U.S. soybean planted acreage has increased since the 1930s, with the 10 million acre mark exceeded in 1940, 20 million acres exceeded in 1956, 30 million in 1964, 40 million in 1967, and 50 million in 1973. Planted soybean acreage has not dropped below 60 million since 1993 (USDA NASS, 2011). Soybean acreage rose rapidly from the end of World War II to the late 1970s, based on increased demand for vegetable oil and higher meat consumption (Ash et al., 2006). U.S. soybeans acres stagnated in the 1980s largely due to farm programs for other corps (Ash et al., 2006). In the 1990s, changes in farm programs, overseas demand, lower production costs associated with herbicide-tolerant crops, increased yields and increased rotations with corn resulted in increased acreage planted to soybeans (Ash et al., 2006).

USDA projections to 2020 show a 2.7% increase in acres planted to soybeans from 2009/10 (77.5 million acres) to 2020/21 (78.5 million acres), with a corresponding increase in production of approximately 11%, based on expected yield increases (USDA, 2011). The expected increase in production is substantially less than occurred in the previous 11 years (1999 to 2000), when U.S. soybean production increased by 25% (USDA NASS, 2011). Major factors affecting future U.S. soybean production include: competition with other crops, especially corn (reduces demand/production); consumer preference for other oils (reduces demand/production); soybean use for biodiesel (increases demand/production), government farm programs, both for soybeans and for other competitor crops (may result in increases or reductions); increases in overseas demand, especially in China (increases production); and competition from major overseas growers, especially in Brazil and Argentina (decreases U.S. demand/production) (USDA ERS, 2010).

During the same time period that soybean acreage has increased, wheat, barley and sorghum acreages have all greatly decreased. USDA attributes a shift to soybeans from wheat, the largest of these three crops, to stagnant yields in spring wheat and the development of better-yielding short season soybeans adapted to more northerly climates (USDA ERS, 2010). As shown in Figure M-2, wheat, the largest of these three crops, has been consistently less profitable than soybeans for many years.

Total U.S. Cropland. While the specific crops vary, and total acreage changes from year to year, total harvested cropland in the U.S. in recent times is about the same as it was 100 years ago (USDA ERS, 2007). In 2006, the most recent year for which data are available, the total agricultural land used for crop in the U.S. was 330 million acres - the same as it was the first year USDA began tracking, in 1910. Peaks occurred in the early 1930s, in 1949, and in the early 1980s, when the area of cropland used for crops reached over 380 million acres (USDA ERS, 2007).

The combined acreage planted to the two largest U.S. crops, corn and soybeans, was at an all-time high of 168.8 million acres in 2011 (USDA NASS, 2011). This was achieved through maximization of existing cropland, reduction of acreage sown to other grain crops and hay, and an increase in the rate of double-cropping (raising two crops in one year on the same land) (Ash, 2011).

M.2.2. Speciality Soybean Production

M.2.1. Certified Seed Production

Certified seed production is discussed in Section VIII.B.2 of the petition and summarized here. Standardized seed production practices are responsible for maintaining high-quality seed stocks, an essential basis for U.S. agriculture. The value of seed quality (including genetic purity, vigor, and minimizing presence of weed seed, seed-borne diseases, and inert materials, such as dirt) has been identified as a major factor in determining crop yields (Oplinger 1986, Pederson 2008a, USDA, 2000).

Soybean seed has four classes: 1) breeder, 2) foundation, 3) registered, and 4) certified (Association of Official Seed Certifying Agencies [AOSCA], 2009). Breeder seed is seed directly controlled by the originating or sponsoring plant breeding organization or firm. Foundation seed is first-generation seed increased from breeder seed and is handled to maintain purity and identity of a specific variety. Registered seed is the progeny of foundation seed that is handled to maintain satisfactory variety purity and identity. Certified seed is the progeny of breeder, foundation or registered seed, and is typically two generations from foundation seed. Not all soybean seed sold is officially certified; however, commercial soybean seed sold and planted for commodity soybean production typically meets or exceeds certified seed standards.

Seed certification programs were initiated in the early 1900s in the U.S. to preserve the genetic identity and variety purity of seed. The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and the establishment of official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Seed certification services are available through various state agencies affiliated with the AOSCA.

Soybean seed is produced throughout most of the U.S. soybean-growing regions by companies that produce and sell seed, and by toll seed producers, or tollers, which are companies that produce but do not sell certified seed. Seed companies and tollers in turn contract acreage with growers to produce the desired amount of soybean seed for particular varieties. Production or processing plants at these seed companies clean, condition, and bag the harvested soybean seed; verify the seeds meet all state and federal seed standards and labelling requirements; as well as monitor and inspect all the processes at the plant. Verification that standards are met includes carrying out certain tests such as germination tests.

The entire seed production process at the majority of the seed companies and tollers operates under standards established by the International Organization for Standardization (ISO), and includes internal and external audits (ISO, 2009). Field inspections are conducted on seed production fields throughout the soybean growing season to evaluate variety purity and ensure soybean plants are developing properly. Management practices in the field are designed with the intent of keeping the fields free of weeds, insects, and diseases. The seed production fields are also mapped to ensure the seed field has the minimum isolation requirement to prevent mechanical mixing of other soybean varieties (AOSCA, 2009). The American Seed Trade Association (ASTA), to which most major seed producers belong, also provides best management practices for seed production (ASTA, 2010).

The field operations and management practices for producing soybean seed are similar to commodity soybean production. However, special attention is needed in certain production practices to produce seed with low levels of weed seed and other foreign material and with high germination rates, and high genetic purity (Helsel and Minor, 1993).

M.2.2.2. Organic Soybean Production

U.S. Organic Soybean Production. Organic soybean acreage by state is reported by USDA AMS from 1997 through 2008 (USDA ERS, 2010). Acreage ranged from 82,143 in 1997 to a high of 174,467 in 2001. In the most recent year available, 2008, USDA ERS reported 125,621 acres of organic soybeans (USDA ERS, 2010); however, the Census of Agriculture reported 98,199 harvested acres of organic soybeans in 2008, yielding 2.58 million bushels, for an average yield of 26 bushels per acre and an average value of $19.45 per bushel (USDA NASS, 2010).

The decline in U.S. organic soybean production from the high in 2001 is likely a result of competition from lower-cost organic soybeans produced primarily in China. The Japanese market for U.S. organic soybeans was largely lost to China; and Chinese imports became more readily available in the U.S. after USDA streamlined the process for other countries to export certified organic products to the U.S. (Organic and Non-GMO Report, 2007).

In 2008, organic soybeans were produced in 28 states, with the largest acreage in Minnesota and Iowa. In 2010, Minnesota and Iowa were the first and fourth largest producers of soybean. The majority of organic soybeans are used for tofu and other soy products in the U.S. Some organic soybean are still exported to Japan and other countries, while other markets for organic soybeans include soybean oil and meal for livestock (ISU, 2003).

In a comparison of organic and non-organic soybean production in 2006, McBride and Greene found that the total economic costs of producing organic soybeans were $6.20 per bushel higher than non-organic, that organic yields were much lower than non-organic, and that the average price premium received for organic soybeans was $9.16 per bushel, which more than offset the added production costs (McBride and Greene, 2009). Other

researchers have found that organic soybean price premiums in 2009 ranged from 65 to 139% and averaged 107% (Clark and Alexander, 2010).

Organic producers must use organic seed if it is available. However, there are exceptions to this rule when comparable organic seed is not available and that exception can apply to a whole range of characteristics including disease resistance profile, quality aspects and fit to a local growing region (USDA AMS, 2011). Several companies produce organically certified soybean seed as well as conventional non-treated soybean seed that can be used by organic growers (Coulter et al., 2010). Many crop management practices are similar to those used by non-organic soybean growers, except that organic growers may not use synthetic fertilizers or pesticides. Because synthetic herbicides are not allowed, organic growers generally need to use more tillage to control weeds (Coulter et al., 2010; Kuepper, 2003).

The National Organic Program. Organic farming operations as described by the National Organic Program (NOP), which is administered by USDA's Agricultural Marketing Service (AMS), requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances (such as pesticides and synthetic fertilizers) or products of excluded methods from adjoining land that is not under an organic production management plan.[16] Excluded methods include a variety of methods used to genetically engineer organisms or influence their growth and development by means that are not possible under natural conditions or processes. The use of biotechnology such as that used to produce MON 87712 is an excluded method under the National Organic Program.[17]

On April 15, 2011, USDA AMS released a Policy Memorandum regarding "Clarification of Existing Regulations Regarding the Use of Genetically Modified Organisms in Organic Production and Handling" (USDA AMS, 2011). This memo was intended to answer questions that have been raised concerning biotechnology-derived crops and organic production and handling. The memorandum reiterates that organic operations must follow a set of production standards and practices which meet the requirements of the Organic Foods Production Act of 1990 and National Organic Program (NOP) regulations. NOP regulations prohibit the use of excluded methods (i.e., "GMOs") in organic operations. Therefore, the use of biotechnology-derived crops remains an excluded method but not a prohibited substance. Organic production operations must also develop and maintain an organic production system plan approved by an accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including avoidance of prohibited substances and the use of excluded methods.[18]

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight

---

[16] 7 CFR 205.202(c)
[17] 7 CFR § 205.2.
[18] 7 CFR Part 205.

includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards (USDA AMS, 2011). The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in an approved organic system plan. Organic certification indicates that organic production and handling processes have been followed, not that the product itself is "free" from any particular substance. As USDA AMS has recently re-iterated in a policy memorandum, organic certification is processed based. The NOP regulations do not allow the use of excluded methods such as biotechnology; however, the inadvertent presence of products of biotechnology "does not constitute a use because there was no intent on the part of the certified operator to use excluded methods" (USDA AMS, 2011).

Organic soybean producers use production practices designed to prevent commingling of their crop with neighboring crops treated with herbicides and other pesticides (spray drift), or that may be using plant varieties produced using excluded methods (pollen movement from biotechnology-derived crops). These well established practices include isolation zones, use of buffer rows surrounding the organic crop, adjusted planting dates, and varietal selection (Kuepper, 2006). The implementation of management practices to avoid pollen from a biotechnology-derived crop in organic or conventional soybean production operations is facilitated by the nature of soybean pollination. Soybean is a highly self-pollinated species and exhibits a very low level of outcrossing (see Section IX.D). Outcrossing is the genetic transmission of a defined heritable characteristic from one group of individuals (population, crop variety) to another. Outcrossing most commonly results from cross-pollination. Since soybean is highly self-pollinating, organic or conventional soybean producers can and have effectively implemented practices (*e.g.*, isolation during the growing season, equipment cleaning during harvest, and post-harvest separation of harvested seed) that allow them to reasonably avoid biotechnology-derived soybean and maintain organic or conventional production status.

M.2.3. Agronomic Practices for Soybeans

M.2.3.1. Production Management Considerations

Production management considerations are described in detail in Section VIII.D of the petition and are summarized here. Refer to Section VIII.D for more detail.

