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March 19, 2009

[redacted]
Biotechnology Regulatory Services, APHIS
4700 River Road, Unit 147
Riverdale, MD 20737–1236

RE: Request for the Determination of Non-Regulated Status for MON 87701

Dear [redacted]

Monsanto Company requests that the USDA/APHIS review the enclosed petition for the determination of non-regulated status in whole for MON 87701.

Monsanto Company has developed biotechnology-derived insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal (Cry) protein (δ-endotoxin) derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. The Cry1Ac protein provides protection from feeding damage caused by targeted lepidopteran pests. The *cry1Ac* gene was transferred into the genome of soybean cells using *Agrobacterium tumefaciens*-mediated transformation. Cry1Ac protein has a long history of safe use, as microbial pesticides containing *B. thuringiensis* Cry1A proteins have been used for more than 50 years and have undergone extensive toxicity testing showing no adverse effects to human or animal health. During the last decade a variety of biotechnology-derived crops containing Cry1 proteins from *B. thuringiensis* have been commercialized; thereby, rendering these crops resistant to several insect pests. Commercially available Bollgard® cotton contains Cry1Ac that has 100% amino acid identity to the MON 87701-produced Cry1Ac, with the exception of four additional amino acids at the N-terminus related to the chloroplast transit peptide. A related protein, Cry1Ab, which has ~90% amino acid identity to the Cry1Ac in MON 87701 and Bollgard cotton, is expressed in YieldGard® Corn Borer (MON 810) corn. These products have been grown commercially for over a decade and are used extensively for feed and food uses.

The data in this petition support the conclusion that MON 87701 is not likely to present an increased plant pest risk or to have a significant environmental impact compared to conventional soybean. The MON 87701 product concept is to reduce or replace current insecticide applications to control lepidopteran pests in tropical and sub-tropical soybean

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production regions, such as South America, where these insects cause significant plant damage and yield loss on approximately 100 million acres of soybean. MON 87701 will offer growers in these regions an effective pest management tool and help to maintain soybean yield potential.

According to USDA-NASS statistics, about 16% of the total 75 million U.S. soybean acreage, those acres grown mainly in the southeastern and Delta states, received insecticide applications annually. Given the limited insecticide applications to control defoliating lepidopteran insects in the major U.S. soybean growing regions, for the foreseeable future, MON 87701 is not expected to be available for commercial production by U.S. soybean growers. Planting of MON 87701 in the U.S. will be limited to breeding and seed multiplication purposes to support commercialization in South America. Monsanto requests a determination from APHIS that MON 87701 and any progeny derived from crosses between MON 87701 and non-regulated soybean be granted non-regulated status under 7 CFR Part 340.

The enclosed "Petition for Determination of Non-regulated Status for MON 87701" contains relevant information upon which to make a determination. This package contains three printed copies and one electronic copy of the petition. A food and feed safety assessment for MON 87701 will be provided to FDA shortly. Meanwhile, to support future breeding and seed multiplication activities in the U.S., Monsanto will file an application with the U.S. EPA for a FIFRA Section 3 seed increase registration of the plant-incorporated protectant *Bacillus thuringiensis* Cry1Ac protein, and the genetic material (vector PV-GMIR9) necessary for its production. USDA may discuss this assessment with representatives of FDA and EPA.

Monsanto will provide similar information to the governments of Canada and Brazil as well as other regulatory agencies in major soybean importing countries.

We would be pleased to meet with you and other USDA officials and scientists to respond to any questions you may have, or to provide you with additional information that you may request. Should you have any questions concerning this letter or the enclosed petition, or wish to set up a meeting to further discuss MON 87701, please contact [redacted] Senior Director, Regulatory Affairs and Policy, Washington DC, [redacted] or me [redacted].

Sincerely,

[redacted]

Regulatory Affairs Manager

cc: [redacted] Monsanto, Washington, DC
Monsanto Regulatory Files

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RECEIVED
By Cynthia A. Eck at 10:06 am, Dec 16, 2009

MONSANTO

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

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

March 19, 2009
(Revised on December 9, 2009)

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

Submitted by

[redacted]

Monsanto Company
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Prepared by

[redacted]

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RELEASE OF INFORMATION

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

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CERTIFICATION

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

[redacted]

Regulatory Affairs Manager

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Saint Louis, MO 63167

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EXECUTIVE SUMMARY

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

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

Product Description

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

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

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

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

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

<u>Data and Information Presented to Assess Plant Pest Potential of MON 87701</u>

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

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

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Weediness Potential of Soybean

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

Molecular Characterization of Inserted DNA

MON 87701 was produced by *Agrobacterium*-mediated transformation of soybean with PV-GMIR9, which is a binary vector containing two T-DNAs. The first T-DNA, designated as T-DNA I, contains the *cry1Ac* gene expression cassette. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* gene cassette. During transformation, both T-DNAs were inserted into the soybean genome at unlinked loci. The *cp4 epsps* gene was used as the selectable marker (glyphosate tolerance) that was needed for the initial selection of transformed cells and plants. After the transformed cells, and subsequently the plants, were identified, the selectable marker gene was no longer needed. Therefore, traditional breeding and segregation was deployed to isolate plants that only contain the *cry1Ac* expression cassette (T-DNA I), thereby producing marker-free MON 87701 plants. Molecular characterization of MON 87701 by Southern blot analyses demonstrated that the DNA inserted into the soybean genome is present at a single locus and contains one functional copy of the *cry1Ac* expression cassette. No T-DNA II (*cp4 epsps* gene expression cassette) genetic elements or backbone sequences from the transformation plasmid were detected in MON 87701. In addition, no partial genetic elements, linked or unlinked to the inserted expression cassette were detected. The stability of the integrated DNA (*cry1Ac* gene) was demonstrated by confirming the Southern blot fingerprint of MON 87701 and was maintained for five generations tested across the breeding history. The stability was further confirmed by the inheritance of the insect-protected trait in MON 87701 that followed the expected Mendelian segregation pattern.

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

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Characterization of the Cry1Ac Protein

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

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

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

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

Allergenicity and Toxicity Potential of the Cry1Ac Protein

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

Composition and Nutrition of Forage and Grain

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

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

Phenotypic and Agronomic Characteristics and Environmental Interactions

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

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

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

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

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

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Non-Target Organisms and Threatened or Endangered Species

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

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

Soybean Agronomic Practices and Land Use

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

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

Conclusion

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

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ABBREVIATION AND DEFINITIONS*

~	Approximately
2T-DNA	Plasmid vector containing two separate T-DNA regions each surrounded by left and right borders of the Ti plasmid
7S α'	3' region of the *Sphas1* gene of *Glycine max* encoding the 7sα' seed storage protein, β-conglycinin, including 35 nucleotides of the carboxyl terminal β-conglycinin coding region with the termination codon and the polyadenylation sequence
35S	The promoter and leader from the cauliflower mosaic virus (CaMV) 35S RNA
aadA	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7
AA	Amino acid
ADF	Acid detergent fiber
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
AOCS	American Oil Chemists Society
AOSA	Association of Official Seed Analysts
AOSCA	Association of Official Seed Certifying Agencies
APS	Analytical protein standard
ASA	America Soybean Association
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
CAPS	N-Cyclohexyl-3-aminopropanesulfonic acid
CAST	Council for Agricultural Science and Technology, USDA
CBI	Confidential business information
CEQ	The Council on Environmental Quality
CEW	Corn earworm [*Helicoverpa zea* (Boddie)]
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
COA	Certificate of analysis
CPB	Cartagena Protocol on Biosafety
Cry	Crystal proteins from *Bacillus thuringiensis*
cry1Ac	Coding sequence for Cry1Ac protein

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

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

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

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OSL	Overseason leaf
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PIP	Plant-incorporated protectant
PMSF	Phenylmethanesulfonyl fluoride
PPA	Plant Protection Act
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
PV-GMIR9	Plasmid vector used to develop MON 87701
RbcS4	Promoter, leader, and 5' non-translated region of the *Arabidopsis thaliana RbcS4* gene encoding ribulose 1,5-bisphosphate carboxylase small subunit 1A
Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA
RK2	Broad host range plasmid of Inc-P1 originally isolated in *Klebsiella pneumonia*
rop	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli*
SAP	Scientific Advisory Panel organized by U.S. EPA
SAS	Statistical Analysis System
SCN	Soybean cyst nematode
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	Standard error
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SOP	Standard operating procedure
TDF	Total dietary fiber
T-DNA I	Transfer DNA containing the *cry1Ac* expression cassette in plasmid vector PV-GMIR9
T-DNA II	Transfer DNA containing the *cp4 epsps* gene cassette in plasmid vector PV-GMIR9
T-DNA	Transfer DNA
TES	Threatened or endangered species
TFA	Trifluoroacetic acid

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

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I. RATIONALE FOR THE DEVELOPMENT OF MON 87701

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The major benefits of MON 87701 are:

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

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

I.C. Submissions to Other Regulatory Agencies

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

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

Submission to FDA

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

Submissions to EPA

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

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

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

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

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

Submissions to Foreign Government Agencies

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

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

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[1] MON 810 × MON 863 label [http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5CND%5Cpesticide%5CProduct%20Label%5C524%5C524-545%5C524-545_YIELDGARD_PLUS_CORN_BORER_ROOTWORM_11_11_2008_5_17_22_PM.pdf]

II. THE SOYBEAN FAMILY

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

II.A. Soybean as a Crop

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

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

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

Source: Soya and Oilseed Bluebook (2008).

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

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

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

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

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

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II.B. History of Soybean

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

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

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

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

II.C. Taxonomy and Phylogenetics of Soybean

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

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

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

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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).

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

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

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

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II.D. The Genetics of Soybean

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

II.E. Pollination of Cultivated Soybean

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

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

II.F. Cultivated Soybean as a Volunteer

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

II.G. Characteristics of the Recipient Plant

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

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

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

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III. DESCRIPTION OF THE TRANSFORMATION SYSTEM

MON 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue utilizing transformation vector, PV-GMIR9 (Section IV, Figure IV-1). PV-GMIR9 is a binary vector that contains well-characterized DNA segments required for selection and replication of the plasmid vector in bacteria and transfer of the T-DNAs into plant cells. Vector PV-GMIR9 contains two separate T-DNAs (hence the descriptor "2T-DNAs") that can be effectively used to generate marker-free plants (Komari et al., 1996). The first T-DNA, designated as T-DNA I, contains the gene cassette bearing the gene of interest *cry1Ac*, and the second T-DNA, designated as T-DNA II, contains the gene cassette of selectable marker gene *cp4 epsps*. During the process of *Agrobacterium*-mediated transformation, the distinct T-DNAs containing the *cry1Ac* and *cp4 epsps* genes were integrated into the soybean genome at independent, unlinked loci, and the rest of the backbone of the vector PV-GMIR9 was not inserted into plant cells. Traditional breeding was then used to isolate plants that only contain the T-DNA I (*cry1Ac* expression cassette) and do not contain the T-DNA II (*cp4 epsps* expression cassette). This resulted in the production of marker-free, insect-protected soybean MON 87701.

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

The R_0 plants generated through this process were self-pollinated to produce the R_1 seed. During subsequent selfing of the R_0 plants to produce the R_1 seed, the unlinked insertions of T-DNA I (*cry1Ac* gene expression cassette) and T-DNA II (*cp4 epsps* gene expression cassette) were segregated. A non-lethal dose of glyphosate herbicide was applied to R_1 plants. The resulting plants with minor injury were selected for further analyses, whereas plants showing no injury, i.e., containing T-DNA II (*cp4 epsps* gene expression cassette), were eliminated from subsequent development. Subsequently, plants containing only a single T-DNA I (*cry1Ac* gene cassette) were identified and selected by a combination of analytical techniques, including ELISA and TaqMan PCR analysis. Only R_1 plants that were homozygous for the T-DNA I cassette and not having the T-DNA II cassette were advanced for development. These R_1 plants were self-pollinated to generate a population of R_2 plants which were repeatedly self-pollinated through subsequent generations. These progeny were subjected to further molecular assessments to ensure the plants contained a single, intact insert and phenotypic assessments to ensure the plants met commercial specifications. MON 87701 was selected as the lead event based on its

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

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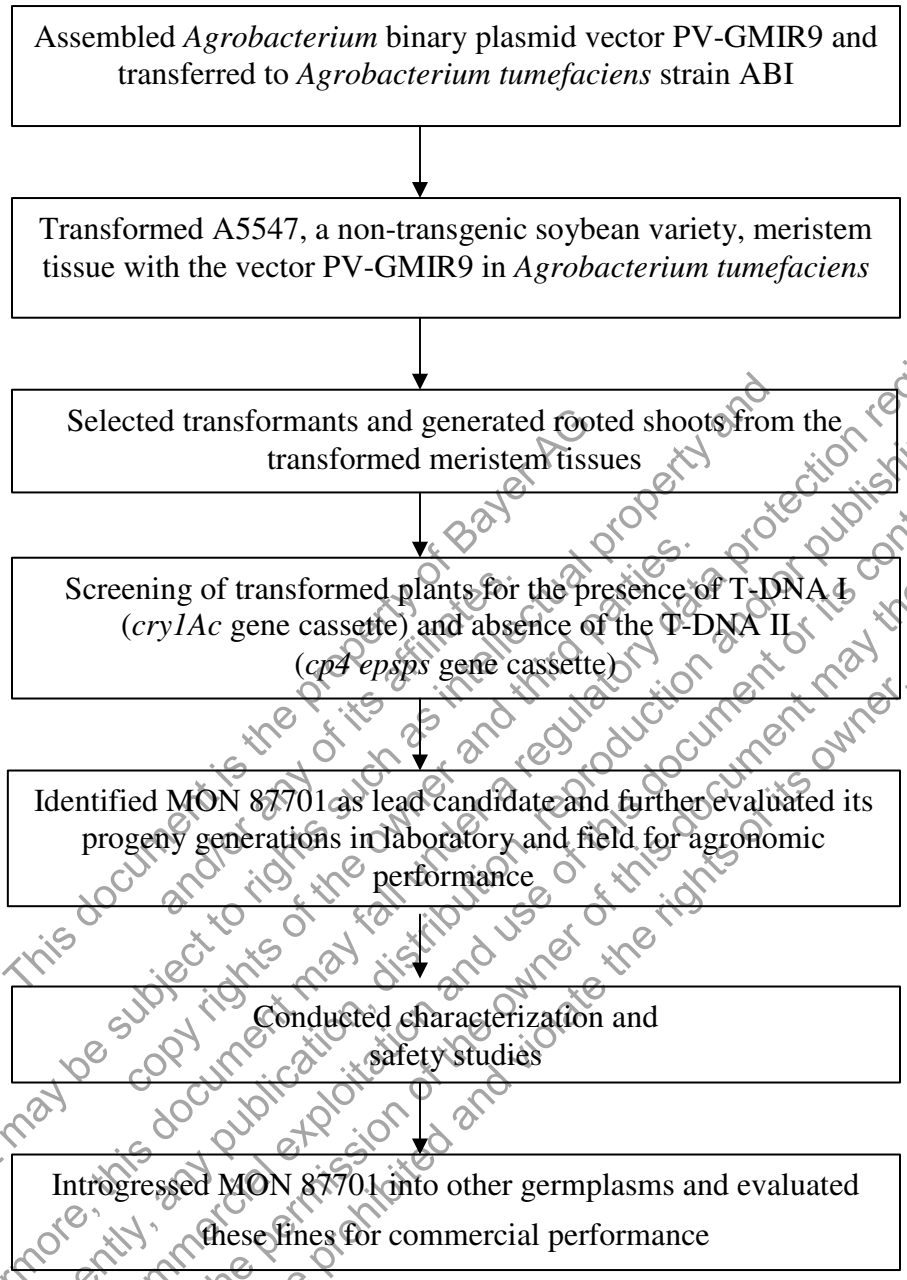

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Figure III-1. Schematic of the Development of MON 87701

IV. GENETIC ELEMENTS

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

IV.A. Vector PV-GMIR9

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

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

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

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

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

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

The *cp4 epsps* gene expression cassette is not present in MON 87701. The *cp4 epsps* gene expression cassette was used as a selectable marker during the transformation to produce MON 87701, but was segregated away by traditional breeding techniques at the R_1 generation. The CP4 EPSPS protein confers tolerance to glyphosate and has been used safely and successfully in many Roundup Ready crops such as canola, corn, cotton, soybean, and sugar beet. The deduced CP4 EPSPS full-length amino acid sequence is shown in Figure IV-3.

IV.D. Regulatory Sequences

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

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

IV.E. T-DNA Borders

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

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

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

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

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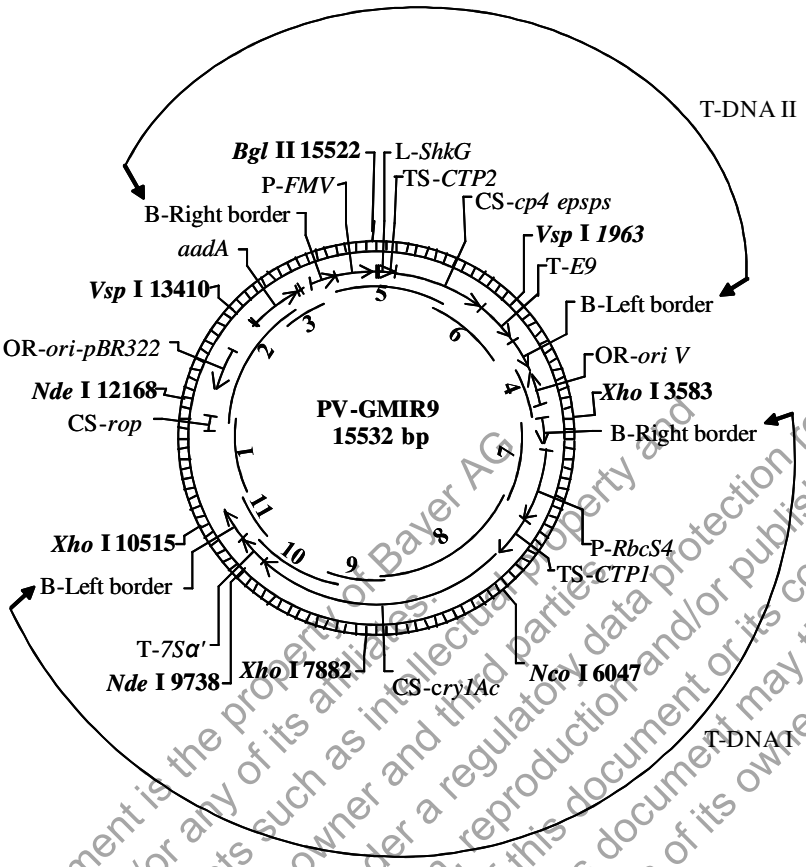

Figure IV-1. Plasmid Map of Vector PV-GMIR9 Showing Probes 1-11

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

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

* Nucleotide 10512 is vector backbone sequence.

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

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

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Genetic Element	Location in Plasmid	Function (Reference)
P[7]-*RbcS4*	4062-5784	Promoter, leader, and 5' non-translated region of the *Arabidopsis thaliana RbcS4* gene encoding ribulose 1, 5-bisphosphate carboxylase small subunit 1A, (Krebbers et al., 1988). Promoter expresses in above ground tissues
TS-*CTP1*	5785-6048	Targeting sequence encoding the transit peptide of the *Arabidopsis RbcS4* encoding small subunit 1A transit peptide, from *Arabidopsis thaliana*, present to direct the *cry1Ac* protein to the chloroplast (Krebbers et al., 1988)
CS-cry1Ac	6049-9585	Codon-modified coding sequence of the Cry1Ac protein of *Bacillus thuringiensis* (Fischhoff and Perlak, 1995)
Intervening Sequence	9586-9594	Sequences used in DNA cloning
T-7S α'	9595-10033	3' region of the *Sphas1* gene of *Glycine max* encoding the 7S *α'* seed storage protein, β-conglycinin, including 35 nucleotides of the carboxyl terminal β-conglycinin coding region with the termination codon and the polyadenylation sequence (Schuler et al., 1982). The element functions to terminate transcription and direct polyadenylation of the mRNA.
Intervening Sequence	10034-10069	Sequences used in DNA cloning
B-Left Border	10070-10511	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Vector Backbone (Continued from bp 3595)		
Intervening Sequence	10512-11786	Sequences used in DNA cloning
CS-*rop*	11787-11978	Coding sequence for repressor of primer protein derived from ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening Sequence	11979-12405	Sequences used in DNA cloning
OR-*ori-pBR322*	12406-12994	Origin of replication from pBR322 for maintenance of plasmid in *Escherichia coli* (Sutcliffe, 1978)
Intervening Sequence	12995-13524	Sequences used in DNA cloning

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

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

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

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

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

1	MAQVSRICNG	VQNPSLISNL	SKSSQRKSPL	SVSLKTQQHP	RAYPISSSWG
51	LKKSGMTLIG	SELRPLKVMS	SVSTACMLHG	ASSRPATARK	SSGLSGTVRI
101	PGDKSISHRS	FMFGGLASGE	TRITGLLEGE	DVINTGKAMQ	AMGARIRKEG
151	DTWIIDGVGN	GGLLAPEAPL	DFGNAATGCR	LTMGLVGVYD	FDSTFIGDAS
201	LTKRPMGRVL	NPLREMGVQV	KSEDGDRLPV	TLRGPKTPTP	ITYRVPMASA
251	QVKSAVLLAG	LNTPGITTVI	EPIMTRDHTE	KMLQGFGANL	TVETDADGVR
301	TIRLEGRGKL	TGQVIDVPGD	PSSTAFPLVA	ALLVPGSDVT	ILNVLMNPTR
351	TGLILTLQEM	GADIEVINPR	LAGGEDVADL	RVRSSTLKGV	TVPEDRAPSM
401	IDEYPILAVA	AAFAEGATVM	NGLEELRVKE	SDRLSAVANG	LKLNGVDCDE
451	GETSLVVRGR	PDGKGLGNAS	GAAVATHLDH	RIAMSFLVMG	LVSENPVTVD
501	DATMIATSFP	EFMDLMAGLG	AKIELSDTKA	A.	

Figure IV-3. Deduced Amino Acid Sequence of the CTP2 Targeting Sequence and the CP4 EPSPS Protein Present in PV-GMIR9

The amino acid sequence of the CTP2 targeting peptide and the CP4 EPSPS protein was deduced from the full-length coding nucleotide sequence present in vector PV-GMIR9. The CTP2 targeting peptide is underlined. The *cp4 epsps* nucleotide sequences are not present in MON 87701, therefore this protein is not expressed. See description in Section III on generating marker free plants using two T-DNA vectors.

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V. GENETIC ANALYSIS

This section details the molecular analyses that characterized the integrated DNA insert in MON 87701. The results confirm that MON 87701 contains a single insert with the intended sequence. The insert is stably maintained over multiple generations, and there are no open reading frames in the immediate vicinity of the insert with similarity to known allergens or toxins. These conclusions are based on several lines of evidence. The entire soybean genome was assayed by Southern blot analyses for the presence of DNA derived from the transformation plasmid PV-GMIR9. These analyses confirmed that a single copy of the *cry1Ac* cassette was inserted at a single site. DNA sequencing analyses determined the exact composition of the inserted DNA. The sequence of the insert was shown to be identical to the sequence to the T-DNA sequence in the transformation vector confirming that only the expected sequences were integrated. A comparison of the soybean genomic DNA flanking the insert in MON 87701 to the sequence of the insertion site in conventional soybean demonstrated that no major rearrangements occurred at the insertion site during transformation. Bioinformatic searches of public databases utilizing the flanking sequences demonstrated that there are no known genes disrupted by the insertion and that no open reading frames exist in the flanking sequences that show similarity to any known toxins or allergens. The stability of the insert was demonstrated by a Southern blot fingerprint analysis covering five generations. Taken together, the characterization of the genetic modification demonstrates that a single copy of the T-DNA was inserted at a single locus of the genome such that no known genes were disrupted.

Southern blot and DNA sequence analyses were used to characterize the T-DNA insert in MON 87701. Southern analysis was used to assay the entire soybean genome for sequences derived from the transformation vector PV-GMIR9. The sequence analysis was used to determine the composition and intactness of the inserted DNA and to evaluate the region of the genomic DNA directly adjacent to the insert. The analyses were performed on the R_5 generation, the same generation used to initiate the integration of MON 87701 into commercial germplasm. The Southern blot strategy was designed to ensure sufficient sensitivity while utilizing probes that span the entire transformation vector. A linear map depicting the restriction sites within the insert DNA sequence, as well as within the soybean genomic DNA immediately flanking the insert in MON 87701 is shown in Figure V-1. A map of plasmid vector PV-GMIR9 annotated with the probes used in the Southern analysis is presented in Figure IV-1. The high level of sensitivity was demonstrated for each blot by including and detecting a $1/10^{th}$ genome equivalent of the positive control. The Southern blots were performed in a way to maximize the resolution of DNA fragments. Two restriction enzyme sets were specifically chosen to minimize the possibility that two DNA fragments could co-migrate on the gel.

For each digest, there were duplicated samples that consisted of equal amounts of digested DNA. 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. For estimating the sizes of bands present in the Long-Run lanes of Southern blots, the molecular weight markers on the left of the figure were used. For estimating the sizes of

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bands present in the Short-Run lanes, the molecular weight markers on the right of the figure were used.

The DNA sequencing analyses compliment the Southern blot analyses. Whereas Southern blots determined that MON 87701 contains T-DNA I-derived sequences at a single insertion site, sequencing of the insert and the flanking genomic DNA determined that T-DNA I inserted as predicted in MON 87701. Each genetic element is intact and the sequence of the insert matches the corresponding sequence in PV-GMIR9. In addition, genomic rearrangements at the insertion site were assessed by comparing the insert and flanking sequence to the insertion site in conventional soybean.

The stability of the DNA insert across multiple generations was also demonstrated by Southern blot fingerprint analyses. Five generations of MON 87701 were digested with one of the enzyme sets utilized for the copy number analysis and were hybridized with probes that would detect restriction fragments that encompass the entire insert (two hybridization bands). This fingerprint strategy consists of two border fragments that assay not only the stability of the insert, but also the stability of genomic DNA directly adjacent to the insert.

The results of these experiments are summarized in the genetic elements table listed in Table V-2. The insert matches the T-DNA sequence of PV-GMIR9 starting with the right border of T-DNA I and ending at left border of T-DNA I. The information and results derived from the molecular analyses were used to construct a linear map of the insert in MON 87701. This linear map, shown in Figure V-1 depicts restriction sites identified in the insert and the soybean genomic DNA flanking the insert, and provides information on the expected banding patterns and sizes of the DNA fragments after restriction enzyme digestions. Based on the insert linear map and the plasmid map, a table summarizing the expected DNA fragments for Southern blot analyses is presented in Table V-1. The probes used in the Southern blot analyses and the map of PV-GMIR9 are presented in Figure IV-1. The materials and methods used in the analyses are presented in Appendix B.

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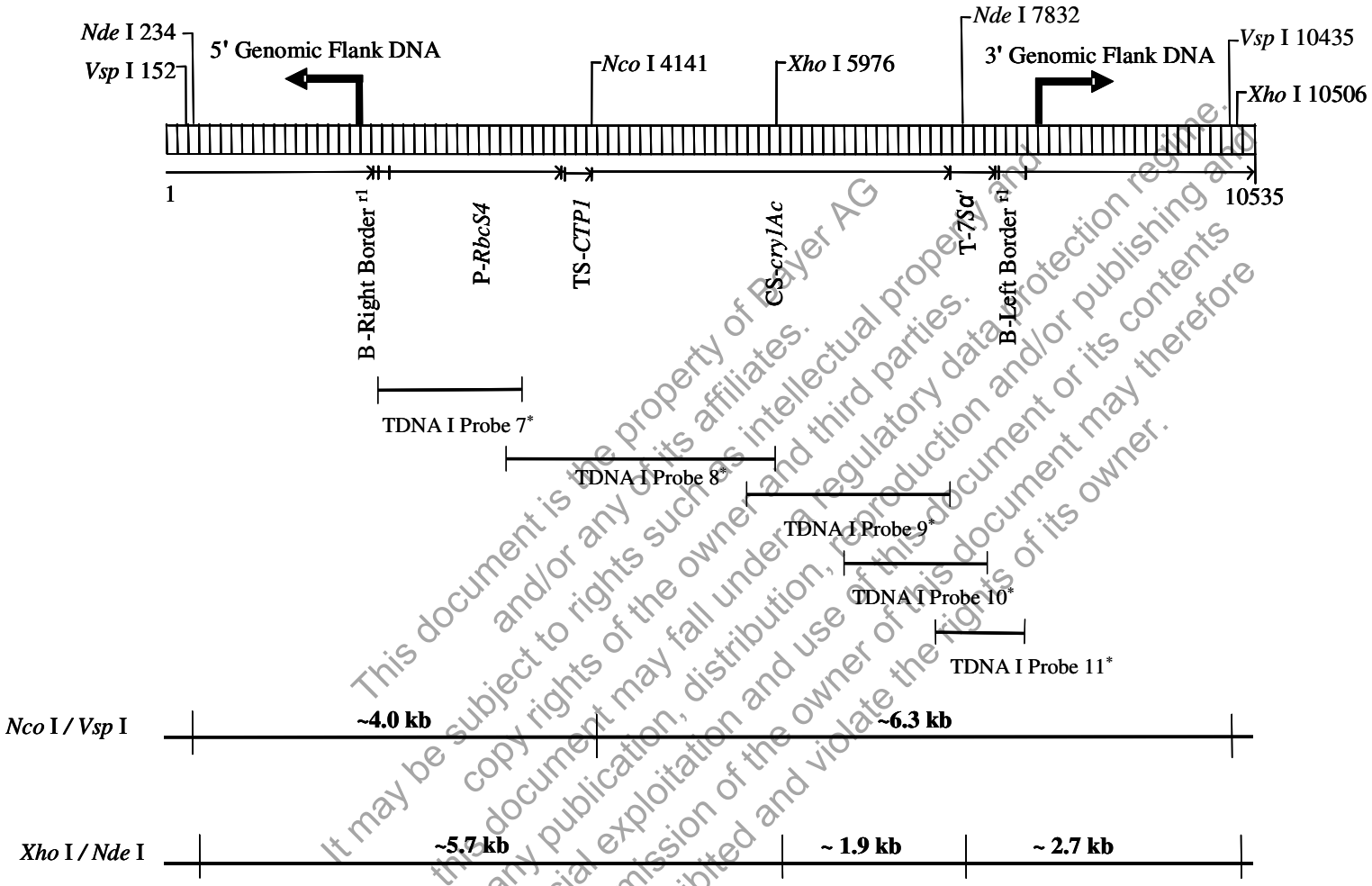

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Figure V-1. Schematic Representation of the Insert and Genomic Flanking Sequence in MON 87701

Linear DNA derived from T-DNA I of vector PV-GMIR9 incorporated into MON 87701. Arrows indicate the end of the insert and the beginning of soybean genomic flanking sequence. Identified on the map are genetic elements within the insert, including restriction sites with positions relative to the size of the genomic flanking sequences and the insert sequence for enzymes used in the Southern analyses.

* These probes are not drawn to scale and are the estimated locations of the T-DNA I probes. Probes are described in Figure IV-1.

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

Probes Used	7	8, 10	9, 11	1, 2, 3	4	5, 6	8
Southern Blot Figure	V-2	V-3	V-4	V-5	V-6	V-7	V-9
Plasmid PV-GMIR9							
***Bgl* II / *Nco* I**	~6.0 kb ~9.5 kb	~6.0 kb ~9.5 kb	~6.0 kb ~9.5 kb	~9.5 kb	~6.0 kb	~6.0 kb ~9.5 kb	~6.0 kb ~9.5 kb
Probe Templates [1]	~~[2]	~1.5 kb ~1.8 kb	~1.2 kb ~1.1 kb	~1.8 kb ~1.5 kb ~1.1 kb	~~[2]	~2.0 kb ~1.2 kb	~1.5 kb
MON 87701							
***Nco* I / *Vsp* I**	~4.0 kb	~4.0 kb ~6.3 kb	~6.3 kb	No band	No band	No band	~6.3 kb ~4.0 kb
Xho I / Nde I	~5.7 kb	~5.7 kb ~1.9 kb	~5.7 kb ~2.7 kb ~1.9 kb	No band	No band	No band	--[3]

[1] probe templates were spiked when multiple probes are used in Southern blot analysis.
[2] '~~' indicates that only the plasmid template was used since the Southern blot was hybridized with one probe.
[3] '--' indicates that the particular restriction enzyme or the combination of enzymes was not used in the analysis.

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Table V-2. Summary of Genetic Elements in MON 87701

Genetic Element	Location in Sequence	Function (Reference)
Sequence flanking 5' end of the insert	1-2000	Soybean genomic DNA
B[1]-Right Border	2001-2045	45 bp DNA region from the right border region remaining after integration (Depicker et al., 1982)
Intervening Sequence	2046-2154	Sequence used in DNA cloning
P[2]- *RbcS4*	2155-3877	Promoter, leader, and 5' non-translated region of the *Arabidopsis thaliana RbcS4* gene encoding ribulose 1,5-bisphosphate carboxylase small subunit 1A (Krebbers et al., 1988)
TS[3]-*CTP1*	3878-4141	Targeting sequence encoding the transit peptide of the *Arabidopsis RbcS4* encoding small subunit 1A transit peptide, from *Arabidopsis thaliana*, present to direct the Cry1Ac protein to the chloroplast, (Krebbers et al., 1988)
CS*[4]*-Cry1Ac	4142-7678	Codon-modified coding sequence of the Cry1Ac protein of *Bacillus thuringiensis* (Fischhoff and Perlak, 1995)
Intervening Sequence	7679-7687	Sequences used in DNA cloning
T[5]-*7S α'*	7688-8126	3' region of the *Sphas1* gene of *Glycine max* encoding the 7S *α*' seed storage protein, β-conglycinin, including 35 nucleotides of the carboxyl terminal β-conglycinin coding region with the termination codon and the polyadenylation sequence (Schuler et al., 1982). The element functions to terminate transcription and direct polyadenylation of the mRNA.
Intervening Sequence	8127-8162	Sequence used in DNA cloning
B[1]-Left Border	8163-8426	264 bp DNA region from the left border region remaining after integration (Barker et al., 1983)
Sequence flanking 3' end of the insert	8427-10535	Soybean genomic DNA

[1]B – Border; [2]P – Promoter; [3]TS – Targeting Sequence; [4]CS – Coding Sequence [5]T –3' non-translated transcriptional termination sequence and polyadenylation signal sequence.

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V.A. Insert and Copy Number of T-DNA I Sequence

The number of copies and insertion sites of T-DNA I sequences in the soybean genome were evaluated by digesting the test and control genomic DNA samples with the two enzyme sets *Nco* I / *Vsp* I and *Xho* I / *Nde* I, which cleave within the insert and known flanking sequences. The enzymes used generate a restriction fragment containing T-DNA I and adjacent plant genomic DNA with a unique banding pattern. If T-DNA I sequences are present at a single integration site in MON 87701 then probing with the sequence from T-DNA I should result in the restriction fragments described in Figure V-1 and Table V-1. Any additional integration sites would be detected as additional bands. The blots were hybridized with overlapping T-DNA I probes spanning the entire inserted DNA sequence (Probes 7-11, Figure IV-1). Each Southern blot contained several controls. Genomic DNA isolated from the conventional soybean control, A5547, was used as a negative control to determine if the probes hybridized to any endogenous sequences. Conventional soybean spiked with either plasmid DNA or probe template was used as a positive hybridization control and to demonstrate sensitivity of the Southern blot. The results of these analyses are shown in Figures V-2 through V-4.

V.A.1. T-DNA I Probe 7

Conventional soybean DNA digested with *Nco* I / *Vsp* I (Figure V-2, lanes 1 and 8) or *Xho* I / *Nde* I (Figure V-2, lanes 3 and 10) and hybridized with Probe 7 (Figure IV-1) showed no detectable hybridization bands as expected for the negative control. Conventional soybean DNA spiked with plasmid PV-GMIR9 DNA, previously digested with *Nco* I / *Bgl* II, (Figure IV-1, Probe 7) produced the expected bands at ~6.0 kb and ~9.5 kb (Figure V-2, lane 7). In Figure V-2, lane 6, the ~0.1 genome equivalent spike produced the expected band at ~6.0 kb, but the ~9.5 kb band is too faint to identify, since only a small portion of probe 7, which spans the right border region, has homology to the 9.5 kb portion of the vector. The ability to detect the spiked controls indicates that the probes are recognizing their target sequences.

MON 87701 DNA digested with *Nco* I / *Vsp* I (Figure V-2, lanes 2 and 9) and hybridized to Probe 7 is expected to produce one band at ~ 4.0 kb. The long run (Figure V-2, lane 2) produced a single band at ~4.1 kb (at or above the 4.1 kb marker) and the short run (Figure V-2, lane 9) also produced a single band of the correct size. MON 87701 DNA digested with *Xho* I / *Nde* I (Figure V-2, lanes 4 and 11) and hybridized with Probe 7 is expected to produce a single band of ~5.7 kb. The long run (Figure V-2, lane 4) produced a single band at ~6.2 kb (at or above the 6.1 kb marker) and the short run (Figure V-2, lane 11) produced a single band at ~5.7 kb. The apparent shift in migration of the bands in the long run versus the short run can be attributed to the method used to record the molecular weight markers on the agarose gel and on the autoradiograph and does not alter the conclusion that a single band was detected of the correct size. Thus, there is a single detectable insert containing Probe 7 sequences. The results presented in Figure V-2 indicate that the sequence covered by Probe 7 resides at a single detectable locus of integration in MON 87701.

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V.A.2. T-DNA I Probes 8 and 10

Conventional soybean DNA digested with *Nco* I / *Vsp* I (Figure V-3, lanes 1 and 8) or *Xho* I / *Nde* I (Figure V-3, lanes 3 and 10) and hybridized with Probes 8 and 10 (Figure IV-1) showed no detectable hybridization bands, as expected for the negative control. Pre-digested conventional soybean DNA spiked with probe template (Figure IV-1, Probes 8 and 10) generated from plasmid PV-GMIR9 produced the expected bands at ~1.5 kb, and ~1.8 kb (Figure V-3, lanes 5 and 6). In lane 6, there is a faint band at ~3.6 kb, which likely represents a minor PCR artifact that was generated during probe template preparation. Conventional soybean DNA spiked with plasmid PV-GMIR9 DNA previously digested with *Nco* I / *Bgl* II produced the two expected bands at ~6.0 kb and ~9.5 kb (Figure V-3, lane 7). Detection of the spiked controls indicates that the probes are recognizing their target sequences.

MON 87701 DNA digested with *Nco* I / *Vsp* I and hybridized with Probes 8 and 10 (Figure V-3, lanes 2 and 9) produced two bands. The ~4.0 kb band is the expected size for the border fragment containing the 5' end of the inserted DNA (T-DNA I) and the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~6.3 kb band represents the 3' border fragment containing the 3' end of the inserted DNA and the adjacent genomic DNA flanking the 3' end of the insert. MON 87701 DNA digested with *Xho* I / *Nde* I (Figure V-3, lanes 4 and 11) produced two bands. The ~5.7 kb band observed in Figure V-3 (lanes 4 and 11) is the expected size for the border fragment containing the 5' end of the inserted DNA (T-DNA I) and the adjacent genomic DNA flanking the 5' end of the insert (Figure V-1). The ~1.9 kb band observed in Figure V-3 (lanes 4 and 11) represents an internal fragment contained in the inserted T-DNA. The results presented in Figure V-3 indicate that sequence covered by Probes 8 and 10 resides at a single detectable locus of integration in MON 87701.

V.A.3. T-DNA I Probes 9 and 11

Conventional soybean DNA digested with *Nco* I / *Vsp* I (Figure V-4, lanes 2 and 11) or digested with *Xho* I / *Nde* I (Figure V-4, lanes 4 and 13) and hybridized with Probes 9 and 11 (Figure IV-1) produced several hybridization signals. This was expected as the 7S α' 3' non-translated region genetic element within T-DNA I was originally isolated from soybean. These hybridization signals result from the probes hybridizing to endogenous targets residing in the soybean genome and are not specific to the inserted DNA. These signals were produced in all lanes, including those containing the conventional soybean DNA material and, therefore, are considered to be endogenous background hybridization. Pre-digested conventional soybean DNA spiked with probe template (Figure IV-1, Probes 9 and 11) generated from plasmid PV-GMIR9 produced the expected bands at ~ 1.2 kb for probe template 9 (Figure V-4, lanes 5 and 6) and ~1.1 kb for probe template 11 (Figure V-4, lanes 7 and 8). Conventional soybean DNA spiked with plasmid PV-GMIR9 DNA previously digested with *Nco* I / *Bgl* II produced the two bands at ~6.0 kb and ~9.5 kb (Figure V-4, lane 9). Detection of the spiked controls indicates that the probes are recognizing their target sequences. There is non-specific hybridization at the bottom of the blot that spans lanes 5-13. This region of the blot

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corresponds to the short run and genomic DNA was not that far in the gel. It is clear when observing the blot that no bands are discernable within this region.

MON 87701 DNA digested with *Nco* I / *Vsp* I and hybridized with Probes 9 and 11 (Figure V-4, lanes 1 and 10) produced one unique band in addition to the endogenous background hybridization. The ~6.3 kb band is the expected size for the border fragment containing the 3' end of the inserted DNA (T-DNA I) and the adjacent genomic DNA flanking the 3' end of the insert (Figure V-1). MON 87701 DNA digested with *Xho* I/ *Nde* I (Figure V-4, lanes 3 and 12) produced three unique bands, as expected, in addition to the endogenous background hybridization. The expected band at ~5.7 kb migrated together with an endogenous hybridization signal observed in Figure V-4, lanes 3 and 12. The ~5.7 kb band represents the 5' border fragment containing the 5' end of the inserted DNA along with the adjacent genomic DNA flanking the 5' end of the insert. The ~1.9 kb band represents a portion of the *cry1Ac* expression cassette. The ~2.7 kb band represents the 3' border fragment containing the 3' end of the inserted DNA and the adjacent genomic DNA flanking the 3' end of the insert. The results presented in Figure V-4 indicate that sequences covered by Probes 9 and 11 reside at a single detectable locus of integration in MON 87701. Taken together, the data presented in Figures V-2, V-3, and V-4 indicate that MON 87701 contains a single copy of T-DNA I at a single insertion site.

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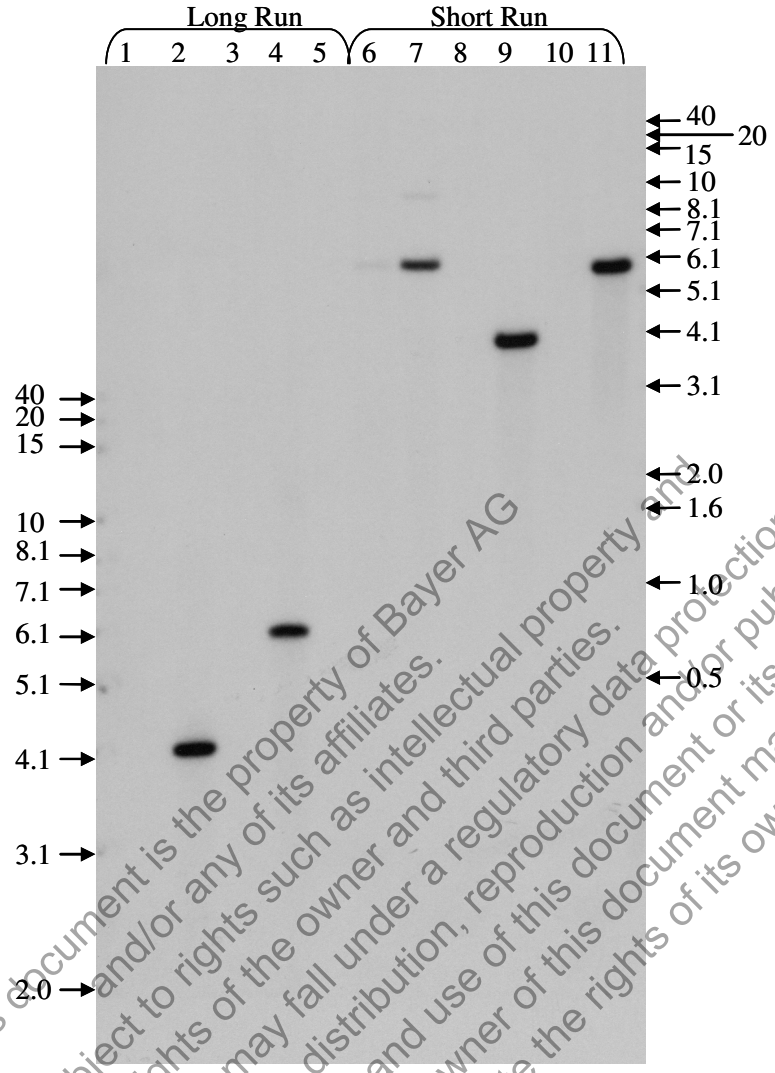

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Figure V-2. Southern Blot Analysis of MON 87701: T-DNA I Copy Number Analysis (Probe 7)

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA I sequence (Figure IV-1, Probe 7). Each lane contains ~10 µg of digested genomic DNA isolated from soybean leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I / *Vsp* I)
2: MON 87701 (*Nco* I / *Vsp* I)
3: Conventional soybean (*Xho* I / *Nde* I)
4: MON 87701 (*Xho* I / *Nde* I)
5: Blank
6: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~0.1 genome equivalent]
7: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~1.0 genome equivalent]
8: Conventional soybean (*Nco* I / *Vsp* I)
9: MON 87701 (*Nco* I / *Vsp* I)
10: Conventional soybean (*Xho* I / *Nde* I)
11: MON 87701 (*Xho* I / *Nde* I)

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

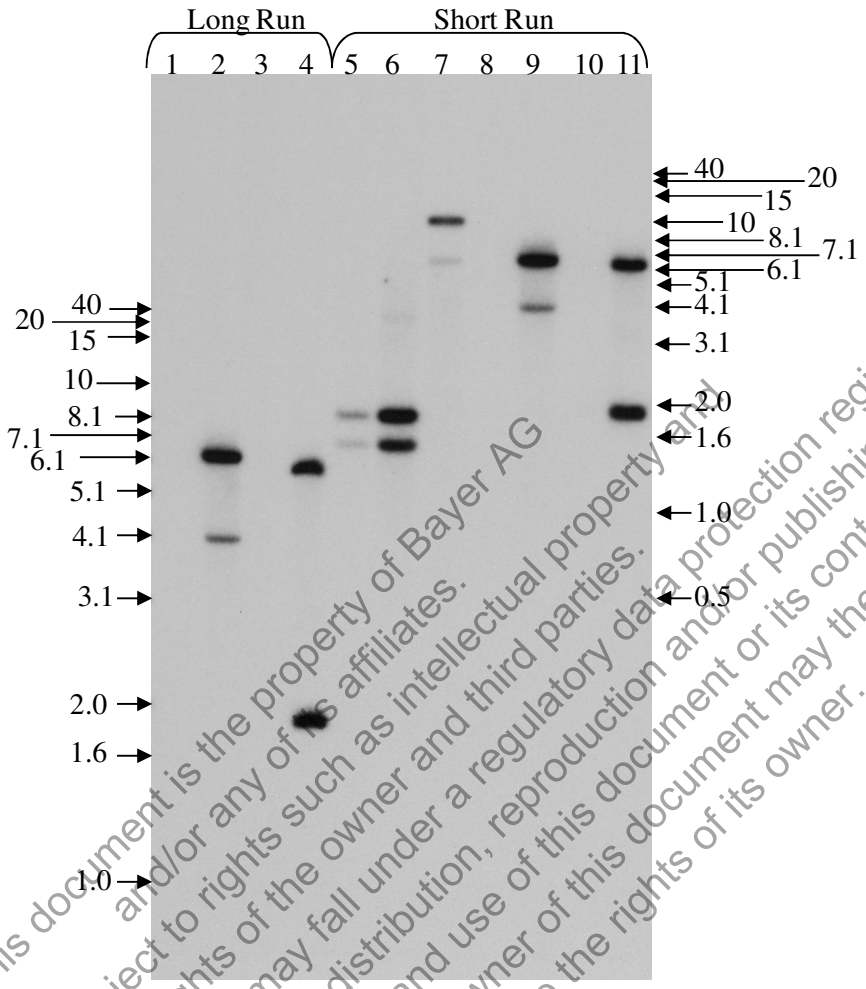

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Figure V-3. Southern Blot Analysis of MON 87701: T-DNA I Copy Number Analysis (Probe 8 and Probe 10)

The blot was hybridized with two ^{32}P-labeled probes that spanned portions of the T-DNA I sequence (Figure IV-1, Probes 8 and 10). Each lane contains ~10 µg of digested genomic DNA isolated from soybean leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I / *Vsp* I)
2: MON 87701 (*Nco* I / *Vsp* I)
3: Conventional soybean (*Xho* I / *Nde* I)
4: MON 87701 (*Xho* I / *Nde* I)
5: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~0.1 genome equivalent]
6: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~1.0 genome equivalent]
7: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~1.0 genome equivalent]
8: Conventional soybean (*Nco* I / *Vsp* I)
9: MON 87701 (*Nco* I / *Vsp* I)
10: Conventional soybean (*Xho* I / *Nde* I)
11: MON 87701 (*Xho* I / *Nde* I)

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

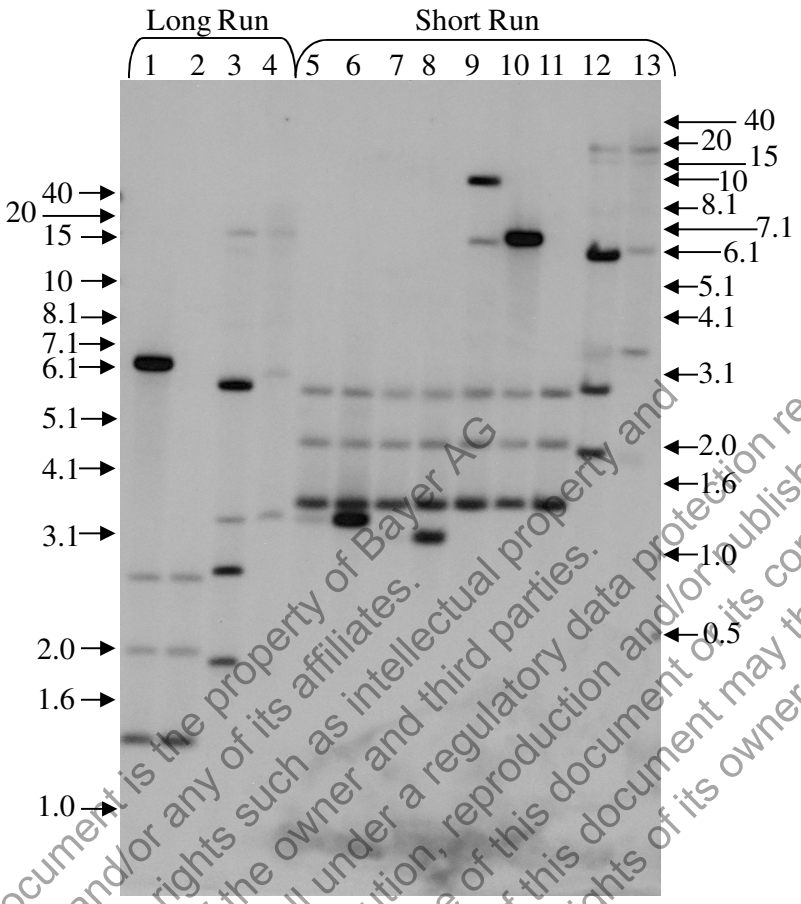

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Figure V-4. Southern Blot Analysis of MON 87701: T-DNA I Copy Number Analysis (Probe 9 and Probe 11)

The blot was hybridized with two ^{32}P-labeled probes that spanned portions of the T-DNA I sequence (Figure IV-1, Probes 9 and 11). Each lane contains ~10 µg of digested genomic DNA isolated from soybean leaf tissue. Lane designations are as follows:

Lane 1: MON 87701 (*Nco* I / *Vsp* I)
2: Conventional soybean (*Nco* I / *Vsp* I)
3: MON 87701 (*Xho* I / *Nde* I)
4: Conventional soybean (*Xho* I / *Nde* I)
5: Conventional soybean spiked with probe 9 template (*Nco* I / *Vsp* I) [~0.1 genome equivalent]
6: Conventional soybean spiked with probe 9 template (*Nco* I / *Vsp* I) [~1.0 genome equivalent]
7: Conventional soybean spiked with probe 11 template (*Nco* I / *Vsp* I) [~0.1 genome equivalent]
8: Conventional soybean spiked with probe 11 template (*Nco* I / *Vsp* I) [~1.0 genome equivalent]
9: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl II*) [~1.0 genome equivalent]
10: MON 87701 (*Nco* I / *Vsp* I)
11: Conventional soybean (*Nco* I / *Vsp* I)
12: MON 87701 (*Xho* I / *Nde* I)
13: Conventional soybean (*Xho* I / *Nde* I)

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

V.B. Southern Blot Analysis to Determine the Presence or Absence of Plasmid PV-GMIR9 Backbone

The presence or absence of plasmid backbone sequences in the soybean genome was evaluated by digesting the test and control genomic DNA samples with *Nco* I / *Vsp* I or *Xho* / *Nde* I and hybridizing with backbone probes spanning the entire backbone sequence of PV-GMIR9 (Figure IV-1, Probes 1, 2, 3, and 4). If backbone sequences are present in MON 87701, then probing with backbone sequences should result in hybridizing bands. The results of this analysis are shown in Figures V-5 and V-6. Each Southern blot contains the same controls as described in Section V.A.

V.B.1. Plasmid Backbone Probe 1, Probe 2, and Probe 3

Conventional soybean control DNA digested with *Nco* I / *Vsp* I (Figure V-5, lanes 1 and 8) or *Xho* I / *Nde* I (Figure V-5, lanes 3 and 10) and hybridized simultaneously with overlapping probes spanning most of the vector backbone of PV-GMIR9 (Figure IV-1, Probes 1, 2, and 3) showed no detectable hybridization bands, as expected for the negative control. Pre-digested conventional soybean DNA spiked with probe template (Figure IV-1, Probes 1, 2, and 3) generated from plasmid PV-GMIR9 produced the expected bands at ~1.5 kb, ~1.8 kb, and ~1.1 kb, respectively (Figure V-5, lanes 5 and 6). Conventional soybean DNA spiked with plasmid PV-GMIR9 DNA previously digested with *Bgl* II / *Nco* I produced the expected size band of ~9.5 kb (Figure V-5, lane 7). Detection of the spiked controls indicates that the probes are recognizing their target sequences.

MON 87701 DNA digested with *Nco* I / *Vsp* I (Figure V-5, lanes 2 and 9) or *Xho* I / *Nde* I (Figure V-5, lanes 4 and 11) and hybridized with Probes 1, 2, and 3 produced no detectable bands. There is a diffuse area of hybridization that overlaps with lane 4. Because this hybridization is not a distinct band, nor is it present in lane 11, which contains the same enzyme set, this area of hybridization is considered non-specific binding. These data indicate that MON 87701 contains no backbone elements from PV-GMIR9 that overlaps Probes 1, 2, and 3.

V.B.2. Plasmid Backbone Probe 4

Conventional soybean control DNA digested with *Nco* I / *Vsp* I (Figure V-6, lanes 1 and 7) or *Xho* I / *Nde* I (Figure V-6, lanes 3 and 9) and hybridized with Probe 4 from the vector backbone of PV-GMIR9 (Figure IV-1, Probe 4) showed no detectable hybridization bands as expected for the negative control. Conventional soybean DNA spiked with plasmid PV-GMIR9 DNA previously digested with *Bgl* II / *Nco* I produced the expected band at ~6.0 kb (Figure V-6, lanes 5 and 6). Detection of the spiked controls indicates that the probes are recognizing their target sequences.

MON 87701 DNA digested with *Nco* I / *Vsp* I (Figure V-6, lanes 2 and 8) or *Xho* I / *Nde* I (Figure V-6, lanes 4 and 10) and hybridized with Probe 4 produced no detectable hybridization bands, indicating that MON 87701 contains no detectable PV-GMIR9 backbone elements that are contained within Probe 4. These data, in combination with

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the data presented in Section V.B.1, indicate that MON 87701 contains no detectable PV-GMIR9 backbone elements.

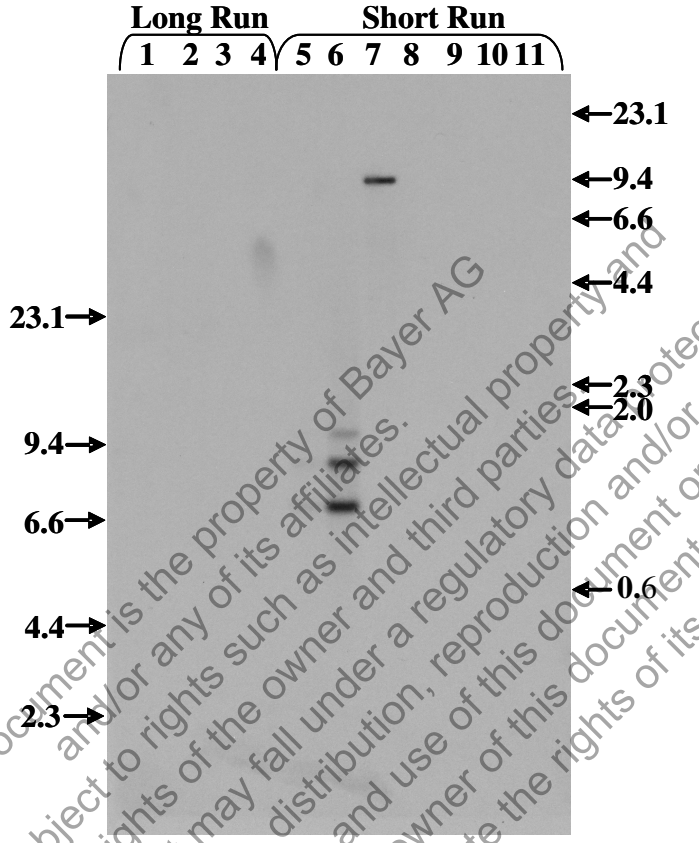

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Figure V-5. Southern Blot Analysis of MON 87701: PV-GMIR9 Backbone Sequence (Probe 1, Probe 2, and Probe 3)

The blot was hybridized with three overlapping ^{32}P-labeled probes that spanned a portion of the PV-GMIR9 backbone sequence (Figure IV-1, Probes 1, 2, and 3). Each lane contains ~10 µg of digested genomic DNA isolated from soybean leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I / *Vsp* I)
2: MON 87701 (*Nco* I / *Vsp* I)
3: Conventional soybean (*Xho* I / *Nde* I)
4: MON 87701 (*Xho* I / *Nde* I)
5: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~0.1 genome equivalent]
6: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~1.0 genome equivalent]
7: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~1.0 genome equivalent]
8: Conventional soybean (*Nco* I / *Vsp* I)
9: MON 87701 (*Nco* I / *Vsp* I)
10: Conventional soybean (*Xho* I / *Nde* I)
11: MON 87701 (*Xho* I / *Nde* I)

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

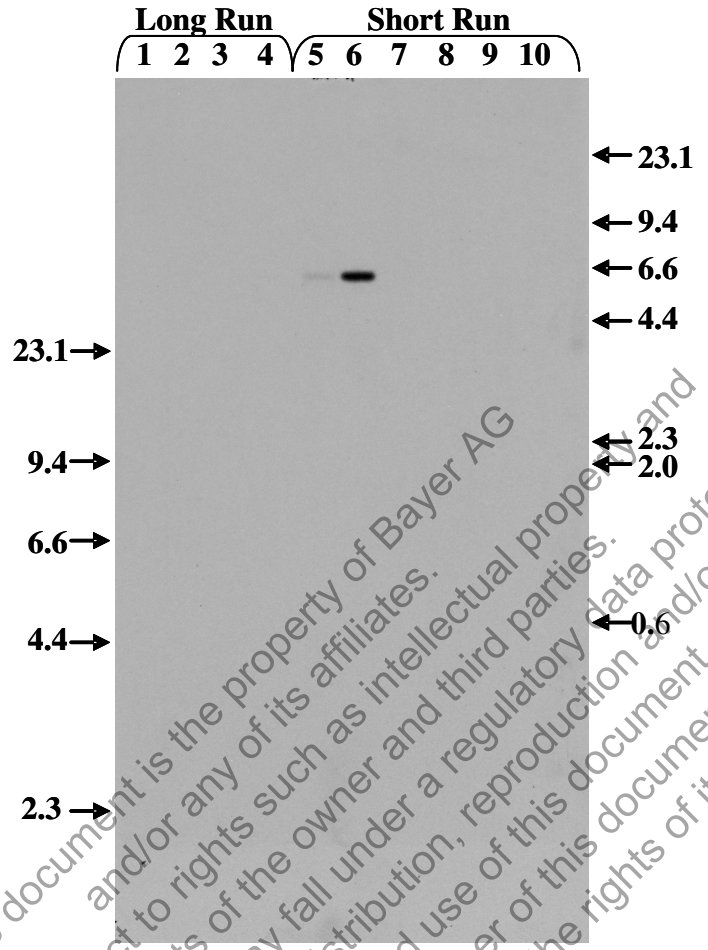

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Figure V-6. Southern Blot Analysis of MON 87701: PV-GMIR9 Backbone Sequence (Probe 4)

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the PV-GMIR9 backbone sequence (Figure IV-1, Probe 4). Each lane contains ~10 µg of digested genomic DNA isolated from soybean leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I / *Vsp* I)
2: MON 87701 (*Nco* I / *Vsp* I)
3: Conventional soybean (*Xho* I / *Nde* I)
4: MON 87701 (*Xho* I / *Nde* I)
5: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~0.1 genome equivalent]
6: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~1.0 genome equivalent]
7: Conventional soybean (*Nco* I / *Vsp* I)
8: MON 87701 (*Nco* I / *Vsp* I)
9: Conventional soybean (*Xho* I / *Nde* I)
10: MON 87701 (*Xho* I / *Nde* I)

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

V.C. Southern Blot Analysis to Determine the Presence or Absence of T-DNA II

The presence or absence of T-DNA II sequences in the soybean genome was evaluated by digesting MON 87701 and control genomic DNA samples with the *Nco* I / *Vsp* I or *Xho* I / *Nde* I enzyme sets and hybridizing with overlapping T-DNA II probes spanning the entire T-DNA II sequence of PV-GMIR9 (Figure IV-1, Probes 5 and 6). The left and right border regions of T-DNA II share 100% homology with those of T-DNA I and thus were not included in the T-DNA II analysis. If T-DNA II sequences are present in MON 87701, then probing with the T-DNA II sequences should result in hybridizing bands. The results of this analysis are shown in Figure V-7. The Southern blot contained the same controls as described in Section V.A.

V.C.1. T-DNA II Probe 5 and Probe 6

Conventional soybean DNA digested with *Nco* I / *Vsp* I (Figure V-7, lanes 1 and 9) or *Xho* I / *Nde* I (Figure V-7, lanes 3 and 11) and hybridized with Probes 5 and 6 (Figure IV-1) showed no detectable hybridization bands, as expected for the negative control. Pre-digested conventional soybean DNA spiked with probe template generated from plasmid PV-GMIR9 produced the expected bands at ~2.0 kb and ~1.2 kb (Figure V-7, lanes 6 and 7). Conventional soybean DNA spiked with plasmid PV-GMIR9 DNA previously digested with *Bgl* II/ *Nco* I produced the expected size bands of ~6.0 kb and ~9.5 kb (Figure V-7, lane 8). Detection of the spiked controls indicates that the probes are recognizing their target sequences.

MON 87701 DNA digested with *Nco* I/ *Vsp* I (Figure V-7, lanes 2 and 10) or *Xho* I / *Nde* I (Figure V-7, lanes 4 and 12) produced no hybridization bands. These results indicate that MON 87701 contains no detectable T-DNA II elements.

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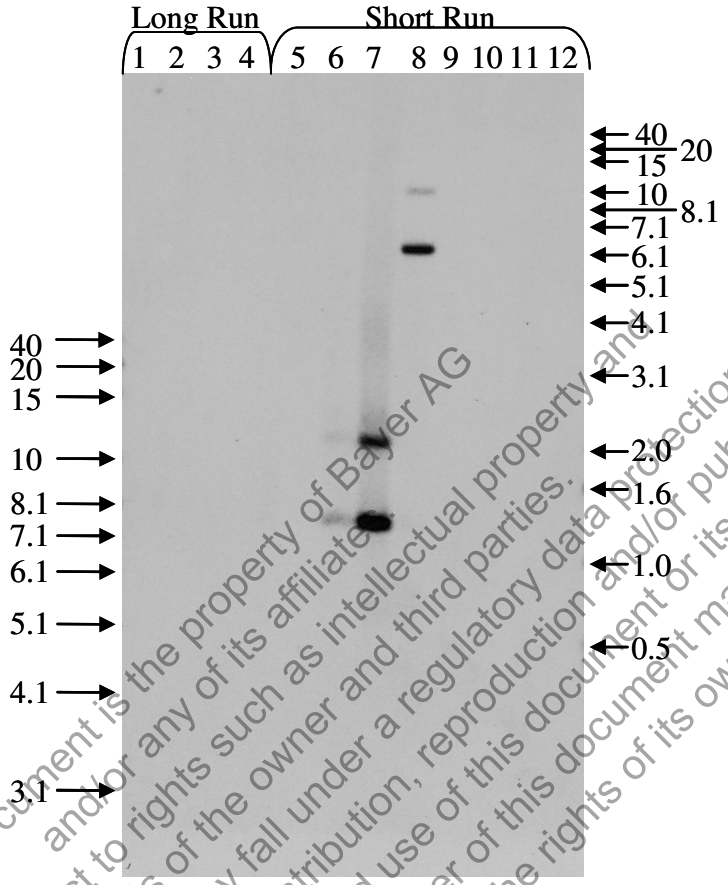

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Figure V-7. Southern Blot Analysis of MON 87701: T-DNA II (Probe 5 and Probe 6)

The blot was hybridized with overlapping ^{32}P-labeled probes that spanned the T-DNA II sequence (Figure IV-1, Probes 5 and 6). Each lane contains ~10 µg of digested genomic DNA isolated from soybean leaf tissue. Lane designations are as follows:

Lane 1: Conventional soybean (*Nco* I / *Vsp* I)
2: MON 87701 (*Nco* I / *Vsp* I)
3: Conventional soybean (*Xho* I / *Nde* I)
4: MON 87701 (*Xho* I / *Nde* I)
5: Blank
6: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~0.1 genome equivalent]
7: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~1.0 genome equivalent]
8: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl II*) [~1.0 genome equivalent]
9: Conventional soybean (*Nco* I / *Vsp* I)
10: MON 87701 (*Nco* I / *Vsp* I)
11: Conventional soybean (*Xho* I / *Nde* I)
12: MON 87701 (*Xho* I / *Nde* I)

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

V.D. Organization and Sequence of the Insert and Adjacent Genomic DNA in MON 87701

The organization of the elements within the MON 87701 insert was confirmed by DNA sequence analyses. Several PCR primers were designed with the intent to amplify nine overlapping regions of DNA that span the entire length of the insert (see Appendix B). The amplified DNA fragments were subjected to DNA sequencing analyses. The DNA sequence of the MON 87701 insert is 6426 base pairs long, beginning at base 3908 of PV-GMIR9 located in the right border region and ending at base 10333 in the left border region of PV-GMIR9. A sequence comparison between the PCR product generated from the conventional soybean and the sequence generated from the 5' and 3' flanking sequences of MON 87701 indicate there was a 32 bp deletion (bases 1441-1472) and a 14 bp insertion (bases 1987-2000) just 5' to the MON 87701 insertion site. This molecular rearrangement likely occurred in the plant during the *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). This analysis confirms that the DNA sequences flanking the 5' and 3' ends of the insert in MON 87701 are native to the soybean genome and that no major unexpected rearrangements occurred during the transformation to produce MON 87701. Results also confirm that the arrangement of the genetic elements in MON 87701 is identical to that in plasmid PV-GMIR9 and is depicted in Figure V-1 and Table V-2.

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V.E. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87701

In order to demonstrate the stability of T-DNA in MON 87701, Southern blot analyses were performed using DNA obtained from multiple generations of MON 87701. For reference, the breeding history of MON 87701 is presented in Figure V-8. The specific generations tested are identified in the legend of Figure V-9. The R_5 generation was used for the molecular characterization analyses shown in Figures V-2 through V-7. To analyze stability, four additional generations were evaluated by Southern blot analysis and compared to the R_5 generation. DNA, isolated from each of the selected generations of MON 87701, were digested with the restriction enzymes *Nco* I / *Vsp* I (Figure V-1) and hybridized with Probe 8 (Figure IV-1). Probe 8 is designed to detect both fragments generated by the *Nco* I / *Vsp* I digest. Any instability associated with the insert would be detected as novel bands within the fingerprint on the Southern blot. The results are shown in Figure V-9. The Southern blot has the same controls as described in Section V.A.

Conventional soybean DNA digested with *Nco* I / *Vsp* I (Figure V-9, lane 4) and hybridized with Probe 8 showed no detectable hybridization bands, as expected for the negative control. Pre-digested conventional soybean DNA spiked with probe template (Figure IV-1, Probe 8) generated from plasmid PV-GMIR9 produced the expected band at ~1.5 kb (Figure V-9, lanes 1 and 2). Pre-digested conventional soybean DNA spiked with plasmid PV-GMIR9 DNA, previously digested with *Bgl* II / *Nco* I, produced the expected size bands of ~6.0 kb and ~9.5 kb (Figure V-9, lane 3). Detection of the spiked controls indicates that the probes are recognizing their target sequences.

DNA extracted from MON 87701 generations R_4, R_5, R_6, R_8, and R_9 digested with *Nco* I / *Vsp* I (Figure V-9, lanes 5-9) and hybridized with Probe 8 each produced two bands of ~6.3 kb and ~4.0 kb. The ~4.0 kb band is the expected size for the 5' border fragment and the ~6.3 kb band is consistent with the expected size of the 3' border fragment. These bands are consistent with the bands detected in Figure V-3 (lanes 2 and 9) indicating that the single copy of T-DNA I in MON 87701 is stably maintained across multiple generations.

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Figure V-8. Breeding History for MON 87701

All generations were self-pollinated (⊗). The R_5 generation seed material was used for regulatory molecular characterization and commercial development. Seed lots from the R_4, R_5, R_6, R_8, and R_9 generations were used in the molecular generational stability analysis.

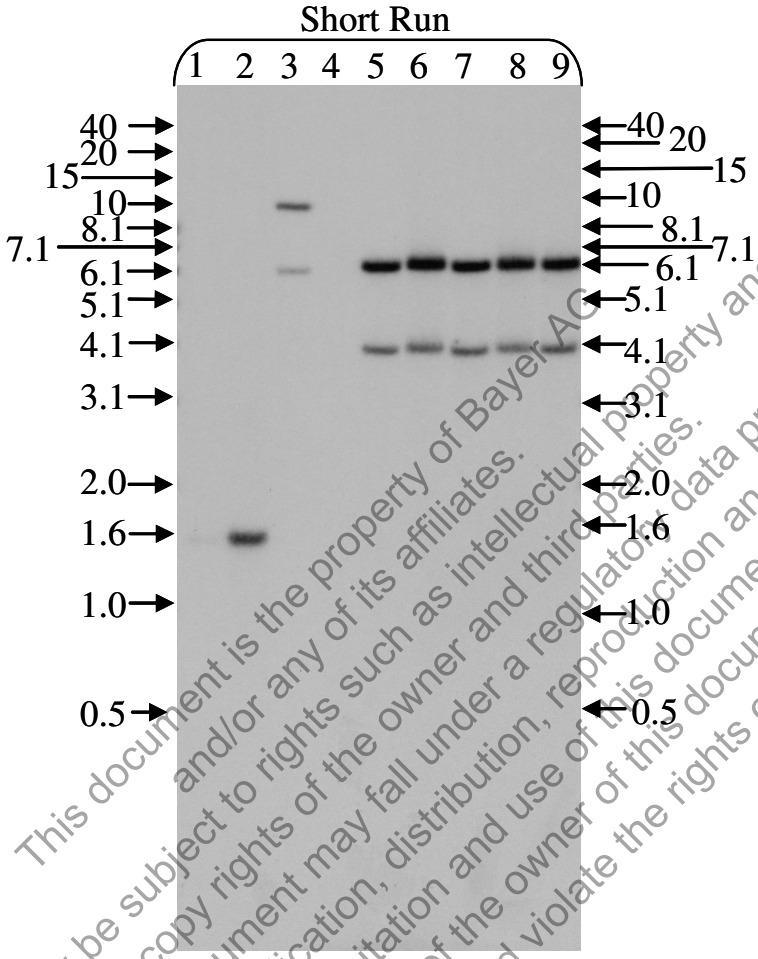

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Figure V-9. Insert Stability of MON 87701: T-DNA I (Probe 8)

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

Lane 1: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~0.1 genome equivalent]
2: Conventional soybean spiked with probe templates (*Nco* I / *Vsp* I) [~1.0 genome equivalent]
3: Conventional soybean (*Nco* I / *Vsp* I) spiked with PV-GMIR9 (*Nco* I / *Bgl* II) [~1.0 genome equivalent]
4: Conventional soybean (*Nco* I / *Vsp* I)
5: MON 87701 (R_4) (*Nco* I / *Vsp* I)
6: MON 87701 (R_5) (*Nco* I / *Vsp* I)
7: MON 87701 (R_6) (*Nco* I / *Vsp* I)
8: MON 87701 (R_8) (*Nco* I / *Vsp* I)
9: MON 87701 (R_9) (*Nco* I / *Vsp* I)

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

V.F. Inheritance of the Genetic Insert in MON 87701

During development of MON 87701, segregation data were recorded to assess the heritability and stability of the *cry1Ac* gene in MON 87701. Chi-square analysis was performed over several generations to confirm the segregation and stability of the *cry1Ac* gene in MON 87701. The Chi-square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The breeding path for generating segregation data for MON 87701 is described in Figure V-10. The transformed R_0 plant was self-pollinated to produce R_1 seed. This seed was planted and the resulting R_1 plants were expected to segregate in a 15:1 ratio of positive to negative individual plants for the insect-protected phenotype. The 15:1 segregation ratio is expected because the *cry1Ac* gene was inserted into the soybean genome (R_0 plant) at two independently segregating loci. An individual plant (#55, designated as MON 87701) homozygous for a single copy of the *cry1Ac* gene was identified from the R_1 segregating population, via Taqman PCR.

The selected R_1 MON 87701 plant was self-pollinated to give rise to a population of R_2 plants that were repeatedly self-pollinated through the R_5 generation. At each generation, the fixed homozygous plants were tested for the expected segregation pattern of 1:0 (positive : negative) for the *cry1Ac* gene using Taqman PCR analysis or for the presence of the Cry1Ac protein via ELISA analysis and/or protein specific lateral flow strips.

At the R_5 generation, homozygous MON 87701 soybean plants were bred via traditional breeding (bi-parental cross) with a soybean variety that did not contain the *cry1Ac* gene. The resulting F_1 plants were then self-pollinated to produce F_2 seed. The subsequent F_2 plants were tested for the presence of the MON 87701 insert by Taqman PCR using an event-specific assay. These plants were predicted to segregate at a 1:2:1 (homozygous positive : hemizygous positive : homozygous negative) ratio according to Mendelian inheritance principles.

The heritability and stability of the *cry1Ac* gene in MON 87701 were further tested in the F_3 generation. Hemizygous positive F_2 plants were selected and self-pollinated to produce F_3 seed. The resulting F_3 plants were tested for the presence of MON 87701 by Taqman PCR using an event-specific zygosity assay. The F_3 generation was predicted to segregate at a 1:2:1 (homozygous positive : hemizygous positive : homozygous negative) ratio.

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

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

where o = observed frequency of the genotype (if PCR used) or phenotype (if ELISA used) and e = expected frequency of the genotype or phenotype. The level of statistical significance was predetermined to be 5% ($p \leq 0.05$).

The results of the χ^2 analysis of the segregating progeny of MON 87701 are presented in Tables V-3 and V-4. The χ^2 value in the R_1 generation indicated no statistically significant difference between the observed and expected 15:1 segregation ratio. The

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insect-protected trait was subsequently fixed in the R_2, R_3, R_4, and R_5 generations and no further segregation occurred in the generations, as expected. Following the crossing of the R_5 generation with a soybean variety that did not contain the *cry1Ac* gene, the resulting F_2 and F_3 progeny were assessed for their heritability of the *cry1Ac* gene. The χ^2 values in the F_2 and F_3 generations indicated no statistically significant difference between the observed and expected segregation ratios. These results support the conclusion that the *cry1Ac* gene in MON 87701 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 87701 contains a single, intact copy of the *cry1Ac* expression cassette that was inserted into the soybean genome at a single locus.