Pre-Season. Well in advance of planting a soybean crop, decisions are made regarding the planned crop rotation, the tillage system and row spacing that will be implemented, the planting equipment that will be used, the seed or variety that will be planted, and soil fertility management requirements. Soybean rotation practices are summarized below. Variety selection has been discussed previously. Soybean requires 16 essential elements for growth and development. Deficiencies in any of these elements can reduce yields

(Hoeft et al., 2000). The primary or major essential nutrients are nitrogen, phosphorus and potassium. The soybean plant is a member of the legume family, like alfalfa and clover, and fixes a significant portion of its own nitrogen through the symbiotic relationship with the nitrogen-fixing Bradyrhizobia bacteria (*Bradyrhizobium japonicum*) that live in the nodules on its roots. Nitrogen fertilizer applications at planting generally do not improve yield and decrease nodulation while increasing the plant's dependency on the soil for nitrogen (Pedersen, 2008d). Therefore, nitrogen fertilizer is seldom applied prior to planting a soybean crop. Soil tests are used to determine the pH, phosphorus, and potassium levels in the soil and assess whether any adjustments need to be made. In corn-soybean rotations in the Midwest, phosphorus and potassium fertilizers are applied prior to a corn crop in accordance with soil test recommendations, but are seldom applied prior to a soybean crop.

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, insect pests, and diseases on soybean yield is dependent on growth stage. The system of soybean growth stages divides plant development into vegetative (V) and reproductive (R) stages (Pedersen, 2004). The vegetative stages begin with VE, which designates emergence. V stages continue and are numbered according to how many fully developed trifoliate leaves are present (*i.e.*, V1, V2, etc.). The reproductive (R) stages begin at flowering (R1) and include pod development and plant maturation. Full maturity is designated as R8.

The time of onset and the duration of the various growth stages in soybean are highly dependent on photoperiod (hours of daylight and darkness) and temperature (Major et al., 1975), and therefore, for the same soybean plant grown at different latitudes, the onset and duration of the growth stages and the total time from planting to maturity would be different.

Good seedbed preparation, soil temperature, soil moisture and planting date impact yield. With planting date having the greatest impact. Highest yields are generally achieved when planting in early to mid-May. Row spacing influences yield because it impacts canopy development and research conducted in the Midwest shows that row spacing of less than 20 inches is optimum. Soybean has the ability to produce good yield over a wide range of plant populations. Most soybean varieties have the ability to branch and adjust the number of pods on branches to compensate for large differences in seeding rate. Maximum yields generally require planting rates that result in about 2.5 to 5 plants per square foot (Hoeft et al., 2000).

In order to maximize yields, weeds must be controlled during the early growth stages of soybean because weeds compete with soybean for water, nutrients, and light.

Mid to Late Season. Weather, day length and management of diseases and insects are the greatest factors impacting yield during the mid to late season stages. Ideal daytime temperatures for soybean growth are between 75ºF and 85ºF (Hoeft et al., 2000). 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. Good soil moisture is most critical during the pod-filling stages to prevent pod abortion and to ensure high yields (Hoeft et al., 2000). Another critical requirement during the seed-filling stages is a high rate of photosynthesis to maximize yield. High humidity and temperatures during seed development and maturity can result in poor seed quality because these conditions promote the development of reproductive-stage diseases.

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

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

M.2.3.2. Crop Rotation

The use of crop rotation in soybean production is discussed in Section VIII.G of the petition. The well-established farming practice of crop rotation is a key management tool for soybean growers. The purposes of growing soybean in rotation include, from Sandretto and Payne (2006) unless otherwise noted:

- improving yield and profitability of one or both crops over time;
- decreasing the need for nitrogen fertilizer on the crop following soybean;
- mitigating or breaking disease, insect, and weed cycles;
- improving soil tilth and soil physical properties;
- increasing residue cover;
- reducing soil erosion;
- increasing soil organic matter; and
- reducing runoff of nutrients, herbicides, and insecticides (Heatherly and Elmore, 2004; Al Kaisi et al., 2003).

According to the USDA Economic Research Service, 95% of the soybean-planted acreage has been in some form of a crop rotation system since 1991, and 5% of soybean-planted acreage is grown in continuous soybean (USDA ERS, 2005). Corn- and wheat-planted acreage has been rotated at a slightly lower level of 75% and 70%, respectively. Although the benefits of crop rotations can be substantial, the grower must make cropping decisions by evaluating both the agronomic and economic returns of various cropping systems. Crop rotations also afford growers the opportunity to diversify farm production in order to minimize market risks.

Agronomic practices such as rotation patterns for soybean vary from state to state. However, there are similarities among states within certain growing regions. The majority of the U.S. soybean acreage (68.6%) is rotated to corn with approximately 14.5% of the subsequent corn acreage rotated back to soybean in the third year of the rotation (soybean-corn-soybean). Wheat follows soybean on approximately 11.2% of the U.S. soybean acreage.

Continuous soybean production is uncommon in the Midwest. Soybean extension specialists encourage growers to avoid the practice as a way to reduce the risk of damage from diseases and nematodes (Hoeft et al., 2000; Al-Kaisi et al., 2003). Corn and soybean occupy more than 80% of the farmland in many of the Midwestern states, and the two-year cropping sequence of soybean-corn is used most extensively in this region. However, a soybean crop sometimes is grown after soybean and then rotated to corn in a 3-year rotation sequence (soybean-soybean-corn) in the Midwest. The yield of both corn and soybean is approximately 10% higher when grown in rotation than when either crop is grown continuously (Hoeft et al., 2000). 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).

M.2.3.3. Irrigation

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

M.2.3.4. Management of Insects

The management of insects in soybean production is discussed in detail in Section VIII.D and summarized here. Although insects are rated as less problematic than weeds in U.S. soybean production, management of insect pests during the growth and development of soybean is important for protecting the yield of soybean (Aref and Pike, 1998). Insect injury can impact yield, plant maturity, or seed quality. Insect injury in soybean seldom reaches levels to cause an economic loss in the primary soybean production areas, as indicated by the low percentage (16%) of soybean acreage that receives an insecticide treatment (USDA NASS, 2007).

Characterizing soybean responses to insect injury is essential in establishing economic injury levels (Higley and Boethel, 1994). Most often, soybean insects pests are categorized or defined by the plant parts they injure, namely root-feeding, stem-feeding, leaf-feeding, or pod-feeding insects. The root- and stem-feeding insect groups are often the hardest to scout and typically are not detected until after they have caused their damage. The leaf-feeding insects comprise the biggest group of soybean insect pests, but not necessarily the most economically damaging insects. Recent research on defoliation has determined that a major effect of leaf injury is to reduce light interception by the soybean canopy which in turn can have a significant effect on yield (Higley and Boethel, 1994). Soybean has an extraordinary capacity to withstand considerable defoliation early in the season without significant yield loss. By contrast, defoliation during the flowering and pod filling stages poses a greater threat to yield, because the soybean plant has less time to compensate for injury compared to other growth stages. Research indicates that the soybean plant can sustain a 35% leaf loss prior to the pre-bloom period without lowering yield (NDSU, 2002). However, from pod-set to maturity, the plant can tolerate only a 20% defoliation level before yield is impacted. Insect damage occurring in the full pod stage (R4) or seed-filling stage (R5) would have the most impact on yield (McWilliams et al 2004).

M.2.3.5. Management of Diseases and Other Pests

The management of diseases and other pests in soybean production is discussed in detail in Section VIII.E. More than 100 pathogens are known to affect soybean, of which 35 are considered to be of economic importance (Heatherly and Hodges, 1999). Pathogens can affect all parts of the soybean plant, resulting in reduced quality and yield. The extent of losses depends upon the pathogen, the state of plant development and health when infection occurs, the severity of the disease on individual plants, and the number of plants affected (Heatherly and Hodges, 1999).

According to field surveys conducted in soybean-producing states during 1996 to 2009, soybean cyst nematode (*Heterodera gylcines*) caused by far the greatest soybean yield losses (Figure M-3) (Wrather et al., 2000). Phytophthora root and stem rot (*Phytophthora sojae*), seedling diseases, charcoal rot (*Macrophomina phaseolina*), sudden death syndrome (*Fusarium virgulifome*), and sclerotinia stem rot (*Sclerotinia sclerotioru)* followed in economical importance. Brown stem rot (*Phialophora gregata*), viruses, anthracnose (*Colletotrichum truncatum*), and pod and stem blight (*Diaporthe phaseolorum*) caused the remainder of the top ten soybean yield losses during 1996 to 2009. As expected, yield losses due to diseases and nematodes varied by region.

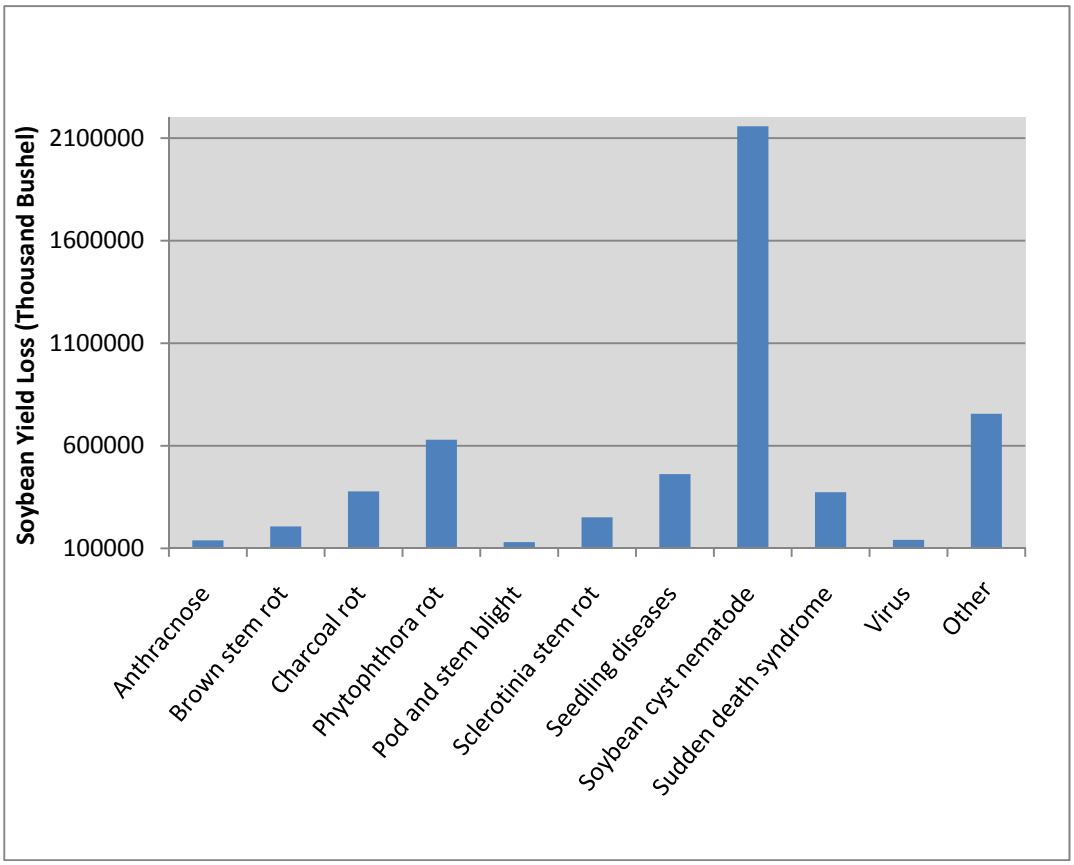


Figure M-3. Soybean Yield Losses in the U.S. Averaged Over the Period 1996 to 2009 Attributable to Pests and Diseases
Source: Wrather and Koenning, 2010

Selection of varieties that are resistant to disease is the primary tool growers have for disease control in soybean (Heatherly and Hodges, 1999). Cultural practices can also play an important role in disease management by reducing initial inoculums or reducing the rate of disease development (Heatherly and Hodges, 1999).