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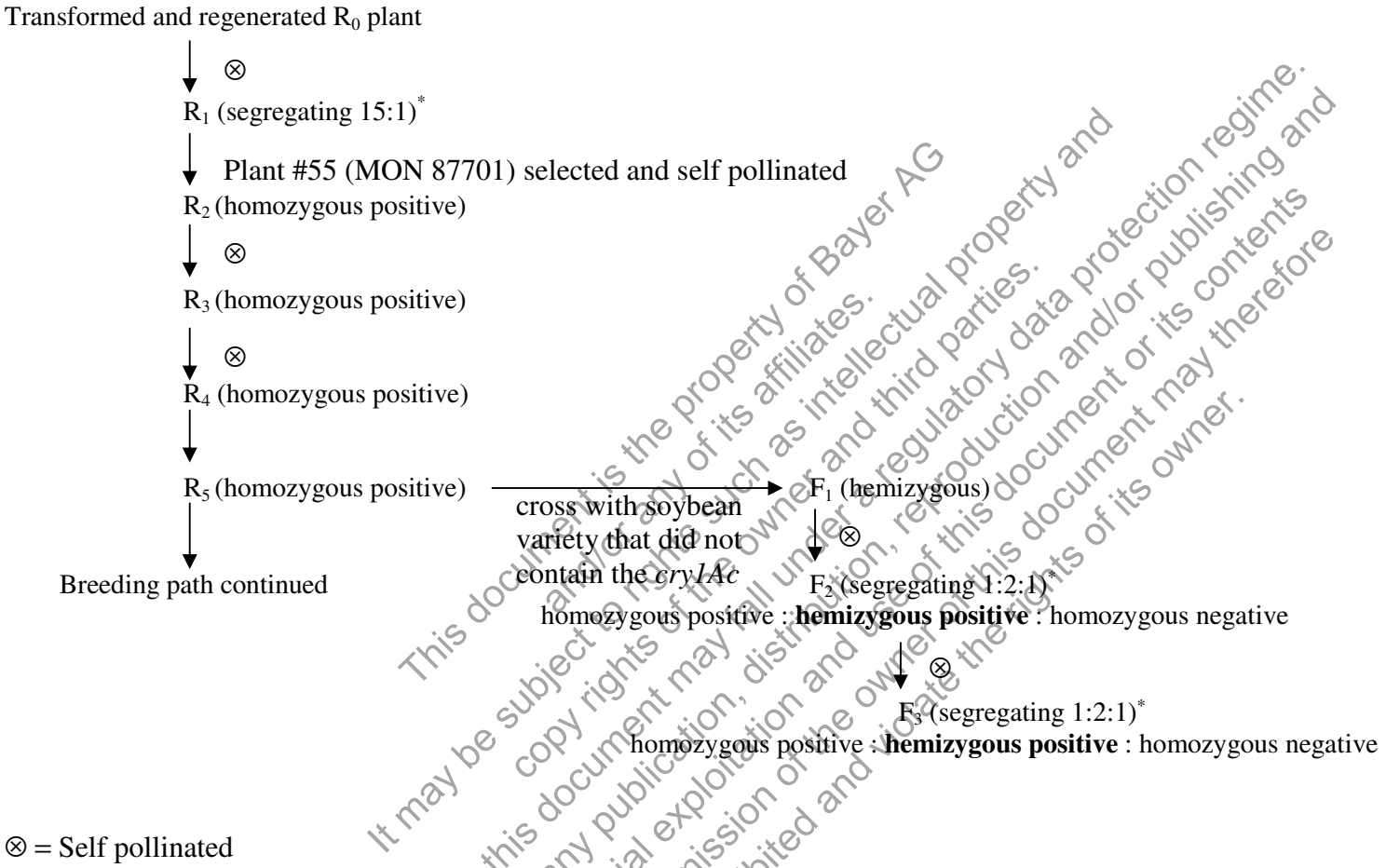

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Figure V-10. Breeding Path for Generating Segregation Data MON 87701

* Chi-square analysis conducted on segregation data from the R_1, F_2, and F_3 generations.

Table V-3. Segregation of the *cry1Ac* Gene during the Development of MON 87701

					Segregation			
Generation	Expected Ratio	Total Plants Tested[1]	Observed # Plants Positive	Observed # Plants Negative	Expected # Plants Positive	Expected # Plants Negative	χ^2	Probability
R_1	15:1	19	18	1	17.8	1.2	0.03	0.8590
R_2	1:0	80	80	0	80	0	Fixed	N/A
R_3	1:0	48	48	0	48	0	Fixed	N/A
R_4	1:0	598	598	0	598	0	Fixed	N/A
R_5	1:0	629	629	0	629	0	Fixed	N/A

[1] Plants were tested for the presence of the *cry1Ac* gene by protein check strips, ELISA analysis, and/or Taqman PCR.
N/A = Not applicable

Table V-4. Segregation of the *cry1Ac* Gene in F_2 and F_3 Progeny from a Cross of MON 87701 with a Soybean Variety that did not Contain the *cry1Ac* Gene

					1:2:1 Segregation				
Generation[1]	Total Plants Tested[2]	Observed # Plants Homozygous Positive	Observed # Plants Hemizygous Positive	Observed # Plants Homozygous Negative	Expected # Plants Homozygous Positive	Expected # Plants Hemizygous Positive	Expected # Plants Homozygous Negative	χ^2	Probability
F_2	297	79	148	70	74.25	148.5	74.25	0.5	0.76
F_3	263	73	121	69	65.75	131.5	65.75	1.8	0.4069

[1] F_2 progeny were from the cross of MON 87701 homozygous positive for the *cry1Ac* gene with a soybean variety that did not contain the *cry1Ac* gene. F_3 progeny were from self-pollinated F_2 plants hemizygous positive for the *cry1Ac* gene.
[2] Plants were tested for the presence of the *cry1Ac* gene by Taqman PCR.

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V.G. Conclusion of Molecular Characterization

Molecular analyses show that one intact copy of the *cry1Ac* expression cassette was integrated at a single chromosomal locus in MON 87701. No additional genetic elements, including backbone sequences from the transformation vector PV-GMIR9, linked or unlinked to the intact DNA insert, were detected in the genome of MON 87701. Generational stability analysis demonstrated that the expected Southern blot fingerprint of MON 87701 has been maintained across five generations of breeding, thereby confirming the stability of the DNA insert over multiple generations. DNA sequence analyses confirmed the sequence identity between the MON 87701 insert and the portion of the T-DNA I from PV-GMIR9 that was integrated into the soybean genome. These results also confirmed the organization of the genetic elements within the *cry1Ac* expression cassettes of MON 87701, which was identical to that in plasmid PV-GMIR9. Analysis of the T-DNA insertion site indicates that there is a 32 bp deletion of genomic DNA and 14 bp insertion at the insert-to-plant DNA junction. Results from segregation analyses show heritability and stability of the *cry1Ac* gene occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the DNA insert at a single chromosomal locus.

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VI. CHACTERIZATION OF THE INTRODUCED Cry1Ac PROTEIN

This section summarizes the assessment of the Cry1Ac protein produced in MON 87701 including: 1) the equivalence of the plant-produced to *E. coli*-produced proteins used in subsequent laboratory and regulatory safety evaluations; 2) the Cry1Ac protein expression levels in MON 87701 soybean tissues; 3) an allergenicity assessment for the Cry1Ac protein; and 4) an evaluation of the potential protein toxicity and human and animal dietary risk assessment for the Cry1Ac protein. Results indicate that the MON 87701-produced Cry1Ac protein is equivalent to *E. coli*-produced protein. Data also support a conclusion of safe consumption based on several lines of evidence, all of which will be submitted to FDA as part of the pre-market consultation.

VI.A. Identity of the Cry1Ac Protein

Cry1Ac protein originates from *Bacillus thuringiensis* (Bt), a ubiquitous gram-positive soil bacterium that accumulates crystal proteins during sporulation. These crystal (Cry) proteins bind to the specific receptors on the midgut epithelium of targeted lepidopteran insects, and form cation selective pores, which lead to the inhibition of the digestive process and result in the insecticidal activity (Hofmann et al., 1988; Slaney et al., 1992; Van Rie et al., 1990). One valuable feature of this activity is that it is targeted to specific categories of insects, and does not impact broader insect populations or other organisms. For example, Cry1A proteins have insecticidal activity specifically against lepidopteran insects, while Cry3 proteins have insecticidal activity specifically against coleopteran insects (Höfte and Whiteley, 1989). Bt Cry1 proteins are synthesized as ~130 kDa prototoxins consisting of a three-domain toxin portion and a C-terminal extension (De Maagd et al., 2003). The C-terminal portion of the Cry1A protein is thought to contribute to crystal formation via disulfide bond formation but is not involved in determining the biological activity or specificity of the toxin toward target insects (Miranda et al., 2001; Rukmini et al., 2000; Schnepf et al., 1998; Diaz-Mendoza et al., 2007). Detailed information on mode of action (MOA) of Cry1Ac protein can be found in Section X.A.2.

The Cry1Ac protein expressed in MON 87701 is targeted to chloroplasts due to the addition of a chloroplast transit peptide (CTP) coding sequence at the 5' end of the coding sequence. Following translation and translocation into chloroplasts, the CTP is cleaved. Experimental analysis of the N-terminus of MON 87701-produced Cry1Ac protein (described below) indicated the presence of four additional amino acids at the N-terminus compared to other Cry1Ac proteins (Figure VI-1, Figure C-4). The additional four amino acids are cysteine (C), methionine (M), glutamine (Q), and alanine (A). While the identities of methionine, glutamine, and alanine were clearly determined by N-terminal sequencing, the identity of the first amino acid, cysteine, was inferred based on the CTP1 coding sequence in MON 87701. The chemistry employed in N-terminal sequencing is known to degrade cysteine (Inglis and Liu, 1970), preventing its clear identification. With the exception of the CTP-derived four added amino acids, the Cry1Ac that accumulates in MON 87701 shares >99% amino acid identity with Cry1Ac from *B. thuringiensis* (Bt) subsp. *kurstaki* and 100% amino acid sequence identity with the Cry1Ac protein present in Bollgard cotton (see Figure VI-1 and Figure C-4). The

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presence of these four additional amino acids at the N-terminus of the MON 87701-produced Cry1Ac protein has no impact on protein activity or toxicity, due to rapid proteolytic excision of the N-terminus during prototoxin activation.

The sequence encoding for the four additional amino acids derived from the CTP in MON 87701-produced Cry1Ac was included in the N-terminus of the *E. coli*-produced Cry1Ac protein that was used in the safety assessment evaluations for MON 87701. This resulted in the production of a full-length Cry1Ac protein of 1182 amino acids (1178 from Cry1Ac and four from the CTP coding region).

```
Bt Cry1Ac       ----MDNNPNINECIPYNCLSNPEVEVLGGERIETGYTPIDISLSLTQFLLSEFVPGAGF
Bollgard        ----MDNNPNINECIPYNCLSNPEVEVLGGERIETGYTPIDISLSLTQFLLSEFVPGAGF
MON87701        CMQAMDNNPNINECIPYNCLSNPEVEVLGGERIETGYTPIDISLSLTQFLLSEFVPGAGF

Bt Cry1Ac       VLGLVDIIWGIFGPSQWDAFLVQIEQLINQRIEEFARNQAISRLEGLSNLYQIYAESFRE
Bollgard        VLGLVDIIWGIFGPSQWDAFLVQIEQLINQRIEEFARNQAISRLEGLSNLYQIYAESFRE
MON87701        VLGLVDIIWGIFGPSQWDAFLVQIEQLINQRIEEFARNQAISRLEGLSNLYQIYAESFRE

Bt Cry1Ac       WEADPTNPALREEMRIQFNDMNSALTTAIPLFAVQNYQVPLLSVYVQAANLHLSVLRDVS
Bollgard        WEADPTNPALREEMRIQFNDMNSALTTAIPLFAVQNYQVPLLSVYVQAANLHLSVLRDVS
MON87701        WEADPTNPALREEMRIQFNDMNSALTTAIPLFAVQNYQVPLLSVYVQAANLHLSVLRDVS

Bt Cry1Ac       VFGQRWGFDAATINSRYNDLTRLIGNYTDYAVRWYNTGLERVWGPDSRDWVRYNQFRREL
Bollgard        VFGQRWGFDAATINSRYNDLTRLIGNYTDHAVRWYNTGLERVWGPDSRDWIRYNQFRREL
MON87701        VFGQRWGFDAATINSRYNDLTRLIGNYTDHAVRWYNTGLERVWGPDSRDWIRYNQFRREL

Bt Cry1Ac       TLTVLDIVALFPNYDSRRYPIRTVSQLTREIYTNPVLENFDGSFRGSAQGIERSIRSPHL
Bollgard        TLTVLDIVSLFPNYDSRTYPIRTVSQLTREIYTNPVLENFDGSFRGSAQGIEGSIRSPHL
MON87701        TLTVLDIVSLFPNYDSRTYPIRTVSQLTREIYTNPVLENFDGSFRGSAQGIEGSIRSPHL

Bt Cry1Ac       MDILNSITIYTDAHRGYYYWSGHQIMASPVGFSGPEFTFPLYGTMGNAAPQQRIVAQLGQ
Bollgard        MDILNSITIYTDAHRGEYYWSGHQIMASPVGFSGPEFTFPLYGTMGNAAPQQRIVAQLGQ
MON             MDILNSITIYTDAHRGEYYWSGHQIMASPVGFSGPEFTFPLYGTMGNAAPQQRIVAQLGQ

Bt Cry1Ac       GVYRTLSSTLYRRPFNIGINNQQLSVLDGTEFAYGTSSNLPSAVYRKSGTVDSLDEIPPQ
Bollgard        GVYRTLSSTLYRRPFNIGINNQQLSVLDGTEFAYGTSSNLPSAVYRKSGTVDSLDEIPPQ
MON87701        GVYRTLSSTLYRRPFNIGINNQQLSVLDGTEFAYGTSSNLPSAVYRKSGTVDSLDEIPPQ

Bt Cry1Ac       NNNVPPRQGFSHRLSHVSMFRSGFSNSSVSIIRAPMFSWIHRSAEFNNIIASDSITQIPA
Bollgard        NNNVPPRQGFSHRLSHVSMFRSGFSNSSVSIIRAPMFSWIHRSAEFNNIIASDSITQIPA
MON87701        NNNVPPRQGFSHRLSHVSMFRSGFSNSSVSIIRAPMFSWIHRSAEFNNIIASDSITQIPA

Bt Cry1Ac       VKGNFLFNGSVISGPGFTGGDLVRLNSSGNNIQNRGYIEVPIHFPSTSTRYRVRVRYASV
Bollgard        VKGNFLFNGSVISGPGFTGGDLVRLNSSGNNIQNRGYIEVPIHFPSTSTRYRVRVRYASV
MON87701        VKGNFLFNGSVISGPGFTGGDLVRLNSSGNNIQNRGYIEVPIHFPSTSTRYRVRVRYASV

Bt Cry1Ac       TPIHLNVNWGNSSIFSNTVPATATSLDNLQSSDFGYFESANAFTSSLGNIVGVRNFSGTA
Bollgard        TPIHLNVNWGNSSIFSNTVPATATSLDNLQSSDFGYFESANAFTSSLGNIVGVRNFSGTA
MON87701        TPIHLNVNWGNSSIFSNTVPATATSLDNLQSSDFGYFESANAFTSSLGNIVGVRNFSGTA

Bt Cry1Ac       GVIIDRFEFIPVTATLEAEYNLERAQKAVNALFTSTNQLGLKTNVTDYHIDQVSNLVTYL
Bollgard        GVIIDRFEFIPVTATLEAEYNLERAQKAVNALFTSTNQLGLKTNVTDYHIDQVSNLVTYL
MON87701        GVIIDRFEFIPVTATLEAEYNLERAQKAVNALFTSTNQLGLKTNVTDYHIDQVSNLVTYL

Bt Cry1Ac       SDEFCLDEKRELSEKVKHAKRLSDERNLLQDSNFKDINRQPERGWGGSTGITIQGGDDVF
Bollgard        SDEFCLDEKRELSEKVKHAKRLSDERNLLQDSNFKDINRQPERGWGGSTGITIQGGDDVF
MON87701        SDEFCLDEKRELSEKVKHAKRLSDERNLLQDSNFKDINRQPERGWGGSTGITIQGGDDVF
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```
Bt Cry1Ac       KENYVTLSGTFDECYPTYLYQKIDESKLKAFTRYQLRGYIEDSQDLEIYLIRYNAKHETV
Bollgard        KENYVTLSGTFDECYPTYLYQKIDESKLKAFTRYQLRGYIEDSQDLEIYSIRYNAKHETV
MON87701        KENYVTLSGTFDECYPTYLYQKIDESKLKAFTRYQLRGYIEDSQDLEIYSIRYNAKHETV

Bt Cry1Ac       NVPGTGSLWPLSAQSPIGKCGEPNRCAPHLEWNPDLDCSCRDGEKCAHHSHHFSLDIDVG
Bollgard        NVPGTGSLWPLSAQSPIGKCGEPNRCAPHLEWNPDLDCSCRDGEKCAHHSHHFSLDIDVG
MON87701        NVPGTGSLWPLSAQSPIGKCGEPNRCAPHLEWNPDLDCSCRDGEKCAHHSHHFSLDIDVG

Bt Cry1Ac       CTDLNEDLGVWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLEW
Bollgard        CTDLNEDLGVWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLEW
MON87701        CTDLNEDLGVWVIFKIKTQDGHARLGNLEFLEEKPLVGEALARVKRAEKKWRDKREKLEW

Bt Cry1Ac       ETNIVYKEAKESVDALFVNSQYDQLQADTNIAMIHAADKRVHSIREAYLPELSVIPGVNA
Bollgard        ETNIVYKEAKESVDALFVNSQYDQLQADTNIAMIHAADKRVHSIREAYLPELSVIPGVNA
MON87701        ETNIVYKEAKESVDALFVNSQYDQLQADTNIAMIHAADKRVHSIREAYLPELSVIPGVNA

Bt Cry1Ac       AIFEELEGRIFTAFSLYDARNVIKNGDFNNGLSCWNVKGHVDVEEQNNQRSVLVVPEWEA
Bollgard        AIFEELEGRIFTAFSLYDARNVIKNGDFNNGLSCWNVKGHVDVEEQNNQRSVLVVPEWEA
MON87701        AIFEELEGRIFTAFSLYDARNVIKNGDFNNGLSCWNVKGHVDVEEQNNQRSVLVVPEWEA

Bt Cry1Ac       EVSQEVRVCPGRGYILRVTAYKEGYGEGCVTIHEIENNTDELKFSNCVEEEIYPNNTVTC
Bollgard        EVSQEVRVCPGRGYILRVTAYKEGYGEGCVTIHEIENNTDELKFSNCVEEEIYPNNTVTC
MON87701        EVSQEVRVCPGRGYILRVTAYKEGYGEGCVTIHEIENNTDELKFSNCVEEEIYPNNTVTC

Bt Cry1Ac       NDYTVNQEEYGGAYTSRNRGYNEAPSVPADYASVYEEKSYTDGRRENPCEFNRGYRDYTP
Bollgard        NDYTVNQEEYGGAYTSRNRGYNEAPSVPADYASVYEEKSYTDGRRENPCEFNRGYRDYTP
MON87701        NDYTVNQEEYGGAYTSRNRGYNEAPSVPADYASVYEEKSYTDGRRENPCEFNRGYRDYTP

Bt Cry1Ac       LPVGYVTKELEYFPETDKVWIEIGETEGTFIVDSVELLLMEE
Bollgard        LPVGYVTKELEYFPETDKVWIEIGETEGTFIVDSVELLLMEE
MON87701        LPVGYVTKELEYFPETDKVWIEIGETEGTFIVDSVELLLMEE
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Figure VI-1. Amino Acid Sequence Alignment for Cry1Ac Proteins

The amino acid sequence alignment for Cry1Ac from *Bacillus thuringiensis* (Cry1Ac, GI-117547*), Cry1Ac expressed in Bollgard cotton, and Cry1Ac expressed in MON 87701. Amino acid sequence differences between Cry1Ac from Bt and the two plant-produced proteins are underlined and highlighted in grey in the Bt Cry1Ac sequence. Four amino acids originating from the CTP1 in MON 87701-produced Cry1Ac are in boldface font.

* Gene identification number used in GenBank.

VI.B. Characterization of the Full Length Cry1Ac Protein from MON 87701

The expression level of Cry1Ac protein in MON 87701 seed was too low and insufficient for use in the subsequent safety evaluations. Therefore, it was necessary to produce the protein in a high-expressing, recombinant microorganism in order to obtain sufficient quantities of the protein for safety evaluations. A recombinant Cry1Ac protein was produced in *Escherichia coli*, the sequence of which was engineered to match that of Cry1Ac protein produced in MON 87701. The equivalence of the physicochemical characteristics and functional activity between the MON 87701-produced and *E. coli*-produced Cry1Ac proteins was confirmed by a panel of analytical techniques, including sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot analysis, matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), glycosylation analysis, and assay of biological activity. The details of the materials, methods, and results are described in Appendix C, while the conclusions are summarized as follows.

The Cry1Ac protein isolated from MON 87701 harvested seed was purified and characterized, and results confirmed the equivalence of MON 87701-produced and *E. coli*-produced Cry1Ac proteins. SDS-PAGE demonstrated that the MON 87701-produced Cry1Ac co-migrated to the same position on the gel as the *E. coli*-produced Cry1Ac protein, indicating the protein from both sources was equivalent in molecular weight. On the basis of Western blot analysis with a polyclonal antibody against Cry1Ac, the electrophoretic mobility and immunoreactivity of the MON 87701-produced Cry1Ac protein were shown to be equivalent to those of the *E. coli*-produced Cry1Ac protein. The intactness of the N-terminus for the MON 87701-produced Cry1Ac protein was confirmed with an antibody which is specific to the N-terminal peptide. Tryptic peptide mapping by MALDI-TOF MS yielded peptide masses consistent with the expected tryptic peptides generated *in silico* based on the predicted trypsin cleavage sites in the Cry1Ac sequence. In addition, the MON 87701-produced and the *E. coli*-produced Cry1Ac proteins were found to be equivalent based on functional activities against a sensitive lepidopteran species and the lack of glycosylation. Taken together, these data provide a detailed characterization of the Cry1Ac protein isolated from MON 87701 and were used in establishing its equivalence to the *E. coli*-produced Cry1Ac protein.

VI.C. Expression Levels of Cry1Ac Protein in MON 87701

The levels of the Cry1Ac protein in various tissues (except pollen/anther) of MON 87701 that are relevant to the risk assessment were assessed by a validated ELISA. The materials and methods for the ELISA analysis, as well as a description of the tissue types, are provided in Appendix D. Tissue samples for analysis were collected from five field trials conducted in the U.S. during 2007. The trial locations were in the states of Alabama, Arkansas, Georgia, Illinois, and North Carolina which represent relevant soybean-growing regions of the U.S. and provide a range of environmental conditions that would be typical of those encountered in the production of soybean. At each site, three replicated plots of MON 87701 and a conventional soybean control (A5547) were planted using a randomized complete block field design. Over-season leaf (OSL), forage,

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root, and harvested seed were collected from each replicated plot at all field sites. A description of the tissues collected is provided below.

Tissue	*Soybean development stage*	*Days after planting (DAP)*
OSL-1	V3-V4	23-34
OSL-2	V6-V8	36-45
OSL-3	V10-V12	43-57
OSL-4	V14-V16	52-70
Forage	R6	85-106
Root	R6	85-106
Mature seed	R8 Harvested at or dried to ~10-15% moisture	139-156
Pollen/Anther	R2	63

Pollen/anther tissue was collected at the R2 growth stage during the 2007 growing season from a field site in Jackson County, IL that was used to generate bulk quantities of MON 87701 and conventional control material. At this site, plots of plants containing MON 87701 as well as the conventional soybean control, were planted using a single plot field design. Pollen/anther* was collected from each plot.

Cry1Ac protein levels were determined in all eight tissue types described above. The results obtained from ELISA analysis are summarized in Table VI-1 for the various tissue types including the tissues collected throughout the growing season. The Cry1Ac protein was determined in over-season leaf (OSL1-4), forage, root, harvested seed, and pollen. The levels of Cry1Ac protein in tissue samples from the conventional soybean control were below the Cry1Ac assay limit of quantitation (LOQ) or limit of detection (LOD) for each tissue type.

In the 2007 U.S. expression assessment, the mean Cry1Ac protein levels across the five sites were highest in leaf (OSL-4, 340 µg/g dwt), followed by forage (34 µg/g dwt) and mature, harvested seed (4.7 µg/g dwt). If present in root, Cry1Ac levels are less than the ELISA assay LOD of 0.347 µg/g fwt. In over-season leaf tissues harvested throughout the growing season, mean Cry1Ac protein levels in MON 87701 across all sites ranged from 220 – 340 µg/g dwt. In general, the mean levels of the Cry1Ac protein in leaf remained relatively constant across sampling time points, but levels of the protein fell within a broader range as the growing season progressed (Table VI-1).

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* Due to the limited quantity of pollen/anther, pollen/anther material was evaluated using a non-validated, but optimized ELISA method.

Table VI-1. Summary of Cry1Ac Protein Levels in Tissue Collected from MON 87701 Produced across Five Sites during the U.S. 2007 Growing Season

Tissue Type	*Cry1Ac µg/g* fwt *(SD)*[1,3]	*Range*[4] *(µg/g* fwt*)*	*Cry1Ac µg/g* dwt *(SD)*[2]	*Range (µg/g* dwt*)*	*LOQ/LOD (µg/g* fwt*)*
OSL-1	30 (8.5)	12-40	220 (70)	110-350	2.5/0.74
OSL-2	38 (16)	18-80	260 (100)	130-500	2.5/0.74
OSL-3	34 (17)	14-77	240(110)	94-480	2.5/0.74
OSL-4	53 (36)	15-110	340 (290)	78-960	2.5/0.74
Root	< LOD	< LOD	NA[5]	NA[5]	0.4/0.347
Forage	9.0 (8.8)	2.5-32	34 (36)	8.2-140	2.0/0.55
Harvested seed	4.2 (0.73)	3.1-5.0	4.7 (0.79)	3.4-5.7	1.0/0.47
Pollen/ anther[6]	2.3 (0.58)	1.8-3.1	NA[7]	NA[7]	ND[8]

1. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
2. Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the fwt value by the dry weight conversion factors obtained from moisture analysis data.
3. The mean and standard deviation were calculated across sites (N=15, except OSL-1 where N=13 and pollen/anther where N=4).
4. Minimum and maximum values were determined for each tissue type across sites.
5. Protein levels that were <LOD on a fwt basis were not converted to dwt values.
6. Due to limited quantity, pollen/anther material was evaluated using a non-validated, but optimized ELISA method.
7. Protein level by dry weight was not calculated due to limited quantities of pollen/anther tissue.
8. Due to limited quantities of pollen/anther tissue the LOD and LOQ were not determined.

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VI.D. Assessment of Potential Allergenicity of the Cry1Ac Protein

According to guidelines adopted by the Codex Alimentarius Commission for the evaluation of the potential allergenicity of novel proteins, the allergenic potential of a novel protein is assessed by comparing the biochemical characteristics of the novel protein to 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 only 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.

The Cry1Ac protein has been assessed for its potential allergenicity according to the recommendations of the Codex Alimentarius Commission (Codex Alimentarius, 2003). The Cry1Ac protein is from *Bacillus thuringiensis,* an organism that is not a source of allergens and has been used commercially in the U.S. since 1958 to produce microbial-derived products with insecticidal activity (EPA, 1988). Bioinformatics analyses demonstrated that the Cry1Ac protein does not share structurally- or immunologically-relevant amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes. Digestive fate experiments conducted with the Cry1Ac protein demonstrated that the full-length protein is rapidly digested in SGF (simulated gastric fluid), although a small, transiently stable fragment is formed. The ~4 kDa Cry1Ac protein fragment that is transiently stable in SGF was degraded within 30 sec after exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length Cry1Ac protein in SGF together with complete degradation of the small, transiently stable fragment in SIF indicates that it is highly unlikely that the Cry1Ac protein or its fragment will reach absorptive cells of the intestinal mucosa. Finally, the Cry1Ac protein represents no more than 0.0012% of the total protein in the harvested seed of MON 87701. Taken together, these data support the conclusion that the Cry1Ac present in MON 87701 does not pose a significant allergenic risk.

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VI.E. Safety Assessment Summary of Cry1Ac Protein

The EPA has previously reviewed and established a tolerance exemption for Cry1Ac protein and the genetic materials necessary for the production of this protein in or on all raw agricultural commodities (40 CFR § 180.1155). The exemption was based on a safety assessment that included rapid digestion in simulated mammalian gastrointestinal fluids, lack of homology to toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. Similar safety assessments were conducted on MON 87701 and the full-length Cry1Ac protein it produced, where a consistent set of data were generated that support the conclusion that the Cry1Ac protein is safe for human and animal consumption. The comprehensive food and feed safety and nutritional assessment of MON 87701 is also scheduled to be submitted to the FDA, which will include the following conclusions:

a) The donor organism, *Bacillus thuringiensis* subspecies *kurstaki* (Bt) is a gram-positive bacterium that is commonly found in soil and has been used commercially in the U.S. since 1958 to produce microbial-derived products (containing Cry proteins as active ingredients) with insecticidal activity. The extremely low

mammalian toxicity of Bt-based insecticide products has been demonstrated in numerous safety studies, and there are no confirmed cases of allergic reactions to Cry proteins in application of microbially-derived Bt products during the past 50 years.

b) A history of safe use of Cry1Ac protein has been established (Cannon, 1993; EPA, 1988; WHO, 1999). Microbial pesticides containing *B. thurigiensis* Cry1A proteins have been used for more than 45 years and subjected to extensive toxicity testing showing no adverse effects to human health (Baum et al., 1999; Betz et al., 2000; EPA, 2000; EPA, 2001; McClintock et al., 1995; Mendelsohn et al., 2003). In addition to its over 45 year history of safe use in bacterial preparations used as biopesticides, Cry1Ac is expressed in commercially available Bollgard cotton. The Bollgard Cry1Ac protein has 100% amino acid identity with the MON 87701-produced Cry1Ac, except for the four additional amino acids at the N-terminus of the MON 87701-produced protein in soybean (Figure VI-1). A related protein, Cry1Ab, which has ~90% amino acid identity to the Cry1Ac in Bollgard and MON 87701, is expressed in YieldGard corn. The U.S. EPA has approved commercial use of the Cry1Ab and Cry1Ac proteins as expressed in corn and cotton (EPA, 2008). An exemption from the requirement for a tolerance was granted in 1996 for Cry1Ab and the genetic material necessary for its production in all plants (40 CFR §180.1173). A tolerance exemption for the Cry1Ac protein and the genetic material necessary for its production in all plants was granted on April 11, 1997 (40 CFR §174.510, newly designated as 40 CFR§180.1155). The history of large scale cultivation of both of these crops without any indication of harmful impact on the environment, non-target insects, or mammals provides additional evidence for the safety of the Cry1Ac protein. Taken together, these data demonstrate that the Cry1Ac protein has a history of safe use and does not pose any adverse effects to human and animal health.

c) Cry1Ac protein does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins which have adverse effects to mammals. This has been demonstrated by extensive assessments with bioinformatic tools, such as FASTA sequence alignment tool and eight-amino acid sliding window search.

d) The full-length Cry1Ac protein is readily digestible in simulated gastric and simulated intestinal fluids. Although a small, transiently stable fragment of ~4 kDa was observed during digestion in SGF, it was very quickly degraded within 30 sec during exposure to SIF. Thus, the Cry1Ac protein is readily susceptible to degradation with the enzymes found in the mammalian gastro-intestinal tracts. Rapid degradation of the full-length Cry1Ac protein in SGF and SIF makes it highly unlikely for the Cry1Ac protein to be absorbed by epithelial cells of the small intestine in a biologically-active form.

e) An acute toxicology study was conducted with the full-length Cry1Ac protein that was produced by an *E. coli*-expression system. This protein was shown to be physicochemically and functionally equivalent to the Cry1Ac protein produced in MON 87701. Results indicate that the Cry1Ac protein did not cause any adverse

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effects in mice, with a No Observable Effect Level (NOEL) of 1460 mg/kg in males and 1290 mg/kg in females, the highest dose levels tested.

f) Potential human health risks from consumption of the Cry1Ac protein in foods derived from MON 87701 were evaluated by calculating a Margin of Exposure (MOE) between the acute mouse NOAEL for Cry1Ac and 95th percentile "eater-only" estimates of acute dietary exposure determined using the Dietary Exposure Evaluation Model (DEEM-FCID version 2.03, Exponent Inc.) and food consumption data from the 1994-1996 and 1998 USDA Continuing Survey of Food Intakes by Individuals (CSFII). The MOEs for acute dietary intake of the Cry1Ac protein were estimated to be 2.93×10^6 and 7.71×10^4 for the general population and non-nursing infants, respectively. These very large MOEs indicate that there is no meaningful risk to human health from dietary exposure to the Cry1Ac protein produced by MON 87701.

g) Potential health risks to animals from the presence of Cry1Ac protein in feed were evaluated by calculating an estimate of daily dietary intake (DDI). Animals such as poultry and pig are expected to be exposed to the Cry1Ac protein through dietary intake of feed derived from MON 87701 soybean meal, and soybean forage in the case of the lactating dairy cow. In the United States, over 93% of soybean is either crushed domestically or traded internationally with less than 7% of the soybean seed used as feed, certified seed or breeding stock. For the soybean meal produced in U.S., approximately 98% is consumed by the livestock industry (ASA, 2008). From a worst case assessment, the percentage of the Cry1Ac protein consumed as part of the daily protein intake for a dairy cow was 0.0498%, and for both the broiler and pig was less than 0.0012%.

Using the guidance provided by the FDA, a conclusion of "no concern" is reached for the donor organism and the Cry1Ac protein. The food and feed products containing MON 87701 or derived from MON 87701 are as safe as soybean currently on the market for human and animal consumption.

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VII. COMPOSITIONAL AND NUTRITIONAL ASSESSMENT OF MON 87701

Compositional analyses were conducted to assess whether the nutrients and anti-nutrient levels in harvested seed and forage derived from MON 87701 are comparable to those in the conventional soybean control, A5547. In addition, twenty commercial conventional soybean varieties were included in the analysis as references to establish a range of natural variability for each analyte, where the range of variability is defined by a 99% tolerance interval for that particular analyte. Compositional analysis included the significant nutrients, anti-nutrients, and key secondary metabolites, consistent with OECD guidelines (OECD, 2001). Results of the comparisons indicate that MON 87701 is compositionally and nutritionally equivalent to conventional soybean that is currently in commerce and has a history of safe human and animal consumption.

Compositional analyses were conducted on forage and harvested seed collected from MON 87701, the conventional soybean control, and twenty unique commercial conventional soybean varieties grown in a 2007 U.S. field production. Forage and harvested seed of MON 87701, the conventional soybean control, and commercial conventional soybean varieties were collected from five replicated U.S. field sites (AL, AR, GA, IL, and NC). Seeds were planted in a randomized complete block design with three replicates per block for MON 87701, the control, and reference soybean varieties. Samples from all three replicates of MON 87701 and control plots were analyzed, whereas one replicate of the twenty unique commercial conventional reference soybean varieties was analyzed. All the samples were collected from plants grown under normal agronomic field conditions for their respective geographic regions.

A total of 64 compositional analytes (seven in forage and 57 in harvested seed) were evaluated. Compositional analyses of the forage samples included proximates (ash, fat, moisture, and protein), carbohydrates by calculation, acid detergent fiber (ADF), and neutral detergent fiber (NDF). Seed samples were analyzed for proximates (ash, fat, moisture, and protein), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), amino acids, fatty acids (C8-C22), trypsin inhibitors, phytic acid, lectin, isoflavones (daidzein, glycitein, and genistein), vitamin E, raffinose, and stachyose. Materials and methods used for compositional analysis of MON 87701, the conventional soybean control, and commercial conventional soybean varieties harvested seed and forage from the 2007 U.S. field production are presented in Appendix E.

The composition of forage and harvested seed of MON 87701 was analyzed and statistically compared to the conventional soybean control. Tolerance intervals calculated from the reference substances (commercial conventional soybean varieties) were also established for each compositional analyte. Each individual analyte for MON 87701 was statistically compared to that of the conventional soybean control across all five sites (combined-site) and within each of the five field sites (individual-site). Of the evaluated components, nine fatty acids in harvested seed had more than 50% of the observations below the assay limit of quantitation (LOQ) and, as a result, were excluded from the statistical analysis.

A statistical summary was generated for each of the remaining 55 compositional analytes (seven in forage and 48 in harvested seed). Least square means, standard errors, and the

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range of observed values for MON 87701 and the conventional soybean control forage and harvested seed from the combined-site and individual-site analyses are presented in Appendix E. The overall data set was examined for evidence of biologically-relevant changes by first examining combined-site differences, followed by the individual-site assessments. Additionally, each mean test value that differed ($p<0.05$) from the control was compared to the 99% tolerance interval generated from the commercial conventional soybean varieties in this evaluation. A summary of these significant differences ($p<0.05$) for MON 87701 and conventional soybean control forage and harvested seed is presented in Table VII-1. Finally, this comparative evaluation also considered the natural ranges in soybean component levels published in the scientific literature and the International Life Sciences Institute - Crop Composition Database (ILSI-CCD) (ILSI, 2006).

Overall, combined-site analyses of both forage and harvested seed samples showed no significant difference ($p\geq0.05$) between MON 87701 and the conventional soybean control for 40 of 55 comparisons. Statistical analyses for MON 87701 from the combined-site analysis for forage detected no differences ($p\geq0.05$). Statistically significant differences between MON 87701 and the conventional soybean control seed were detected ($p<0.05$) for 15 analytes: alanine, 22:0 behenic acid, carbohydrates, daidzein, glycine, histidine, isoleucine, leucine, lysine, protein, serine, threonine, trypsin inhibitor, valine, and vitamin E. The magnitude of these differences were generally small (most <5%).

Biological relevance of these differences was assessed based on magnitude of the difference, reproducibility across sites, and comparison of mean analyte values to the 99% tolerance interval. It was noted that the protein content in MON 87701 had an increased level, but at a small order of magnitude (3.87%) compared to the conventional soybean control. Likewise, all nine amino acids are slightly increased in magnitude (2.15-4.63%) when compared to the conventional soybean control. Based on the relatively high total protein content (35-45%) in commercially available conventional soybean, and the relatedness of amino acid content to total protein content, it is not unexpected that several of the amino acids in MON 87701 also had elevated levels that are significantly different from the conventional soybean control. Considering the generally small increase in these component levels, that neither protein nor any of the amino acids analytes were statistically different at more than two of the five sites in the individual-site analyses, and that the mean levels of protein and amino acids were well within the 99% tolerance interval established by commercial conventional soybean varieties grown concurrently at the same time and field sites, these differences are not considered to be biologically relevant changes in composition.

The remaining five components for harvested seed with statistical differences in the combined-site analysis were also assessed for biological relevance. The magnitude of difference between MON 87701 and the conventional soybean control for carbohydrates and 22:0 behenic acid was considered to be relatively small (-6.10 and 4.33%, respectively). Similarly, the trypsin inhibitor magnitude of difference was small at - 8.79%, where the trypsin inhibitor level was lower for MON 87701 compared to the conventional control. Daidzein is one of the three basic types of isoflavones present in soybean seed. It is well documented that isoflavone levels in soybean seed are highly variable and are greatly influenced by many factors (OECD, 2001). Subsequently, the

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increased content (approximately 10%) of daidzein in MON 87701 compared to the conventional soybean control is not unexpected for a highly variable component. The mean levels of these four components (22:0 behenic acid, carbohydrates, daidzein, and trypsin inhibitors) were well within the 99% tolerance interval established by commercial conventional soybean varieties grown concurrently at the same time and field sites. Additionally, these same four analytes were within the ranges reported in ILSI-CCD (ILSI, 2006). Considering the generally small increase/decrease relative to the conventional soybean control in these component levels, that the 22:0 behenic acid, carbohydrates, daidzein, and trypsin inhibitors analytes were not statistically different at more than two of the five sites in the individual-site analyses, and that the mean levels of these analytes were well within the 99% tolerance interval, these differences are not considered to be biologically relevant.

Vitamin E levels were higher in MON 87701 compared to the conventional soybean control at a 23.26% difference. Likewise, vitamin E was statistically higher than the conventional soybean control in four of the individual-site analyses. From a nutritional perspective, vitamin E is not listed as a key nutrient in soybean by OECD (2001) for food and feed uses. Meanwhile, the MON 87701 mean levels in the combined-site and individual-site analyses were all well within the 99% tolerance interval established by commercial conventional soybean varieties grown concurrently at the same time and field sites; therefore, these differences are not considered to be biologically relevant changes in composition.

In conclusion, compositional data were generated and statistical analyses performed on the forage and harvested seed from MON 87701, the conventional soybean control, and 20 commercial conventional soybean varieties. The overall dataset was evaluated for evidence of biologically meaningful changes from a food and feed safety and nutritional perspective. Overall, statistical analyses of both forage and harvested seed showed no significant difference ($p \geq 0.05$) between MON 87701 and the conventional soybean control for 40 of 55 comparisons from the combined-site analyses and 243 of 275 comparisons from the individual-site analyses. For those few comparisons for which a significant difference ($p < 0.05$) was detected, the analyte mean values for MON 87701 were generally of similar to lower magnitude of difference to those for the conventional soybean control. Additionally, the analyte mean values for MON 87701 were within the calculated 99% tolerance interval for the population of commercial conventional soybean reference varieties grown concurrently at the same time and field sites and, therefore, were not regarded to be biologically meaningful. Forage and harvested seed analytical component values were also comparable to published scientific literature and the ILSI Crop Composition Database, further supporting the conclusion that soybean forage and harvested seed produced from MON 87701 are compositionally equivalent to those of conventional soybean.

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Table VII-1. Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean	A5547 Mean	Mean Difference (Test minus Control) Mean Difference (% of A5547)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in *Combined Site* Analysis						
Seed Proximate (% DW)						
Protein	39.27	37.80	3.87	0.023	[36.49 – 42.23]	[35.30, 45.38]
Carbohydrates	34.22	36.44	-6.10	0.037	[21.58 - 39.61]	[28.17, 40.99]
Seed Amino Acid (% DW)						
Alanine	1.72	1.69	2.15	0.027	[1.66 - 1.84]	[1.49, 2.02]
Glycine	1.75	1.70	2.88	0.007	[1.63 - 1.89]	[1.49, 2.09]
Histidine	1.12	1.08	3.94	<0.001	[1.05 - 1.18]	[0.94, 1.31]
Isoleucine	1.81	1.76	2.94	0.031	[1.68 - 1.99]	[1.54, 2.14]
Leucine	3.04	2.94	3.23	0.046	[2.82 - 3.36]	[2.64, 3.52]
Lysine	2.74	2.62	4.63	0.012	[2.48 - 2.99]	[2.05, 3.47]
Serine	2.03	1.96	3.08	0.004	[1.90 - 2.19]	[1.75, 2.38]
Threonine	1.60	1.55	2.95	0.024	[1.50 - 1.72]	[1.40, 1.83]
Valine	1.92	1.86	2.85	0.040	[1.80 - 2.07]	[1.64, 2.22]
Seed Fatty Acid (% Total FA)						
22:0 Behenic Acid	0.56	0.54	4.33	0.022	[0.46 - 0.65]	[0.30, 0.67]

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Table VII-1 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean	A5547 Mean	Mean Difference (Test minus Control) Mean Difference (% of A5547)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in *Combined Site* Analysis						
Seed Vitamin (mg/100g DW)						
Vitamin E	7.69	6.24	23.26	<0.001	[6.36 - 9.62]	[0, 11.09]
Seed Antinutrient (TIU/mg DW)						
Trypsin Inhibitor	26.06	28.57	-8.79	0.014	[21.65 - 32.53]	[13.58, 46.02]
Seed Isoflavone (mg/kg DW)						
Daidzein	667.54	604.88	10.36	0.040	[188.96 - 983.26]	[0, 1585.14]
Statistical Differences Observed in *More than One Individual Site*						
Seed Amino Acid (% DW)						
Arginine Site GA	2.80	2.57	8.75	0.011	[2.72 - 2.91]	[2.22, 3.25]
Arginine Site IL	2.61	2.44	6.88	0.045	[2.49 - 2.70]	
Histidine Site GA	1.15	1.09	5.17	0.019	[1.13 - 1.16]	[0.94, 1.31]
Histidine Site IL	1.11	1.05	4.90	0.036	[1.09 - 1.13]	
Tyrosine Site AL	1.32	1.20	9.95	0.034	[1.28 - 1.33]	[0.85, 1.48]
Tyrosine Site IL	1.10	1.01	9.14	0.003	[1.07 - 1.13]	

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Table VII-1 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean	A5547 Mean	Mean Difference (Test minus Control) Mean Difference (% of A5547)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in *More than One Individual Site*						
Seed Fatty Acid (% Total FA)						
22:0 Behenic Acid Site AR	0.47	0.46	3.08	0.037	[0.46 - 0.48]	[0.30, 0.67]
22:0 Behenic Acid Site GA	0.60	0.55	8.24	0.029	[0.58 - 0.62]	
Seed Vitamin (mg/100g DW)						
Vitamin E Site AR	6.88	5.03	36.69	<0.001	[6.77 - 7.08]	[0, 11.09]
Vitamin E Site GA	9.16	7.77	17.81	0.011	[8.51 - 9.62]	
Vitamin E Site IL	6.72	5.31	26.56	<0.001	[6.36 - 7.27]	
Vitamin E Site NC	7.83	6.14	27.55	0.017	[7.59 - 8.19]	
Seed Antinutrient (%DW)						
Stachyose Site AL	1.84	2.37	-22.36	0.024	[1.83 - 1.89]	[0.99, 7.93]
Stachyose Site NC	4.56	5.50	-17.12	0.006	[4.32 - 4.72]	
Seed Isoflavone (mg/kg DW)						
Daidzein Site AR	767.90	658.21	16.67	0.031	[747.32 – 793.95]	[0, 1585.14]
Daidzein Site IL	890.96	803.42	10.90	0.042	[834.82 – 983.26]	

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Table VII-1 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean	A5547 Mean	Mean Difference (Test minus Control) Mean Difference (% of A5547)	Significance (p-Value)	Test Range	Commercial Tolerance Interval[2]
Statistical Differences Observed in *One Individual Site*						
Forage Fiber (% DW)						
Neutral Detergent Fiber Site AR	49.83	38.62	29.02	0.023	[46.69 - 55.99]	[21.51, 66.01]
Seed Proximate (% DW)						
Ash Site IL	5.42	5.29	2.42	0.039	[5.20 – 5.55]	[3.74, 6.45]
Carbohydrates Site IL	36.65	39.17	-6.45	0.024	[35.60 – 37.72]	[28.17, 40.99]
Seed Amino Acid (% DW)						
Isoleucine (% DW) Site GA	1.81	1.74	4.23	0.029	[1.77 - 1.84]	[1.54, 2.14]
Leucine Site GA	3.04	2.91	4.59	0.014	[2.98 - 3.09]	[2.64, 3.52]
Proline Site GA	2.00	1.94	3.56	0.025	[1.99 - 2.02]	[1.73, 2.35]
Tryptophan Site NC	0.49	0.47	4.75	0.006	[0.47 - 0.51]	[0.43, 0.59]
Valine Site GA	1.91	1.84	3.96	0.035	[1.88 - 1.94]	[1.64, 2.22]
Seed Fatty Acid (% Total FA)						
16:0 Palmitic Acid Site IL	11.53	11.71	-1.48	0.025	[11.39 - 11.63]	[8.88, 13.53]
16:1 Palmitoleic Acid Site NC	0.09	0.10	-13.81	0.012	[0.084 - 0.089]	[0.04, 0.15]

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Table VII-1 (continued). Summary of Differences (p<0.05) for the Comparison of Soybean Component Levels for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean	A5547 Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[2]
			Mean Difference (% of A5547)	Significance (p-Value)		
Statistical Differences Observed in *One Individual Site*						
Seed Fatty Acid (% Total FA)						
18:0 Stearic Acid Site NC	4.42	4.79	-7.62	0.038	[4.34 - 4.49]	[1.88, 6.25]
18:1 Oleic Acid Site NC	19.78	21.60	-8.42	0.047	[19.21 - 20.21]	[5.01, 42.01]
18:2 Linoleic Acid Site NC	54.21	52.62	3.03	0.046	[53.89 - 54.61]	[38.57, 66.94]
20:1 Eicosenoic Acid Site GA	0.24	0.22	5.27	0.035	[0.23 - 0.24]	[0.16, 0.33]
Seed Antinutrient (TIU/mg DW)						
Trypsin Inhibitor Site GA	23.28	29.27	-20.48	0.005	[21.65 - 25.24]	[13.58, 46.02]
Seed Isoflavone (mg/kg DW)						
Genistein Site AR	807.35	680.07	18.72	0.007	[771.77 - 840.99]	[0, 1352.86]

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

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VIII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides an evaluation of the phenotypic and agronomic characteristics (including plant-symbiont associations), and the environmental interactions of MON 87701 compared to the conventional soybean control, A5547. The conventional soybean control is a variety that has background genetics similar to MON 87701 but does not contain the *cry1Ac* gene cassette. As a soybean variety in maturity group V, the conventional soybean control is most suitable for production in the Mid-South region.

These data support a determination that MON 87701 is no more likely to pose a plant pest risk or to have a significant environmental impact compared to conventional soybean. The conclusions are based on the results of the multiple evaluations reported herein.

Phenotypic and agronomic characteristics of MON 87701 were evaluated in a comparative manner to assess plant pest potential (OECD, 1993). These assessments included 14 plant growth and development characteristics, five seed germination parameters, two pollen characteristics, and observations for plant-insect and plant-disease interactions and plant responses to abiotic stressors. Results from the phenotypic and agronomic assessments indicate that MON 87701 does not possess characteristics that would confer a plant pest risk compared to conventional soybean, nor would it have a significant environmental impact on the affected environment. Data on environmental interactions also indicate that MON 87701 does not confer any increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors.

VIII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 87701, data were collected to evaluate specific aspects of altered plant pest potential based on requirements of USDA-APHIS set forth at 7 CFR § 340.6. The MON 87701 plant characterization encompasses six general data categories: 1) germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant; 5) plant-symbiont associations; and 6) plant interactions with insect, disease, and abiotic stressors. An overview of the characteristics assessed is presented in Table VIII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated on the 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 87701 was compared to an appropriate conventional control, which had a genetic background similar to MON 87701 but did not possess the Cry1Ac tolerance trait. In addition, multiple commercial soybean varieties (see Appendix E and Table F-1) were included to provide a range of baseline values that are common to existing commercial soybean varieties for each measured phenotypic, agronomic, and environmental interaction characteristic. Data collected from the commercial reference varieties reflect a range of selection and

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breeding for desirable characteristics and can therefore provide context for interpreting experimental results.

Table VIII-1. Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials during 2007

Characteristic	Characteristics measured	Evaluation timing	Evaluation description (measurement endpoints)
Plant phenotypic and agronomic characteristics	Seedling vigor	V2 - V4	Rated on a 1-9 scale, where 1-3 = excellent, 4-6 = average, and 7-9 = poor vigor
	Early stand count	V2 - V4	Number of emerged plants per plot, standardized to 20 ft rows
	Growth stage assessment	Every two-three weeks, V2-R8	Average soybean plant growth stage per plot
	Days to 50% flowering	Flowering, R1-R2	Calendar day number when approx. 50% of the plants in each plot were flowering
	Pollen viability	Flowering, R1-R2	Viable and nonviable pollen based on pollen grain staining characteristics
	Pollen morphology	Flowering, R1-R2	Diameter of viable pollen grains
	Flower color	Flowering, R1-R2	Color of flowers: purple, white, or mixed
	Plant pubescence	Maturity, R8	Pubescence on plants in each plot categorized as hairy, hairless, or mixed
	Plant height	Maturity, R8	Distance from the soil surface to the uppermost node on the main stem of five representative plants per plot
	Lodging	Maturity, R8	Rated on 0-9 scale, where 0 = completely erect and 9 = completely flat or lodged
	Pod shattering	Maturity, R8	Rated on 0-9 scale, where 0 = no shattering and 9 = completely shattered
	Final stand count	Maturity, R8	Number of plants per plot, standardized to 20 ft rows
	Seed moisture	Harvest	Percent moisture content of harvested seed
	100 seed weight (g)	Harvest	Mass of 100 harvested seeds
	Test weight (lb/bu)	Harvest	Mass of a bushel of harvested seed
	Yield (bu/ac)	Harvest	Bushels of harvested seed per acre, adjusted to 13% moisture
Plant environmental interactions	Plant response to abiotic stressors	Four times per growing season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Disease damage	Four times during growing season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Arthropod damage	Four times during growing season	Qualitative assessment of each plot, with rating on a 0-5 scale, where 0 = no symptoms and 5 = severe symptoms
	Arthropod abundance	Three times during growing season	Quantitative assessment or pest and beneficial arthropods

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VIII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are, by OECD (1993) standard, comparative assessments. Familiarity is a useful approach to evaluate the potential environmental impact of a biotechnology-derived plant. 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. Thus, assessment of phenotypic and agronomic characteristics and environmental interactions was essential to compare the biotechnology-derived plant to the conventional counterpart. An overview of the characteristics assessed is presented in Table VIII-1. A subset of the data relating to well-understood weediness criteria (e.g., dormancy, pre-harvest seed loss characteristics, lodging) was used to assess whether there is an increased weediness potential, an element of APHIS's plant pest determination. Data on abiotic stress tolerance from the greenhouse and growth chamber assays were used to characterize the extent of stress tolerance imparted by the insertion of the *cry1ac* gene and determine whether any potential changes in tolerance required additional evaluation as a component of the plant pest risk assessment. Based on all of the data collected, an assessment was made whether the biotechnology-derived plant is likely to pose an increased plant pest risk compared to the conventional counterpart.

During the processes of data collection, summarization, and analysis, experienced scientists familiar with each experimental design and evaluation criteria were involved in all steps. This level of oversight ensured that the evaluation system was functioning appropriately, measurements were taken properly, and data were consistent with expectations based on experience with the crop. In addition, the overall dataset was evaluated for evidence of biologically-relevant changes, and for possible evidence of an unexpected plant response. These were no unexpected observations or issues in the course of these evaluations. Data were then submitted for statistical analysis.

VIII.C. Interpretation of Detected Differences Criteria

Comparative plant characterization data between a biotechnology-derived crop and the 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 the step-wise method (Figure VIII-1). All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest potential of the biotechnology-derived crop. Ultimately, a weight of evidence approach considering all characteristics and

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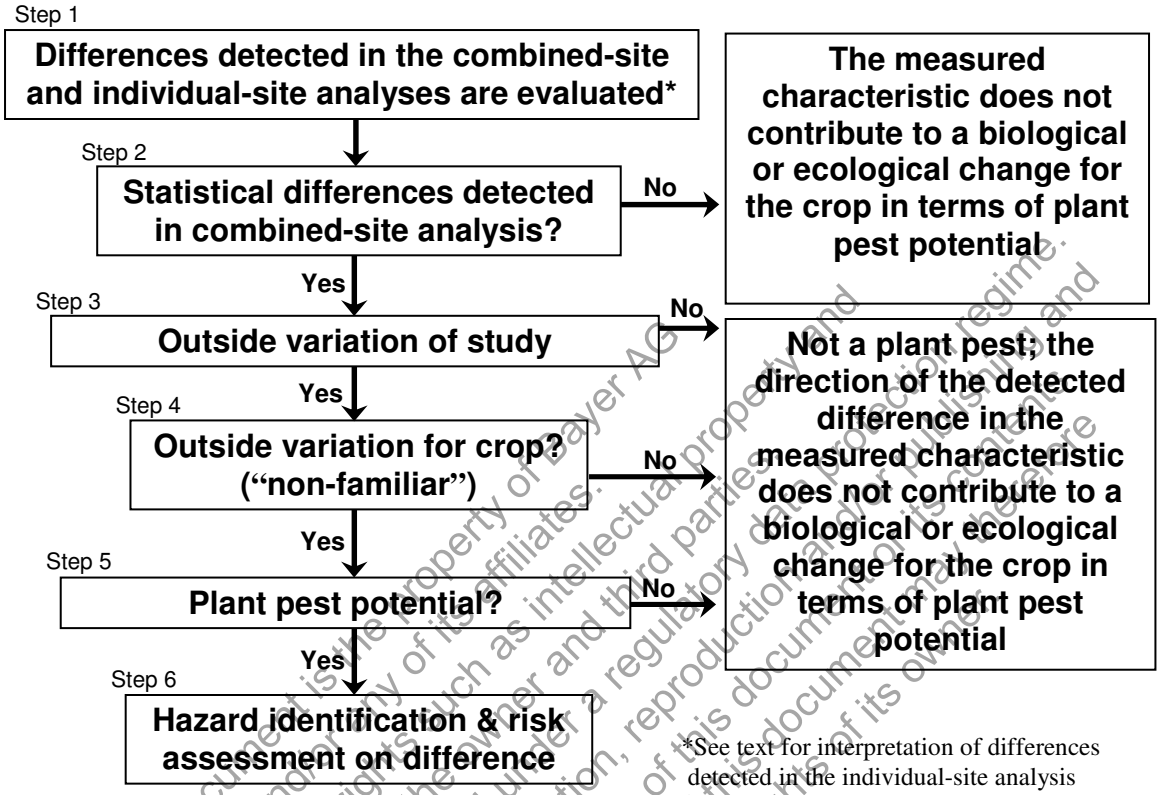
studies was used for the overall risk assessment of differences and their significance. In detail, Figure VIII-1 illustrates the stepwise assessment process employed:



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

Figure VIII-1. Schematic Diagram of Data Interpretation Methods

- **Steps 1 & 2. Evaluate Detected Statistical Differences.** Combined-site and individual-site statistical analyses are conducted and evaluated on each measured characteristic. Differences detected in the individual-site analysis must be observed in the combined-site analysis to be considered further for plant pest potential. Any difference detected in the combined-site analysis is further assessed.
- **Step 3. Evaluate Differences Relative to Reference Range.** If a difference is detected in the combined-site analysis across multiple environments, then the test substance mean value is assessed relative to the reference substances.
- **Step 4. Evaluate Differences in the Context of the Crop.** If the test substance mean is outside the variation of the reference substances (e.g., reference range), the test substance mean is considered in the context of known values common for the crop.
- **Step 5. Plant Pest Potential.** If the test substance mean is outside the range of values common for the crop, the detected difference is then assessed for 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

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differences detected in other measured characteristics, and potential for and effects of trait transfer to feral populations of the crop or a sexually compatible species.

VIII.D. Phenotypic, Agronomic and Environmental Interactions Characteristics

As a significant part of the evaluation of MON 87701, plant phenotypic and agronomic characteristics including seed dormancy and germination, phenotypic, agronomic and environmental interactions, pollen characteristics, and symbiont interactions were evaluated. The phenotypic, agronomic, and environmental interaction evaluations are based on replicated laboratory, greenhouse, and/or multi-site field trials and experiments. In evaluating the phenotypic and agronomic characteristics of MON 87701, data were collected that address specific plant pest risks, as defined by APHIS.

VIII.D.1. Seed Dormancy and Germination Characteristic

APHIS considers the potential for weediness to constitute a plant pest factor (7 CFR § 340.6). Seed germination and dormancy mechanisms, relevant to a weediness assessment because they control a plant's ability to overwinter and become feral, vary with species and their genetic basis tends to be complex. Seed dormancy (e.g., hard seed) also improves a plant's ability to overwinter, and so is also an important characteristic that is often associated with plants that are considered as weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003), and in soybean it is not uncommon to observe low levels of hard seed (Mullin and Xu, 2001; Bradford and Nonogaki, 2007). 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) (Table VIII-2). In addition, five other temperature regimes of 10, 20, 30, 10/20, and 10/30°C were used to assess other seed germination properties. The temperature regimes and types of observation are listed in Table VIII-2. For the alternating temperature regimes of 10/20, 10/30, or 20/30°C, the lower temperature was maintained for 16 hours, and the higher temperature for eight hours.

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 87701 and A5547, where A5547 served as a comparable control with background genetics similar to MON 87701 but does not contain the *cry1Ac* gene cassette. In addition, four commercially available soybean varieties were included as references to provide baseline values common to soybean. The seed lots for MON 87701, the conventional soybean control and reference varieties were produced during 2007 at Texas (TX), Mississippi (MS), and South Carolina (SC), which represent environmentally relevant conditions for soybean production for this product.