Preplant tillage can bury crop residue, which encourages the decomposition of fungal-resting structures. Crop rotation is often recommended as a disease-management strategy. Rotating crops interrupts the disease cycle and allows time for the decomposition of inoculums. One exception is *Rhizoctonia*, a soil-inhabitant pathogen that grows on a wide variety of crops and can survive sufficiently in the soil to make crop rotation an impractical means of controlling this pest. Row spacing, plant population, and planting date can also be changed to manage soybean diseases.

Soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most damaging pathogens of soybean throughout the soybean growing regions of the U.S. with losses estimated to be about $1.5 billion (Pedersen, 2008b). The simplest, least expensive method to reduce populations of this pest is to rotate soybean with a non-host crop such as corn, small grains, or sorghum. However, planting resistant varieties is regarded as the best and most effective management practice to prevent losses from this pest.

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

Asian soybean rust is a foliar fungal disease that typically infests soybean during reproductive stages of development and can cause defoliation and reduce yields significantly in geographies such as Brazil (Dorrance et al., 2007). Soybean rust is caused by the fungus *Phakopsora pachyrhizi*. This disease in the U.S. was first detected in Louisiana in 2004 (LSU, 2010). At this time, foliar application of fungicides is the standard disease-management practice to limit yield losses due to soybean rust.

M.2.3.6. Weed Management

The management of weeds in soybean production is discussed in Section VIII.F. Weed control in soybean is essential to optimizing yields because weeds compete with soybean for light, nutrients, and soil moisture. Weeds can also harbor insects and diseases, and also can interfere with harvest, causing extra wear on harvest equipment (Pedersen, 2008). Approximately 98% of the soybean acreage received an herbicide application in 2006, indicating the importance of excellent weed control in maximizing soybean yield (USDA NASS, 2007).

Herbicide-tolerant soybean was introduced to provide growers with additional options to improve crop safety and/or improve weed control. The Roundup Ready soybean system (planting Roundup Ready soybean and applying glyphosate in crop to provide primary weed control) was introduced in 1996 and has become the standard weed control program in U.S. soybean production and was utilized on 91% of U.S. soybean acreage in 2008 (USDA-NASS, 2009).

The primary factors that affect a potential yield loss in soybean from weed competition are the weed species, weed density, and the duration of the competition. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001).

Cultural and mechanical weed control practices can be important components of an effective weed management program (Loux et al., 2010). Cultural practices such as crop rotation, narrow row spacing and planting date are a few of the crop management practices that are implemented to provide the crop with a competitive edge over weeds. Mechanical methods of weed control including tillage have been used for centuries to control weeds in crop production. Spring or fall preplant tillage and in-crop shallow cultivation can effectively reduce the competitive ability of weeds by burying the plants, disturbing or weakening their root systems, or causing sufficient physical injury to kill the plants. A consequence of in-crop cultivation for weed control is that it can injure crop roots and cause moisture loss.

M.2.4. Human Health and Worker Safety

Humans safely consume soybean and have done so for thousands of years. The current primary food use is soybean oil (OECD, 2001). Nutrients in soybeans and soy products include protein, fats, carbohydrates and fiber (OECD, 2001). The essential amino acids required by humans are contained in soybeans (OECD, 2001). Anti-nutrients include trypsin inhibitors (interfere with digestion), lectins (inhibit growth), isoflavones (also referred to as phytoestrogens that may affect reproduction), stachyose and raffinose (produce gas), and phytic acid (binds mineral nutrients, making them unavailable) (OECD, 2001). Soybeans also contain allergenic proteins that may cause reactions in hypersensitive individuals (OECD, 2001). Toasting or heating the soybeans during processing reduces the content of trypsin inhibitors and lectins (OECD, 2001). All biotechnology-derived soybean with new traits produced by biotechnology have been reviewed by the FDA through FDA's consultation process. Biotechnology-derived soybean on the market pose no unique risks relative to other soybean developed using traditional breeding methods. Most soybeans currently grown are herbicide tolerant and have been shown to be as safe and nutritious as their conventional counterparts.

Agriculture ranks among the most hazardous industries in the nation (USBLS, 2011) Fatal injuries constitute a significant burden on the agricultural sector, as indicated by the 456 farmers and farm workers who died from a work-related injury for a fatality rate of 25.1 deaths per 100,000 workers during that same year, compared to the 5,214 fatal injuries (for a fatality rate of 3.7 deaths per 100,000 workers) that happened across all industries in 2008 (USBLS, 2011 and USDOL-OSHA, 2011). No fatalities were reported in 2008 directly associated with soybean farming, although four fatalities were reported in 2007 associated with soybean farming (USBLS, 2011). According to OSHA in 1986, farm tractors (rollovers and run-overs) represented 51% of all farm related fatalities, followed by fatalities associated with buildings and structures (including grain suffocation and silo-gas), and farm trucks (non-highway accidents), with 11% and 6%, respectively. Other farm related hazards related to crop workers (including soybean) include injuries related to ergonomics, noise, respiratory (including dust hazards), and chemicals (including pesticides and fertilizers), These hazards can be minimized, among other things, through the use of hazard recognition, hazard control and the use of personnel protective equipment (USDOL-OSHA, 2011). Genetically engineered soybean with new traits produced by biotechnology pose no unique worker safety issues relative to other soybean developed using traditional breeding methods.

M.2.5. Animal Health

Animals have consumed soybeans as forage and meal for many years. Soybean meal is currently the primary animal feed from soybeans and is fed to animals primarily as a protein source. Soybean meal contains relatively high levels of essential amino acids that are deficient in other common feed (OECD, 2001). Trypsin inhibitors, lectins and phytic acid are the primary anti-nutrients in soybeans that should be minimized in animal diets. Toasting or heating the soybeans during processing reduces the content of trypsin inhibitors and lectins (OECD, 2001). All biotechnology-derived soybean with new traits produced by biotechnology have been reviewed by the FDA through FDA's consultation process. Biotechnology-derived soybean with new traits produced by biotechnology pose no unique risks relative to other soybean developed using traditional breeding methods. Soybeans currently grown are largely herbicide tolerant and have been shown to be as safe and nutritious as their conventional counterparts.

M.2.6. Animal and Plant Communities

M.2.6.1. Animal Communities

Soybean production systems in agriculture are host to many animal species. Mammals and birds may seasonally consume grain, and invertebrates can feed on the plant during the entire growing season. Animals that feed primarily on soybean are seed-feeding insects and rodents found in agricultural fields. Rodents, such as mice or squirrels, may seasonally feed exclusively on soybean seeds. Thus, these animals may have a diet containing significant amounts of soybean seeds. Deer may also browse in soybean fields on the forage and on seed left after harvest. The USDA reports that soybean has minor to moderate value as a food source for large animals; low/minor value as a food source for small mammals, water birds and terrestrial birds; and moderate value as cover for terrestrial birds (USDA NRCS, 2011).

M.2.6.2. Plant Communities

The affected environment for growing soybean plants can generally be considered the agroecosystem (managed agricultural fields) plus some area extending beyond the intended plantings that might be affected by agricultural operations. Plants, extraneous to the crop, which grow in planted fields can be considered weeds and are dealt with in a separate section in this document. Plants not growing in a field amongst the soybeans would be considered in this section. These plants could be in ditches, hedge rows, fence rows, wind breaks, yards, etc. These plants could be annuals, biennials or perennials. Regardless of the agricultural operation, these plants may be impacted, both positively and negatively, by agricultural operations. Fertilizers and/or water may run off into adjacent lands, resulting in increased plant growth outside the agroecosystem. Negative impacts on plants adjacent to production fields can occur from herbicide drift, however, measures can be implemented to minimize drift.

M.2.7. Physical Environment

M.2.7.1. Surface Water and Groundwater Quality

Surface water may be impacted from soybean production by runoff from soybean fields that carries soil particles and herbicides or other pesticides to streams, rivers, lakes, wetlands and other water bodies. As discussed below, based on existing data, the soil component of runoff is a much more important contributor to surface water impacts than is the pesticide component.

Tillage causes widespread soil disturbance. Thus, erosion, topsoil loss and the resulting sedimentation and turbidity in streams are likely to increase with increased tillage. In 2009, based on the states' water quality reports, EPA identified sedimentation and turbidity as two of the top 10 causes of impairment to surface water in the U.S. in general; in 2007, EPA identified sedimentation/siltation as a leading cause of impairment to rivers and streams in particular (U.S. EPA, 2009; U.S. EPA, 2007). Although a comprehensive data set has not yet been developed to confirm its assertion. EPA has projected conservation tillage to be "the major soil protection method and candidate best management practice for improving surface water quality" (U.S. EPA, 2002). EPA identifies conservation tillage as the first of its CORE4 agricultural management practices for water quality protection (U.S. EPA, 2008a).

Based on the states' water quality reports to EPA, which EPA makes available through its National Assessment Database, pesticides in general and herbicides in particular are a relatively minor contributor to impairment of surface water in the U.S., compared to sedimentation/siltation and turbidity (U.S. EPA, 2008b). Pesticides accounted for less than one percent of reported causes of surface water impairment in all but four of the 17 leading U.S. soybean-producing states. In those four states, pesticides accounted for two to eight percent of reported causes of impairment. Of the pesticides that were reported as contributing to impairment among the 17 leading soybean-producing states, almost all are previously used, highly persistent chemicals that are no longer registered for use in the U.S. (U.S. EPA, 2008b).

Groundwater impacts may be of potential concern in some areas where nitrogen levels are either approaching or have exceeded the maximum contaminant level (10 milligrams/liter) (Klocke et al., 1999). In areas, such as Nebraska, where soybean and corn are grown in rotation and where ground water is a principle source of water for human consumption, this can be an important issue. However, as a legume, soybean generally does not require addition of nitrogen fertilizer, and therefore does not contribute appreciably to nitrogen levels in groundwater.