After completion of the study, it was determined the seed produced at the MS and SC field sites had high incidences of seed-borne disease (Phomopsis, Cercospora) that are documented to adversely affect seed germination (Pathan et al., 1989; Zorilla et al., 1994). Further study of these incidences of disease is documented in Section VIII.D.2.2 (see below). Therefore, it was determined that the data generated on the seed produced at the MS and SC sites were not appropriate for assessing the potential effects of the insect-

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protected trait on seed dormancy and germination characteristics. Thus, only data on seed from the TX field site were used to assess whether the introduction of the insect-protected trait altered the dormancy and germination characteristics of MON 87701 compared to the conventional control. The details of the materials, experimental methods, and data from all individual production sites are presented in Appendix F.

There were no statistically significant differences detected between MON 87701 and the control for percent germinated seed (normal and abnormal germinated in the AOSA temperature regime), dead seed, or viable firm-swollen seed in any temperature regime for seed produced at the TX site (Table VIII-3). One significant difference was detected out of 24 comparisons for seed produced at the TX site. MON 87701 had no hard seed detected compared the control which had two hard seeds (0.0 vs. 0.5%) at the 20º C temperature condition. The mean value for hard seed from MON 87701 was within the conventional reference range (0.0 – 4.3 %) and the difference was not detected in the other temperature regimes. This suggests the difference was not indicative of a consistent plant response associated with the trait. Furthermore, a decrease in hard seed would not contribute to increased plant pest potential of MON 87701 compared to the control.

The biological characteristics evaluated in this study were used to characterize MON 87701 in the context of a plant pest risk assessment. The results of this study, in particular the absence of hard seed, support a conclusion that there is no increased weed potential of MON 87701 compared to conventional soybean based on the germination and dormancy parameters assessed.

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Table VIII-2. Seed Dormancy and Germination Parameters Evaluated

Evaluation Timing[1]	Temperature Regimes and Seed Characteristics Evaluated	
	AOSA[2]	Additional Temperatures
	20/30°C	10 °C, 20 °C, 30 °C, 10/20 °C and 10/30 °C
Day 5	Normal germinated	Germinated
	Dead	Dead
Day 8	Normal germinated	Germinated
	Abnormal germinated	Dead
	Dead	
	Hard viable and non-viable[3]	
	Firm swollen viable and non-viable[3]	
Day 13	No data collected	Germinated
		Dead
		Hard viable and non-viable[3]
		Firm swollen viable and non-viable[3]

[1] Seed in the 20/30°C temperature regime were evaluated in Days 5 and 8 (according to AOSA guidelines), while seed in the additional temperatures regimes were evaluated on Days 5, 8, and 13.
[2] Association of Official Seed Analysts (AOSA, 2007).
[3] Hard and firm swollen were confirmed to be viable or non-viable by Tetrazolium (Tz) test (AOSA, 2000).

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Table VIII-3. Germination Characteristics of MON 87701 and A5547

Temp. Regime	Category	Mean % (SE)[1]		
		MON 87701	A5547	Reference Range[2]
10°C	Germinated	96.7 (0.3) §	93.8 (1.4)	86.0 – 98.8
	Viable Hard	0.3 (0.3) §	0.8 (0.3)	0.3 – 4.3
	Dead	2.7 (0.3) §	5.5 (1.3)	0.8 – 9.0
	Viable Firm- Swollen	0.3 (0.3) §	0.0 (0.0)	0.0 – 0.8
20°C	Germinated	95.0 (0.7)	94.5 (1.2)	89.3 – 98.3
	Viable Hard	0.0 (0.0)*	0.5 (0.3)	0.0 – 4.3
	Dead	5.0 (0.7)	5.0 (0.9)	1.8 – 6.3
	Viable Firm- Swollen	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
30°C	Germinated	97.5 (0.3)	97.3 (0.9)	90.8 – 99.3
	Viable Hard	0.3 (0.3)	0.0 (0.0)	0.0 – 0.3
	Dead	2.3 (0.3)	2.8 (0.9)	0.8 – 6.3
	Viable Firm- Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.3
10/20°C	Germinated	94.8 (1.4)	93.8 (1.6)	86.3 – 99.5
	Viable Hard	0.5 (0.3)	0.3 (0.3)	0.0 – 6.5
	Dead	4.8 (1.7)	6.0 (1.5)	0.5 – 7.3
	Viable Firm-Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.0
10/30°C	Germinated	94.8 (1.1)	98.8 (0.6)	92.3 – 98.3
	Viable Hard	0.8 (0.5)	0.5 (0.3)	0.0 – 3.5
	Dead	4.5 (0.9)	0.8 (0.8)	1.8 – 4.0
	Viable Firm-Swollen	0.0 (0.0) †	0.0 (0.0)	0.0 – 0.3
20/30°C (AOSA)	Normal Germinated	72.3 (3.9) §	67.3 (4.4)	52.3 – 84.5
	Abnormal Germinated	25.7 (4.4) §	30.0 (3.7)	14.0 – 39.0
	Viable Hard	0.7 (0.3) §	0.8 (0.5)	0.0 – 4.3
	Dead	1.3 (0.7) §	1.8 (0.5)	1.3 – 4.3
	Viable Firm-Swollen	0.0 (0.0) §	0.3 (0.3)	0.0 – 0.3

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on four replicates (N=4) of 100 seed except where denoted by §, in which means are based on three replicates (N=3) of 100 seeds.

*Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

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

[1] In some instances, the total percentage for both MON 87701 and the control did not equal exactly 100% due to numerical rounding of the means.

[2] Minimum and maximum means determined from among the four commercially-available reference soybean varieties produced at the Texas site.

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VIII.D.2. Field Phenotypic, Agronomic Characteristics and Environmental Interactions

Plant growth, development, and yield characteristics were assessed under field conditions as part of the plant characterization assessment of MON 87701. These data were developed to provide APHIS with a detailed description of any unintended phenotypic differences in MON 87701 relative to the conventional soybean control, A5547, and commercially available soybean. Environmental interactions were also assessed as an indirect indicator of phenotypic changes to MON 87701 relative to the same comparator described above. The purpose of these field evaluations was to assess whether the introduction of the insect-protected trait altered the phenotypic and agronomic characteristics or the plant-insect, plant-disease, or plant-abiotic stressor interactions of MON 87701 compared to the control. Certain growth, reproduction, and pre-harvest seed loss characteristics (such as lodging and pod shattering) can be used in the assessment of whether MON 87701 has enhanced plant pest potential.

Data were collected at 16 field locations during 2007 to thoroughly evaluate phenotypic, agronomic, and environmental interaction characteristics. These 16 locations provided a diverse range of environmental and agronomic conditions representative of commercial soybean production areas in the U.S. (Table VIII-4). A randomized complete block design with three replications was employed for the comparisons and interaction analyses. The categories of phenotypic characteristics and environmental interactions evaluated are listed in Table VIII-1. Plant growth stage was assessed several times during the growing season. In addition, observational data on the presence of abiotic and responses to biotic (pests and disease) stressors were collected. The observations of plant response to abiotic stressors, disease damage, and arthropod damage were performed four times during the growing season at 11 of the 16 sites, and arthropod abundance was assessed from collections performed three times during the growing season at four of the 16 sites. The methods and detailed results of these individual site data comparisons are presented and discussed in Appendix G, while the across-site analyses are summarized below. The results of this assessment showed the insect-protected trait did not unexpectedly alter MON 87701 compared to conventional soybean. The lack of differences in plant response to abiotic stressors, disease damage, arthropod damage, and arthropod pest and beneficial insect abundance indicate that the introduction of the insect-protected trait is unlikely to increase plant pest potential.

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VIII.D.2.1. Field Phenotypic and Agronomic Characteristics

A total of 14 phenotypic and agronomic characteristics were evaluated. For the across-site analyses, no significant differences were detected between MON 87701 and the control for early stand count, seedling vigor, days to 50% flowering, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, test weight, and yield (Table VIII-5). Flower color, plant pubescence, and plant growth stage data were categorical and, therefore, were not statistically analyzed; however, all plants of MON 87701 and the control had white flowers and hairy pubescence at each site as expected. The range of growth stages overlapped for all replications of MON 87701 and its control during the 116 growth stage observations among the 16 sites (Appendix G;

Table G-4). Thus, there were no developmental differences observed between MON 87701 and the control.

The phenotypic and agronomic characteristics evaluated in this study were used to provide a detailed description of any differences between MON 87701 compared to the nontransformed control (A5547). A subset of these characteristics were useful to assess the weediness potential of MON 87701 compared to the conventional soybean control. Based on the measured phenotypic and agronomic characteristics, the results support a conclusion of no unexpected changes in the phenotype and no increased plant pest potential of MON 87701 compared to the conventional soybean control.

Table VIII-4. Field Phenotypic Evaluation Sites for MON 87701 during 2007

Location	Location Code	USDA-APHIS Notification Number
Baldwin Co, Alabama	AL	07-059-112n
Independence Co., Arkansas	AR1	07-054-105n
Crittenden Co., Arkansas	AR2	07-054-105n
Jackson Co., Arkansas	AR3	07-059-112n
Tift Co., Georgia	GA1	07-054-105n
Clarke Co., Georgia	GA2	07-059-112n
Jackson Co., Illinois	IL	07-059-112n
Posey Co., Indiana	IN	07-054-105n
Pawnee Co., Kansas	KS	07-054-105n
St. Landry Parish., Louisiana	LA1	07-054-105n
Rapides Parish., Louisiana	LA2	07-054-105n
Washington Co., Mississippi	MS	07-054-105n
Wayne Co., North Carolina	NC	07-059-112n
Barnwell Co., South Carolina	SC	07-054-105n
Armstrong Co., Texas	TX1	07-054-105n
Hockley Co., Texas	TX2	07-054-105n
Louisa Co., Virginia	VA*	07-054-105n

* Data not reported due to wild animal damage early in the season.

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Table VIII-5. Plant Growth and Development Data across 16 Locations during 2007

Phenotypic Characteristic (units)	MON 87701 Mean (SE)	A5547 Mean (SE)	Reference Range[1] Minimum	Reference Range[1] Maximum
Early stand count (#/plot) [2]	230.7 (7.90)	233.1 (6.19)	135.9	298.0
Seedling vigor (1-9 scale)[3]	3.7 (0.20)	3.8 (0.20)	2.3	5.8
Days to 50% flowering [3]	206.5 (1.69)	206.7 (1.67)	197.6	219.7
Plant height (in) [3]	31.9 (0.61)	30.7 (0.52)	19.4	40.3
Lodging (0-9 scale)[3]	2.0 (0.31)	1.8 (0.24)	0.0	7.3
Pod shattering (0-9 scale)[2]	0.6 (0.15)	0.4 (0.09)	0.0	2.0
Final stand count (#/plot) [2]	206.2 (8.09)	211.7 (7.01)	111.5	284.8
Seed moisture (%)[5]	13.1 (0.40)	12.7 (0.36)	10.0†	14.7†
100 seed weight (g)[4]	16.8 (0.36)	16.5 (0.34)	13.2†	20.6†
Test weight (lb/bu)[4]	54.9 (0.75)	55.6 (0.83)	50.3†	60.9†
Yield (bu/ac)[4]	48.8 (3.14)	50.4 (2.79)	18.7†	73.2†

Note: The experimental design was a randomized complete block. SE = Standard Error.
No significant differences were detected between MON 87701 and the conventional soybean control A5547 (p>0.05).
† Minimum and maximum mean values among the 23 commercially-available reference soybean varieties evaluated.
[1] Reference range = Minimum and maximum mean values among the 24 commercially-available reference soybean varieties evaluated except where denoted by †.
[2] Mean based on N=41 for MON 87701 and 42 for A5547.
[3] Mean based on N=47 for MON 87701 and 48 for A5547.
[4] Mean based on N=38 for MON 87701 and 39 for A5547.
[5] Mean based on N=36 for MON 87701 and A5547.

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VIII.D.2.2. Environmental Interaction Analyses

Evaluations of environmental interactions were conducted as part of the plant characterization for MON 87701. In the 2007 U.S. field trials conducted for evaluation of phenotypic and agronomic characteristics of MON 87701, observational data on plant response to abiotic stressors (drought, wind, nutrient deficiency, etc.), disease damage, arthropod damage, and arthropod abundance (Appendix G; Tables G-5, G-6, G-7, and G-8, respectively) were also collected at select sites. These data are used as part of the environmental risk assessment to evaluate plant pest potential and impact on non-target organisms (NTOs) for MON 87701 compared to the conventional soybean control. In addition, multiple commercial soybean varieties were included in the analysis to establish a range of natural variability for each characteristic. The environmental interactions evaluation included data collected in the phenotypic studies (plant-insect, plant-disease, and plant-environment interactions). The results of this assessment showed the insect-protected trait did not unexpectedly alter MON 87701 compared to conventional soybean. The lack of differences in plant response to abiotic stressors, disease damage, arthropod damage, and arthropod pest and beneficial insect abundance indicate that the introduction of the insect-protected trait is unlikely to be biologically meaningful in terms of increased pest potential. In these trials, the observations of plant response to abiotic stressors, disease damage, and arthropod damage were performed four times during the growing season at 11 of the 16 sites, and arthropod abundance was assessed from collections performed three times during the growing season at four of the 16 sites. The observed stressors were at "natural" levels (i.e., no artificial infestation or interference was used). Therefore, the same stressors were not necessarily observed at each field site.

Environmental interactions were assessed qualitatively, and for selected sites, arthropod abundance data were collected quantitatively. For the plant-insect interactions, plant-disease interactions, and plant responses to abiotic stressors, the reported values represent the range of ratings observed across the three replications at each site. MON 87701 and the control were considered qualitatively different in response to a stressor if the ratings between MON 87701 and the conventional soybean control did not overlap across all replications for that particular stressor (e.g., "none" rating vs. "slight-moderate" rating). The ratings observed among the commercial reference soybean varieties provide qualitative assessment data common to the crop for each stressor assessed.

In an assessment of abiotic stress response, disease damage, and arthropod damage, no significant differences were detected between MON 87701 and the conventional soybean control for 367 of 373 comparisons (including 109 abiotic stressor, 131 disease damage, and 133 arthropod damage comparisons) among all observations at the 16 sites (Appendix G; Tables G-5, G-6, and G-7). The six observed differences were in the arthropod damage category. Four of the detected differences were from observations where MON 87701 had less damage caused by lepidopteran pests than the control and, thus, were expected since the insect-protected trait controls certain lepidopteran pests. For the other two detected differences, MON 87701 had less damage than the conventional soybean control from bean leaf beetles during one observation at two separate sites. Bean leaf beetle damage was not consistent across sites or observations. Therefore, the detected differences in arthropod damage ratings are unlikely to be biologically meaningful in terms of increased plant pest potential of MON 87701

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compared to the conventional soybean control. Overall, except for the intended change in resistance to selected lepidopteran insects, the response to abiotic stress, disease, and arthropod damage of MON 87701 are consistent with those of conventional soybean. This is further supported by the restricted spectrum of activity of Cry1 proteins against insect pests within the order *Lepidoptera* (Section X.A.3).

As discussed above, no differences were observed between MON 87701 and the control in their susceptibility or tolerance to the assessed diseases in the field assessments. However, visual assessment of seed produced from the MS and SC sites for the germination study revealed a high incidence of purple staining. Such purple staining is known to be caused by the seed-borne fungal pathogen *Cercospora kikuchii* (Hartman et al., 1999). This observation prompted a further analysis that indicated seed-borne disease infection of the seed from these sites by *Cercospora kikuchii* and *Phomopsis* complex (Section VIII.D.1; Appendix F). Seed germination results from these two sites were not used to assess potential effects of the insect-protected trait on dormancy and germination characteristics because these diseases are documented to adversely affect seed germination (TeKrony et al., 1987). Additional visual evaluations of the seed from the MS and SC sites were conducted that indicated potential differences in the level of purple seed staining (that is an indicator of the prevalence of *C. kikuchii* infection) between MON 87701 and the conventional control (A5547). While it is not expected that the insect-protected trait would be associated with increased disease susceptibility or resistance, a follow-up disease evaluation was conducted. This assessment utilized MON 87701 crossed into additional breeding lines representing other maturity group genetics (maturity groups V and VIII, A5602 and M-Soy 8329) as well as additional seed lots of MON 87701 in the A5547 transformant line that were subsequently compared to either the negative isogenic line or the conventional soybean control (A5547).

The follow-up disease evaluation utilized a standard laboratory assay and is accepted by USDA-National Seed Health System (USDA-NSHS) and the seed industry for assessing the incidence of *Phomopsis* complex or *C. kikuchii* (McGee and Nyvall, 1984). Results from seed screened from two maturity groups (Groups V and VIII) and three genetic backgrounds (A5547, A5602, and M-Soy 8329) indicated no consistent trend in percent seed infected by *Phomopsis* complex or *C. kikuchii* between MON 87701 and its conventional control (Appendix F; Tables F-4 and F-5). Results from these field and laboratory assessments did not indicate a consistent association of disease susceptibility with the trait between MON 87701 and the appropriate control. Furthermore, infection from *Phomopsis* complex and *C. kikuchii* is not uncommon in soybean seed produced in the southern United States (TeKrony et al., 1987), particularly in seed of varieties grown beyond its region of adaptation in later-maturing zones (Mayhew and Caviness, 1994). Thus, it was not unexpected to observe seed-borne diseases in the maturity group V seed materials produced in MS and SC, which are areas where maturity group VI or VII soybean varieties are most adapted.

In an assessment of pest and beneficial arthropod abundance, no statistical differences were detected between MON 87701 and the conventional soybean control for 70 out of 80 comparisons (including 26 out of 34 arthropod pest comparisons and 44 out of 46 beneficial arthropod comparisons) among the collection intervals at the four sites (Appendix G; Tables G-8 and G-9). Seven of the 10 statistical differences between

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MON 87701 and the conventional soybean control in arthropod abundance were for lepidopteran pests including corn earworms, green cloverworms, soybean loopers, and webworms. These differences were not unexpected since the insect-protected trait expressed in MON 87701 effectively controls specific lepidopteran pests. The remaining three statistical differences were for stink bug, *Orius*, and ladybird beetle abundance. None of the statistical differences in arthropod abundance were consistent across collection intervals or field sites. Thus, the differences are unlikely to be biologically meaningful in terms of increased plant pest potential.

These results indicate that compared to conventional soybean, the environmental interactions between MON 87701 and arthropod pest and beneficial organisms, diseases, and abiotic stressors were not altered except for the introduced lepidopteran-protection trait. The lack of meaningful biological differences in plant response to abiotic stressors, disease damage, arthropod damage, and arthropod pest and beneficial insect abundance indicate that MON 87701 and its Cry1Ac protein are unlikely to increase plant pest potential.

VIII.D.3. Pollen Characteristics

In determining the potential for a biotechnology-derived plant to increase weedy or invasive characteristics in other plants, APHIS considers the potential for gene flow and introgression of the biotechnology-derived trait into other plant varieties or wild relatives. Therefore, pollen morphology and viability of MON 87701 were assessed. Morphological characterization of pollen produced by MON 87701 and the conventional soybean control are relevant to APHIS's plant pest risk assessment because they add to the detailed description of the phenotype of MON 87701 compared to the conventional soybean.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from MON 87701 compared to a conventional soybean control. Pollen was collected from MON 87701, the control (A5547), and four commercially-available reference soybean varieties grown under similar agronomic conditions in a field trial in Illinois. The trial was arranged in a randomized complete block design with three replications. 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 three replications of MON 87701, the control, and the reference soybean varieties (see Appendix H).

No statistically significant differences were detected ($p>0.05$) between MON 87701 and the control for percent viable pollen or pollen grain diameter (Table VIII-6). Furthermore, no visual differences in general pollen morphology were observed between MON 87701 and the control. These results demonstrate that the introduction of the insect-protected trait and the expression of the Cry1Ac protein did not alter the overall morphology or viability of MON 87701 pollen compared to conventional soybean control. The lack of statistically significant differences between the pollen collected from

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MON 87701 compared to the conventional soybean control for the assessed characteristics demonstrate that the observed values were within the range of observations expected for soybean. Thus, these data further support no change in plant pest potential for MON 87701 compared to the non-transformed control and other soybean varieties.

Table VIII-6. Pollen Grain Diameter and Viability Analyses

Pollen Characteristic	MON 87701 Mean (SE)	A5547 Mean (SE)	Reference Range[1] Minimum	Reference Range[1] Maximum
Viability (%)	99.7 (0.3)	99.3 (0.7)	97.4	98.9
Diameter (µm)	24.6 (0.3)	24.5 (0.3)	24.9	25.4

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3).
No significant differences were detected betweenMON 87701 and the conventional soybean control A5547 ($p>0.05$).
[1] Reference range is the minimum and maximum mean value observed among the four reference soybean varieties.

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VIII.D.4. Symbiont Interactions

Members of the bacterial family *Rhizobiaceae* and *Bradyrhizobiaceae* form a highly complex and specific symbiotic relationship with leguminous plants, including soybean (Gage, 2004). The *Rhizobium*-legume symbiosis results in the formation of root nodules, providing an environment in which differentiated bacteria called bacteroids are capable of reducing or "fixing" atmospheric nitrogen. The product of nitrogen fixation, ammonia, can then be utilized by the plant. In soybean, atmospheric nitrogen is fixed into organic nitrogen through a symbiotic association with the bacterium *Bradyrhizobium japonicum*. As a result of this relationship, no nitrogen inputs are needed for agricultural production of soybean.

As part of the plant pest risk assessment, APHIS considers the impact of the biotechnology-derived crop to agricultural or cultivation practices (7 CFR §340.6). Changes in the symbiotic relationship with *Rhizobiaceae* and *Bradyrhizobiaceae* could directly impact cultivation practices (i.e., need to add additional nitrogen to soybean production). Thus, the purpose of this evaluation was to assess whether the *B. japonicum*-soybean symbiosis of MON 87701 had been altered as a result of the introduction of the *cry1Ac* gene and the Cry1Ac protein production compared to a conventional soybean control.

The relative effectiveness of the symbiotic association between a leguminous plant and its rhizobial symbiont can be assessed by various methods. Assessment of nodule number and mass along with plant growth and nitrogen status are commonly used to assess differences in the symbiotic association between a legume and its associated rhizobia (Israel et al., 1986). It should be noted, however, that nodule number relative to nodule dry weight may be variable in soybean experiments because some nodules may be

larger in diameter and less numerous while others are not as developed (smaller) but more abundant (Israel et al., 1986; Appunu et al., 2006).

MON 87701, a conventional soybean control (A5547), and reference soybean varieties were produced from seeds planted in pots containing nitrogen-deficient potting medium and grown in a 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, then shoot and root plus nodule material were removed from the pots. Nodules were separated from roots prior to enumeration and determination of dry weight (dwt). Detailed information on materials and methods used for symbiont evaluation is presented in Appendix I.

No significant differences were detected between MON 87701 and the control for each measured parameter, including nodule number, shoot total nitrogen (percent and mass), and biomass (dwt) of nodules, shoot material, and root material (Table VIII-7). In addition, each measurement endpoint evaluated for MON 87701 was within the range of the conventional reference soybean varieties. Based on the assessed characteristics, the results support the conclusion that the introduction of the insect-protected trait does not alter the symbiotic relationship between *B. japonicum* and MON 87701 compared to conventional soybean. Thus, there is no expected impact to cultivation practices relative to nitrogen inputs and no increased plant pest potential for MON 87701 compared to the non-transformed control or other soybean varieties.

Table VIII-7. Symbiont Interaction Assessment of MON 87701 and the Control

Measurement Endpoint	MON 87701 Mean (SE)*	A5547 Mean (SE)	p-Value*	Reference Range[1]	
				Min	Max
Nodule Number (per plant)	178 (12)	212 (25)	0.131	105	228
Nodule Dry Wt (g/plant)	0.58 (0.03)	0.58 (0.03)	0.853	0.42	0.59
Root Dry Wt (g/plant)	2.33 (0.13)	2.16 (0.13)	0.304	1.42	2.35
Shoot Dry Wt (g/plant)	8.63 (0.52)	8.00 (0.42)	0.381	5.80	8.89
Shoot Total Nitrogen (% dwt)	2.70 (0.07)	2.76 (0.09)	0.724	2.33	3.30
Shoot Total Nitrogen (g)	0.23 (0.01)	0.22 (0.09)	0.457	0.16	0.27

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on eight replicates (N=8).

* No significant differences were detected between MON 87701 and the conventional soybean control A5547 ($p > 0.05$).

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

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VIII.E. Overall 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 the insect-protected trait and the expression of the Cry1Ac protein altered the plant pest potential of MON 87701 compared to the conventional soybean control, which had a genetic background similar to MON 87701 but did not possess the insect-protected trait. Phenotypic and agronomic characteristics of MON 87701 were evaluated and compared to those of the conventional soybean control. These assessments included 14 plant growth and development characteristics; five seed dormancy and germination parameters under six different temperature regimes; two pollen characteristics; more than 500 observations for the abiotic stressor, disease damage, arthropod damage, arthropod abundance, and plant-symbiont interactions; and compositional evaluation (Section VII) of 64 different components (seven in forage, and 57 in harvested seed).

Results from the phenotypic and agronomic assessments demonstrate that MON 87701 does not possess characteristics that would confer a plant pest risk compared to conventional soybean. Data on environmental interactions indicate that MON 87701 does not confer any biologically-meaningful increased susceptibility or tolerance to specific disease, insect, or abiotic stressors, or changes in agronomic and phenotypic characteristics with the exception of protection against certain lepidopteran pests. Taken together, these data conclude that MON 87701 is not likely to pose increased plant pest risk compared to conventional soybean.

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IX. U.S. AGRONOMIC PRACTICES

IX.A. Introduction

This section provides a summary of the key agronomic practices in the U.S. for producing soybean. Discussions will include soybean production, growth and development, general management practices, management of weeds, insects and diseases, soybean rotational crops, and volunteer soybean management. Special emphasis is placed on insect management and the pest management targets for MON 87701 and the anticipated impacts on agronomic practices from the deregulation of MON 87701.

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

Annual and perennial weeds are perceived to be the greatest pest problem in soybean production. Economic thresholds for controlling weeds in soybean require some form of weed management practice on all soybean acreage. Approximately 98% of the soybean acreage receives an herbicide application. Soybean insects and diseases are generally considered less problematic, although infestations do reach economic thresholds requiring treatment.

Soybean insect problems are variable from year to year due largely to variability in environmental conditions (Higley and Boethel, 1994). Generally, insect infestations are greater in the Southern U.S. soybean growing region. Insects can feed on soybean and cause damage throughout the growing season. However, insects that feed on leaves, stems and pods of soybean during the reproductive stages in mid- to late-season cause the most damage and can result in yield losses. In areas where insect pests are routinely present, soybean fields must be scouted frequently during this period to determine whether insect populations have reached economical threshold levels to warrant an insecticide application. About 16% of the U.S. soybean acreage received an insecticide application in 2006.

Disease problems in soybean are also extremely variable because of variability in environmental conditions. Selecting soybean varieties with disease resistance is the first line of defence against most common diseases. In the past fungicides were rarely used in soybean production, however, uses of fungicides, especially as seed treatments, has recently begun to increase (Miles et al., 2003; Mueller, et al., 1999).

Volunteer soybean is not considered a significant concern in rotational crops primarily because of climatic conditions and adequate control from tillage practices. Additionally, mechanical and chemical control methods are available to manage the occasional volunteer soybean plant. Due to the lack of weediness potential, introduction of MON 87701 in the soybean production system would have a negligible impact on managing soybean volunteer plants in rotational crops such as corn, cotton, and rice

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because control measures are available for volunteer soybean when they arise. Preplant tillage is the first management tool for control of emerging volunteer soybean in the spring. If volunteer soybean should emerge after planting, shallow cultivation will control most of the plants and effectively reduce competition with the crop. Several post emergence herbicides also are available to control volunteer soybean (conventional or glyphosate-tolerant soybean) in each of the major rotational crops.

As shown in Sections VII and VIII, with the exception of the intended insect-protected trait, no phenotypic, compositional, or environmental differences between MON 87701 and conventional soybean have been observed. Therefore, it is not anticipated that commercialization of MON 87701 in the U.S. would have a notable impact on current soybean cultivation practices including the management of weeds, diseases, and insects except for the control of lepidopteran insect pests.

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

IX.B.1. Soybean Production

Soybean first entered North America in the 18th century (Hoeft et al., 2000). Sometime during the 1930s, soybean started to be processed industrially in the U.S. for edible oil and protein meal. Currently, the U.S. produces approximately 32% of the global soybean supply (ASA, 2008). In 2007, the U.S. exported 1 billion bushels (27.9 million metric tons) of soybean, which accounted for 37 percent of the world's soybean exports (ASA, 2008). In total, the U.S. exported $[redacted] USD worth of soybean and soybean products globally in 2007 (ASA, 2008). China is the largest export market for U.S. soybean with purchases totalling $[redacted]. Japan is the second largest export market with sales of $[redacted] in the same year. Other significant markets include the European Union and Mexico.

The production of 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 eastern half of the U.S. provide sufficient water supplies 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 acreage to supplement the water supply during dry periods in the western and southern soybean growing regions (ASA, 2008).

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

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moisture. Economic factors include expected soybean price and anticipated economic return (Boerma and Specht, 2004).

The U.S. soybean acreage in the past ten years has varied from approximately 63.6 to 75.5 million acres, with the lowest acreage recorded in 2007 and the highest in 2006 (Table IX-1). Average soybean yields have varied from 33.9 to 43.3 bushels per acre over this same time period. Soybean production ranged from 2.45 to 3.19 billion bushels over the past ten years, with 2006 being the largest production year on record. According to data from USDA-NASS (2008a), soybean was planted on approximately 63.6 million acres in the U.S. in 2007, producing 2.6 billion bushels of soybean (Table IX-1). Soybean acreage and production in 2007 was down significantly from 2006, mainly due to a large increase in corn acreage. The average yield in 2007 of 41.5 bushels per acre is slightly below the highest average yield (43.3 bu./acre) on record in 2005. The value of soybean reached [redacted] in the U.S. in 2007 (ASA, 2008; Soya and Oilseed Bluebook, 2008). In comparison, corn and wheat values in 2007 were $[redacted] and $[redacted], respectively (USDA-NASS, 2008b).

For purposes of this agronomic practices discussion, soybean production is divided into three major soybean growing regions accounting for 99.5% of the 2007 U.S. soybean acreage – Midwest/Great Plains region (IL, IN, IA, KS, KY, MI, MN, MO, NE, ND, OH, SD, and WI), Southeast region (AL, AR, GA, LA, MS, NC, SC, and TN) and the Eastern Coastal region (DE, MD, NJ, NY, PA, and VA) (Table IX-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 13.0% and 2.8% of the acreage, respectively. Among the three regions, the Midwest region produced the highest average yield at 43.1 bushels per acre in 2007, and average state yields in this region ranged from 26.0 to 51.5 bushels per acre. The average yield in the Southeast region was 31.0 bushels per acre, with states within this region averaging from 18.0 to 42.0 bushels per acre. The average yield in the Eastern Coastal region was 31.7 bushels per acre, with individual state averages ranging from 24.0 to 41.0 bushels per acre.

Managing input costs is a major component to the economics of producing a soybean crop. Key decisions on input costs include choosing what seed or soybean varieties to plant, amounts of fertilizer to apply, and what herbicide program to use. The average operating cost for producing soybean in the U.S. in 2006 was $[redacted] per acre according to statistics compiled by the USDA-Economic Research Service (USDA-ERS, 2006). The value of the production less operating cost was reported to be $[redacted] per acre. A summary of typical potential production costs and returns from this farmer survey are presented in Table IX-3.

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Table IX-1. Soybean Production in the U.S., 1998 – 2007

Year	Acres Planted[1] (×1000)	Acres Harvested[1] (×1000)	Average Yield[1] (bushels/acre)	Total Production[1] (×1000 bushels)	Value[2] (billions $)
2007	63,631	62,820	41.2	2,585,207	[redacted]
2006	75,522	74,602	42.7	3,188,247	[redacted]
2005	72,142	71,361	43.3	3,086,432	[redacted]
2004	75,208	73,958	42.2	3,123,686	[redacted]
2003	73,404	72,476	33.9	2,453,665	[redacted]
2002	73,963	72,497	38.0	2,756,147	[redacted]
2001	74,075	72,975	39.6	2,890,682	[redacted]
2000	74,266	72,408	38.1	2,757,810	[redacted]
1999	73,730	72,446	36.6	2,653,758	[redacted]
1998	72,025	70,441	38.9	2,741,014	[redacted]

[1] Source: USDA-NASS, 2008a.
[2] Source: USDA-NASS, 2008b.

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Table IX-2. U.S. Soybean Production by Region and State in 2007

Region/State	Acres Planted[1] (thousands)	Acres Harvested[1] (thousands)	Average Yield[1] (bushels/acre)	Total Production[1] (×1000 bushels)	Value[2] (billions $)
Midwest Region					
Illinois	8,200	8,150	43.0	350,450	[redacted]
Indiana	4,700	4,680	45.0	210,600	[redacted]
Iowa	8,550	8,520	51.5	438,780	[redacted]
Kansas	2,600	2,550	33.0	84,150	[redacted]
Kentucky	1,100	1,080	26.0	28,080	[redacted]
Michigan	1,750	1,740	39.0	67,860	[redacted]
Minnesota	6,250	6,150	41.0	252,150	[redacted]
Missouri	4,600	4,550	37.0	168,350	[redacted]
Nebraska	3,800	3,770	50.5	190,385	[redacted]
North Dakota	3,050	2,990	35.0	104,650	[redacted]
Ohio	4,150	4,130	47.0	194,110	[redacted]
South Dakota	3,200	3,180	42.0	133,560	[redacted]
Wisconsin	1,350	1,330	39.0	51,870	[redacted]
Region Totals	**53,300**	**52,820**	**43.1**	**2,274,995**	[redacted]
Southeast Region					
Alabama	190	180	21.0	3,780	[redacted]
Arkansas	2,830	2,790	36.0	100,440	[redacted]
Georgia	285	275	30.0	8,250	[redacted]
Louisiana	605	590	42.0	24,780	[redacted]
Mississippi	1,450	1,420	40.0	56,800	[redacted]
North Carolina	1,420	1,360	21.0	28,560	[redacted]
South Carolina	450	425	19.0	8,075	[redacted]
Tennessee	1,040	970	18.0	17,460	[redacted]
Region Totals	**8,270**	**8,010**	**31.0**	**248,145**	[redacted]
Eastern Coastal Region					
Delaware	150	145	24.0	3,480	[redacted]
Maryland	400	380	27.0	10,260	[redacted]
New Jersey	81	79	31.0	2,449	[redacted]
New York	205	203	38.0	7,714	[redacted]
Pennsylvania	425	420	41.0	17,220	[redacted]
Virginia	500	480	27.0	12,960	[redacted]
Region Totals	**1,761**	**1,707**	**31.7**	**54,083**	[redacted]

[1] Source: USDA-NASS, 2008a.
[2] Source: USDA-NASS, 2008b.

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Table IX-3. U.S. Soybean Production Costs and Returns in 2006

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

Supporting Information: Yield = 46 bushels/acre, Price = $[redacted]/bushel, Enterprise size = 268 planted acres, Irrigated = 9%, Dry land = 91%.

Source: USDA-ERS, 2006.

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IX.B.2. Soybean Seed Production

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 to maintain specific varietal purity and identity. 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 two generations from foundation seed. All soybean seed sold may not be officially certified; however, commercial soybean seed sold and planted for normal soybean production is predominately produced to meet or exceed certified seed standards. This section of the Petition will provide a broad overview of the practices utilized in producing certified seed.

Soybean seed breeders and producers have put in place practical measures to assure the quality and genetic purity of soybean seed varieties 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 United States to preserve the genetic identity and variety purity of seed varieties. There are special land requirements, seed stock eligibility requirements, field inspections and seed labelling standards for seed certification. Seed certification services are available through various state agencies affiliated with the Association of Official Seed Certifying Agencies (AOSCA). Large seed producers implement their own seed quality assurance programs. However, large seed producers will utilize the services of state certifying agencies as a third party source to perform certain field inspections and audits.

The U.S soybean production for all purposes according to USDA-NASS statistics has varied from approximately 63.6 to 75.5 million acres in the past ten years, with the lowest acreage recorded in 2007 and the highest in 2006 (USDA-NASS, 2008a; Table IX-1). This range of soybean acreage would require between 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.

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 IX.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 companies which produce and sell seed, such as Monsanto Company, Pioneer Hi-Bred Intl., Syngenta Seeds, Kruger Seed Co., Becks Hybrids, and tollers, which are companies that produce but do not sell certified seed, such as

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Remington Seeds LLC and Precision Soya. Seed companies and tollers in turn contract acreage with growers to produce the required amount of soybean seed. Production or processing plants at these seed companies identify top soybean growers to produce the seed and also monitor and inspect seed fields throughout the growing season. The 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 is ISO (International Organization for Standardization) certified and therefore includes internal and external audits (ISO, 2009). The 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 must not only meet the customer's requirements and applicable seed regulatory requirements, but they aim to enhance customer satisfaction and achieve continual improvement of its performance in pursuit of these objectives.

The field operations and management practices for producing soybean seed are very 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. The seed production field should not have been planted to soybean the previous crop in order to avoid volunteer soybean plants (even though the risk of soybean volunteer plants is negligible) and ensure genetic purity.

Very early planting should be 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 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 during the reproductive stages 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 minimize genetic contamination. Certain 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 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

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requirement of five feet (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 identity of the seed is maintained throughout the handling and storage operation. Bin inspections and sample collections are conducted at storage locations at the plant to examine the physical characteristics of the soybean seed and 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.

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

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 which were distributed to growers for use. Seed were 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, presence of weed seed, seed borne diseases and inert materials, such as dirt) was quickly identified as a major factor in 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 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 in the U.S., and includes international member countries in North and South America, as well as Australia and New Zealand.

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IX.C. Production Management Considerations

Pre-Season

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

The benefits of conservation tillage or no-till systems are well documented and include reduced soil erosion, reduced fuel and labor costs, and conserving soil moisture. In 2004, approximately 29.3 million acres (38.6%) of soybean were planted in a no-till system (CTIC, 2004). Slow soybean emergence and growth plus lower yields have been some of the concerns associated with adoption of conservation tillage systems in soybean, especially no-till. Research in Wisconsin and Minnesota shows that soil temperatures can be four to five degrees colder in no-till than conventional tillage systems which can slow emergence, but have little effect on soybean yield (Pedersen, 2008a). Improved planters for establishment of good soybean populations and planting Roundup Ready soybean to effectively control weeds in no-till fields have made no-till a viable production system for soybean. Researchers still recommend some spring tillage on fine-textured and poorly drained soils for proper seedbed preparation (Pedersen, 2008a).

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

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Soil tests are the only reliable way to determine the pH, phosphorus, and potassium levels in the soil. Liming and fertilizer requirements are subsequently 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, for soybean plants which require large amounts of phosphorus and potassium, fertilizer is often needed in some of the southern growing areas due to differences in crop rotations and soil types.

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

Soybean varieties are developed and adapted to certain geographical zones and are separated into ten maturity groups – Group 00 to Group VIII (Pimentel, 1991; Zhang et al., 2004). Groups 00 and 0 are the earliest maturity groups and are adapted best to the area north of latitude 46º north. Succeeding groups are adapted further south with Groups I and II within latitudes 41º and 46º north, and Group III within latitudes 38º and 41º North. Group 00 through Group IV soybean varieties are planted in the Midwest and Eastern Coastal regions. Groups II, III and IV account for approximately 75% (24%, 36%, and 16%, respectively) of the soybean planted in the U.S., with Group III having the largest acreage (Schlueter, 2008). Groups IV through VIII are planted in the southern states with Groups V, VI and VII representing 7%, 2%, and 2% of the planted soybean, respectively (Schlueter, 2008).

Soybean variety selection is crucial for high yield and quality, and is the foundation of an effective management plan (Pedersen, 2008a). Soybean 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.

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

Planting and Early Season

An understanding of the growth stages of soybean is also important for the proper timing of certain management practices, such as herbicide and insecticide applications. In addition, the impact of certain weather conditions, insect pests, and diseases on soybean

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

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

Planting date has the greatest impact on yield according to research conducted in the northern states (Hoeft et al., 2000). Highest yields are generally obtained when planting is in early to mid May. Yields begin to drop off quite rapidly when planting is delayed until late May. For example, the optimum planting dates for soybean in Iowa are the last week of April in the southern two-thirds 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. Soybean has the ability to produce good yield over a wide range of plant populations. Most soybean varieties have the ability to branch and adjust the number of pods on branches to compensate for large differences in seeding rate. Maximum yields generally require planting rates that result in about 2.5 to five plants per square foot (Hoeft et al., 2000). Therefore, a full stand of soybean is approximately eight to 10 plants per foot of row at harvest for 40-inch rows, six to eight plants per foot of row in 30-inch rows, four to six plants in 20-inch rows, and two to three plants in 10-inch rows. This translates to 109,000 to 218,000 plants per acre at harvest. Higher populations are recommended in narrow rows for maximum yields because plants are more uniformly spaced in narrow rows. Seeding rates are generally 10 to 25% higher than the desired harvest population, especially in no-till, to account for the losses in germination, emergence, and seedling diseases. The accuracy of the planting equipment can also impact the decision on seeding rate. Soybean seed has traditionally been sold by weight. Therefore, the farmer must know the number of seeds per pound for the particular soybean varieties being planted for accurate seeding rates.