M.2.7.2. Air

Many agricultural activities, including those associated with soybean production, affect air quality including exhaust emissions from tillage, planting, and harvesting equipment; and nitrous oxide emissions from the use of nitrogen fertilizer. Approximately 80% of the global CO_2 emissions attributable to human activity are derived from the combustion

of fossil fuels and cement production, and ~20% are derived from land use change and deforestation related to agriculture (Burney et al., 2010; IPCC, 2007).

M.2.7.3. Soil and Soil Microorganisms

Microbial populations and associated biochemical processes are critical to maintaining soil health and quality. Soil microbial communities are highly complex and are often characterized by high microbial diversity (Tiedje et al., 1999). The occurrence and abundance of soil microorganisms are affected by 1) soil characteristics like tilth, organic matter, nutrient content, and moisture capacity, 2) typical physico-chemical factors such as temperature, pH, and redox potential, and 3) soil management practices. Agricultural practices such as fertilization and cultivation may also have profound effects on soil microbial populations, species composition, colonization, and associated biochemical processes (Buckley and Schmidt, 2001; Buckley and Schmidt, 2003). Consequently, significant variation in microbial populations is expected in agricultural fields.

Maintaining soil pH in the range of 6.0 to 7.0 will enhance the availability of inherent and fertilizer nutrients; reduce the availability of toxic elements, particularly aluminium and manganese; and enhance microbial activity (Hoeft, 2000). The increased microbial activity that is associated with the optimum pH level results in oxidation of organic matter and increased release of nutrients from the organic matter. The increased microbial activity applies also to the rhizobia bacteria that are responsible for symbiotic nitrogen fixation in soybeans and other legumes (CAST, 2009). Properly nodulated soybeans grown at the proper pH will fix about two-thirds of the nitrogen contained in the harvested crop (Hoeft, 2002). Results of most studies show that application of nitrogen fertilizer does not improve yields in soybeans (CAST, 2009 p. 25). Soybean removes approximately 0.85 pounds of phosphate and 1.2 pounds of potash (Potassium) for each bushel of seeds harvested (CAST, 2009). Micronutrient deficiencies are uncommon and maintaining proper pH prevents most micronutrient problems (Heatherly and Elmore, 2004).

Members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae* form a highly complex and specific symbiotic relationship with leguminous plants, including soybean (Gage, 2004). The nitrogen-fixing plant-microbe symbiosis results in the formation of root nodules, which provide 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. As a result of this relationship, nitrogen inputs are typically not necessary for agricultural production of soybeans.

M.2.7.4. Adjacent Agricultural Crops and Non-Agricultural Plants

Soybean is widely grown throughout the U.S. on land devoted to agricultural use. The biology of soybean is discussed in Section II. Soybean (*Glycine max* (L.) Merr.) originated in east Asia and is not native to the Americans (USDA ARS, National Genetic Resources Program [GRIN]). *Glycine max* is not listed as an invasive or noxious weed by USDA (USDA NRCS, 2011). Soybean does not have any related wild relatives in the

U.S. with which it can hybridize. Soybean is not found outside of cultivation and is not invasive or weedy (OECD, 2000).

Soybean is primarily grown adjacent to other large acre crops such as corn, wheat, and alfalfa. It may also be grown near vegetables, orchards, pastures, and adjacent to non-agricultural lands, such as forests, grasslands, streams, lakes, rivers and occasionally near urban lands.

Soybean is self-pollinating and shows low rates of outcrossing. The potential for cross-pollination in soybean is limited. This is recognized in certified seed regulations for foundation seed in the U.S., which permit any distance between different soybean cultivars in the field as long as the distance is adequate to prevent mechanical mixing (USDA APHIS, 2006). Numerous studies on soybean cross-pollination have been conducted, and the published results, with and without supplemental pollinators, are summarized in Table IX-1, Section IX. Under natural conditions, cross-pollination among adjacent plants in a row or among plants in adjacent rows ranged from 0 to 6.3%.

M.3. Alternatives

The decision-making process of deregulation is governed by 7 CFR § 340.6 (d)(3)(i) which states that APHIS may approve the petition in whole or in part, resulting in three possible outcomes in response to Monsanto's petition, described below.

M.3.1. Alternatives Studied in Detail

M.3.1.1. Deregulation in Whole Alternative

Under the "deregulation in whole" alternative, MON 87712 would no longer be a regulated article under 7 CFR Part 340 and would be widely available for planting, movement and importation without prior authorization in a permit or notification. MON 87712 is expected to be gradually adopted by many of those growers who are already growing biotechnology-derived soybeans. The rate of adoption will depend on the expectation of yield increase, the price of the seed, and other factors. The rate of adoption is expected to be gradual partly because of the difficulty to a grower of assessing a yield increase within the inherent temporal and spatial variability in yield due to many other factors, as discussed in Appendix M.2.1.2. Unlike some other traits (e.g., herbicide tolerance), where the trait and benefits are immediately obvious, the yield increase may not always be readily apparent and in some cases may be masked by other yield-inhibiting factors such as disease, drought stress or insect pressure. Maximum trait adoption is expected to be approximately 50 to 60%, based on Monsanto's estimate. This estimate assumes that other seed companies will offer soybean varieties that do not contain MON 87712 and these varieties will compete for market share with varieties that contain MON 87712. In addition, it is assumed that there will be demand for conventional, organic soybean as well as other specialty soybean and these soybean varieties will also compete for acreage with MON 87712.

No Action Alternative

Under the "no action" alternative, MON 87712 would remain a regulated article under 7 CFR Part 340. MON 87712 could be grown under USDA notification or permit under confined release conditions. However, currently deregulated biotechnology-derived soybean would continue to be available and would be expected to continue to be widely grown. It is expected that higher yielding soybean varieties developed through conventional breeding would continue to be introduced and that growers will continue to adopt these new varieties for use on their farm.

M.3.2. Alternatives Considered and Dismissed

M.3.2.1. Deregulation in Part

Approval in Part Based on Plant Pest Risk

The 'approval in part" alternative would be dependent upon a finding of the potential for a plant pest risk for MON 87712 in certain geographies or under certain conditions. APHIS may impose conditions upon the cultivation or use of MON 87712 in specific

geographies or conditions to mitigate potential plant pest risk. For example, APHIS could impose conditions that require assurance of the integrity and purity of the material containing MON 87712, or conditions requiring the implementation of stewardship practices in the use of MON 87712 that formed the basis of an APHIS decision that MON 87712 does not pose a plant pest risk. MON 87712 has been thoroughly characterized and extensive information presented in Sections I through IX of demonstrates that MON 87712 does not present a plant pest risk in any of the geographies or under any conditions where MON 87712 may be grown. Therefore, from a plant pest risk perspective, there is no basis for imposing geographic or other restrictions on MON 87712. Monsanto has requested a determination of non-regulated status without conditions.

M.4. Environmental Consequences

This section describes the environmental consequences of the no action and the deregulation in whole alternatives.

M.4.1.1. Commercial Soybean Production and Use

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Also under the no action alternative, soybean acres planted in the U.S. are expected to experience modest increases, based on USDA projections (Appendix M.2.1). General locations of production and uses of soybean would be expected to continue as they are now, potentially with continued westward and northward expansion and slight decreases in production in the south and east.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. As summarized in the petition, MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its yield trait. Introduction of MON 87712 is not expected to result in an increase in percent of soybeans planted that are biotechnology-derived, as over 90% of soybean currently grown are biotechnology-derived. Due to a lack of phenotypic characteristics that would make it suitable to be grown in regions outside of where soybeans are currently grown, the MON 87712 trait is not expected to have an impact on where and how soybean is grown in the U.S.

M.4.1.2. Soybean Varieties and Maturity Groups

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Also

under the no action alternative, soybean variety development, selection and evaluation will continue to be used, and may be adjusted based on new data and practices.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual commercial introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the increased yield resulting from use of commercial soybean varieties that contain MON 87712. Data presented in the petition showed that MON 87712 displayed a delay in days to 50% senescence and days to physiological maturity (Table VII-4). Farmers select soybean varieties for planting on their farm based upon the correct maturity group for their region. Leaf senescence and physiological maturity are two of several characteristics that breeders use to help assign a variety to a correct maturity group (Heatherly and Elmore, 2004). A delay in physiological maturity of a variety may impact when the soybeans can be harvested. Soybean yield and quality are affected if a season ending freeze occurs before a variety reaches its physiological maturity.

It is not expected that the delay in physiological maturity or leaf senescence observed will impact the maturity group designation of soybean varieties that are commercialized using MON 87712 for the following reasons. The majority of soybean varieties on the market today are produced from a breeding process termed forward breeding. In this process new soybean varieties are created by crossing two existing varieties. The varieties crossed are not necessarily from the same maturity group. The progeny of the crosses are tested in field trials to evaluate a number of parameters including yield, maturity, flowering time, leaf senescence, overall plant health, and seed characteristics like protein, and oil levels (Heatherly and Elmore, 2004). Varieties that yield better than the checks of the same maturity group are selected for commercialization. The entire process from initial cross to introduction of a new variety usually takes several years during which each new variety is repeatedly evaluated against the best commercially available varieties of the same maturity group. When MON 87712 is used in soybean breeding programs, there may be an impact on leaf senescence and physiological maturity in new soybean varieties containing the trait. However, the system that breeders use to evaluate new varieties and assign a variety to a correct maturity group will be used to place the new variety into the correct maturity group. Breeders consider many characteristics of the new variety to determine a maturity group designation for a new soybean variety. As with any new soybean variety, whether it is biotechnology-derived or conventional, the interplay of numerous characteristics ultimately contributes to maturity group assignments and breeders will continue to rely on these characteristics during the variety development process.

M.4.1.3. Yield

No Action Alternative Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Under the no action alternative, continued yield increases would be expected in soybean, at least for the foreseeable future, before a true yield plateau is reached (Appendix

M.2.1.3.). As discussed in Appendix M.2.1., USDA projects average annual yield increases from 2010 to 2021 of 0.33 bu/acre (USDA, 2011). These yield increases may be expected to result from development of higher-yielding biotechnology-derived and conventionally bred varieties, and from continued improvements in agronomic and cultural practices.

Deregulation in Whole Alternative. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. The actual yield gain attributable to MON 88712 will continue to be evaluated as the event is utilized and introgressed into a broader range of elite germplasm and new varieties are developed and released.

Yield is a complex parameter impacted by many variables and varies from year to year and by location. Because adoption of the technology is expected to occur over a period of several years and maximum market adoption is expected to reach 50 to 60% over time (Appendix M.3.1.1) the use of commercial soybean varieties containing MON 87712 would likely result in a gradual increase in the slope of the average soybean yield curve shown in Figure M-1. The change would still be within the range of scatter shown in Figure M-1. As discussed in Appendix M.2.1.2, changes of 20% or more between one year and the next in U.S. average annual per-acre soybean yield are not uncommon, and large local temporal and geographic variation is common. As discussed in Appendix M.2.1.2, because of the high variability in crop production, producers, processors and transporters are accustomed to dealing with harvested crops in excess of system capacity.

M.4.1.4. Economics

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Under the no action alternative, growers would be expected to continue to choose crops for planting based on expected profitability, and profitability would likely continue to vary from year to year and crop to crop (Appendix M.2.1.3). Seed prices are likely to continue to be a major operating cost in soybean production, with overhead cost the majority of the total costs.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The only difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield from the use of commercial MON 87712 soybean varieties, in addition to the incremental yield opportunity resulting from the use of other technologies or conventional breeding. The economic benefit of higher yield is well understood and is compelling to growers as it helps them increase productivity and profitability on their farm. To the U.S. economy as a whole, increased productivity (increased value of production without increasing inputs) is of value as well as it helps

U.S. businesses to be more competitive in the global economy. How that value will be apportioned between consumers (in the form of reduced prices for soybeans or derived products) and growers (in the form of increased profits), will vary depending on the government policies and global market conditions, as described in Appendices M.2.1.3 and M.2.1.4.

M.4.1.5. Land Use

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Also, under the no action alternative, based on USDA projections, acres planted in soybean in the U.S. are expected to experience a small increase over the next ten years. However, based on the historic stability of overall U.S. cropland acres, any increases in soybean acreage in the U.S. would likely occur with corresponding decreases in some other crops, and increases in the rate of double-cropping (Appendix M.2.1.4). Growers will produce the crops they anticipate to be most profitable. Any changes in soybean production would not be expected to expand outside the range of agricultural land used for crops in the U.S. for the past 100 years (Appendix M.2.1.4). As discussed in Appendix M.2.1.4, corn and soybean combined planted acreage is at an all-time high in 2011. This was achieved partly through maximization of existing cropland. Other factors included decreases in other crop acreage and double-cropping.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. The presence of the MON 87712 trait is not expected to affect the overall land used for crop production in the U.S., based on the historic stability of U.S. cropland acreage. While the yield increases expected with MON 87712 may encourage increased soybean planting to some extent, many factors other than yield affect planted acreage, and yield is not necessarily a good predictor of crop acreage. In the other major U.S. crops, corn, production has increased much more dramatically than soybeans in recent years, and has not affected the overall range of acreage of cropland; and corn acreage is well below historical corn acreage in spite of dramatically increased yields (USDA NASS, 2011).

M.4.1.6. Seed Production

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Under the no action alternative, soybean seed production would be expected to continue as described in Appendix M.2.2.1.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of

MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. MON 87712 will also be a trait in soybean grown for seed production. As summarized in Section VII, MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its increased yield trait. It is expected that MON 87712 soybean grown for seed production purposes would yield more seed. Seed growers are accustomed to variations in seed yield due to the same factors that impact grain yield (e.g., climate, pest pressure, etc.). Therefore, seed production practices are not expected to change.

M.4.1.7. Organic Soybean Production

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Under the no action alternative, organic soybean production would be expected to continue as described in Appendix M.2.2.2. and it is expected that biotechnology-derived soybean varieties will continue to be grown on over 90% of the soybean acres. As described in Appendix M. 2.2.2., organic soybean producers employ practices that allow them to reasonably avoid biotechnology-derived soybeans.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. As summarized in the petition, (Section VII), MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its yield trait. Soybean is principally self pollinated; however, low levels of cross pollination occur. The pollen from MON 87705 has been characterized and compared to pollen produced by conventional soybean. No statistically significant differences were detected between MON 87712 and the conventional control A3525 for percent viable pollen or pollen grain diameter (Table VII-5). Furthermore, no visual differences in general pollen morphology were observed between MON 87712 and the conventional control A3525. Thus, the potential for cross pollination from MON 87712 is expected to be the same as conventional soybean and the practices that organic farmers are using to avoid biotechnology-derived traits are not expected to change with the introduction of MON 87712. MON 87712 is not expected to result in increases in biotechnology-derived soybean, as over 90% of U.S. soybean is currently biotechnology-derived. For these reasons, MON 87712 is not expected to have an impact on organic soybean production any different than the no action alternative.

M.4.1.8. Agronomic Practices

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Soybean breeders will continue to develop higher yielding soybean varieties and farmers will likely use these new varieties on their farm due to their economic returns. Under the no action alternative, agronomic practices are expected to continue as described in Section VIII.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its yield trait (See Section VII). Field assessments included observations for plant responses to abiotic stressors, disease, and plant-arthropod interactions. The observed phenotypic characteristics were similar between MON 87712 and the control (Section VII). Therefore, MON 87712 is not expected to result in changes in agronomic practices and no impacts on tillage, crop rotation, irrigation, management of insects, disease management, weed management or herbicide tolerant weeds are expected with the introduction of MON 87712.

The expected yield increase associated with MON 87712 will predictably require more potassium and phosphorous from the soil. As mentioned previously, soybean fixes its own nitrogen and does not benefit from added nitrogen; therefore, no additional nitrogen inputs are expected for MON 87712. Potassium and phosphorous are rarely applied to soybean because soybean follows well fertilized crops like corn and wheat in a rotation and residual levels of potassium and phosphorous are sufficient to produce a soybean crop. The application of fertilizers to corn has not changed substantially from the late 70's even though higher yielding soybean varieties have continued to be adopted (See Figure M-2 and M-4). As noted in Section VII, detailed observations were taken of MON 87712 and the control over the growing season at multiple locations. No signs of nutrient deficiency were noted for MON 87712. Therefore, the use of MON 87712 in breeding programs and the opportunity to increase yield is not expected to change fertilization practices for soybean compared to the no action alternative.

M.4.1.9. Human Health and Worker Safety

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Human health and worker safety issues are described in Appendix M.2.4.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of

MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. As summarized in the petition, (Section VI) MON 87712 soybean is compositionally comparable to conventional soybean. Detailed compositional analyses were conducted in accordance with OECD guidelines to assess levels of key nutrients and anti-nutrients in MON 87712 compared to levels present in the parental conventional soybean control of a similar genetic background as well as conventional commercial reference varieties. These compositional comparisons were made by analyzing the seed and forage harvested from plants grown at each of eight field sites in the U.S. during the 2009 field season. The conventional commercial reference varieties used to establish a range of natural variability for the key nutrients and anti-nutrients in conventional commercial soybean varieties have a history of safe consumption. Nutrients assessed in this study included proximates, fiber, amino acids, fatty acids, and vitamin E in seed, and proximates and fiber in forage. The anti-nutrients assessed in seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitors, and isoflavones..

Assessment of the analytical results confirmed that the differences observed in the combined-site analysis were not meaningful to food and feed safety or the nutritional quality of MON 87712 soybean. In addition, the levels of assessed components in MON 87712 were compositionally equivalent to the conventional control and within the range of variability of the conventional commercial reference varieties that were grown concurrently in the same field trial. Therefore, it is concluded that soybean seed and forage produced from MON 87712 are compositionally equivalent to that of the conventional soybean and that the high yield trait in MON 87712 does not have a meaningful impact on the composition and therefore on the food and feed safety or nutritional quality of MON 87712 compared to conventional soybean.

The food, feed and environmental safety of the BBX32 protein has been addressed in the petition (Section V). The BBX32 protein is produced by a gene obtained from the *Arabadopsis* plant. The BBX32 protein is similar to other plant produced BBX proteins with know histories of food, feed and environmental safety. The B-box zinc finger family is found in many plant species including soybean, where the B-box family contains 61 genes. The protein represents an extremely minor fraction of the total protein produced by the plant and has no characteristics associated with or similar to toxic or allergenic proteins.

Therefore, soybeans and the processed fraction produced from MON 87712 are expected to be as safe and nutritious as soybeans and processed fractions produced from existing commodity soybeans. Practices for use of herbicides and other pesticides are not expected to be affected. Therefore, MON 87712 is expected to have the same impact as commodity soybean to human health and worker safety.

M.4.1.10. Animal Health

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. As discussed in Appendix M.2.5., soybean meal is currently the primary animal feed from soybeans and is fed to animals as a protein source. Anti-nutrients of potential concern in soybean meal are trypsin inhibitors, lectins and phytic acid, which are generally controlled by heating during processing.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. As summarized in Section VII, MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its high yield trait. As discussed above in Appendix M.4.1.6, MON 87712 has been found to be compositionally equivalent to conventional soybeans in terms of both nutritional and anti-nutritional composition. Therefore, soybean as forage and soybean meal produced from MON 87712 are expected to be as safe and nutritious as soybean forage and meal produced from existing conventional and GE soybeans. As discussed above, the BBX32 protein is produced by a gene obtained from the *Arabadopsis* plant. The BBX32 protein is similar to other plant proteins with know histories of food, feed and environmental safety. The protein represents an extremely minor fraction of the total protein produced by the plant and has no characteristics associated with or similar to toxic or allergenic proteins. Therefore, MON 87712 is expected to have the same impact as commodity soybean on animal health.

M.4.1.11. Animal and Plant Communities

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Animal and plant communities are discussed in M.2.6. There are no native species in the U.S. related to soybeans; therefore, outcrossing to native species is not a concern with soybean.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. As summarized in Section VII, MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its increased yield trait. Agronomic practices that may affect animal and plant communities are not expected to change as a result of the introduction of MON 87712. Therefore, MON 87712 is expected to impact animal and plant communities the same as commodity soybean under the no action alternative.

M.4.1.12. Surface Water, Groundwater and Air Quality

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Surface water and groundwater are discussed in Appendix M.2.7.1. and air is discussed in Appendix M.2.7.2.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the increased yield in commercial soybean varieties that contain MON 87712. The presence of the MON 87712 trait is not expected to affect agronomic practices such as tillage or herbicide or pesticide application. As described in Appendix M.2.7.3., the increased yield that will occur with MON 87712, or increases in soybean yield from any other source, may result in the removal of more phosphate and potash from the soil, and therefore may require the replacement of these nutrients at a slightly higher rate than would be needed with lower-yielding soybeans. For the same volume of soybeans produced, however, the phosphate and potash needs would be the same. Because agronomic practices will be negligibly affected by the introduction of MON 87712, the introduction of MON 87712 is expected to have negligible impacts on surface water, groundwater or air quality, in terms of per acre impacts compared to existing soybean varieties. Because MON 87712 offers the potential to increase productivity of soybean, production a small positive impact to surface water and air quality may be expected in terms of impacts per bushel of soybean produced. That is, on average, the impacts as described in Appendices M.2.7.1. and M.2.7.2 will be essentially the same per acre while more soybeans may be produced on that acre. Therefore, the impact per bushel of soybean production will be slightly less than before the introduction of MON 87712.