Treating soybean seed with a fungicide (e.g., pyraclostrobin, metalaxyl, mefenoxam) to prevent damping-off diseases may be beneficial when planting in cold, wet soils, using

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reduced till and no-till planting systems, and when planting seed with a low germination rate (<80%) or low seed vigor.

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

Mid to Late Season

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

Soybean is photoperiod sensitive, which means that it transitions from vegetative to flowering stage in direct response to length of daylight (Scott and Aldrich, 1970). Most soybean varieties begin flowering soon after the day length begins to shorten. Flowering of southern varieties is initiated by a shorter day than that of varieties adapted to the 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. These are grown in the northern and central U.S. Determinate varieties increase their height very little after flowering and are generally grown in the southern U.S. Indeterminate and determinate varieties also differ in flowering characteristics. Indeterminate plants generally bloom first at the fourth or fifth node and progress upward. Flowering on determinate plants begins at the eight or tenth node and progresses both downward and upward.

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

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

IX.D. Weed Management

Annual weeds are perceived to be the greatest pest problem in soybean production, followed by perennial weeds (Aref and Pike, 1998). Soybean insects and diseases are rated less problematic but may reach economic thresholds requiring treatment. Weed control in soybean is essential to optimizing yields. Weeds compete with soybean for light, nutrients, and soil moisture. Weeds can harbor insects and diseases, and can also interfere with harvest, causing extra wear on harvest equipment (Pedersen, 2008a). The primary factors affecting soybean yield loss from weed competition are the weed species, weed density, and the duration of the competition. When weeds are left to compete with soybean for the entire growing season, yield losses can exceed 75% (Dalley et al., 2001). Generally, the competition increases with increasing weed density. The time period that weeds compete with the soybean crop influences the level of yield loss. In general, the later the weeds emerge, the less impact the weeds will have on yield. Soybean plants withstand early season weed competition longer than corn, and the canopy closes earlier in soybean than corn. In addition, canopy closure is much sooner when soybean is drilled or planted in narrow rows.

Crop rotations and environment have a significant impact on the adaptation and occurrence of weeds in soybean. Foxtail spp. (foxtail species group), pigweed, velvetleaf, lambs quarters, and cocklebur are common weeds in Midwest corn and soybean fields. However, growers consider giant ragweed (*Ambrosia artemisiifolia*), lambs quarters (*Chenopodium album*), Canada thistle (*Cirsium arvense)*, cocklebur (*Xanthium strumarium*), and velvetleaf (*Abutilon theophrasti*) to be the top five most problematic weeds in corn and soybean because of the difficulty to control these weeds (Nice and Johnson, 2005). The most frequently reported common weeds in the Southeast region are morning glory (*Ipomoea spp.*), prickly sida (*Sida spinosa*), johnsongrass (*Sorghum halepense*), sicklepod (*Cassia obtusifolia*), and broadleaf signalgrass (*Brachiaria platyphylla*)(Webster et al., 2005).

Cultural and mechanical weed control practices are important components of an effective weed management program (Baumann et al., 2008). Crop rotation, narrow row spacing,

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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 percent of the soybean acreage received an herbicide application in 2006 indicating the importance of excellent weed control in maximizing soybean yield (USDA-NASS, 2007b). Herbicide-tolerant soybean were introduced to provide growers with additional options to improve crop safety and/or improve weed control. The Roundup Ready soybean system – that is, planting Roundup Ready soybean and applying glyphosate in crop – has become the standard weed control program in U.S. soybean production. Currently, Roundup Ready soybean is planted on 91 percent of the soybean acreage (USDA-NASS, 2007a). Consequently, glyphosate is the most widely used herbicide in soybean, being applied on 96 percent of the soybean acreage in 2006 (USDA-NASS, 2007b).

IX.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, 1998). Understanding the impact of insects on soybean growth is essential for proper management (Higley and Boethel, 1994). It is important to understand the way that insects injure soybean as well as how the soybean plant responds to insect injury. Insect injury can impact yield, plant maturity, and seed quality. Injury is defined as a stimulus producing an abnormal change in plant physiological processes. Injury may produce stress which is a departure from optimal physiological conditions (Higley and Boethel, 1994). The ultimate impact of injury is damage – a measurable reduction in plant growth development or reproduction. 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 (Table IX-4).

Characterizing soybean responses to insect injury is essential in establishing economic injury levels (Higley and Boethel, 1994). Most often, soybean insects are categorized or defined by the plant parts they injure, namely root-feeding, stem-feeding, leaf-feeding, or pod-feeding insects. The root- and stem-feeding insect groups are often the hardest to scout and typically are not detected until after they have caused their damage. The leaf-feeding insects comprise the biggest group of insects, but not necessarily the most damaging insects. Recent research on defoliation has determined that a major effect of 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. but 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 Extension Service, 2002). However, from pod-set to maturity, the plant can tolerate only a 20% defoliation level before yield is impacted.

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The most damaging defoliating insects are velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), green cloverworm (*Plathypena scabra*), Mexican bean beetle (*Epilachna varivestis*), and bean leaf beetle (*Cerotoma trifurcate*) (Higley and Boethel, 1994). The pod-feeding insects are generally the most detrimental to yield since they directly affect the reproductive parts.

Soybean response to increasing levels of insect injury varies among insects. For example, stink bug feeding on soybean pods produces a linear yield loss response; where each increase in injury causes a corresponding increase in yield loss (Higley and Boethel, 1994). In comparison, stand reductions caused by seedcorn maggot (*Delia platura*) feeding on germinating soybean seeds do not affect yields until levels of injury become very high (Higley and Boethel, 1994). The environment also impacts the response of soybean to insect injury. The most important environmental factor is water stress. In general, yield reductions from insect injury are more severe under water stress.

Another approach to characterization of insect injury is on the basis of how it impacts soybean physiology (Higley and Boethel, 1994). The identification of physiological responses to injury is important in developing insect pest management programs that are efficient and accurate. One approach is the concept of insect guilds. A guild is a collection of insect pest species that attack the same soybean parts and produce the same types of soybean responses to injury. It is usually more practical to estimate the impact of the collection of pest species and make management decisions based on the combined effect of the guild rather than manage each species independently. One guild of soybean insects is the defoliating caterpillars; soybean looper, velvetbean caterpillar, and green cloverworm. These lepidopteran species commonly occur together and procedures have been developed for making management decisions based on the combined action of these pests.

Soybean is attacked by numerous insects throughout the growing season, but only a few pose a significant economic threat, and not to all production regions (Higley and Boethel, 1994). Eight species typically account for most insect damage in U.S. soybean production, namely: velvetbean caterpillar, soybean looper, green cloverworm, Mexican bean beetle, bean leaf beetle, southern green stink bug (*Nezara viridula*), green stink bug (*Acrosternum hilare*), and corn earworm (*Helicoverpa zea*)(Higley and Boethel, 1994). Some species may cause damage every year, but even a relatively minor pest can seriously affect soybean production if the insect occurs in sufficient numbers. Soybean insect pest populations and soybean damage from insect pests varies annually and regionally due to differences in climatic and weather conditions, species distributions and environmental tolerances and production practices (Higley and Boethel, 1994).

The occurrence of soybean insects follows a north-south gradient (Higley and Boethel, 1994). Generally, soybean insect pest problems are less severe in the Midwest states than in other soybean producing areas (Higley and Boethel, 1994). However, minor pest problems can have a serious economic impact because of the large acreage of soybean grown in the Midwest. Table IX-5 lists the important soybean insect pests in the Midwest region. Green cloverworm is the only lepidopteran insect that occurs frequently in the Midwest. Cutworms (*Agrostis ipsilon, Peridroma saucia*) and Painted Lady (*Cynthia cardui*) are other lepidopteran pests found in the Midwest region, but occur less

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frequently. While economic insect problems were quite rare in the northern Midwest region during the 1970s, they became more frequent in the 1990s (Hammond, 1996). For example, Mexican bean beetle and bean leaf beetle have caused severe soybean damage in the Midwest region during the past two decades. However, insect problems in the Midwest seldom reach economic threshold levels to justify an insecticide treatment.

Soybean production in the Eastern Coastal region is relatively free of insect pests. However, some soybean fields may require insect control on occasion. Eight insects occasionally are abundant in numbers high enough to cause economic losses in soybean (Penn State University, 2008). Seven are foliage feeding insects: soybean aphid (*Aphis glycines*), green cloverworm, Japanese beetle (*Popillia japonica*), potato leafhopper (*Empoasca fabae*), Mexican bean beetle, bean leaf beetle, and grasshopper (*Melanoplus spp.*). The seedcorn maggot is a seed-feeding insect that can reduce the stands of soybean especially when a living, green cover crop is incorporated into the soil prior to planting and when conditions are cool and moist for long periods after the seed is planted.

Insect pressure is generally greatest in the Southeast region, particularly in the southern states bordering the Gulf of Mexico and the Atlantic Ocean. Table IX-6 lists the soybean pests in the southern states. Four of these major insects are lepidopteran insect pests. Velvetbean caterpillar and soybean looper infestations are greatest in the southeastern states because of their close proximity to the tropics where these insect pests overwinter and because the warm climate facilitates multiple generations per year (Heatherly and Hodges, 1999). Numerous other insect pests are present in the Southeast region, but occur less frequently and are of lesser economic importance.

Stink bugs (*Nezara viridula, Acrosternum hilare, Euschistus servus*), which are of the hemiptera insect order, are the number one soybean insect pest in the southeastern states in terms of infestations and economic losses (McPherson et al., 1999). They account for approximately 50% of losses attributable to insects in soybean in the southeastern states. Nevertheless, several of the economically significant insect pests of soybean are lepidopterans (Higley and Boethel, 1994). The lepidopteran insects, primarily soybean looper, velvetbean caterpillar, corn earworm (soybean podworm), and lesser cornstalk borer (*Elasmopalpus lignosellus*) are typically responsible for most of the remaining economic insect damage in the southeastern states (McPherson et al., 1999). Velvetbean caterpillar and soybean looper are considered the most damaging defoliating insects in the South, accounting for over $[redacted] in damage and control costs in the southeastern states in 1984 (Higley and Boethel, 1994). Insecticides are used on approximately 50% of the soybean acreage in Georgia for lepidopteran pests with velvetbean caterpillar being the most targeted pest (Gianessi et al., 2002). Approximately 40% of the soybean acreage in Louisiana is treated with insecticides for lepidopteran pests, with soybean looper being the main target (Gianessi et al., 2002). Based on the extensive damage to soybean production in the Southeast region, additional acreage potentially could be treated for lepidopteran pests.

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Table IX-4. Insecticide Applications Registered for Soybean Use in AR, IL, IN, IA, KS, KY, LA, MI, MN, MS, MO, NE, NC, ND, OH, SD, TN, VA, and WI in 2006[1]

Insecticide	Chemical Family	Mode of Action (MOA)	Area Applied (Percent)	Total Area Applied (Percent/MOA)	Quantity Applied (1000 lbs)	Total Quantity Applied (1000 lbs/MOA)
Acephate	organophosphate	Acetylcholine esterase inhibitors	1	6	546	2,275
Chlorpyrifos	organophosphate		5		1,663	
Methyl parathion	organophosphate		*		66	
Benzoic acid			*	<0.5%	9	9
Carbaryl	carbamate	Acetylcholine esterase inhibitors	*	<1%	91	130
Thiodicarb	carbamate		*		39	
Cyfluthrin	pyrethroid	Sodium channel modulators	*	10	10	206
Esfenvalerate	pyrethroid		3		70	
Gamma-cyhalothrin	pyrethroid		*		3	
Lambda-cyhalothrin	pyrethroid		6		97	
Permethrin	pyrethroid		*		12	
Zeta-cypermethrin	pyrethroid		1		14	
Diflubenzuron	benzoylureas	Inhibitors of chitin biosynthesis	*	<0.5%	10	10

* Area receiving application is less than 0.5 percent. Planted acreage for the 19 primary soybean production states was 71.9 million acres, which represents 95% of total planted acres.

[1] USDA-NASS, 2007b.

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Table IX-5. Important Soybean Pests in the Midwest Region of the U.S.

Common name	Scientific Name/Order	Primary Feeding Site
	Frequently Occurring Pests[1]	
Soybean aphid	*Aphis glycines* / H [2]	Leaf
Japanese beetle	*Popillia japonica* / C	Leaf
Bean leaf beetle	*Cerotoma trifurcata* / C	Leaf
Mexican bean beetle	*Epilachna varivestis* / C	Leaf
Twospotted spider mite	*Tetranychus urticae* / A	Leaf
	Less Frequently Occurring Pests	
Blister beetles	*Epicauta spp.* / C	Leaf
Cutworms,		
Black,	*Agrotis ipsilon* / L	Stems
Southern armyworm	*Spodoptera eridania* / L	Stems and leaf
Variegated	*Peridroma saucia* / L	Stems
Grasshoppers,		
Redlegged	*Melanoplus femurrubrum*/ O	Leaf, pods, seeds
Differential	*Melanoplus* differentialis /O	Leaf, pods, seeds
Green cloverworm	*Plathypena scabra* /L	Leaf
Painted Lady	*Cynthia cardui* /L	Leaf
Potato leafhopper	*Empoasca fabae* / H	Leaf and veins
Seedcorn maggot	*Delia platura* / D	Seed
Soybean thrips	*Sericothrips variablis* /T	Leaf
Stink bugs,		
Green	*Acrosternum hilare* / H	Pods, seeds
Brown	*Euschistus servus* / H	Pods, seeds
Grey garden slugs	*Derocerus reticulatum*	Seed

[1] Order of importance ([redacted] 2008, Personal communication).
[2] Insect Orders: A-Acari; C-Coleoptera; D-Diptera; H-Hemiptera; I-Isoptera; L-Lepidoptra; O-Orthoptera; T-Thysanoptera (Pimentel, 1991).

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Table IX-6. Important Soybean Pests in the Southeast Region of the U.S.

Common name	Scientific name/Order	Plant parts injured
	Frequently Occurring Pests[1]	
Southern green stink bug	*Nezara viridula* / H[2]	Pods, seeds
Green stink bug	*Acrosternum hilare* / H	Pods, seeds
Brown stink bug	*Euschistus servus* / H	Pods, seeds
Bean leaf beetle	*Cerotoma trifurcate* / C	Roots, leaf blades, pods,
Three cornered alfalfa	*Spissistilus festinus* / H	Lower stems
Soybean looper	*Pseudoplusia includens* / L	Leaf blades
Corn earworm	*Helicoverpa zea* / L	Leaf blades, pods, seeds
Velvetbean caterpillar	*Anticarsia gemmatalis* / L	Leaf blades
Lesser cornstalk borer	*Elasmopalpus lignosellus* / L	Lower stems
	Less Frequently Occurring Pests	
Silverleaf whitefly	*Bemisia argentifolii* / H	Leaf blades
Banded winged whitefly	*Trialeurodes abutiline* / H	Leaf blades
Fall armyworm	*Spodoptera frugiperda* / L	Leaf blades
Yellow striped armyworm	*Spodoptera ornithogalli* / L	Leaf blades
Beet armyworm	*Spodoptera exigua* / L	Leaf blades
Green cloverworm	*Plathypena scabra* / L	Leaf blades
Mexican bean beetle	*Epilachna varivestis* / C	Leaf blades
Redlegged grasshopper	*Melanoplus femurrubrum* / O	Leaf blades, pods, seeds
Differential grasshopper	*Melanoplus differentialis* / O	Leaf blades, pods, seeds
Two spotted spider mite	*Tetranychus urticae* / A	Leaf blades
Wireworms	*Melanotus* species / C	Roots
Grubs	*Phyllophaga* species / C,	Roots
Soybean nodule fly	*Rivellia quadrifasciata*[2] / D	Roots, nodules
Potato leafhopper	*Empoasca fabae* / H	Leaf blades and veins

[1] Order of importance ([redacted], 2008).

[2]: Insect Orders: A-Acari; C-Coleoptera; D-Diptera; H-Hemiptera; I-Isoptera; L-Lepidoptra; O-Orthoptera; T-Thysanoptera (Heatherly and Hodges, 1999).

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Management of insects in soybean involves agronomic, economic and biological factors (Higley and Boethel, 1994). Preventive pest management practices are most important where the pest problem can be anticipated each year. Changes in cultural practices can adversely affect pest species or aid beneficial species. Variety selection, crop rotation, tillage, planting dates, and adjacent crops all play a role in pest outbreaks in a particular field or influence the degree to which natural enemies are effective in suppressing pest populations.

Efforts to develop elite insect resistant soybean lines with high yield and desirable agronomic characteristics through conventional breeding and selection of germplasms exhibiting inherent insect resistance have not been very successful (Boethel, 1999). There has been limited success over the past 30 years in the development of superior soybean cultivars with insect resistance. A resistant conventional soybean variety could have many potential advantages in insect management including effectiveness, selectivity against pest, relatively long stability, compatibility with other tactics and human and environmental safety (Pedigo, 1996). In addition, resistant varieties can be adopted into crop production systems easily and economically. There have only been three cultivars released with soybean insect resistance derived from a plant introduction, and none of these cultivars has been widely accepted by growers because of inadequate resistance levels, inferior seed yield, or poor agronomic characteristics (Lambert and Tyler, 1999). The success may be hindered by the quantitative nature of resistance and by linkage drag from resistant plant introduction donor parents (Narvel et al., 2001). The length of time to develop conventionally-bred insect resistant varieties is also a significant limitation. Some new resistant wheat varieties have required 15 to 20 years for development (Pedigo, 1996). However, new techniques in selection and breeding have shortened this time of development. For example, soybean varieties with resistance to a certain biotype of soybean aphid are expected to be introduced in 2009 which will have taken approximately eight to nine years to develop ([redacted] 2008, Personal communication).

Alternative insect control strategies such as biological insecticides and natural enemies are available, but are not widely used due to cost and limited efficacy (Luttrell et al., 1998; Moscardi, 1999). Subsequently, insecticidal pesticides generally have provided the most effective and economical means of control or suppression of soybean insect pests that reach economic thresholds. Integrated Pest Management (IPM) programs which integrate preventive pest management with insecticidal control have proven most effective in managing pest problems and reducing insecticide usage (Pedigo, 1996).

Despite the advances in developing resistant varieties and the availability of insecticides, it has not been possible or feasible to eliminate all economic losses attributable to insects in soybean (Heatherly and Hodges, 1999). IPM programs are implemented to minimize economic losses from insects. These IPM programs involve scouting or monitoring fields during periods of risk for insect damage. Fields are monitored for growth stage, insect development and population density, and occasionally natural enemy development and population density. Management decisions for insect populations in individual fields are based on economic injury level, which is defined as the lowest population density of each insect likely to cause economic damage. The economic injury level usually changes during the growing season. For example, control of velvetbean caterpillar and similar caterpillars is normally not warranted until greater than 30% of the foliage is destroyed

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prior to bloom, or when 20% of the foliage is destroyed during the bloom, pod set, or fill stages (NDSU Extension Service, 2002). This usually requires an average infestation of four to eight caterpillar larvae per row foot. IPM programs integrate chemical control and biological control, cultural control, and plant resistance to minimize insecticide resistance and reduce dependence on insecticides.

According to USDA-NASS statistics, about 16% of the U.S. soybean acreage in 2006 received an insecticide treatment (Table IX-4). Three insecticides (chlorpyrifos, esfenvalerate, lambda-cyhalothrin) account for almost all the soybean treated acreage. Each of these insecticides controls a similar broad spectrum of insect pests including lepidopteran and non-lepidopteran pests. USDA-NASS statistics are not available to determine the targeted insect pests for these insecticide treatments; however, they do indicate that approximately 15% of the soybean acreage in the Midwest region received an insecticide treatment in 2006 (USDA-NASS, 2007b). These insecticide treatments were predominately for the control of bean leaf beetles and soybean aphids ([redacted] 2008, Personal communication). Approximately 25% of the soybean acreage in the Southeast and Eastern Coastal regions received an insecticide treatment in 2006 with some states in the Southeast requiring treatments on up to 75% of their acreage (USDA-NASS, 2007b). Based on frequency of pest problems in Table IX-6, these treatments are predominately for stink bugs, bean leaf beetle, three cornered alfalfa hopper (*Spissistilus festinus*) and various lepidopteran insect pests.

Chemical insecticides are used for controlling lepidopteran infestations in soybean, but are not always effective. Narrow application windows, the emergence of insecticide resistance, and public pressure for reduced pesticide use limit the desirability of this approach to pest management (Thomas and Boethel, 1994). Soybean looper has developed resistance to every synthetic class of insecticide used against it (Thomas and Boethel, 1994), and resistance to pyrethroids is widespread across the southern U.S. (Felland et al., 1990; Leonard et al., 1990). Insecticides remain effective against velvetbean caterpillar. However, infestations can quickly reach damaging levels and cause economic loss if insecticides are not applied promptly.

MON 87701 offers an efficient and environmentally sound alternative to chemical insecticides for control of lepidopteran pests in soybean. Since the introduction in other crops, biotechnology-derived crop products have become important tools for effective insect pest management. The adoption of insect-protected (Bt) cotton has not only enabled more effective management of lepidopteran pests but also significantly reduced chemical insecticide use (Carriere et al., 2003; Perlak et al., 2001). Likewise, the adoption of insect-protected (Bt) corn has reduced the impact of lepidopteran stalk borers while reducing insecticide use (Armstrong et al., 1995; Pilcher and [redacted], 2003).

As described above in Section X.A.3, MON 87701 provides protection from of a variety of lepidopteran soybean pests. MON 87701 has demonstrated control of velvetbean caterpillar and soybean looper in multiple research trials and geographies which are two of the most important lepidopteran pests in U.S. soybean production (MacRae et al., 2005). Additional studies have shown nearly complete control of corn earworm and green cloverworm and suppression of fall armyworm (*Spodoptera frugiperda*). MON 87701 has the potential to provide similar economic and environmental benefits in soybean fields that experience economic thresholds of lepidopteran pests to those realized

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with the commercialization of insect-protected corn and cotton. These benefits include lower insecticide use, better insect pest control with less scouting, preservation of beneficial insect populations, reduced risks of losses due to suboptimal timing of an insecticidal application, convenience to the grower, safety to the applicator, and more consistency in year-to-year performance in a farm pest management program.

Insect-protected soybean MON 87701 would provide growers an additional option for the control of economically important lepidopteran pests. However, the market fit for MON 87701 is presently limited to the southeastern U.S., where the lepidopteran insect pressure is greatest and economic thresholds for insecticide treatment are reached most frequently. Due to this limited commercial potential in the U.S., the initial commercial production of MON 87701 is targeted for South America. In the U.S., Monsanto is only seeking EPA registration of MON 87701 to allow for domestic breeding and seed multiplication activities. Because the economic threshold for insecticide treatment for these activities is lower than for commercial soybean production, breeders will continue to use existing IPM systems, and the benefits of MON 87701 will not factor into those systems.

In the event that Monsanto eventually seeks and obtains EPA registration of MON 87701 to allow for commercial planting within the U.S., commercial soybean growers in this country could utilize the benefits of MON 87701. As with other insect-protected crops, MON 87701 would not address all pest control problems in soybean and thus, not all insecticide applications would be eliminated. IPM would remain a necessary approach for total insect pest management, where MON 87701 would integrate well into an IPM framework due to its specific nature and consequent positive interaction with classic biological control methods.

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IX.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 (Heatherly and Hodges, 1999). The estimated yield losses to soybean diseases in the U.S. were 10.9, 11.9, and 14.0 million metric tons in 1996, 1997, and 1998, respectively (Wrather et al., 2000). Pathogens can affect all parts of the soybean plant resulting in reduced quality and yield. The extent of losses depends upon the pathogen, the state of plant development and health when infection occurs, the severity of the disease on individual plants, and the number of plants affected (Heatherly and Hodges, 1999).

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

According to field surveys conducted in fifteen soybean producing states during 1996 to 1998, soybean cyst nematode (*Heterodera gylcines*) caused the greatest soybean yield losses (Wrather et al., 2000). Phytophthora root and stem rot (*Phytophthora sojae*), brown stem rot (*Phialophora gregata*), sclerotinia stem rot (*Sclerotinia sclerotiorum*), and seedling diseases followed in economical importance. As expected, yield losses

varied by region. Sclerotinia stem rot caused yield losses in several northern states, but not in other states. *Rhizoctonia* foliar blight losses were greatest in Arkansas, Louisiana, and Texas where humidity and temperature conditions are suitable for disease development.

Selecting resistant varieties is the primary tool growers have for disease control (Heatherly and Hodges, 1999). Resistant varieties may have morphological or physiological characteristics that provide immunity, resistance, tolerance or avoidance to certain pathogens. Cultural practices play an important role in disease management by reducing initial inoculums or reducing the rate of disease development (Heatherly and Hodges, 1999). Preplant tillage can bury crop residue which encourages the decomposition of fungal-resting structures. Crop rotation is routinely recommended as a disease management strategy. Rotating crops interrupts the disease cycle and allows time for the decomposition of inoculums. One exception is *Rhizontonia*, 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 can also be changed to manage soybean diseases.

Soybean cyst nematode (SCN) is one of the most damaging pathogens of soybean throughout the soybean growing regions of the U.S. (Pedersen, 2008b). Losses have been estimated to be at about $[redacted] in the U.S. (Pedersen, 2008a). SCN can cause yield losses up to 50%, where this pest in 2004 alone caused an estimated loss of 50 million bushels in Iowa (Pedersen, 2008b). 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 is also beneficial.

High quality seed is essential for controlling seedling diseases. The most important seedling diseases in soybean are *Phytophthora, Pythium, Rhizoctonia, and Fusarium* (Pedersen, 2008a). Many soybean varieties have race-specific resistance to *Phytophthora.* Treating soybean seed with a fungicide (e.g., pyraclostrobin, metalaxyl, 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. Foliar application of fungicides is the standard disease management practice to limit yield losses due to soybean rust at this time.

Foliar fungicide applications can effectively reduce the incidence of many diseases (Heatherly and Hodges, 1999). However, the economic return from a fungicide application may be limited to select production programs; for instance, primarily to high-yield environments or when producing soybean seed. According to USDA-NASS

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statistics, fungicides were applied on approximately 4% of the soybean acreage in 2006 (USDA-NASS, 2007b).

IX.G. 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 with other crops 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 reduce runoff of nutrients, herbicides, and insecticides (Al-Kaisi et al., 2003; Boerma and Specht, 2004). According to USDA Economic Research Service (ERS) crop residue management studies, 95% of the soybean-planted acreage has been in some form of a crop rotation system since 1991 (USDA-ERS, 2006). Corn- and wheat-planted acreage has been rotated at a slightly lower level of 75% and 70%, respectively. Although the benefits of crop rotation can be substantial, the farmer must make cropping decisions by evaluating both the agronomic and economic returns on various cropping systems. Crop rotations also afford 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 diseases and nematodes (Al-Kaisi et al., 2003; Hoeft et al., 2000). Corn and soybean occupy more than 80% of the farmland in many of the Midwestern states, and the two-year cropping sequence of a soybean-corn rotation is used most extensively in this region. However, a soybean crop sometimes is grown after soybean and then rotated to corn in a three-year rotation sequence (soybean-soybean-corn) in the Midwest. Compared to corn, soybean shows a greater response to being grown after a number of years without soybean. The yields of both corn and soybean are approximately 10% higher when grown in rotation than when either crop is grown continuously (Hoeft et al., 2000).

A combination of conservation tillage practices and crop rotation has been shown to be very effective in improving soil physical properties. Long-term studies in the Midwest indicate that a 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 region and the mid-south states, soybean is grown in a double-cropping system. Double-cropping refers to the practice of growing two crops in one year. This practice can improve income and reduce soil and water losses by having the soil covered with a plant canopy most of the year (Hoeft et al., 2000). In the Midwest, winter wheat is harvested in late June or July, and then soybean is planted into the wheat stubble in a no-till system to conserve moisture. Due to the uncertainty of double-cropping yields, farmers sometimes do not plant if soils are too dry at the time of wheat harvest. Soybean is typically grown in a corn-wheat-soybean rotation sequence when grown in a double-cropping system. In the northern soybean growing areas, wheat will typically follow soybean in the rotation.

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IX.H. Soybean Volunteer Management

Volunteer soybean is defined as a plant that has germinated and emerged unintentionally in a subsequent crop. Soybean seeds can remain in a field after soybean harvest as a result of pods splitting before or during harvest. Soybean seeds also can remain in a field when pod placement on the plants is too close to the ground for the combine head to collect all the pods or the combine is improperly adjusted for efficient harvesting. Volunteer soybean in rotational crops is typically not a concern in the Midwest region because the soybean seed is typically not viable after the winter period (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 is normally not a concern in rotational crops such as corn, cotton, rice, and wheat that are the significant rotational crops following soybean due to control measures that are available for volunteer soybean when they arise (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 post emergence herbicides also are available to control volunteer soybean (conventional or glyphosate-tolerant soybean) in each of the major rotational crops. Table IX-7 provides control ratings on volunteer glyphosate-tolerant soybean for several herbicides used in the major rotational crops.

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

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

Volunteer soybean in rice is rarely a concern due to the combination of preplant tillage, flooding practices, and herbicides utilized in producing rice ([redacted] 2006, Personal communication). If volunteer plants should emerge in rice, the post emergence applications of Grasp (penoxsulam), Permit (halosulfuron) and Regiment (bispyribac) typically used for weed control in rice will effectively alleviate competition from volunteer soybean (Dillon et al., 2006).

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Table IX-7. Ratings for Control of Volunteer Glyphosate-Tolerant Soybean in Labeled Rotational Crops[1]

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

NA denotes "not applicable"

[1] Weed control ratings: E = Excellent (90 to 99% control), G = Good (80 to 90% control), F = Fair (65 to 80 control), and P = Poor (40 to 65% control).

[2] Zollinger, 2005.

[3] York et al., 2005.

[4] Dillon et al., 2006.

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IX.I. Insect Resistance Management

In agricultural production systems, an insect resistance management (IRM) program is seen as a critical part of prolonging the product life cycle of insect-control technologies (Jutsum et al., 1998). Over forty years of experience with conventional insecticides have shown that the development of insect resistance to any widely used insect control tactic is inevitable, whether this involves chemical use, cultural controls, or biological control tactics. Resistance will lead to increased insecticide use either by increasing applied dose rate or application frequency, and most likely will end up with both, forcing a change in cropping practice or even total crop failure. However, through proper adoption of a resistance management strategy, grower education, and other measures, insect resistance development can be significantly delayed.

A critical component for the long-term use of biotechnology-derived Bt crop products containing insecticidal Cry proteins is to implement IRM programs to prevent or delay the onset of resistance in the target insect species. IRM programs for biotechnology-derived Bt crop products are dependent upon many variables, including: the nature of the product, its performance, how it is used, the pattern of Cry protein expression, and particularly the magnitude and consistency of the Cry protein expression, which will dramatically affect resistance development. As recommended by independent scientific experts (the Scientific Advisoty Panel or SAP) advising EPA on the subject (EPA, 1998a), the preferred Cry protein pattern is a season-long, high level expression (referred to as "high dose") that is sufficient to control target insects that are heterozygous for any resistance genes.

MON 87701 was evaluated using the SAP expert adviser-recommended approaches (EPA, 1998a) for determining a high-dose expression pattern and was shown to meet two of the high dose expression pattern criteria (MacRae et al., 2005). The first approach is demonstration of an effective Cry protein expression level in the crop product through the use of dilution bioassays, where it was demonstrated that the Cry1Ac expression level in MON 87701 leaf tissue was at least 25× higher than the level necessary for complete mortality of *A. gemmatalis* and *P. includens* first instars. The second method involved multiple field and screenhouse tests demonstrating virtually complete efficacy against target insect pests in tropical and sub-tropical soybean production regions. Based on these evaluations, MON 87701 has been demonstrated to have the preferred season-long, high-dose expression pattern that serves as an effective tool in managing potential insect resistance to the Cry1Ac protein and, thereby, would prolong the durability of this product.

The EPA is responsible for the regulation of pesticides, including plant incorporated protectants (PIPs) such as Cry proteins, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). All PIPs must be registered by EPA for a particular crop and use pattern (e.g., experimental, breeding and seed multiplication, or commercial planting). To determine whether a PIP is appropriate for registration, EPA must complete a thorough review of the product application, which includes a significant safety data package, and, based on that review, determine that the PIP will not cause "unreasonable adverse effects on the environment" under the proposed terms of use. The phrase is defined, in relevant part, to mean "any unreasonable risk to

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man or the environment" [7 U.S.C. § 136(bb)]. If no such effect is found, EPA will register the PIP for the requested terms and conditions of use.

As described previously in this Petition, given the limited commercial fit of this product in the major soybean growing regions in the U.S., for the foreseeable future, U.S. plantings of MON 87701 will be limited to breeding and seed multiplication purposes to support the commercial introduction of MON 87701 in South America. MON 87701 will initially not be available for commercial use by U.S. growers. For this reason, Monsanto intends to file an application with the EPA for a Section 3 seed increase registration of the plant-incorporated protectant *Bacillus thuringiensis* Cry1Ac protein, and the genetic material (vector PV-GMIR9) necessary for its production in soybean. This registration will only allow for breeding and seed multiplication activities in the U.S. Commercial planting under this EPA registration would be prohibited by law.

Based on past experience and current practices, it is estimated that total annual seed multiplication of MON 87701 in the U.S. will not exceed 15,000 acres, which is less than 1% of the acres devoted to soybean certified seed production in the U.S. On a per county basis, the area of MON 87701 will not exceed 1,000 acres. Thus, MON 87701 non-commercial plantings will be small and scattered and will not pose a significant increase in the potential for resistance development by lepidopteran pests, and therefore no IRM plan will be required[1]. In addition, where significant pest pressure is present, insecticide applications will be made to protect the valuable seed multiplication and breeding lines, thereby making the risk of Bt resistance evolving due to MON 87701 plantings negligible. In summary, the intended limited plantings of MON 87701 in the U.S. will not increase the overall likelihood of lepidopteran insect resistance development based on the following:

- *P. includens* and *A. gemmatalis*, are the key lepidopteran insect pests of soybean in the U.S. Southeast region where MON 87701 seed multiplication will occur. It was demonstrated that the Cry1Ac expression level in MON 87701 leaf tissue was at least 25× higher than the level necessary for complete mortality of *A. gemmatalis* and *P. includens* first instars.
- MON 87701 displays a high-dose expression pattern of the Cry1Ac protein that effectively provides season-long control of the targeted lepidopteran insect pests. High dose is the strategy recommended by independent scientific experts advising EPA on insect-resistance management (EPA, 1998a).
- Alternative hosts, in the form of wild plants, other crop hosts or non-Bt soybean plantings, will be present for the targeted pest species. Thus, these alternative hosts can serve as a natural source of refuge and can further reduce the risk of resistance development in these lepidopteran species.

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[1] MON 810 × MON 863 label [http://www.kellysolutions.com/erenewals/documentsubmit/KellyData%5CND%5Cpesticide%5CProduct%20Label%5C524%5C524-545%5C524-545_YIELDGARD_PLUS_CORN_BORER_ROOTWORM_11_11_2008_5_17_22_PM.pdf]

In the event that Monsanto decides to seek commercial registration of MON 87701 in the U.S. at some future date, an IRM plan for MON 87701 in the U.S. would be submitted to the EPA as part of the registration process in compliance with EPA guidance for the commercial registration of PIPs. Monsanto's proposed IRM plan would be fully reviewed by the agency prior to commercial sale or distribution of the product. This IRM program would be designed to address the specific characteristics of U.S. soybean production (e.g., agricultural practices being utilized to control insect pests) at that time, taking into account the scale and purpose for which MON 87701 would be grown.

In conclusion, the growing of MON 87701 for U.S. breeding programs and seed multiplication under a limited EPA registration will not constitute a significant resistance risk for lepidopteran pests and structured refuges will not be needed because of the large areas of non-Bt soybean that will be present. If Monsanto decides to commercialize MON 87701 in the U.S., Monsanto would develop an IRM plan for MON 87701 in those geographies where the product will be grown commercially and include the IRM program with the required Section 3 commercial use registration application to the EPA.

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IX.J. Stewardship of MON 87701

Monsanto Company is firmly committed to its legal, ethical and moral obligation to ensure that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment through product stewardship. Monsanto's product stewardship programs span the entire lifecycle of a product, including discontinuation. These policies and practices include rigorous field compliance and quality management systems and auditing, which are audited by third parties through our commitment to the Excellence Through Stewardship program.

As with all of our products, Monsanto is committed to the rigorous product stewardship of MON 87701. This includes having a process in place to restrict the use of MON 87701 to breeding and seed multiplication in the U.S., unless and until the product is registered by the EPA for commercial production. All breeding or seed multiplication work will be done directly by Monsanto or its licensees under a contract. The limitations on the EPA registration, combined with contractual production of seed, will serve to effectively limit use of MON 87701 in the U.S. to breeding and seed multiplication for so long as the limitation is in place.

In keeping with past practice, before commercially launching MON 87701 in any country, Monsanto will gain regulatory approval from the key soybean import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. Monsanto also commits to best industry practices on seed quality assurance and control to assure the purity and integrity of MON 87701. Before commercializing MON 87701 in any country, a detection method will be made available to soybean producers, processors, and buyers.

IX.K. Impact of the Introduction of MON 87701 on Agricultural Practices

With the exception of potentially less insecticide applications against targeted lepidopteran pests, no impact is expected from the introduction of MON 87701 on current cultivation and management practices for soybean. MON 87701 has been shown to be no

different from conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics (refer to Sections VII, VIII, IX), and has the same levels of resistance to insects and diseases as current commercial soybean, except for the introduced trait of enhanced protection from feeding damage caused by certain lepidopteran pests. Like the other Bt-based crops, such as Bt cotton and Bt corn which have been cultivated and consumed in U.S. since 1996 (EPA, 2008), insect-protected soybean MON 87701 would improve the current agricultural practices by eliminating or reducing insecticide use for targeted lepidopteran pests, reduce the risks to non-target species, and improve the efficiency in soybean production system by increasing or maintaining yield potential while reducing insecticide and labor costs.

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X. ENVIRONMENTAL CONSEQUENCES AND IMPACT ON AGRONOMIC PRACTICES

This section provides a brief review and assessment of the plant pest potential of MON 87701 and its impact on agronomic practices. USDA-AHPIS has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

The definition of "plant pest" in the Plant Protection Act (PPA) includes living organisms that could directly or indirectly injure, damage, or cause disease in any plant or plant product [7 U.S.C. § 7702(14)]. Information in this Petition related to plant pest risk characteristics include disease and pest susceptibilities, expression and characteristics of the gene product (Cry1Ac), impacts to non-target organisms, changes to plant metabolism, weediness of the regulated article, impacts on agronomic practices, any impacts on the weediness of any other plant with which it can interbreed, and the transfer of genetic information to organisms with which it cannot interbreed.

The regulatory end-point under the PPA for biotechnology-derived crop products is not zero risk but rather a determination that deregulation of the regulated article is not likely to pose a plant pest risk. As part of the plant pest risk assessment, the genetic construct inserted into MON 87701 was evaluated to determine if those sequences cause plant disease. Morphological characteristics of MON 87701 were analyzed to determine if it will become weedy or invasive. Agronomic practices associated with MON 87701 were considered relative to potential changes that could lead to increased plant pest potential. The potential for gene flow and introgression of the genetic construct into, other plant varieties or wild relatives was also evaluated to determine the potential of increased weedy or invasive characteristics in other plant species. Finally, the propensity of MON 87701 to become a greater reservoir of plant pests (insects or pathogens) compared to conventional plants and the potential for horizontal gene transfer were evaluated. Using this risk assessment process, the data and analysis presented in this Petition leads to a conclusion that MON 87701 is unlikely to be a plant pest and, therefore, should no longer be subject to regulation under 7 CFR Part 340.

The assessment of the impact of MON 87701 and the introduced protein on threatened and endangered species and other NTOs concludes that risk to these organisms from the use of MON 87701 is negligible. This risk assessment took into consideration several components, including familiarity with the mode of action of Cry proteins, the activity spectra of the Cry1Ac protein, the expression level of the Cry1Ac protein in MON 87701, the environmental fate of the Cry1Ac protein, and feeding tests with the Cry1Ac protein or MON 87701 soybean materials to representative NTOs.

The evaluation of weediness potential and gene flow concluded that MON 87701 is no more likely to become a weed than conventional soybean, and MON 87701 is expected to

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be similar to conventional soybean regarding the potential for and impact from gene flow. Due to lack of sexually compatible relatives in the U.S., pollen-mediated gene flow is expected to occur only within cultivated soybean. Given the reproductive biology of soybean, pollen-mediated gene flow is expected to be negligible within cultivated soybean. The probability for horizontal gene flow is exceedingly small. Even if it were to occur, the consequences would be negligible since the gene introduced into MON 87701 is of bacterial origin and the Cry1Ac protein produced has no meaningful toxicity to humans and other NTOs under the conditions of use.

An assessment of current soybean agronomic practices in the U.S. indicates that the introduction of MON 87701 will not impact current U.S. soybean cultivation practices and the management of weeds, diseases, and insects, except for the benefit of effective control of target lepidopteran insect pests (see Section IX). In addition, the observed high dose expression pattern in MON 87701 would provide an effective tool in managing potential insect resistance to the Cry1Ac protein, and, thereby prolong the durability of this product. As an outcome of the Plant Pest Assessment and lack of significant impact to the human environment, Monsanto has developed an Environmental Assessment for MON 87701 in Appendix K. In this appendix, it has been concluded that the requested action of deregulation in whole does not present a significant environmental impact.

APHIS has recently proposed to amend 7 CFR Part 340 to include its noxious weed authority. Because the data show that MON 87701 has no potential to cause injury, damage or disease to any protected interest, MON 87701 would also not be considered a "noxious weed" as defined by the Plant Protection Act.

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X.A. Plant Pest Assessment of the Genetic Insert and Its Cry1Ac Protein

X.A.1. Characteristics of the Genetic Insert

MON 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using the binary transformation plasmid PV-GMIR9 (Section IV; Figure IV-1 and Table IV-1). MON 87701 contains one copy of the insert at a single integration locus. No additional genetic elements from the transformation vector were detected in the genome of MON 87701, including backbone sequence from plasmid PV-GMIR9. Additionally the data confirm the organization and sequence of the insert, demonstrate the stability of the insert over several generations, and demonstrate that the genomic DNA sequences flanking the 5' and 3' ends of the insert are native to the soybean genome. On the basis of these data, it is concluded that only the expected Cry1Ac protein is produced from the inserted DNA.

The inserted T-DNA I in MON 87701 contains left and right border sequences from *Agrobacterium-tumefaciens,* a plant pest. These sequences are well characterized and are only non-coding regions. These regions will not cause MON 87701 to promote plant disease (refer to Table IV-1).

X.A.2. Mode of Action of the Cry1Ac Protein

The history of safe use of *Bacillus thuringiensis* (Bt) microbial pesticides and Bt crops and the well understood mode of action of Bt Cry proteins are important considerations in

the environmental safety assessment of these proteins. Sprays of sporulated *B. thuringiensis* have a long history of safe use for pest control in agriculture, especially in organic farming (Cannon, 1993; EPA, 1988; WHO, 1999). Microbial pesticides containing Bt Cry1A proteins have been used for more than 45 years and subjected to extensive toxicity testing showing no adverse effects to human health (Baum et al., 1999; Betz et al., 2000; EPA, 2000; EPA, 2001; McClintock et al., 1995; Mendelsohn et al., 2003). During the last decade a variety of biotechnology-derived crops containing Bt Cry1 proteins have been commercialized, thus, rendering these plants resistant to several insect pests (De Maagd et al., 1999; Mendelsohn et al., 2003). For example, corn that produces the Cry1Ab (YieldGard, Bt11) and Cry1F (Herculex® I) proteins, as well as cotton producing the Cry1Ac and Cry2Ab2 (Bollgard and Bollgard II) proteins are currently registered and sold on the market (Mendelsohn et al., 2003). Moreover, corn DBT418 which produced the tryptic core of Cry1Ac was previously registered (EPA, 1997). Compositional equivalence of these products to conventional varieties has been demonstrated (Berberich et al., 1996). Detailed human and animal safety assessments and almost a decade of safe human and animal consumption of these crops confirm their safety (Betz et al., 2000; Mendelsohn et al., 2003; Siegel, 2001).

As discussed in Section VI, Bt Cry1 proteins are synthesized as ~130 kDa protoxins consisting of a three-domain toxin portion and a C-terminal extension (OECD, 2007). The Cry1Ac protein expressed in MON 87701 shares >99% amino acid identity with Cry1Ac from Bt (Cry1Ac, gi 117547) and 100% amino acid sequence identity with the Cry1Ac protein present in Bollgard and Bollgard II cotton products, with the exception of four additional amino acids at the N-terminus that are derived from a chloroplast targeting sequence. Therefore, the Cry1Ac protein contained in MON 87701 shares a high degree of functional and structural characteristics with the Cry1Ac protein in biotechnology-derived crop products with a demonstrated history of environmental safety in cotton agricultural systems (Mendelsohn et al., 2003).

The general mode of action of Cry proteins is well understood. The bacterially-produced crystal proteins are first solubilized in the insect midgut, followed by activation of the protoxins (full-length proteins) to active toxins (proteolytic-resistant cores) by midgut proteases. A similar process occurs when Cry proteins are expressed in plants. The activated proteins then bind to midgut membrane receptors in susceptible insects, insert into the apical membrane, and form pores. Formation of the pores causes loss of osmotic regulation, and eventually leads to cell lysis, which is thought to be responsible for insect death (Gill et al., 1992; Schnepf et al., 1998; Zhuang and Gill, 2003).

Cry1 protoxins (such as Cry1Ac, Cry1Ab, and Cry1F) are 130 to 140 kDa in size, and are activated by proteases to active cores of 65 to 70 kDa. The crystal solubilization is facilitated by an alkaline pH. The typical midgut pH is between 9-11 in lepidopteran larvae. During the solubilization and activation of Cry1 proteins, an N-terminal peptide of 25-30 amino acids and approximately half of the sequences from the C-terminus are cleaved (Bravo et al., 2002; Choma et al., 1990; Gill et al., 1992; Schnepf et al., 1998; Zhuang and Gill, 2003). The role of the C-terminal domain is believed to be in the

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formation of crystalline inclusion bodies within the Bt bacterium and is not required for insecticidal activity (De Maagd et al., 2001; Park and Federici, 2000). The 25-30 amino acid residues at the N-terminus play a role in promoting crystallization of the protoxin in the bacterium, but do not contribute to toxicity to insects (Choma et al., 1990; Gill et al., 1992; Schnepf et al., 1998). In fact, it was shown with Cry1Ac that proteolytic removal of the N-terminal peptide is essential before the protein becomes fully active (Bravo et al., 2002).

The 3-dimensional structures of three members of the Cry protein family, which may well prove to be representative of all Cry proteins, reveal the presence of three structural domains (Grochulski et al., 1995; Li et al., 1991; Morse et al., 2001). Domain I, consisting of seven α-helices, is involved in membrane insertion and pore formation. Domain II, consisting of three β-sheets in a Greek key conformation, is involved in specific receptor recognition and binding. Domain III, which consists of two β-sheets in a jellyroll conformation, has been suggested to maintain the structural integrity of the protein molecule (Li et al., 1991) and also to contribute to specificity (De Maagd et al., 2001; De Maagd et al., 2000). All three domains are included in the N-terminal portion of the protoxins during the formation of active toxins in the insect gut. Since domains II and III can both contribute to the specificity, the difference in these domains among different Cry proteins would account for the diversity of insecticidal activities. These domains may dictate whether and how binding occurs between the Cry proteins and the insect midgut. Only those insects with specific receptors are affected by Cry proteins and no toxicity is observed in species that lack these receptors (Crickmore et al., 1998; De Maagd et al., 2001).

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X.A.3. Efficacy against Target Pests

Information presented in this section is relevant to the plant pest assessment for MON 87701 because it describes the spectrum of activity of the Cry1Ac protein and its impact on non-target organisms. According to 7 CFR Part 340.6, this information is part of the required information needed for evaluation of plant pest potential.

X.A.3.1. Laboratory Tests on Activity of the Cry1Ac Protein against Target Lepidopteran Pests

Lepidopteran pest larvae feed on the leaves, axils, and pods of soybean and can significantly affect yield. The major lepidopteran insect pests of soybean include velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean axil borer (*Epinotia aporema*), and sunflower looper (*Rachiplusia nu*) in soybean production regions from southern U.S. to Argentina. Velvetbean caterpillar and soybean looper are the primary lepidopteran pests of soybean in the U.S. (MacRae et al., 2005) and throughout soybean growing regions of South America.

Studies have previously been conducted to evaluate the spectrum of insecticidal activity of Cry1Ac protein produced from *B. thuringiensis* var. *kurstaki* HD-73 against a variety of agronomically-important insects and one non-insect arthropod taxon (MacIntosh et al., 1990). Species tested included seven species of *Lepidoptera*: beet armyworm (*Spodoptera exigua*), black cutworm (*Agrotis ipsilon*), cabbage looper (*Trichoplusia ni*),

corn earworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*), tobacco budworm (*Heliothis virescens*), and tobacco hornworm (*Manduca sexta*); five species of *Coleoptera*: alfalfa weevil (*Hypera postica*), cotton boll weevil (*Anthonomis grandis*), horseradish flea beetle (*Phyllotreta armoraciae*), southern corn rootworm (*Diabrotica undecimpunctata howardi*), and Japanese beetle (*Popillia japonica*); one species of *Diptera*: yellow fever mosquito (*Aedes aegypti*); one species of *Blattodea*: German cockroach (*Blatella germanica*); one species of *Hemiptera*: green peach aphid (*Myzus persicae*); one species of *Isoptera*: termite (*Reticulitermes flavipes*); and one species of mite: two-spotted spider mite (*Tetranychus urticae*). The results showed that the Cry1Ac protein had activity against all seven of the representative lepidopteran insects. However, there was no indication of activity of Cry1Ac against any of the ten non-lepidopteran species (MacIntosh et al., 1990). The results from these assays suggest that, under expected agricultural use, the Cry1Ac protein has an effective range of insecticidal activity against lepidopteran insect pests and does not have insecticidal activity against the non-lepidopteran pests that were tested.

Additional studies had been conducted to quantify the level of insecticidal activity of Cry1Ac protein produced from *B. thuringiensis* subsp. *kurstaki* HD-73 against a variety of lepidopteran insect species of importance in soybean. Species tested include velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean axil borer (*Epinotia aporema*), sunflower looper (*Rachiplusia nu*), soybean podworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), lesser cornstalk borer (*Elasmopalpus lignosellus*), alfalfa caterpillar (*Colias lesbia*), and "gata peluda norteamericana" (*Spilosoma virginica*). The insects were exposed for seven days to a range of concentrations of wild-type Cry1Ac protein using diet-incorporation bioassay procedures (Luttrell et al., 1999; MacIntosh et al., 1990; MacRae et al., 2005). The results showed that all nine of these species were sensitive to the Cry1Ac protein. Fall armyworm was less sensitive than the other species, exhibiting severe stunting at concentrations of the Cry1Ac protein up to 100 µg of protein per mL of diet. The remaining eight species were all highly sensitive to the Cry1Ac protein, exhibiting LC_{50} values less than 10 µg/mL. The results from these assays confirm that, under typical agricultural use, the Cry1Ac protein has the required insecticidal activity against the important targeted lepidopteran insect pests of soybean (Luttrell et al., 1999; MacIntosh et al., 1990; MacRae et al., 2005).

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X.A.3.2. Field Efficacy Trials

The efficacy of MON 87701, in both screenhouse and open field trials against major lepidopteran pests of soybean, was evaluated at multiple locations in the U.S. and Argentina from 2002 to 2003. The 2002 U.S. screenhouse trials were conducted at two sites in Jerseyville and Monmouth, Illinois. At each site, screenhouse trials were infested separately with *A. gemmeatalis* and *P. includens*. The 2002 U.S. field trials were conducted at five locations each season in Alabama, Georgia, Louisiana, and Mississippi. The 2002/03 Argentina screenhouse trials were conducted at two locations in Fontezuela and Oliveros. At each site, screenhouse trials were infested separately with *R. nu* and *S. virginica* (MacRae et al., 2005). MON 87701 was tested along with isogenic parental soybean lines as negative controls. The experimental design for all 2002 U.S. field trials

at all locations was a split-plot randomized complete block, with whole plots consisting of soybean entry and subplots consisting of preventive insecticide treatments versus no insecticides. All treatments were replicated four times in each trial. Experimental units were 4-row plots measuring 10m or 30ft in length, with approximately 23-25 seeds/m and row spacing of 91-100 cm, depending upon location. All subplots were buffered by four rows of conventional soybean to eliminate edge effects, and the entire trial areas were surrounded by at least four rows of conventional soybean to eliminate border effects. Open field trials relied upon natural insect pressure for infestation, with different lepidopteran species being encountered at different locations in different years. The most frequently encountered target lepidopteran pests were *Anticarsia*, *Pseudoplusia*, *Helicoverpa*, *Epinotia*, *Rachiplusia*, and *Spodoptera* spp. Insect damage was assessed by counting in each subplot within the trial by one of two means: 1) the number of target pest larvae per m row from two or more 1-m samplings or (for *Epinotia*) the number out of ten randomly selected plants with live larvae, and 2) visually estimating in each subplot the percentage of defoliation or (for *Epinotia*) counting in each subplot the number out of ten randomly selected plants per plot with damage.

The results from 2002/2003 U.S. and Argentina screenhouse trials demonstrated that MON 87701 provided nearly complete control of lepidopteran pests including *A. gemmatalis, P. includens, R. nu,* and *E. aporema* across all sites, and Cry1Ac-negative or the conventional soybean control line exhibited up to 94% defoliation; whereas, a maximum 6.3% defoliation was observed in MON 87701 plots at the Oliveros site when infested with *S. virginica* (MacRae et al., 2005). Meanwhile, up to 451 larvae (cumulative) per m row were found in parental control line plots, while only 1-5 larvae (cumulative) per m row were found in MON 87701 plots. In addition, the results from 2002 U.S. open field trials where *A. gemmatalis, P. includens, H. zea, Playthpena scabra, Spodopteran exigua,* and *Spodopteran ornithogalli* were observed at one or more locations, revealed that the parental control line exhibited up to 83.3% defoliation, whereas less than 3.3% defoliation was observed for MON 87701. Likewise, up to 260 larvae (cumulative) per m row were found in parental control line treatments, while only 1-8.7 larvae (cumulative) per m row were observed for MON 87701 (MacRae et al., 2005). In conclusion, the screenhouse and field efficacy evaluations show that MON 87701 is highly efficacious in controlling the most common and economically important lepidopteran pests of soybean.

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X.A.4. Impact on Non-target Organisms

Evaluation of the potential risks to NTOs is an important component of APHIS's plant pest risk assessment of a biotechnology-derived crop. Assessment of the potential risks to NTOs associated with the introduction of a biotechnology-derived crop producing an insecticidal protein is based on the characteristics of the crop and the introduced protein. Since risk is a function of hazard and exposure, it is critical to determine the potential hazards and exposure scenarios that are most likely and that require evaluation through experimental studies. Selection of the test organisms and test material are important decisions that are based on the characteristics of the trait and the product (Romeis et al., 2008). In the U.S., regulatory guidelines for NTO risk assessment of insect-protected crops were developed by the EPA. The testing is conducted according to a tier-based

system (EPA, 1998b; 2001a-d; 2004a-b). Additionally, the EPA has convened several Scientific Advisory Panel (SAP) meetings to make recommendations and provide guidance for NTO testing and risk assessment for agricultural products produced by methods of biotechnology (EPA, 2001a-b; 2002a-b; 2004a-b).

For the Bt Cry proteins tested in laboratory assays to date, potentially significant adverse effects have been observed for only a very few NTO species that are closely related to the target species (Mendelsohn et al., 2003; Romeis et al., 2006). In addition, field evaluations conducted over the past decade by industry and the academic community on registered insect-protected crops that produce a variety of Bt Cry proteins have confirmed that these crops pose a negligible risk to tested populations of natural enemies, and other ecologically important non-target arthropods (Bhatti et al., 2005; Bitzer et al., 2005; Daly and Buntin, 2005; Dively, 2005; Head et al., 2005; Lopez et al., 2005; Lozzia et al., 1998; Naranjo et al., 2005; Naranjo, 2005a; Naranjo, 2005b; Orr and Landis, 1997; Pilcher et al., 1997; Pilcher et al., 2005; Torres and Ruberson, 2005; Whitehouse et al., 2005). No unexpected adverse effects were observed in meta-analyses of the effects of Bt cotton and corn on non-target invertebrates (Marvier et al., 2007) and of the effects of Bt crops on honeybees (Duan et al., 2008).

The potential risk posed to NTOs by MON 87701 can be initially assessed from the information already available from previously approved biotechnology-derived crop products expressing the Cry1Ac protein such as Bollgard and Bollgard II cotton. A standard set of NTO tests was completed for these biotechnology-derived cotton products that used a full-length Cry1Ac protein (*Btk* HD-73) produced in *E. coli* that shares greater than 99% amino acid identity to the Cry1Ac protein expressed by MON 87701. It should be noted that the dose levels tested previously were a function of the Cry1Ac protein expression levels in Bollgard and Bollgard II cotton. While the dose levels for the cotton products were appropriate for the NTO risk assessment, these levels do not provide the desired margin of exposure (MOE) for the levels of Cry1Ac expressed by MON 87701. Furthermore, two of the NTO evaluations conducted for Bollgard cotton (Green Lacewing and Parasitic Wasp) have been updated or revised in the NTO testing battery to support MON 87701. Nevertheless, results from the previously conducted evaluations to support the evaluation of potential impact of Cry1Ac-expressing Bt cotton on NTOs, support a conclusion of negligible risk posed to NTOs by MON 87701 based on the published EPA level of concern of 50% mortality at 5× the maximum expected exposure concentration (MEEC) (EPA, 1998b) (Table X-1).

The NTO risk assessment of the Cry1Ac protein produced in MON 87701 was a multi-step process taking into consideration hazard identification and characterization, exposure assessment, and risk characterization. The hazard identification and characterization included testing the spectrum of insecticidal activity of the Cry1Ac protein and efficacy of MON 87701 against target soybean insect pests. These evaluations demonstrate that the Cry1Ac protein produced in MON 87701 performs in the expected manner based on the extensive knowledge and experience with Cry proteins.

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Table X-1. Relevant Toxicity Testing of Cry1Ac[1] Protein for Bollgard Cotton at Levels of Cry1Ac Protein Produced in MON 87701

Test Organism	MEEC	NOEC[2]	MOE[3]
Honeybee larvae (*Apis mellifera*)	3.1 µg/g fwt (pollen)	≥20 µg/ml as a single dose	>6
Honeybee adult (*Apis mellifera*)	3.1 µg/g fwt (pollen)	≥20 µg/ml	>6
Green Lacewing (*Chrysoperla carnea*)	3.1 µg/g fwt (pollen)	≥20 µg/g	>6
Ladybird beetle (*Hippodamia convergens*)	3.1 µg/g fwt (pollen)	≥20 µg/ml	>6
Parasitic wasp[4] (*Nasonia vitripennis*)	3.1 µg/g fwt (pollen)	≥20 µg/ml	>6

[1] The test substance was a full-length Cry1Ac protein (*Bik* HD-73) produced in *Escherichia coli* that shares greater than 99% amino acid similarity to Cry1Ac expressed in MON 87701.

[2] No Observed Effect Concentration.

[3] Non-target arthropod testing was conducted as part of risk assessment for Bollgard cotton. Published U.S. EPA guidance states the following: The dose margin can be less than 10× where uncertainty in the test system is low. High dose testing also may not be necessary where many species are tested or tests are very sensitive, although the test concentration used must exceed 1× MEEC. The published EPA level of concern is 50% mortality at 5× MEEC (U.S. EPA, 1998b).

[4] This organism, an endoparasitoid of dipteran pupae, has limited ecological relevance to soybean.

The exposure assessment of the NTO risk assessment is comprised of three components: 1) estimation of the expression level of the Cry1Ac protein in tissues from MON 87701; 2) a conservative calculation of margins of exposure based on the maximum amount of soybean tissue that might be exposed to NTOs; and 3) assessment of the environmental fate of the Cry1Ac protein in soil. Cry1Ac expression values from several tissue types were used to determine the appropriate doses to be used in the NTO toxicity tests, while the results from soil degradation tests were used to characterize the potential Cry1Ac exposure to soil organisms and the likelihood that the Cry1Ac protein could persist and accumulate in agricultural soils.

The insecticidal activity spectrum of the Cry1Ac protein was found to be typical for the Cry1 class of Bt proteins; the activity was only evident against insect pests within the order *Lepidoptera*. Exposure information was developed to determine the maximum expected exposure concentration (MEEC) for the Cry1Ac protein produced in MON 87701. Dosing in the NTO tests was based on the estimated MEEC of the Cry1Ac protein present in the tissue(s) likely to be ingested by the representative NTO. A targeted MOE of at least 10-times greater than the MEEC was used in the tests. The maximum expression level for the Cry1Ac protein in pollen (3.1 µg/g fwt) was used to determine the dose levels for honeybee, ladybird beetle, a parasitic wasp, and minute pirate bug, for which pollen represents the major route of exposure.

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The principal route of exposure for soil macro-organisms (Collembola and earthworm) was assumed to be from decomposing plant tissue containing the Cry1Ac protein. The use of a soil MEEC to calculate MOEs is appropriate because these organisms feed on detritus that is made up of soil and decaying plant and other material. Therefore, the maximum amount of plant tissue entering the soil environment and the maximum concentration of the Cry1Ac protein in the plant tissue were considered in determining the MEEC for the Cry1Ac protein in the top 15 cm of soil. The model[1] assumes that soybean plants in the field are tilled into the top 6 inches (15 cm) of soil, and the plants uniformly express the introduced protein at the maximum concentration. However, because expression of Cry proteins varies in a tissue-specific and temporal manner, this hazard assessment was generated based on the maximum *in planta* expression of the Cry1Ac (960 µg Cry1Ac/g dwt) protein at a late developmental stage (V14-V16, R3) of MON 87701 soybean leaves. The Cry1Ac levels in leaf tissue of a late plant developmental stage represent a worst-case exposure scenario. These data as well as additional parameter estimates were used to calculate the soil MEECs for Collembola and earthworm.

The August 2002 EPA SAP report (EPA, 2002a) recommended that non-target testing should be focused on species exposed to the crop being evaluated (i.e., for MON 87701 beneficial insects or avian species found in soybean fields). Effects tests on aquatic species were not conducted for MON 87701 since there is no meaningful, ecologically-relevant exposure to aquatic organisms from soybean other than through purposeful feeding of processed soybean products, such as soybean meal. According to OECD consensus document (2007), aquatic species (fish, *e.g.* rainbow trout, and aquatic invertebrates, *e.g.* daphnia) testing may be useful if they are likely to be exposed, but often, there may be no significant aquatic exposure from substances produced in transgenic plants with the exception of transgenic Bt rice. EPA (2000a) also concluded that potential for accidental aquatic exposure from Bt crops is extremely small, and there is no evidence for sensitivity of aquatic species to Bt proteins. In a recent study by Rosi-Marshall et al. (2007), conducted on Bt corn pollen, it was suggested that Bt producing crops grown in close proximity to headwater streams may enter the stream, potentially exposing aquatic organisms. Since soybean is highly self-pollinated and pollen is essentially all contained in the flower (Caviness, 1966), the exposure of fish or aquatic invertebrates to the Cry1Ac protein in MON 87701 pollen is negligible in agricultural settings. Therefore, a static-renewal freshwater fish toxicity evaluation and an aquatic invertebrate acute toxicity test using the fresh water *Daphnia magna* were not justified and, subsequently, were not performed. Furthermore, it is unlikely that sufficient plant litter from MON 87701 could enter streams to adversely affect lepidopteran invertebrate populations in nearby fresh water streams. Based on minimal exposure, cultivation of MON 87701 poses negligible risk to aquatic invertebrate species.

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[1] The soil MEEC for Collembola and earthworm was calculated using the following assumptions: 175,000 soybean plants/acre; a soybean plant dry weight is 71.2 g/plant; a bulk density of soil of 1500 kg/cubic meter; a soil depth is 0.15 m (about 6 inches) and a soil volume in a one-hectare 0.15 m layer is 1500 cubic meters. The Cry1Ac maximum expression values were used for leaves at the V14-V16, R3 stage and were 960 µg/g dwt.

Based on the results from the product characterization and exposure assessment, an evaluation of the potential toxicity to selected NTOs (hazard assessment) was conducted. The detailed hazard assessment included toxicity testing against one mammalian species (mice); an avian species (bobwhite quail); soil decomposers, including two species of Collembola (*Folsomia candida* and *Xenylla grisea*) and an earthworm (*Eisenia fetida*); and four beneficial insect species [honeybee (*Apis mellifera*), parasitic wasp (*Pediobius foveolatus*), ladybird beetle (*Coleomegilla maculata*), and minute pirate bugs (*Orius albidipennis*)]. A published report was used in the hazard assessment to evaluate potential effects of the Cry1Ac protein on minute pirate bugs (Gonzalez-Zamora et al., 2007). The test substance was trypsinized Cry1Ac from *Bt* strain EG11070 that shares >98.9% amino acid identity to the Cry1Ac produced in MON 87701. The test materials were selected for each study based on the species being evaluated and whether more ecologically-relevant exposures (plant tissues) could be achieved without compromising the performance of the study. In many cases, *E. coli*-produced Cry1Ac protein was used because ingestion of the material could be ensured using artificial diets containing high levels of the Cry1Ac protein. The NOECs (no observed effect concentrations) determined for each of the tests used in the NTO risk assessment for MON 87701 are summarized in Table X-2.

Table X-2. No Observed Effect Concentrations (NOECs) of Cry1Ac for Each of the Evaluations Used in the NTO Risk Assessment for MON 87701

Test Organism	NOEC
Collembola (*two species*)	≥200 µg/g
Earthworm	≥250 mg/kg dry soil
Honeybee larvae	≥410 µg/ml as a single dose[1]
Honeybee adult	≥175 µg/ml
Minute pirate bugs	≥1000 µg/g[2]
Ladybird beetle	≥60 µg/g
Parasitic wasp	≥250 µg/ml
Mouse	≥1292 mg/kg
Quail	≥20% raw soybean seed from MON 87701 in diet

[1] The NOEC for the honeybee larval assay is based on the Cry1Ac concentration of the dosing solution.
[2] Gonzalez-Zamora et al., (2007).

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MOEs for the non-target arthropods were also calculated based on the ratio of the NOECs to the MEECs. The calculated MOEs were at least ≥15 fold of the potential maximum exposure level for these NTOs (Table X-3). MOEs that exceed 10 are considered as indicative of minimal risk by many regulatory authorities. Therefore, as with other Cry proteins, the Cry1Ac protein present in MON 87701 is not likely to produce adverse effects at field exposure levels on tested representative terrestrial beneficial invertebrate species. This conclusion is in agreement with prior published literature which reported no adverse effects on non-target organisms from insect-protected crops that produce Cry1 proteins (Daly and Buntin, 2005; Dively, 2005; Mendelsohn et al., 2003; Naranjo et al.,

2005; Pilcher et al., 2005). A summary of non-target organism evaluations can be found in Appendix J.

Table X-3. Estimated Margins of Exposure (MOE) to Non-Target Arthropods for Levels of Cry1Ac[1] Protein Produced in MON 87701

Test Organism	MEEC[2]	NOEC	MOE[3]
Collembola[4] (*Folsomia candida*) (*Xenylla grisea*)	13.2 mg/kg dry soil	≥200 µg/g	≥15[5]
Earthworm (*Eisenia fetida*)	13.2 mg/kg dry soil	≥250 mg/kg dry soil	≥18
Honeybee larvae (*Apis mellifera*)	3.1 µg/g fwt (pollen)[6]	≥410 µg/ml as a single dose[7]	≥132[6]
Honeybee adult (*Apis mellifera*)	3.1 µg/g fwt (pollen)	≥175 µg/ml	≥56
Minute pirate bugs[8] (*Orius albidipennis)*	3.1 µg/g fwt (pollen)	≥1000 µg/g	≥322
Ladybird beetle (*Coleomegilla maculata)*	3.1 µg/g fwt (pollen)	≥60 µg/g	≥19
Parasitic wasp (*Pediobius foveolatus)*	3.1 µg/g fwt (pollen)	≥250 µg/ml	≥80

[1] *E. coli*-produced Cry1Ac protein derived from *Bacillus thuringiensis* (Bt) var. *kurstaki* that it is identical to the Cry1Ac protein expressed in MON 87701.

[2] Based on Cry1Ac expression levels determined for MON 87701.

[3] Margins of Exposure (MOE) were calculated based on the ratio of the No Observed Effect Concentration (NOEC) to MEEC. The MOE was determined based on the expression level of the Cry1Ac protein in the MON 87701 tissue deemed most relevant to non-target insect exposure.

[4] The test substance was a full-length Cry1Ac protein (*Btk* HD-73) produced in *E. coli* that shares greater than 99% amino acid similarity of Cry1Ac expressed in MON 87701.

[5] The MOE for collembola and earthworm was calculated using the following parameter assumptions: 175,000 soybean plants/acre; soybean plant dry weight of 71.2 g /plant; soil bulk density of 1500 kg/cubic meter; soil depth of 0.15 m (about 6 inches); soil volume in a one-hectare 0.15 m layer or 1500 cubic meters; and a Cry1Ac expression value of 960 µg/g dwt for leaves at the V14-16, R3 growth stage.

[6] Due to limited tissue availability, pollen/anther material was evaluated using a non-validated, but optimized ELISA method.

[7] The NOEC for the honeybee larval assay is based on the confirmed Cry1Ac protein concentration of the dosing solution.

[8] *E. coli*-produced Cry1Ac protein derived from *Bacillus thuringiensis* (Bt) var. *kurstaki.* The *E. coli*-produced Cry1Ac test substance used for non-target arthropod testing shares >98.9% amino acid identity to the Cry1Ac protein produced in MON 87701.

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X.A.5. Impact on Threatened and Endangered Species

As discussed in the above sections, Cry proteins are known to have biological activity exclusively toward insect species. Extensive literature references support the observation that Cry proteins have a high degree of specificity and will not pose a significant hazard to non-insect animals (Federici, 2002; Romeis et al., 2006). This observation has been confirmed through testing with a standard battery of terrestrial and aquatic non-target organisms, including mammals, birds, water fleas, earthworms, and beneficial insects, for Bt crop registrations (Mendelsohn et al., 2003). These data establish that the Cry proteins pose negligible risk to non-insect animals and the vast majority of non-target insects. Based on the demonstrated low hazard of Cry proteins to non-insect animals, no adverse effects are expected for threatened or endangered mammals, birds, non-insect aquatic animals, and non-insect soil organisms. This conclusion has been affirmed in earlier regulatory decisions for other commercial Bt-based crop products containing Cry1 proteins (i.e., Bollgard and Bollgard II cotton, and YieldGard corn).

Monsanto has conducted extensive evaluations testing the Cry1Ac protein for activity against a range of both target and non-target insect species (Sections X.A.3 and X.A.4). The results also show that the Cry1Ac protein is highly specific in insecticidal activity against lepidopteran insects and has no activity against non-lepidopteran insects. These data taken together indicate that the only potential adverse effects to threatened and endangered species reside with endangered butterflies and moths in the order *Lepidoptera*.

Threatened and endangered species risk assessments were conducted by USDA and EPA for Cry1Ac-containing cotton products (Bollgard and Bollgard II) and Cry1-containing corn (YieldGard, Herculex I, YieldGard VT Pro) and cotton (WideStrike®), all indicating negligible risk to threatened or endangered *Lepidoptera*. Soybean is highly self-pollinated and its pollen is essentially contained in the flower (Caviness, 1966). Yoshimura et al. (2006) measured the highest level of exposure to pollen from soybean in a soybean field to be 0.368 grains/cm^2/day with an average value being 0.18 grains/cm^2/day. Due to this low pollen level, non-target lepidopteran species will have an exceedingly low likelihood of exposure to Cry1Ac produced in pollen from MON 87701. Furthermore, according to information found in the U.S. Fish and Wildlife Service's website on threatened and endangered species (http://www.fws.gov/endangered/wildlife.html#Species), no threatened or endangered *lepidoptera* are known to feed on soybean nor are soybean fields considered suitable habitats for these organisms. Given that soybean fields are not a critical habitat for threatened and endangered *Lepidoptera* and the lack of exposure to threatened and endangered *Lepidoptera* in general through soybean tissues, for example pollen, it is reasonable to conclude no impact to threatened and endangered lepidopteran species. This conclusion is further supported by results obtained for risk assessments conducted for corn products containing Cry1A proteins that indicate negligible risk to endangered species even when low exposure is possible (Stanley-Horn et al., 2001; Sears et al., 2001;

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Dively et al., 2004). Therefore, it is concluded that MON 87701 is not likely to adversely affect threatened and endangered species, including lepidopteran species.

X.A.6. Environmental Fate of Cry1Ac and Impact on Soil-dwelling Organisms

Soil organisms may be exposed to the Cry1Ac protein by contact with roots, incorporation of above ground plant tissues into soil after harvest, or by root exudation of the protein. Exposure may occur by feeding on living or dead soybean biomass or by ingestion or absorption of the Cry1Ac protein after its release into the soil. Several soil factors (e.g., pH and clay content) have been reported to influence the degradation rate of Cry proteins. Published studies on the effect of Cry proteins on soil-dwelling organisms show little or no impact on the soil microflora from the use of biotechnology-derived crops producing Bt proteins. For example, a season-long field study conducted with the Cry3A protein expressed in biotechnology-derived potato, showed no adverse effects towards soil-dwelling microorganisms (Donegan et al., 1996). In a study conducted in Kansas during the 2000 and 2001 growing seasons, the numbers of soil mites, Collembola, and nematodes observed in plots planted with Cry3Bb1-producing corn were similar to those observed in plots planted with conventional corn (Al-Deeb et al., 2003). Other published reports showed that Cry proteins had no microbiocidal or microbiostatic activity *in vitro* against selected bacteria, fungi, and algae (Koskella and Stotzky, 2002), and had no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi (Saxena and Stotzky, 2001). Specific studies on the degradation rate of the Cry1Ac protein expressed in Bt cotton showed a half-life (DT_{50}) of 16 days when incorporated into soil under laboratory conditions (Sims and Ream, 1997). In addition, there was no detection, persistence or accumulation of the Cry1Ac protein in field soils where Bt cotton was grown consecutively for three or more years (Head et al., 2002). This lack of field persistence or accumulation of the Cry1Ac protein under agronomic field conditions is consistent with that found for other Bt proteins such as Cry1Ab and Cry3Bb1 (Ahmad et al., 2005; Dubelman et al., 2005). These published results strongly suggest that the Cry1Ac protein produced in MON 87701 will not persist or accumulate under soybean production conditions.

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X.B. Weediness Potential of MON 87701

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; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). Soybean does not possess any of the attributes commonly associated with weeds (Baker, 1965), such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Due to the lack of dormancy (a trait that has been removed through commercial breeding), soybean seed can germinate quickly under adequate temperature and moisture and can potentially grow as volunteer plants. However, volunteer plants likely would be killed by frost during autumn or winter of the year they were produced. If they did become established, volunteer plants would not compete well with the succeeding crop, and could be

controlled readily by either mechanical or chemical means (OECD, 2000). In addition, since the wild populations of *Glycine* species are not known to exist in the U.S., the potential does not exist for MON 87701 to outcross to wild or weedy relatives and alter their weediness potential.

Empirical data used to assess the weed potential of MON 87701 include an evaluation of the dormancy and germination of the seed, and phenotypic characteristics of the plants (Section VIII). Results of these evaluations indicate that there is no fundamental difference in MON 87701 for traits associated with weediness. Collectively, these findings support the conclusion that MON87701 has no increased weed potential compared to conventional soybean. Data on environmental interactions also indicate that MON 87701 does not confer any biologically-meaningful increased susceptibility or tolerance to specific disease, insect, or abiotic stressors, with the excepted protection against certain lepidopteran pests.

Data presented in Section VII summarize the composition of forage and the harvested seed from MON 877701, the conventional control and from 20 commercial soybean varieties. Compositional analyses compared a total of 64 compositional analytes, seven in forage and 57 in harvested seed, between MON 87701 and a conventional soybean control with genetics comparable to MON 87701, but lacking the introduced trait. Data presented in Section VII indicate that there are no meaningful differences in compositional or nutritional quality of MON 87701 compared to conventional soybean. Compositional data were statistically analyzed and while there were some statistical differences between MON 87701 and the conventional control, it is concluded that the statistical differences represent the natural variability for these soybean analytes such that they were not regarded as biologically meaningful. Harvested seed and forage analytical component values were also comparable to published scientific literature and the ILSI Crop Composition Database, further supporting the conclusion that harvested seed and forage from MON 87701 are compositionally equivalent to those of conventional soybean. Thus, the composition of MON 87701 is not different from conventional soybean.

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X.C. Potential for Pollen-mediated Gene Flow

X.C.1. Vertical Gene Flow

X.C.1.1. Hybridization with Cultivated Soybean (*Glycine max*)

Although soybean is a largely self-pollinated species, low levels of natural cross-pollination can occur (Caviness, 1966; OECD, 2000). In studies with cultivated soybean where conditions have been optimized to ensure close proximity and flowering synchrony, natural cross-pollination has been found to be generally very low. Most outcrossing occurred with surrounding plants and cross-pollination frequencies vary depending on growing season and genotype. Insect activity does increase the outcrossing rate, but soybean generally is not a preferred plant for pollinators (Erickson, 1975; Erickson, 1984).

Numerous studies on soybean cross-pollination have been conducted, and the published results (with and without supplemental pollinators) are summarized in Table X-4. Under

natural conditions, cross pollination among adjacent plants in a row or among plants in adjacent rows ranged from 0.03 to 3.62%. In experiments where supplemental pollinators (usually bees) were added to the experimental area, cross pollination ranged from 0.5 to 7.74% in adjacent plants or adjacent rows. However, cross pollination does not occur at these levels over long distances. Cross-pollination rates decrease to less than 1.5% beyond one meter from the pollen source, and rapidly decrease with greater distances from the source. The following cross-pollination rates at extended distances have been reported: 0.02% at 8.2 m (Caviness, 1966), 0.05% at 5.4 m (Ray et al., 2003), and 0% at 6.5 m (Abud et al., 2003).

The potential for cross-pollination 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).

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Table X-4. Summary of Published Literature on Soybean Cross-Pollination

Distance from Pollen Source	% Cross-Pollination	Comments	Reference
0.3 m	0.04% (estimated per pod)	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties. Percent outcrossing calculated per pod rather than per seed.	Woodworth, 1922
0.8 m	0.07 to 0.18%	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	Garber and Odland, 1926
0.1 m	0.38 to 2.43%	Adjacent plants within a row. Experiment conducted in a single year. Several male and female parental varieties.	Cutler, 1934
0.1 m	0.2 to 1%	Adjacent plants within a row. Experiment conducted in single year at two locations. Several male and female parental varieties.	Weber and Hanson, 1961
0.9 m 2.7 – 4.6 m 6.4 – 8.2 m 10 – 15.5 m	0.03 to 0.44 % 0.007 to 0.04% 0 to 0.02% 0 to 0.01%	Frequency by distance was investigated. Experiment conducted over three years. Single male and female parental varieties.	Caviness, 1966
0.8 m	0.3 to 3.62%	Various arrangements within and among adjacent rows. Experiment conducted over three years. Several male and female parental varieties.	Beard and Knowles, 1971
One row (undefined)	1.15 to 7.74%	Bee pollination of single-row, small-plots of pollen receptor surrounded by large fields (several acres) of pollen donor soybean. Soybean is not a preferred flower for honeybee.	Abrams et al., 1978
0.1 – 0.6 m	0.5 to 1.03% (depending on planting design)	Bee pollination of soybean grown in various spatial arrangements. Experiment conducted over four years. Several soybean cultivars.	Chiang and Kiang, 1987
1.0 m	0.09 to 1.63%	Adjacent rows. Experiment conducted over two years. Several male and female parental varieties.	Ahrent and Caviness, 1994
0.5 m 1.0 m 6.5 m	0.44 to 0.45% 0.04 to 1.4% none detected	Frequency by distance was investigated. Experiment conducted in a single year. Single male and female parental varieties.	Abud et al., 2003
0.9 m 5.4 m	0.29 to 0.41% 0.03 to 0.05%	Frequency by distance was investigated. Experiment conducted in a single year. Single male and female parental varieties.	Ray et al., 2003
0.15 m	1.8%	Interspaced plants within a row. Experiment conducted in a single year. Single male and female parental varieties.	Ray et al., 2003

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X.C.1.2. Hybridization with the Wild Annual Species within Subgenus *Soja*

The subgenus *Soja* includes the cultivated soybean *G. max* and the wild annual species *G. soja.* *G. soja* is found in China, Taiwan, Japan, Korea, and Russia and can hybridize naturally with the cultivated soybean, *G. max* (Hymowitz, 2004). Hybridization between female *G. soja* and male *G. max* was less successful then hybridization in the opposing direction (Dorokhov et al., 2004), where frequency of spontaneous cross pollination in reciprocal combinations of *G. max* and *G. soja* varied from 0.73 (♀ *G. soja* × ♂ *G. max*) to 12.8% (♀ *G. max* × ♂ *G. soja*). Species relationships in the subgenus *Soja* indicated that F1 hybrids of *G. max* and *G. soja* carry similar genomes and are fertile (Singh and Hymowitz, 1989).

As described earlier, the subgenus *Soja* also contains an unofficial species, *G. gracilis* (Hymowitz, 2004). *G. gracilis* is known only from Northeast China, and is considered a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja*. *G. gracilis* may be a hybrid between *G. soja* and *G. max* (Hymowitz, 1970). 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). Given that, although hybridization between *G. max* and members of the subgenus *G. soja* can take place, because *G. soja* is not found in North or South America, it is highly unlikely that gene transfer will occur.