The introduction of MON 87712 is not expected to impact air quality differently from the no action alternative. In the future it is expected that more food will need to be produced to feed a growing and increasingly affluent global population. This can be accomplished by either increasing crop yield per acre, or expanding the amount of land used for agriculture. Under the no action alternative and deregulation in whole alternative, it is likely that crop yields will increase. The increased yield associated with MON 87712 is expected to allow for less land-use change compared to the no action alternative, and is not expected to have any direct impact on exhaust emissions or fertilizer applications. Therefore, MON 87712 is not expected to have a deleterious effect on air quality. Of the global anthropogenic CO_2 emissions related to climate change, ~80% are derived from the combustion of fossil fuels and cement production, and ~20% are derived from land use change and deforestation related to agriculture (IPCC, 2007). Higher yielding crop varieties will likely help to meet the higher food demands in the 21st century without expanding the amount of land used in agriculture, therefore leading to less land-use change and potentially lower anthropogenic CO_2 emissions (Burney et al., 2010).

M.4.1.13. Soil and Soil Microorganisms

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Soil and soil microorganisms are described in Appendix M.2.7.3.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. One critical interaction between soybeans and microbes in the soil is in the development of a symbiotic relationship between soybean and rhizobia. As noted in Section VII.C, measurement of nodule number and mass along with plant growth and nitrogen status are commonly used to assess differences in the symbiotic relationship between a legume and its associated rhizobia. As described in Section VII.C, no statistically significant differences were detected (5% level of significance) between MON 87712 and the conventional control for each measured parameter related to assessing this relationship, including nodule number, shoot percent total nitrogen, shoot total nitrogen (g), and dry weight of nodules, shoot material, and root material. In addition, field assessments included observations for plant responses to abiotic stressors, disease, and the observed phenotypic characteristics were similar between MON 87712 and the control.

The introduction of MON 87712 is expected to have minmal impact on agronomic practices compared to the no action alternative. As described in Appendix M.2.7.3, the use of MON 87712 in soybean varieties offers the opportunity for increased yield. As with any higher yielding soybean variety, increases in soybean yield may result in the removal of more phosphate and potassium from the soil, and may require the replacement of these nutrients at a slightly higher rate than would be needed with lower-yielding soybeans. Soybean is typically rotated with corn in crop rotations and benefits from the fertilizers applied to corn requiring very little fertilizer inputs. According to the USDA NASS data base, fertilizer inputs on corn the primary rotational crop for soybean have leveled off or slightly decreased since the 1980's (Figure M-4). In this same period, yields of soybean have continued to increase suggesting that higher yields in soybean are not related to increased fertilizer inputs. Similarly, the expected yield increase in MON 87712 soybean varieties is unlikely to change this trend. Rather, improved crop genetics and more precise farming methods are expected to provide increased yield on the same or reduced land base using the same or reduced inputs.

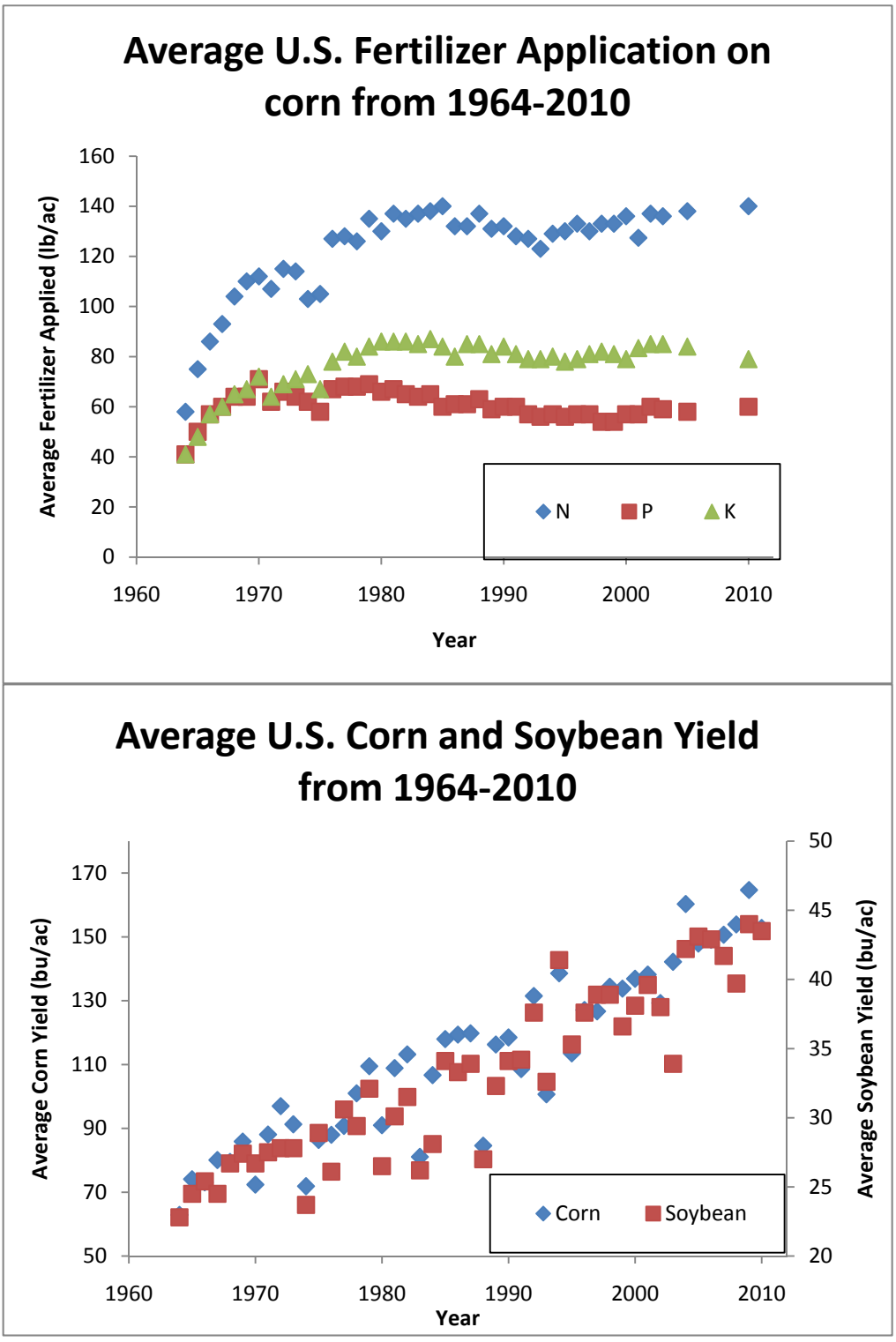


Figure M-4: Average historical U.S. fertilizer application to corn crop and corn and soybean yields over time.
Source: USDA NASS

M.4.1.14. Adjacent Agricultural Crops and Non-Agricultural Plants

No Action Alternative. Under the no action alternative, MON 87712 would continue to be regulated and would only be grown on limited acres under notification or permit. Adjacent agricultural crops and non-agricultural plants are discussed in Appendix M. 2.7.4.

Deregulation in Whole Alternative. The difference between the no action and the deregulation in whole alternative is expected to be the gradual introduction of MON 87712 into soybean varieties and use in soybean production. The primary difference between the no action and the deregulation in whole alternative attributable to the introduction of MON 87712 into a broad base of soybean germplasm would be the opportunity for increased yield in commercial soybean varieties that contain MON 87712. As summarized in Section VII, MON 87712 soybean is agronomically, phenotypically, and compositionally comparable to conventional soybean with the exception of its high yield trait. Because MON 87712 impacts the yield of soybean, it may be speculated that the trait could impact the invasiveness or persistence of soybean. The assessment of the volunteer potential of MON 87712 is presented in the petition (Section VII.C.5). Based on the assessed data, the results of the study support a conclusion that the introduction of the yield trait did not alter the volunteer potential of MON 87712 compared to conventional soybean. Furthermore, these results demonstrate that the increased yield trait in MON 87712 confers no biologically meaningful change to the invasiveness or potential for soybean to persist in the environment (petition p. 158). Therefore, MON 87712 is expected to have no impact on adjacent agricultural crops and non-agricultural plants.

M.5. Cumulative Effects

CEQ regulations define cumulative impacts as "the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (federal or non-federal) or person undertakes such other action." [19] Thus, cumulative impacts are assessed only for those resources that are impacted by the proposed action.

As described in Appendix M.4.0, the primary impact of dereculation in whole of MON 87712 is increased yield in soybeans. Therefore, the only cumulative impacts assessed are those associated with increased yield. The no action alternative (Appendix M.4.1.3) already includes yield increases in soybean that will result from other past, present and reasonably foreseeable future actions , and based on the trend of incremental annual average soybean yield gains observed over several decades, these are expected to occur whether or not MON 87712 is introduced. Note that these projected yield increases in soybean are not based on specific events or activities by others, but are based on USDA ERS projections. The impacts as described in Appendix M.4.1.3 includes projected yield increases under the no action alternative plus those resulting from

[19] 40 CFR 1508.7

deregulation of MON 87712; thus, there are no other foreseeable cumulative yield impacts to discuss.

Conventional Breeding with Other GE or Conventional Soybean

As previously mentioned, several biotechnology-derived soybean products have been deregulated or are under consideration for deregulation, and a list of the events deregulated or under review by USDA is presented in Table M-2.

Table M-2. Deregulated or Submitted Biotechnology-derived Soybean Products

Phenotype	ID Code(s)	Institution	Date Deregulated
High Oleic Acid, Low Saturated Fat	MON 87705	Monsanto	Submitted
Omega 3 Fatty Acid	MON 87769	Monsanto	Submitted
Lepidopteran Resistant	MON 87701	Monsanto	Submitted
Herbicide-tolerant (*Glyphosate*/*Isoxaflutole)*	FG72	Bayer Crop Sciences	Submitted
Herbicide-tolerant (*Imidazolinone*)	BPS-CV127-9	BASF Plant Science	Submitted
Dicamba-tolerant	MON 87708-9	Monsanto	Submitted
High Oleic Acid	DP-3Ø5423-1	Pioneer	June, 2010
Glyphosate- and ALS-tolerant	DP-356Ø43-5	Pioneer	July, 2008
Glyphosate-tolerant	MON 89788	Monsanto	July, 2007
Phosphinothricin-tolerant	GU262	AgrEvo	October, 1998
Phosphinothricin-tolerant	A5547-127	AgrEvo	April, 1998
Altered Oil Profile	G94-1, G94-19, G-168	DuPont	May, 1997
Phosphinothricin-tolerant	W62, W98, A2704-12, A2704-21, A5547-35	AgrEvo	July, 1996
Glyphosate-tolerant	40-3-2	Monsanto	May, 1994

Source is website: http://www.aphis.usda.gov/brs/not_reg.html. and
http://www.aphis.usda.gov/brs/status/petday.html

Once deregulated, MON 87712 may be bred with these deregulated biotechnology-derived soybean products as well as with conventional soybean, creating new improved varieties. APHIS has determined that none of the individual biotechnology-derived soybean products it has previously deregulated displays increased plant pest characteristics and that any progeny derived from crosses of these soybean products with other conventional or biotechnology-derived soybean are unlikely to exhibit new plant pest properties.

An assessment of the stability of the genetic insert in MON 87712 is discussed in Section IV of and summarized here. Data have demonstrated that MON 87712 is stable in its progeny. Having established that the genetic material is stable and inherited in a Mendelian fashion, and based on data and observations taken on soybean varieties

containing MON 87712 in Monsanto's plant breeding program, it is concluded that the phenotype of MON 87708 is likewise stable. Traditional soybean breeding has an established history of safe use, and use of MON 87712 in breeding programs is expected to behave in a manner similar to other conventional traits and biotechnology-derived traits. Given that there have been no plant pest characteristics associated with MON 87712, or with any of the previously deregulated events listed in Table 2, no significant impacts are expected to other soybean through the use of MON 87712 in breeding programs and in combination with any of the previously deregulated biotechnology-derived soybean products.

All biotechnology-derived soybean products on the market today have satisfactorily completed the FDA consultation process established to review the safety of foods and feeds derived from biotechnology-derived crops for human and animal consumption (see Table M-2). Since MON 87712 is expected to be utilized broadly in the future for Monsanto's soybean products, it will likely be bred with other biotechnology-derived soybean products that Monsanto has petitioned APHIS for deregulated status (e.g., MON 89788, MON 87701, MON 87705). No impacts to public health (e.g., food or feed safety) are expected due to combination of these events through conventional breeding because the deregulated events have all completed a safety consultation with FDA and on the basis of knowledge of the type of modifications made to each of the deregulated events, and to the events under review, the biochemical pathways are not likely to unexpectedly interact or result in the production of novel constituents.

The decision to deregulate MON 87712 would also allow for breeding of this product with conventional soybean of diverse genetic background. No impacts to public health (e.g., food or feed safety) or environmental safety are expected due to the breeding of MON 87712 with these other soybean because these varieties have an established history of safe use.

Furthermore, the process of conventional breeding to combine biotechnology-derived traits or biotechnology-derived and conventional soybean to produce combined trait products is designed to identify and remove off-types (i.e., plants that lack the intended phenotype or that do not show expected agronomic or phenotypic characteristics) during development of new varieties. Breeders use standard testing and assessment procedures to further examine and confirm the equivalence of the combined-trait products, compared to the single trait products, in terms of phenotypes, agronomic characteristics, and the efficacy of the individual traits when present in combination. This screening process is a means to assure and confirm that the traits present in stacked products are performing as expected and thus do not display novel phenotypes. Given this evaluation it can be concluded that no significant impacts are expected through the use of MON 87712 in combination with previously deregulated biotechnology-derived soybean products.

References for Appendix M

Al-Kaisi, M. 2001. Value of crop rotation in nitrogen management. Iowa State University, Ames, Iowa. http://www.ipm.iastate.edu/ipm/icm/2001/4-23-2001/valuen.html [Accessed May 12, 2010].

Al-Kaisi, M., M. Hanna and M. Tidman. 2003. Crop rotation considerations for 2004 management season rotation. Iowa State University, Ames, Iowa. http://www.ipm.iastate.edu/ipm/icm/2003/12-15-2003/croprotation.html [Accessed June 19, 2006].

Alsager, M. 2010. 2010 yield contest release. Missouri Soybean Merchandising Council, Missouri Soybean Association, Jefferson City, Missouri. http://mosoy.org/2010-yield-contest-release/ [Accessed July 14, 2011].

AOSCA. 2009. Seed certification handbook: Including genetic and crop standards, procedures, and AOSCA service programs. Association of Official Seed Certifying Agencies, Moline, Illinois.

Aref, S. and D.R. Pike. 1998. Midwest farmers' perceptions of crop pest infestation. Agronomy Journal 90:819-825.

ASA. 2008. Introduction. American Soybean Association, St. Louis, Missouri. http://www.soystats.com/2008/Default-frames.htm [Accessed October 7, 2008].

Ash, M. 2011. Oil crops outlook: Larger foreign crops temper a sharp decline in U.S. soybean stocks. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://usda.mannlib.cornell.edu/usda/ers/OCS//2010s/2011/OCS-04-11-2011.pdf.

Ash, M., J. Livezey and E. Dohlman. 2006. Soybean backgrounder. U.S. Department of Agriculture, Economic Research Service, Washington, D.C.

ASTA. 2010. Guide to seed quality management practices. American Seed Trade Association, Alexandria, Virginia.

Buckley, D.H. and T.M. Schmidt. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecology 42:11-21.

Buckley, D.H. and T.M. Schmidt. 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology 5:441-452.

Burney, J.A., S.J. Davis and D.B. Lobell. 2010. Greenhouse gas mitigation by agricultural intensification. Proceedings of the National Academy of Science of the United States of America 107:12052-12057.

CAST. 2009. Sustainability of U.S. soybean production: Conventional, transgenic, and organic production systems. Council for Agricultural Science and Technology, Ames, Iowa.

Clark, S. and C. Alexander. 2010. The profitability of transitioning to organic crops in Indiana. Purdue University Department of Agricultural Economics, West Layfayette, Indiana. http://www.agecon.purdue.edu/extension/pubs/paer/2010/february/alexander.asp [Accessed July 13, 2011].

Coulter, J., K. Moncada and C. Sheaffer. 2010. Soybean production. Risk Management Guide for Organic Producers. University of Minnesota, St. Paul, Minnesota.

Dalley, C.D., K.A. Renner and J.J. Kells. 2001. Weed competition in Roundup Ready soybean and corn. Michigan State University, Department of Crop and Soil Science, East Lansing, Michigan. http://www.ipm.msu.edu/CAT05_fld/pdf/5-26FactSheet.pdf [Accessed June 15, 2010].

Dorn, T.W. 2011. Computations on grain stored in various configurations. University of Nebraska - Lincoln, Lincoln, Nebraska. http://lancaster.unl.edu/ag/crops/inservice/outside.htm [Accessed July 7, 2011].

Dorrance, A.E., M.A. Draper and D. Hershman. 2007. Using foliar fungicides to manage soybean rust. The Ohio State University, Colombus, Ohio. http://oardc.osu.edu/soyrust/2007edition/fungisoyrust.pdf [Accessed May 12, 2010].

Funderbunk, J., R. McPherson and D. Buntin. 1999. Soybean disease management and Soybean insect management. Pages 231-290 in Soybean Production in the Midsouth. L.G. Heatherly and H.F. Hodges (eds.). CRC Press, Inc., Boca Raton, Florida.

Gage, D.J. 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing Rhizobia during nodulation of temperate legumes. Microbiology and Molecular Biology Reviews 68:280-300.

Harner, J.P., T.J. Herrman and C. Reed. 1998. Temporary grain storage considerations. MF-2362. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Pittsburg, Kansas.

Heatherly, L.G. and R.W. Elmore. 2004. Managing inputs for peak production. Pages 451-536 in Soybeans: Improvement, Production, and Uses. H.R. Boerma and J.E. Specht (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Hellevang, K.J. 1998. Temporary grain storage. North Dakota State University Extension, Fargo, North Dakota. http://www.ag.ndsu.edu/pubs/ageng/grainsto/ae84-1.htm [Accessed July 7, 2011].

Helsel, Z.R. and H.C. Minor. 1993. G4410: Soybean production in Missouri. University of Missouri Extension, Columbia, Missouri. http://extension.missouri.edu/explore/agguides/crops/g04410.htm [Accessed November 11, 2009].

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000. Soybean as a crop. Pages Pages 31, 36, 38, 39, 40, 41, 47, 86, 87, 89, 90, 93, 94, 96, 97,120,121, 208, 209 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Holshouser, D.L. 2010. Days to soybean physiological maturity. 3009-1459. Virginia Cooperative Extension, Blacksburg, Virginia.

Hurburgh, C.R. 2005. Grain storage challenges in Iowa for the 2005 crop. Iowa State University Cooperative Extension, Ames, Iowa. http://www.extension.iastate.edu/agdm/articles/others/HurOct05.htm [Accessed July 7, 2011].

Iowa State University. 2003. Growing organic soybeans on conservation reserve program land. PM 1881. Iowa State University Extension, Ames, Iowa. http://www.extension.iastate.edu/Publications/PM1881.pdf.

IPCC. 2007. Climate change 2007: Working group I: The physical science basis. World Health Organization, Intergovernmental Panel on Climate Change, Geneva, Switzerland. http://www.ipcc.ch/publications_and_data/ar4/wg1/en/ch7s7-3-1-3.html#7-3-2 [Accessed May 14, 2009].

ISO. 2009. Selection and use of the ISO 9000 family of standards. International Standards Organization, Geneva, Switzerland.

Kantolic, A.G. and G.A. Slafer. 2005. Reproductive development and yield components in indeterminate soybean as affected by post-flowering photoperiod. Field Crops Research 93:212-222.

Klocke, N.L., D.G. Watts, J.P. Schneekloth, D.R. Davison, R.W. Todd and A.M. Parkhurst. 1999. Nitrate leaching in irrigated corn and soybean in a semi-arid climate. Transactions of the American Society of Agricultural Engineers 42:1621-1630.

Koger, T. 2010. Soybean growth stages. Plant Management Network, St. Paul, Minnesota. http://www.plantmanagementnetwork.org/edcenter/seminars/SoybeanGrowthStages/ [Accessed July 11, 2011].

Kuepper, G. 2003. Organic soybean production. Appropriate Technology Transfer for Rural Areas, National Sustainable Agriculture Information Service, Fayetteville, Arkansas. https://attra.ncat.org/attra-pub/organicsoy.html [Accessed July 11, 2011].

Loux, M.M., D. Doohan, A.F. Dobbels, W.G. Johnson, G.R.W. Nice, T.N. Jordan and T.T. Bauman. 2010. Weed control guide for Ohio and Indiana. Bulletin 789. Purdue University and Ohio State University Extension, West Lafayette, Indiana.

LSU. 2010. Asian soybean rust: Background. Louisiana State University, Baton Rouge, Louisiana. http://www.agctr.lsu.edu/en/crops_livestock/crops/soybeans/Asian_Soybean_Rust/ [Accessed June 17, 2010].

Maier, D.E. and W.F. Wilcke. 2011. Temporary grain storage considerations. Purdue University, Grain Quality Task Force, Purdue, Indiana. http://www.extension.purdue.edu/extmedia/GQ/GQTF38/GQTF-38.html [Accessed July 7, 2011].

Major, D.J. 1980. Photoperiod response characteristics controlling flowering of nine crop species. Canadian Journal of Plant Science 60:777-784.

Major, D.J., D.R. Johnson, J.W. Tanner and I.C. Anderson. 1975. Effects of daylength and temperature on soybean development. Crop Science 15:174-179.

McBride, W.D. and C. Greene. 2009. The profitability of organic soybean production. American Agricultural Economics Association Annual Meeting, Orlando, Florida.

McWilliams, D.A., D.R. Berglund and G.J. Endres. 2004. Soybean growth and management: Quick guide. North Dakota State University Extension Service, Fargo, North Dakota.

Moore, M. 2009. Approaching a yield plateau? Corn & Soybean Digest, Minneapolis, Minnesota. http://cornandsoybeandigest.com/seed/approaching-yield-plateau [Accessed July 14, 2011].

NDSU. 2002. Soybean production field guide for North Dakota and northwestern Minnesota. North Dakota State University Extension Service and North Dakota Soybean Council, Fargo, North Dakota. http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a1172.pdf [Accessed November 11, 2009].

OECD. 2000. Consensus document on the biology of *Glycine max* (L.) merr. (soybean). ENV/JM/MONO(2000)9. Series on harmonization of regulatory oversight in biotechnology No.15. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2001. Consensus document on compositional considerations for new varieties of soybean: Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2001)15. Series on the Safety of Novel Foods and Feeds No.2. Organisation for Economic Co-operation and Development, Paris, France.

Oplinger, E.S. and E. Amberson. 1986. Soybean seed quality and certified seed. University of Wisconsin Extension, Madison, Wisconsin.

Pedersen, P. 2004. Soybean growth and development. Iowa State University, Ames, Iowa.

Pedersen, P. 2008a. Soybean variety selection. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/production_varietyselect.html [Accessed April 19, 2011].

Pedersen, P. 2008b. Soybean cyst nematode. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/diseases_SCNbiology.html [Accessed July 14, 2011].

Pedersen, P. 2008c. Damping off and seed decay. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/diseases_dampoff.html [Accessed July 14, 2011].

Pedersen, P. 2008d. Soil fertility. Iowa State University Extension, Ames, Iowa. http://extension.agron.iastate.edu/soybean/production_soilfert.html [Accessed July 14, 2011].

Pedersen, P. 2010. How to maximize soybean yield. Plant Management Network, Fayetteville, Arkansas. http://www.plantmanagementnetwork.org/infocenter/topic/focusonsoybean/ [Accessed July 11, 2011].

Pedersen, P. 2011. Managing soybean for high yield. Iowa State University Department of Agronomy, Ames, Iowa.

Sandretto, C. and J. Payne. 2006. Soil management and conservation. Pages 96-106 in Agricultural Resources and Environmental Indicators. K. Wiebe and N. Gollehon (eds.). U.S. Department of Agriculture, Economic Research Service, Washington, D.C.

Scott, W.O. and S.R. Aldrich. 1970. Modern soybean production. The Farm Quarterly: 16-152.

Soyatech. 2010. Soy facts. Soyatech, Inc., Southwest Harbor, Maine. http://72.32.142.180/soy_facts.htm [Accessed October 13, 2010].

Tiedje, J.M., S. Asuming-Brempong, K. Nusslein, T.L. Marsh and S.J. Flynn. 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology 13:109-122.

Tylka, G.L., G.D. Gebhart, C.C. Marett, M.P. Mullaney and S.N. Wiggs. 2010. Evaluation of soybean verieties resistant to soybean cyst nematode in Iowa - 2010. Iowa State University Extension, Ames, Iowa.

U.S. EPA. 2002. Environmental implications of conservation tillage: A systems approach. EPA/600/D-85/147 (NTIS PB85237089). U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Lab, Washington, D.C. http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=39100&fed_org_id=770&SIType=PR&TIMSType=&showCriteria=0&address=nerl&view=citation&keyword=Pesticides&sortBy=pubDateYear&count=100&dateBeginPublishedPresented [Accessed May 19, 2010].

U.S. EPA. 2007. National water quality inventory: Report to Congress, 2002 reporting cycle. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/305b/2002report/ [Accessed May 19, 2010].

U.S. EPA. 2008. Agricultural management practices for water quality protection. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/owow/watershed/wacademy/acad2000/agmodule/ [Accessed August 27, 2010].

U.S. EPA. 2008. Water quality assessment and total maximum daily loads information. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/waters/ir/ [Accessed July 11, 2011].

U.S. EPA. 2009. Findings: Rivers and streams. U.S. Environmental Protection Agency, Washington, D.C.

USBLS. 2011. Census of fatal occupational injuries. U.S. Bureau of Labor Statistics, Washington, D.C. http://data.bls.gov/cgi-bin/drsv [Accessed May 10, 2011].

USDA. 2000. Crop profile for soybeans in Illinois. U.S. Department of Agriculture, Washington, D.C. http://www.ipmcenters.org/cropprofiles/docs/ilsoybeans.pdf [Accessed July 11, 2011].

USDA. 2010. Outlook for U.S. agricultural trade: FY 2010 exports expected to rise to $104.5 billion; Imports drop to $76.5 billion. U.S. Department of Agriculture, Economic Research Service and Foreign Agricultural Service, Washington, D.C. http://www.fas.usda.gov/cmp/outlook/2010/May-10/May2010_fullreport.pdf [Accessed July 11, 2011].

USDA. 2011. USDA agricultural projections to 2020. U.S. Department of Agriculture, Washington, D.C. http://www.usda.gov/oce/commodity/archive_projections/USDAAgriculturalProjections2020.pdf.

USDA-AMS Organic. 2011. Clarification of existing regulations regarding the use of genetically modified organisms in organic production and handling Policy Memo 11-13. U.S. Department of Agriculture, Agricultural Marketing Service, Washington, D.C.

USDA-APHIS. 2006. Soybean. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C. http://www.aphis.usda.gov/br/soybean.html [Accessed March 2, 2006].

USDA-ARS. 2011. Taxon: *Glycine max* (L.) Merr. U.S.Department of Agriculture, Agricultural Research Service, Beltsville, Maryland. http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?17711 [Accessed April 20, 2011].

USDA-ERS. 2005. Briefing room - Agricultural chemical and production technology: Soil management. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http:///www.ers.usda.gov/Briefing/AgChemicals/soilmangement.htm [Accessed July 11, 2011].

USDA-ERS. 2007. Data sets: Major land uses. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/data/majorlanduses [Accessed July 11, 2011].

USDA-ERS. 2008. Data sets: U.S. and regional cost and return estimates in soybean for 2008. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/data/CostsandReturns/testpick.htm [Accessed January 18, 2010].

USDA-ERS. 2010. Data sets: Organic production. U.S. Department of Agriculture, Economic Research Service, Washington D.C. http://www.ers.usda.gov/Data/organic/ [Accessed May 5, 2010].

USDA-ERS. 2011. Data sets: Commodity costs and returns: Data. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://transcoder.usablenet.com/tt/http://www.ers.usda.gov/data/CostsandReturns/testpick.htm [Accessed July 11, 2011].

USDA-FAS. 2011. Oilseeds: World markets and trade. U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C

USDA-NASS. 2007. Agricultural chemical usage - 2006 field crops summary. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2007. Acreage. June 2007. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2009. Acreage - June 2009. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000 [Accessed May 12, 2010].

USDA-NASS. 2010. Crop production: 2009 summary, January 2010. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2011. Crop production: Historical track records, April 2011. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2011. Crop production: 2010 summary, January 2011. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NRCS. 2011. PLANTS profile: *Glycine max* (L.) Merr. soybean. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, D.C. http://plants.usda.gov/java/profile?symbol=GLMA4 [Accessed April 20, 2011].

USDOL-OSHA. 2011. Agricultural operations. U.S. Department of Labor Occupational Safety & Health Administration, Washington, D.C. http://www.osha.gov/SLTC/agriculturaloperations/index.html [Accessed May 10, 2011].

Voosen, P. 2009. Molecular breeding makes crops hardier and more nutrutious: Markers, knockouts and other technical advances improved breeding without modifying genes. Scientific American, London, United Kingdom. http://www.scientificamerican.com/article.cfm?id=molecular-breeding-crops-genetics-rice-soy-corn-wheat [Accessed April 20, 2011].

Way, M.O. 1994. Status of soybean insect pests in the United States. Pages 15-16 in Handbook of Soybean Insect Pests. L.G. Higley and D.J. Boethel (eds.). The Entomological Society of America, Lanham, Maryland.

Wrather, J.A. and S. Koenning. 2010. Soybean disease loss estimates for the United States, 1996-2009. University of Missouri Delta Research Center, Agricultural Experiment Station, Portageville, Missouri. http://aes.missouri.edu/delta/research/soyloss.stm [Accessed April 20, 2011].

Zhang, L.X., S. Kyei-Boahen, J. Zhang, M.H. Zhang, T.B. Freeland, C.E. Watson, Jr. and X. Liu. 2007. Modifications of optimum adaptation zones for soybean maturity groups in the USA. Plant Management Network, Fayetteville, Arkansas. http://www.plantmanagementnetwork.org/sub/cm/research/2007/zone/ [Accessed September 27, 2007].

MONSANTO
imagine

MONSANTO COMPANY
800 N. LINDBERGH BLVD.
ST. LOUIS, MISSOURI 63167
http://www.monsanto.com

March 28, 2012

RECEIVED
By APHIS BRS Document Control Officer at 3:13 pm, Mar 29, 2012

Dr. John Turner
United States Department of Agriculture
Biotechnology Regulatory Services, APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1236

RE: Waiver of Confidential Business Information (CBI) Claim for Petition Number 11-202-01p (Determination of Non-regulated Status for MON 87712 Soybean)

Dear Dr. Turner:

Monsanto Company has developed the biotechnology-derived soybean line MON 87712, which will be used in traditional breeding programs to produce commercial soybean varieties with increased yield opportunity. The yield increase in MON 87712 is achieved using the *BBX32* gene from the plant *Arabidopsis thaliana* that produces a protein that regulates the plant's day/night processes and results in an extended period of reproductive development in soybean. Monsanto has requested a determination from APHIS that MON 87712 and any progeny derived from crosses between MON 87712 and non-regulated soybean be granted non-regulated status under 7 CFR Part 340. In support of this request Monsanto submitted Petition #11-202-01p on July 21st, 2011 which is currently under review by APHIS.

Monsanto does not object to APHIS publishing for public comment, the un-redacted version of Monsanto's petition for the determination of non-regulated status for MON 87712 that Monsanto submitted to APHIS on July 21st, 2011 and APHIS deemed complete on September 16th, 2011. As we explained in our letter and supporting analysis provided to APHIS on February 22nd, 2012, Monsanto's Confidential Business Information (CBI) claim for certain information in our draft petition extends until such time as: 1) APHIS determines the petition to be "complete"; and 2) APHIS makes the final petition available for public comment. Therefore, we hereby waive all prior CBI claims related to this completed petition upon APHIS' publication of the same for public comment.

In addition, as requested by BRS, please find attached a signed copy of page 3 of petition #11-202-01p. Should you have any questions concerning Petition #11-202-01p, or wish to set up a

meeting for further discussion, please contact Daniel Jenkins, U.S. Agency Regulatory Affairs Lead, Washington D.C., at 202-383-2851, or me at 314-694-9879 or at dennis.phillion@monsanto.com.

Sincerely,

Dennis P. Phillion
Regulatory Affairs Manager

cc: Daniel Jenkins/Monsanto
Cynthia Eck/USDA
Tonya Woods/USDA
Regulatory files/11-SY-217U

Enclosure:
Page 3 of petition #11-202-01p