X.C.1.3. Hybridization with Wild Perennial Species of Subgenus *Glycine*

The wild perennial species of *Glycine* subgenus occur in Australia, West Central and South Pacific Islands, China, Papua New Guinea, Philippines, and Taiwan. Therefore, the only opportunities for inter-subgeneric hybridization would occur in areas where those species are endemic (Hymowitz et al., 1992; Hymowitz and Singh, 1992). Nonetheless, the likelihood of interspecific hybridization between *G. max* and the wild perennial *Glycine* species is extremely low, because they are genomically dissimilar (see Table II-2) and pod abortion is common. From time to time, immature seeds of the crosses could be 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; Singh and Hymowitz, 1989). In North and South America, it is not possible for gene transfer between cultivated soybean and wild perennial species of *Glycine* subgenera, as these wild species do not exist in these regions.

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

Monsanto is not aware of any reports regarding the unaided transfer of genetic material from soybean species to other species with which soybean cannot sexually interbreed. The probability for horizontal gene flow to occur is judged to be exceedingly small. Even if it were to occur, the consequences would be negligible since the genes introduced into MON 87701 are of bacterial origin and the Cry1Ac protein produced has no

meaningful toxicity to animals, including humans, and other NTOs under the conditions of use.

X.D. Summary of Environmental Consequences and Impact on Agronomic Practices

Plant pests are defined in the Plant Protection Act as certain living organisms that can directly or indirectly injure, cause damage to, or cause disease to any plant or plant product [7 U.S.C. § 7702(14)]. Characterization data presented in Sections III through X of this Petition confirm that MON 87701 expresses the Cry1Ac protein, a protein with an established history of safe use to the environment and to human health. On the basis of plant characterization data, other than the expression of Cry1Ac, MON 87701 is no different from conventional soybean in its phenotype, environmental interactions, or susceptibility to disease. An assessment of MON 87701 was conducted to assess the potential impact of the introduced Cry1Ac protein on non-target organisms, endangered species and soil-dwelling organisms, the potential for gene flow, and the pest and weed potential of MON 87701. Based on the results of this assessment, it is concluded that the potential risk of MON 87701 and the Cry1Ac protein to cause adverse effects on NTOs and endangered species is negligible. MON 87701 is no more likely to become a weed than conventional soybean, and MON 87701 is also expected to be similar to conventional soybean regarding the potential for gene flow. With the exception of insecticide applications, there are no changes expected in agronomic practices for MON 87701. The changes in insecticide application are not expected to impact the plant pest potential of MON 87701. Thus, compared to conventional soybean, there are no increased plant pest characterisitics associated with MON 87701.

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XI. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto knows of no study results or observations associated with MON 87701 or Cry proteins indicating that there would be an adverse environmental consequence from the introduction of MON 87701. MON 87701 provides protection from feeding damage caused by lepidopteran insect pests. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 87701 and conventional soybean is the presence of the Cry1Ac protein.

The data and information presented in this Petition demonstrate that MON 87701 is unlikely to pose an increased plant pest potential or to have an adverse environmental consequence compared to conventional soybean. This conclusion is reached 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 and protein analyses, which confirmed the insertion of a single functional copy of *cry1Ac* expression cassette at a single locus within the soybean genome and that the Cry1Ac protein was expressed in tissues at levels that are efficacious for the control of target insect pests. Extensive characterization of the plant phenotype including compositional analysis of key nutrient and antinutrients also indicated that MON 87701, with the exception of intended modification, was unchanged compared to conventional soybean. Allergenicity assessment and history of safe use of the Cry1Ac protein concluded that the Cry1Ac protein is unlikely to be an allergen for humans. Toxicity tests including an acute mouse oral gavage and other selected non-target organisms with equivalent protein produced by recombinant strains of *E. coli* or MON 87701 tissues showed no signs of adverse effects at high doses. An endangered species risk assessment also concluded that MON 87701 is unlikely to have adverse effects on these organisms, including endangered lepidopteran insects. Therefore, the risks for humans, animals, and other non-target organisms from MON 87701 are negligible under the conditions of use.

The introduction of MON 87701 will not impact cultivation practices and the management of weeds, diseases, and insects except for the control of targeted lepidopteran insect pests in soybean production systems. Successful adoption of MON 87701 would be expected to improve the current agricultural practices by eliminating or reducing insecticide use for targeted lepidopteran pests, reduce the risks for non-target species, and improve the soybean production efficiency by increasing yield potential while reducing insecticide costs.

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APPENDICES

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Appendix A: USDA Notification

Field trials of MON 87701 have been conducted in the U.S. since 2001. 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 phenotypic assessment data provided for MON 87701, observational data on pest and disease stressors were collected from these product development trials. All final reports have been submitted to the USDA. A list of trials conducted under USDA notification are provided in Table A-1.

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Table A-1. USDA Notifications Approved for MON 87701 and Status of Trials Conducted under These Notifications

USDA Reference Number	Effective Date	Approved Release Site (by State) Covered by Notification
	2001 Field Trials	
01-242-01n	9/29/01	PR (2)
	2002 Field Trials	
02-077-21n	4/17/02	NC
02-077-08n	5/15/02	AL, AR(3), GA(2), IL(2), MD, MS(2)
02-077-17n	4/26/02	LA(2)
02-113-02n	5/23/02	NC
02-214-10n	9/12/02	HI
02-220-05n	9/11/02	PR(2)
	2003 Field Trials	
03-052-55n	3/23/03	PR(2)
03-052-54n	4/4/03	AL, GA, IL(2), LA(2), MS
03-052-53n	3/23/03	MS
03-052-51n	3/23/03	NC
03-052-50n	3/23/03	LA(2)
03-052-60n	3/23/03	TN
03-058-05n	4/4/03	AL, AR(3), GA, MD, MS(2)
03-323-02n	12/19/03	PR
	2004 Field Trials	
04-148-01n	6/28/04	PR
	2005 Field Trials	
05-067-03n	4/4/05	PR(3)
05-067-04n	4/26/05	AL, AR(2), IL(2), MD, MS, NC
05-067-05n	5/3/05	GA, LA
05-151-07n	6/28/05	PR(2)
05-347-02n	1/10/06	PR
	2006 Field Trials	
06-059-09n	4/3/06	IL
06-061-05n	5/11/06	AL, AR(2), GA, LA, MD, MS, NC
06-069-11n	4/24/06	PR(4)
06-166-107n	7/12/06	PR
06-201-106n	9/11/06	AL, GA, PR
06-222-102n	9/9//06	PR
06-226-101n	9/13/06	PR(2)
06-310-102n	12/6/06	PR(4)

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Table A-1. (continued). USDA Notifications Approved for MON 87701 and Status of Trials Conducted under These Notifications

USDA Reference Number	Effective Date	Approved Release Site (by State) Covered by Notification
2007 Field Trials		
07-023-101n	3/17/07	AL, AR(3), GA, IA, IL, LA, MD, MS, NC
07-045-103n	4/9/07	AR(2), GA, IN, KS, LA(2), MS, OK(2), SC, TN, TX(2), VA
07-054-105n	4/4/07	AR(2), GA, IN, KS, LA(2), MS, OK(2), SC, TN, TX(2), VA
07-057-102n	3/28/07	IL(2)
07-057-103n	3/28/07	IL(2)
07-059-112n	4/18/07	AL, AR, GA, NC,IL,MO
07-060-101n	4/2/07	AL, AR, GA, IL, NC
07-094-110n	5/2/07	LA(2)
07-094-114n	5/10/07	AL, AR(3), GA(2), IL, LA(3), MD, MS, NC
07-127-101n	6/6/07	IL
07-157-101n	7/6/07	PR(4)
07-250-104n	10/7/07	AR(3), GA, KS, LA(2), MS, SC, TX(2)
07-275-111n	11/1/07	PR
07-304-104n	11/30/07	MS, SC, TX
07-312-102n	12/6/07	PR
2008 Field Trials		
08-002-101n	2/1/08	AR(2), PR(2)
08-017-109n	2/20/08	AR(2), GA, IN, LA, MS, SC, TX(2)
08-024-107n	2/23/08	AR, MD
08-080-111n	4/21/08	AR(2)
08-084-101n	4/25/08	IL
08-261-101n	10/17/08	PR

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Appendix B. Materials and Methods Used for Molecular Analyses of MON 87701

Materials

The DNA used in molecular analyses was isolated from leaf tissue of MON 87701 collected in 2007 harvested from Production Plan 07-01-59-05 (Seed lot: GLP-0705-18705-S). Additional DNA extracted from various MON 87701 generations of leaf tissues were used in generation stability analyses. The control DNA was isolated from the leaf tissue of a conventional soybean variety, A5547. The reference substance, plasmid PV-GMIR9, was used in the transformation process to develop MON 87701. Digested whole plasmid and probe templates generated from this plasmid served as positive hybridization controls. The plasmid was isolated prior to the study and its identity confirmed by restriction enzyme digestion. The 1 kb DNA extension ladder and λ DNA/*Hin*d III fragments from Invitrogen (Carlsbad, CA) were used for size estimations on agarose gels for Southern analyses. Additionally, the 500 bp ladder from Invitrogen and GeneRuler 1kb DNA ladder Plus from Fermentas (Burlington, Ontario) were used for size estimations on agarose gels.

Characterization of the Materials

The quality of the source materials from MON 87701 and A5547 were verified by PCR analysis to confirm the presence or absence of MON 87701 except the materials used in the generational stability analyses where the identity of the materials was confirmed by the generation stability Southern blots themselves. The stability of the genomic DNA was confirmed in each Southern analysis by observation of the digested DNA sample on an ethidium bromide-stained agarose gel.

DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA from the test and control substances was isolated from soybean leaf tissue. The leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. DNA was extracted from the processed leaf tissue using the following method. Approximately 5-6 grams of soybean leaf tissue was processed in liquid nitrogen using a mortar and pestle on dry ice. To each sample, 25 milliliters (ml) of a pre-warmed lysis solution was added [24.25 ml pre-warmed (50-60°C) CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl) pH 8.0, 0.5 ml 2-mercaptoethanol (2-ME), and 0.25 ml of 10 mg/ml proteinase K for a final concentration of 2% 2-ME and 100 µg/ml proteinase K]. The tube was incubated for at least 60 minutes at 50-60°C, with periodic shaking. Twenty ml of a phenol: chloroform: isoamyl alcohol (PCI 25:24:1) mixture was added to each tube and vigorously mixed by hand. The tubes were centrifuged for 10 minutes at 13,000 x g at 15-25°C and the supernatant was transferred to a pre-spun 50 ml MaXtract High Density conical tube (Qiagen, Carlsbad, CA). Twenty ml of PCI 25:24:1 was added to each tube and vigorously mixed by hand. The tubes were centrifuged for 10 minutes at 1500 x g at 15-25°C. This was repeated for a total of two MaXtract High Density extractions. After the last extraction, the upper aqueous phase was transferred to a clean 50 ml conical tube and approximately two times the volume of -20°C 100% ethanol was added. The tube was gently inverted by hand several times to mix. To precipitate the DNA, the tubes were placed in a -20°C

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freezer for at least 30 minutes. To pellet the DNA, the tubes were centrifuged at 13,000 x g for 20 minutes at 1-9°C. The DNA was rinsed at least twice with 70% ethanol and residual ethanol was removed by heating at 37-65°C. The pellets were redissolved in 3 ml of TE (10 mM Tris HCl, 1 mM EDTA), pH 8.0. The tubes were incubated at 60-70°C for at least 1 hour to resuspend the pellets completely. The tubes were then centrifuged at 15,000 x g for 10 minutes at 15-25°C to remove undissolved material. The supernatants were transferred to a 13 ml Sarstedt tube and approximately 4 µl of 100 mg/ml RNase A was added to each tube. The tubes were then incubated at 60°C for 15 minutes. To remove residual polysaccharide compounds, the DNA was PEG precipitated according to draft SOP with the exception of using a smaller volume of TE buffer to resuspend the pellet, which created a more concentrated DNA solution for use in the Southern analyses.

Genomic DNA from the test substance samples used in the insert stability analyses was isolated according to draft SOP. Some of the genomic DNA from the test substance used in the T-DNA I copy number analyses was also isolated according to this SOP, except that the amount of processed leaf tissue was increased and the other volumes of material were increased accordingly. This was acknowledged in the raw data as a protocol deviation. All extracted DNA was stored in a 4°C refrigerator and/or -20°C freezer until use.

Quantification of Genomic DNA

Quantification of DNA samples was performed using a Hoefer (Holliston, MA) DyNA Quant 200 Fluorometer with Roche (Indiannapolis, IN) molecular size marker IX as a DNA calibration standard.

Restriction Enzyme Digestion of Genomic DNA

Ten micrograms (µg) of genomic DNA extracted from the test and control substances was digested with the appropriate restriction enzymes according to the draft SOP in a total volume of ~500 µl using ~100 units of the restriction enzyme with the exception of the reactions presented in Figure V-3, which used ~50 units of restriction enzyme. For the purpose of running positive hybridization controls, ~10 µg of genomic DNA extracted from the control substance was digested and the appropriate positive hybridization control(s) were added to these digests and loaded.

DNA Probe Preparation for Southern Blot Analyses

Probes were prepared by PCR amplification of the PV-GMIR9 template using a standard procedure based on Sambrook and Russell (2001). The probes were designed based on the nucleotide content (%GC) so that the entire probe would hybridize under the conditions used. Approximately 25 ng of each probe template were radiolabeled with either ^{32}P-deoxycytidine triphosphate (dCTP) or ^{32}P-deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using the random priming method (RadPrime DNA Labeling System, Invitrogen) or by PCR. Probe locations relative to the genetic elements in plasmid PV-GMIR9 are depicted in Figure IV-1.

Southern Blot Analyses of Genomic DNA

Digested genomic DNA isolated from test and control materials was evaluated using Southern blot analyses. When multiple probes were used for the analysis, the appropriate

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probe templates were used as positive hybridization controls (Figure IV-1). The plasmid DNA was digested with *Bgl* II / *Nco* I and added to conventional soybean genomic DNA as an additional positive hybridization control. The DNA was then separated by agarose gel electrophoresis. Southern blots were hybridized and washed at 50°C, 55°C, 60°C, or 65°C depending on the melting temperature of the probe. The table below lists the temperature and radiolabeling conditions of the probes used in this study. Multiple exposures of each blot were then generated using Kodak Biomax MS film in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

Probe	DNA Probe	Labeling Method	Probe labeled with dNTP (^{32}P)	Hybridization/ Wash Temperature (°C)
1	Backbone Probe 1	RadPrime	dATP	60
2	Backbone Probe 2	RadPrime	dATP	60
3	Backbone Probe 3	RadPrime	dATP	60
4	Backbone Probe 4	RadPrime	dCTP	65
5	T-DNA II Probe 5	RadPrime	dATP	55
6	T-DNA II Probe 6	RadPrime	dATP	55
7	T-DNA I Probe 7	RadPrime	dATP	50
8	T-DNA I Probe 8	RadPrime	dATP	60
9	T-DNA I Probe 9	RadPrime	dATP	55
10	T-DNA I Probe 10	RadPrime	dATP	60
11	T-DNA I Probe 11	RadPrime	dATP	55

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DNA Sequence Analyses of the Insert

Overlapping PCR products were generated that span the insert and adjacent 5' and 3' flanking genomic DNA sequence in MON 87701 (Figure B-1). These products were sequenced to determine the nucleotide sequence of the insert in MON 87701, as well as determining the nucleotide sequence of the genomic DNA flanking the 5' and 3' ends of the insert. The PCR analysis was performed to amplify nine overlapping DNA fragments (Products A, B, C, D, E, F, G, H, and I) spanning the entire length of the insert. The table below lists the PCR reaction and cycling conditions used in this study.

Product Name	Product A		Product B		Product C		Product D	Product E		Product F	Product G		Product H	Product I
DNA Template (ng) in Reaction Volume (µl)	30 ng in 50 µl		48 ng in 25 µl		96 ng in 25 µl		48 ng in 25 µl	96 ng in 25 µl		48 ng in 25 µl	48 ng in 25 µl		96 ng in 25 µl	30 ng in 50 µl
Reaction Conditions	1♠		2♣		2		2	2			2		2	1
Cycling Conditions	Cycles	Temp Time	Cycles	Temp Time	Cycles	Temp Time	Same Cycling conditions as Product C	Cycles	Temp Time	Same Cycling conditions as Product C	Cycles	Temp Time	Same Cycling conditions as Product E	Same Cycling conditions as Product A
	1 cycle	95°C 2 min	1 cycle	94°C 2 min	1 cycle	94°C 2 min		1 cycle	94°C 2 min		1 cycle	94°C 2 min		
		94°C 30 sec		94°C 45 sec		94°C 45 sec			94°C 45 sec		10 cycles	94°C 45 sec		
	40 cycles	65°C 30 sec	35 cycles	69°C 45 sec	35 cycles	68°C 45 sec		35 cycles	65°C 45 sec		Decrease 1°C per cycle	70°C 45 sec		
		72°C 1.5 min		72°C 5 min		72°C 5 min			72°C 5 min			72°C 5 min		
	1 cycle	72°C 5 min	1 cycle	72°C 10 min	1 cycle	72°C 10min		1 cycle	72°C 10 min			94°C 45sec		
											35 cycles	60°C 45 sec		
												72°C 5 min		
											1 cycle	72°C 10 min		

1♠ 2 Mm $MgSO_4$, 0.2 µM of each primer, 0.1 mM each dNTP, 1M Betaine, and 0.2 U KOD Hot Start DNA Polymerase

2♣ 1 Mm $MgSO_4$, 0.8µM of each primer, 0.1 mM each dNTP, 1M Betaine, and 0.02 U KOD Hot Start DNA Polymerase

Aliquots of each PCR product were separated on 0.8 % (w/v) agarose E-gels (Invitrogen) and visualized by ethidium bromide staining to verify that the products were of the expected size prior to sequencing. To remove residual excess primer following PCR amplification, Products B and C were treated with a 2 µl mixture of Exonuclease I (EXO) (USB Cleveland, OH) and Shrimp Alkaline Phosphatase (SAP) (USB) (0.1 Units (U)/ µl each) per 5µl of PCR product and cycled as follows: one cycle at 37°C for 15 minutes and one cycle at 80°C for 15 minutes. Not all products were treated with EXO-SAP prior to sequencing as documented in the raw data. The PCR products were sequenced using multiple primers, including some of the primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry (ABI, Foster City, CA).

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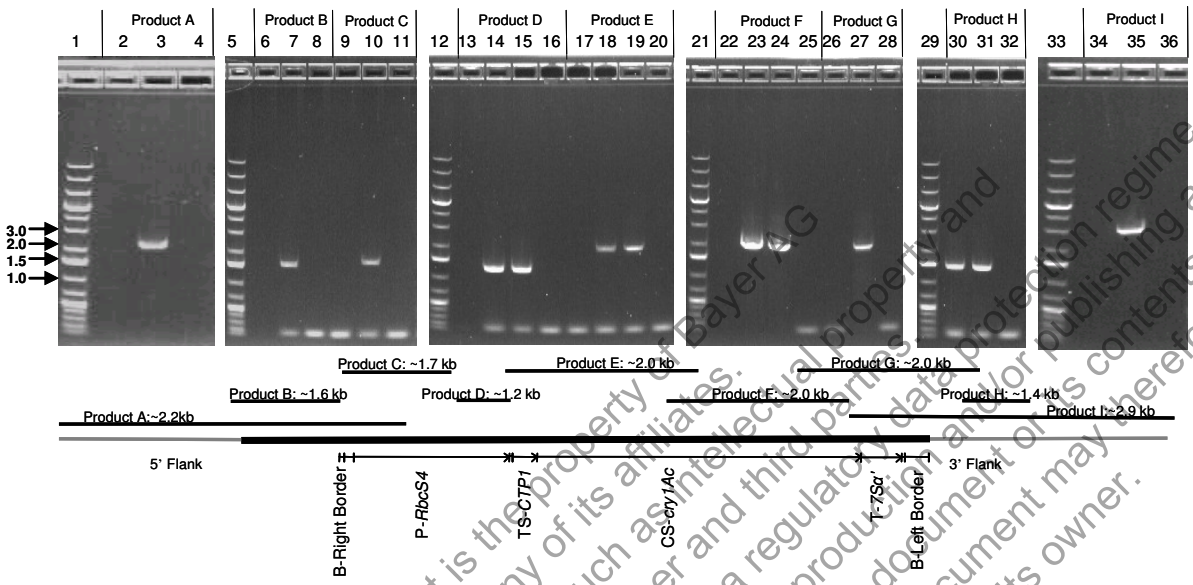

Figure B-1. Overlapping PCR Analysis across the Insert in MON 87701

PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 87701 were performed on MON 87701 genomic DNA extracted from leaf tissue (Lanes 3, 7, 10, 14, 18, 23, 27, 31 and 35). Lanes 2, 6, 9, 13, 17, 22, 26, 30, and 34 contain reactions with conventional soybean control DNA extracted from leaf tissue. Lanes 4, 8, 11, 16, 20, 25, 28, 32, and 36 are reactions containing no template DNA. Lanes 15, 19, and 24 contain reactions with PV-GMIR9 control DNA. Lanes 1, 5, 12, 21, 29, and 33 contain Fermentas GeneRuler™ 1 kb Plus DNA Ladder. Lanes are marked to show which product has been loaded and is visualized on the agarose E-gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 87701 that appears at the bottom of the figure. Three to six µl of each of the PCR products was loaded on the gel. PCR amplicons reported in this figure were not necessarily used in sequencing, but are representative of the study data.

Lanes	Lanes	Lanes	Lanes
1.) GeneRuler™ 1 kb Plus DNA Ladder	10.) MON 87701 genomic DNA	19.) PV-GMIR9 control DNA	28.) No template DNA control
2.) Conventional soybean control DNA	11.) No template DNA control	20.) No template DNA control	29.) GeneRuler™ 1 kb Plus DNA Ladder
3.) MON 87701 genomic DNA	12.) GeneRuler™ 1 kb Plus DNA Ladder	21.) GeneRuler™ 1 kb Plus DNA Ladder	30.) Conventional soybean control DNA
4.) No template DNA control	13.) Conventional soybean control DNA	22.) Conventional soybean control DNA	31.) MON 87701 genomic DNA
5.) GeneRuler™ 1 kb Plus DNA Ladder	14.) MON 87701 genomic DNA	23.) MON 87701 genomic DNA	32.) No template DNA control
6.) Conventional soybean control DNA	15.) PV-GMIR9 control DNA	24.) PV-GMIR9 control DNA	33.) GeneRuler™ 1 kb Plus DNA Ladder
7.) MON 87701 genomic DNA	16.) No template DNA control	25.) No template DNA control	34.) Conventional soybean control DNA
8.) No template DNA control	17.) Conventional soybean control DNA	26.) Conventional soybean control DNA	35.) MON 87701 genomic DNA
9.) Conventional soybean control DNA	18.) MON 87701 genomic DNA	27.) MON 87701 genomic DNA	36.) No template DNA control

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

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Appendix C. Materials, Methods and Results for Characterization of Cry1Ac Protein Produced in MON 87701

Materials

The MON 87701-produced Cry1Ac protein (Orion lot 10000801) was purified as described below from harvested seed of MON 87701 prior to the initiation of this study. The seed used for the isolation of Cry1Ac protein, lot GLP-0612-17898-S, was produced under protocol IP036 by the Monsanto Trait Development group. The identity of the harvested seed containing MON 87701 was confirmed by event-specific PCR; a copy of the Certificate of Analysis for this seed lot is archived in the Monsanto Regulatory archives with the records documenting protein isolation. The purified MON 87701-produced protein was stored in a -80 ºC freezer in a buffer solution containing 50 mM CAPS, pH 10.8, 1 mM EDTA, 2 mM DTT, 1 mM benzamidine HCl, ~30 mM NaCl, ~1% ethylene glycol, and a trace amount of PMSF. The records describing the purification of this MON 87701-produced protein are archived under the Orion lot 10000801 in the Monsanto Regulatory archives.

The *E. coli*-produced Cry1Ac reference protein (Orion lot 10000804) was purified from the fermentation of *E. coli* transformed plasmid. The DNA sequence encoding this Cry1Ac reference protein was confirmed both prior to and following fermentation of *E. coli*. Records pertaining to the purification of this *E. coli*-produced reference protein are archived under Orion lot 10000804. The *E. coli*-produced Cry1Ac reference standard was previously characterized (APS Characterization Plan 20-100133) and a copy of the Certificate of Analysis (COA) is included as in Monsanto archives. The *E. coli*-produced Cry1Ac protein was stored in a -80 ºC freezer in a buffer solution (50 mM CAPS, 1 mM benzamidine-HCl, 1 mM EDTA, and 2.5 mM DTT, pH 10.25) at a total protein concentration of 1.4 mg/ml.

The *E. coli*-produced Cry1Ac protein was used as a reference protein for the immunoblot assay, the functional activity assay, and the purity and molecular weight evaluation, and as a negative control in the glycosylation analysis.

Description of Assay Control

Protein molecular weight standards (BioRad, Hercules, CA) were used to calibrate SDS-PAGE gels and verify protein transfer to PVDF membranes. A peptide mixture (CalMix2 from the Sequazyme Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass analysis. A PTH-amino acid standard mixture (Applied Biosystems) was used to calibrate the sequencer for N-terminal sequence analysis. Dilutions of an amino acid standard (NIST) were used to generate a standard curve for determining protein concentration. Transferrin and horseradish peroxidase (both from Sigma, St. Louis, MO) were used as positive controls for glycosylation analysis. CandyCane Glycoprotein Molecular Weight Standards (Molecular Probes, Eugene, OR) were used as molecular weight markers and positive and negative controls for glycosylation analysis.

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Protein Purification

The MON 87701-produced Cry1Ac protein was purified from harvested seed of MON 87701 prior to initiation of this study. The purification procedure was not performed under a GLP protocol or plan; however, all procedures were documented on worksheets and, where applicable, SOPs were followed. The Cry1Ac protein was purified at 4 °C from an extract of ground seed using a combination of ammonium sulfate fractionation, anion exchange chromatography, and immunoaffinity chromatography.

Approximately one kilogram of MON 87701 seed was ground to a powder using a Perten Laboratory Mill. Ground material was stored in a -80 °C freezer until use. To de-fat the seed powder, two ~500 g batches were extracted four times with warm hexane (~50 °C) added at a ratio of ~3 ml of hexane per gram of ground seed, and then air dried. The final weight of the de-fatted seed powder was ~760 g.

The Cry1Ac protein was purified from a total of four ~100 g aliquots of the ground, de-fatted MON 87701 seed in four separate runs that were pooled to generate the final MON 87701-produced Cry1Ac protein sample.

Each run included the following series of extraction and chromatography steps:

PBS wash – To promote extraction of neutral pH-soluble proteins, seed powder was stirred in cold PBS pH 7.0, 1 mM benzamidine HCl, 0.5 mM PMSF, 1% PVPP at 7.5 ml/g powder for about 1 h. The Cry1Ac-containing washed ground seed pellets were collected by centrifugation.

CAPS solubilization – Cry1Ac protein was extracted from the washed ground seed pellet with CAPS solubilization buffer (100 mM CAPS, pH 10.8, 1 mM benzamidine HCl, 0.5 mM PMSF, 1 mM EDTA, 10 mM DTT) added at 5 ml/g of starting powder. The suspension was stirred for 1-2 h, and solubilized proteins, including Cry1Ac, were separated from insoluble material by centrifugation.

$(NH_4)_2$ SO_4 precipitation and re-solubilization – An ammonium sulfate precipitate was prepared by the addition of ammonium sulfate salt to the CAPS solubilization supernatant to a final saturation of 40%. After mixing for 2-4 h, precipitated proteins were collected by centrifugation, and were re-solubilized in 50 mM Bis-Tris propane, pH 9.0, 150 mM NaCl, 1 mM EDTA, 2 mM benzamidine HCl, 1 mM PMSF, 5 mM DTT at 0.75 ml per starting ml of CAPS supernatant. Insoluble material was removed by centrifugation at 37000×g for 1 h, and the supernatant was diluted with 50 mM Bis-Tris propane, pH 9.0, 1 mM EDTA, 2 mM benzamidine HCl, 1 mM DTT to bring the NaCl concentration to 50 mM.

Anion exchange chromatography – The diluted Cry1Ac-containing protein solution (18-26 column volumes, depending on the run) was loaded at a flow rate of 1.4-2.4 ml/min onto a CaptoQ (GE Healthcare, Piscataway, NJ) anion exchange column (100 ml, 50 x 50 mm) equilibrated with 50 mM Bis-Tris propane, pH 9.0, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 2 mM benzamidine HCl buffer. After loading, the column was washed with 2.7-3.5 column volumes of the equilibration buffer. Proteins were then eluted in two steps, the first consisting of 4-7 column volumes of 50 mM Bis-Tris propane, pH 9.0, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 2 mM benzamidine HCl buffer, and the second

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consisting of 3-4 column volumes of 50 mM Bis-Tris propane, pH 9.0, 600 mM NaCl, 1 mM EDTA, 1 mM DTT, 2 mM benzamidine HCl buffer. Cry1Ac protein was predominantly present in the second elution step, which was collected as a single fraction. All wash and elution steps were carried out at a flow rate of 6 ml/min.

Immunoaffinity chromatography – For immunoaffinity chromatography, resin was prepared by binding and then chemically cross-linking a monoclonal anti-Cry1Ac antibody to protein A agarose (Sigma, St. Louis, MO). The Cry1Ac-containing fraction from the anion exchange column (~300-400 ml, depending on the run) was loaded on to the immunoaffinity column (6 ml; 20 × 15 mm, h × d) equilibrated with 5-10 column volumes of 50 mM Bis-Tris propane, pH 9.0, 600 mM NaCl, 1 mM EDTA, 5 mM DTT, 2 mM benzamidine HCl, 1 mM PMSF. To maximize Cry1Ac binding to the immunoaffinity column, the load solution was recirculated through the column overnight. Following the load, the column was washed with 4-6 column volumes of the equilibration buffer. Proteins were then eluted in two elution steps, the first consisting of 4-7 column volumes of 50 mM Bis-Tris propane, pH 9.0, 800 mM NaCl, 1 mM EDTA, 5 mM DTT, 2 mM benzamidine HCl, 1 mM PMSF buffer, and the second consisting of ~7 column volumes of 50 mM Bis-Tris propane, pH 9.0, 800 mM NaCl, 30% (v/v) ethylene glycol, 1 mM EDTA, 5 mM DTT, 2 mM benzamidine HCl, 1 mM PMSF. Cry1Ac protein was predominantly present in the second elution step, which was collected as several ~ 4 ml fractions. Equilibration, load, wash, and first elution step were carried out at a flow rate of ~2 ml/min; the flow rate for the final elution step was ~0.7 ml/min. Fractions collected from the final elution step were evaluated for the presence and amount of Cry1Ac by quantitative immunoblot, and fractions with the highest amounts of Cry1Ac protein were pooled for each run.

All operations described above were carried out at 4°C. Following the final immunoaffinity chromatography run, the four batches of purified Cry1Ac protein were pooled. The pooled sample (~140 ml) was concentrated ~9-fold by diafiltration using a polysulfone hollow fiber cartridge with a 30 kDa molecular weight cut-off, diluted ~10-fold with a buffer containing 50 mM CAPS, pH 10.8, 1 mM EDTA, 2 mM DTT, 1 mM benzamidine HCl, and re-concentrated by diafiltration using the same cartridge. The final concentrated solution of the Cry1Ac protein (~1 ml) was diluted with the same buffer to a final volume of ~4 ml. This material was submitted to the APS program and assigned lot number 22-100135. The lot number was later reassigned as Orion lot 10000801 due to adoption of a new tracking database. The physical appearance of the protein solution was a clear liquid.

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Molecular Weight and Purity Estimation-SDS-PAGE

Aliquots of the *E. coli*-produced and MON 87701-produced Cry1Ac proteins were mixed with 5 × sample buffer (0.31 M Tris-HCl, pH 6.8, 50% (v/v) glycerol, 10% (w/v) SDS, 25% (v/v) β-mercaptoethanol, 0.025% (w/v) bromophenol blue) to a final total protein concentration of 22 ng/µl and 17 ng/µl, respectively. The MON 87701-produced protein was analyzed in duplicate at 95, 189, and 284 ng of total protein per lane. The *E. coli*-produced Cry1Ac reference standard was loaded at 198 ng total protein, in a single lane. The Broad Range Molecular Weight marker (Bio-Rad, Hercules, CA) was loaded at 360 ng total protein. All samples were heated in a thermo-block at 95.8 °C for 5 min and

applied to a pre-cast tris-glycine 4-20% gradient polyacrylamide gel. Electrophoresis was performed at a constant voltage of 125 V for 105 min.

The gel was stained using a Silver Staining Kit from Owl Separation Systems (Portsmouth, NH) according to the manufacturer's protocol. The gel was fixed for 15 min in 100 ml of Fixing Solution I. This was followed by incubation for 15 min in 100 ml of Fixing Solution II. Next, the gel was incubated in 100 ml of Pretreatment Solution for 10 min, followed by a 5 min wash in 100 ml of deionized water. The gel was stained using 100 ml of Silver Staining Solution for 12 min, followed by three 3-5 min washes, each in 100 ml of deionized water. Next the gel was incubated in 100 ml of Developer for 5 min, followed by addition of 5 ml of Stopper Solution to the Developer and incubation for 15 min. Finally, the gel was washed twice for 10 min each in 100 ml of deionized water. All incubations occurred at room temperature with gentle shaking.

Immunoblot Analysis-Immunoreactivity

Immunoblot analysis was performed to confirm the identity of the MON 87701-produced Cry1Ac protein and compare immunoreactivity of the MON 87701-produced and *E. coli*-produced proteins. The MON 87701-produced Cry1Ac protein and the *E. coli*-produced Cry1Ac reference protein (each corrected for the purity of the full-length protein), were loaded on gels at 10, 20, or 30 ng per lane. Each protein was mixed with 5× sample buffer, heated at 96.2 °C for 5 min, and applied to a pre-cast tris-glycine 4-20% gradient polyacrylamide gel. Electrophoresis was performed at a constant voltage of 200 V for 60 min. Precision Plus Dual Color molecular weight marker (Bio-Rad, Hercules, CA) was used to verify electrotransfer of protein to the membrane and estimate the size of the immunoreactive bands. Electrotransfer to a PVDF membrane was performed at a constant voltage of 100 V for 44 min.

The membrane was blocked overnight with 5% (w/v) NFDM in 1× PBST. The membrane was probed with a 1:500 dilution of goat affinity-purified anti-Cry1Ac antibody (Orion lot 10000963) in PBST containing 1% (w/v) NFDM for 1 h. Excess antibody was removed using three 5 min washes with PBST. Finally, the membrane was probed with HRP-conjugated anti-goat IgG (Pierce, Rockford, IL) at a dilution of 1:10000 in PBST containing 1% (w/v) NFDM for 2 h. Excess HRP-conjugate was removed using three washes, each at least 5 min, with PBST. The blocking step was performed at 4°C. All other incubations were performed at room temperature. Immunoreactive bands were visualized using the ECL detection system (GE Healthcare, Piscataway, NJ) and exposed (1, 2, and 5 min) to Hyperfilm ECL high performance chemiluminescence film (GE Healthcare, Piscataway, NJ). Films were developed using a Konica SRX-101A automated film processor.

Analysis of the film was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software.

MALDI-TOF Tryptic Mass Map Analysis

Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was used to confirm the identity of the MON 87701-produced Cry1Ac protein. The MON 87701-produced Cry1Ac protein was subjected to electrophoresis on an SDS-polyacrylamide gel. The protein sample was mixed with 5× DTT-containing

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sample buffer (250 mM Tris-HCl, pH 6.8, 50% (v/v) glycerol, 10% (w/v) SDS, 0.25% (w/v) bromophenol blue, 0.5 M DTT) heated at 98.6°C for 5 min, and loaded across eight lanes of a pre-cast tris-glycine 4-20% gradient polyacrylamide gel. Precision Plus Dual Color molecular weight marker was loaded to enable estimation of molecular weight. Electrophoresis was performed at a constant voltage of 125 V for 105 min. Proteins were stained with Brilliant Blue G Colloidal stain (Sigma, St. Louis, MO) for 1 h, and destained according to manufacturer's protocol with 3 h of destaining in Destain Solution B prior to gel scanning.

The band representing full-length MON 87701-produced Cry1Ac protein was excised from several lanes of the gel, destained, reduced, and alkylated. Briefly, each excised gel band was destained for 30 min by incubation in 100 µl of destain solution in a microfuge tube. Following destaining, each excised gel band was incubated in 100 µl of 100 mM ammonium bicarbonate buffer for 40 min at room temperature. Each gel band was reduced in 100 µl of 10 mM DTT solution for 2 h at 37°C. Each band was alkylated by the addition of 100 µl of 20 mM iodoacetic acid. The alkylation reaction was allowed to proceed at room temperature for 25 min in the dark. Each gel band was subsequently washed in 200 µl of 25 mM ammonium bicarbonate buffer for 15-45 min at room temperature. This step was repeated two additional times, following which each gel band was dried using a Speed Vac concentrator. Three gel bands were combined and rehydrated with 60 µl of 0.02 µg/µl trypsin in 25 mM ammonium bicarbonate, 10% acetonitrile, and the sample was incubated for about 1 h at room temperature. Next, excess liquid was removed and the sample was incubated overnight at 37 °C in 120 µl of 25 mM ammonium bicarbonate, 10% acetonitrile. The following day, the sample was sonicated for 5 min, and the supernatant was transferred to a new tube and dried using a Speed Vac concentrator (Extract 1). The gel material was resuspended in 90 µl 60% acetonitrile, 0.1% TFA, 0.1% octyl-β-D-glucopyranoside solution, and sonicated for 5-10 min. After transfer of the supernatant to a new tube, this step was repeated one time, and the combined supernatants were dried using a Speed Vac concentrator (Extract 2). Extracts 1 and 2 were each resuspended in 20 µl 0.1% TFA and then dried using a Speed Vac concentrator. Extract 1 was resuspended in 5 µl of 50% acetonitrile, 0.1% TFA, while Extract 2 was resuspended in 10 µl of the same solution. Each extract was sonicated for 5 min. The extracts were then ready for loading onto the MALDI-TOF sample plate.

Mass spectral analyses were performed as follows. Mass calibration of the instrument was performed using an external peptide mixture (CalMix 2; Applied Biosystems, Foster City, CA). Extract 1 and Extract 2 samples (0.1-0.25 µl) were co-crystallized with 0.75 µl each of the following matrix solutions: α-cyano-4-hydroxy cinnamic acid (α-cyano), dihydroxybenzoic acid (DHB), and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) at separate locations on the analysis plate. The samples in α-cyano matrix were analyzed in the 500 to 6000 Da range using 200 shots at a laser intensity setting of 2511. The laser intensity setting is a unit-less MALDI-TOF instrument-specific value. The samples in DHB matrix were analyzed in the 550 to 6000 Da range using 200 shots at a laser intensity setting of 3101. The samples in sinapinic acid matrix were analyzed in the 900 to 8000 Da range using 200 shots at a laser intensity setting of 3247. Protonated (MH+) peptide masses were observed monoisotopically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993), except above 3000 Da, where mass-averaged values were

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observed. GPMAW32 software (Applied Biosystems, Foster City, CA) was used to generate a theoretical trypsin digest of the expected Cry1Ac protein sequence, which was based upon the nucleotide sequence of the inserted cry1Ac gene and the N-terminal sequence analysis that identified the amino terminus of the protein. Masses were calculated for each theoretical fragment and compared to the raw mass data. Experimental masses (MH+) were assigned to ion peaks in the 500 to 1000 Da range if there were two or more isotopically resolved ion peaks, and in the 1000 to 8000 Da range if there were three or more isotopically resolved ion peaks in the spectra. Ion peaks were not assessed if the ion peak heights were less than approximately twice the baseline noise, or when a mass could not be assigned due to overlap with a stronger signal ±2 Da from the mass analyzed. Known autocatalytic fragments from trypsin digestion were identified in the raw data.

N-terminal Sequence Analysis

N-terminal sequence analysis was used to confirm the identity of the MON 87701-produced Cry1Ac protein. The MON 87701-produced Cry1Ac protein was subjected to electrophoresis on an SDS-polyacrylamide gel. MON 87701-produced Cry1Ac protein was mixed with 5 × DTT-containing sample buffer, heated at 98.6 °C for 5 min and then loaded across eight lanes of a pre-cast tris-glycine 4-20% gradient polyacrylamide gel. Precision Plus Dual Color molecular weight marker was used to estimate molecular weights and verify protein transfer to a PVDF membrane. Electrophoresis was performed at a constant voltage of 125 V for 105 min. Electrotransfer to a PVDF membrane was performed at a constant voltage of 25 V for 2 h. After transfer the blot was washed in deionized water three times for 2-5 min each, then briefly (≤2 min) stained in Coomassie Brilliant Blue R-250 Staining Solution (Bio-Rad, Hercules, CA). The blot was destained in Coomassie Brilliant Blue R-250 Destaining Solution (Bio-Rad, Hercules, CA) for ~5 min, and the blot image was captured using a Bio-Rad GS-800 densitometer with the supplied Quantity One software.

Two bands were excised from the stained membrane: a band with a molecular weight of ~133 kDa, corresponding to full-length Cry1Ac protein, and a band with a molecular weight of ~75 kDa that, by purity analysis, represented ~10% of the total protein. N-terminal sequence analysis was performed on each of the excised bands for 15 cycles using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise Sequencing System with 140C Microgradient system and 785A Programmable Absorbance Dectector and Procise Control Software (version 1.1a) was used. Chromatographic data were collected using Atlas software (Thermo Scientific, Woburn, MA). A PTH-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to chromatographically calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A control protein (β-lactoglobulin, Applied Biosystems, Foster City, CA) was analyzed before and after the test protein to verify that the sequencer met acceptable performance criteria for repetitive yield and sequence identity.

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Glycosylation Analysis

Glycosylation analysis was used to determine whether the MON 87701-produced Cry1Ac protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 87701-produced Cry1Ac protein, the *E. coli*-produced Cry1Ac reference protein, and the positive controls, transferrin and horseradish peroxidase (both from Sigma, St. Louis, MO), were each mixed with 5× sample buffer. These samples were heated at 96 °C for 4 min, cooled, and loaded on a tris-glycine 4-20% gradient polyacrylamide gel. Both *E. coli*- and MON 87701-produced Cry1Ac proteins were loaded at 50 and 100 ng purity-corrected for the full-length protein. The Precision Plus Dual Color pre-stained protein molecular weight was loaded to verify electrotransfer of the proteins to the membrane and as markers for molecular weight, and the CandyCane Glycoprotein Molecular Weight Standard (Molecular Probes, Eugene, OR) was loaded as markers for molecular weight and to provide additional positive and negative controls for glycosylation. Electrophoresis was performed at a constant voltage of 150 V for 15 min, then 200 V for 55 min. Electrotransfer to a PVDF membrane was performed at a constant voltage of 25 V for 80 min.

Carbohydrate detection was performed directly on the PVDF membrane using the Pro-Q Emerald 488 Glycoprotein Gel and Blot Stain Kit (Molecular Probes, Eugene, OR). All steps were performed at room temperature. The PVDF membrane was fixed in two changes of 25 ml each of a solution containing 50% methanol and 5% glacial acetic acid, with the first fix step for 60 min and the second overnight. Two 15 min washes (50 ml each) of 3% (v/v) glacial acetic acid (wash solution) were followed by a 20 min oxidation in 25 ml of the kit-supplied oxidizing solution. After oxidation, three 15 min washes in wash solution prepared the membrane for staining. The blot was incubated in 25 ml of Pro-Q Emerald Staining Solution prepared as recommended for blot staining. After 75 min of staining in the dark, two 15 min washes were followed by one 20 min wash, all in 50 ml of wash solution. The final wash cycles included two 1 min deionized water washes followed by three 25 ml, 5 min washes in 100% methanol. Last, the blot was washed for 10 min in deionized water. The blot was then scanned using the BioRad Molecular Imager FX using the Alexa 488 illumination setting in order to visualize fluorescent signal from the glycosylated proteins.

After glycosylation analysis, the blot was stained to visualize the proteins present on the membrane. The blot was stained for 2 min in Coomassie Brilliant Blue R-250 Staining Solution (BioRad, Hercules, CA). The blot was destained in Coomassie Brilliant Blue R-250 Destaining Solution (BioRad) for ~15 min, and the blot image was captured using a BioRad GS-800 densitometer with the supplied Quantity One software.

Functional Activity Assay

The functional activities of the MON 87701-produced Cry1Ac protein and the *E. coli*-produced Cry1Ac reference protein were compared using an insect bioassay. Aliquots of MON 87701-produced Cry1Ac protein and *E. coli*-produced Cry1Ac reference protein were transferred to the Monsanto Ecological Technology Center for testing in an assay using corn earworm (CEW; Helicoverpa zea), an insect species known to be susceptible to Cry1Ac protein (MacIntosh et al., 1990). Dose-response assays were performed for Cry1Ac proteins from both sources in parallel and assays were repeated on three separate

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days to estimate the mean EC_{50} value, the effective concentration necessary to inhibit CEW growth by 50% relative to the control response.

CEW Bioassay

Materials:

Plant-Produced Cry1Ac protein, *E. coli*-produced Cry1Ac Reference Standard Protein and Control Substance:

The reference standard, an *E. coli*-produced Cry1Ac protein (Orion ID: 10000804) and a plant-produced Cry1Ac protein (Orion ID: 10000801) from the harvested seed of MON 87701, were received from the Monsanto Product Characterization Center (PCC). The total protein concentration of the *E. coli*-produced Cry1Ac protein aliquots was 1.4 mg/mL, with a purity of 80%, and a purity corrected concentration of 1.1 mg Cry1Ac/mL. The total protein concentration of the plant-produced Cry1Ac protein aliquots were 42 µg/mL with a purity of 77%, and a purity corrected concentration of 32 µg Cry1Ac/mL. The *E. coli*-produced Cry1Ac protein was suspended in 50 mM CAPS, pH 10.25, 1 mM EDTA, 2.5 mM DTT, 1 mM benzamidine-HCl buffer, while the plant-produced Cry1Ac protein was suspended in 50 mM CAPS, pH 10.8, 1 mM EDTA, 2 mM DTT, 1 mM benzamidine, and <1% ethylene glycol buffer. Additionally, the buffers used to store the *E. coli*-produced and the plant-produced proteins, 50 mM CAPS, pH 10.25, 1 mM EDTA, 2.5 mMDTT, and 1 mM benzamidine-HCl (lot # G-826331-A), and "Soy Cry1Ac Sample Buffer" (lot # G-824555B) were received from the PCC. The plant-produced and *E. coli*-produced Cry1Ac proteins were stored in a -80°C freezer and the buffers were stored in a 4°C refrigerator.

Methods:

Insects. CEW were obtained from Benzon Research Inc (Carlisle, PA). Insect eggs were incubated at temperatures ranging from 10^{o} C to 27^{o} C, to achieve the desired hatch time.

Bioassays. CEW were used to measure biological activity of the MON 87701-produced and *E. coli*-produced Cry1Ac protein samples. The bioassay was replicated three times on separate days with separate batches of insects. The MON 87701-produced and *E. coli*-produced substances were run in parallel during each bioassay. Each bioassay replicate for the *E. coli*-produced and MON 87701-produced Cry1Ac proteins consisted of a series of six dilutions yielding a dose series with a two-fold separation factor ranging from 0.00065 – 0.020 µg Cry1Ac protein/mL diet and two buffer controls. All dose levels, including the buffer controls, contained an equal volume and composition of buffer. The Cry1Ac protein dosing solutions were prepared by diluting the protein with purified water and incorporating the dilution into a Southland agar-based insect diet (Lake Village, AR). This dose series in diet was chosen to adequately characterize the dose-effect relationship on CEW weight gain for the proteins from both sources. The diet mixture was then dispensed in 1 mL aliquots into a 128 well tray (# BAW128, Bio-Serv, Frenchtown, NJ). Insect larvae were placed on these diets using a fine paintbrush, with a target number of 16 insects per treatment. The infested wells were covered by a

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ventilated adhesive cover (# BACV16, Bio-Serv, Frenchtown, NJ) and the insects were allowed to feed for a period of approximately seven days in an environmental chamber programmed at 27° C, ambient relative humidity and a lighting regime of 14 light:10 dark. The number of surviving insect and the combined weight of the surviving insects at each dose level was recorded at the end of the 7-day incubation period.

Results of Cry1Ac Molecular Weight Equivalence

For molecular weight analysis, MON 87701-produced Cry1Ac protein was separated using SDS-PAGE and stained using a Silver Staining Kit (Owl Separation Systems) (Figure C-1, lanes 3-8). The full-length MON 87701-produced Cry1Ac protein had an estimated molecular weight of 133.4 kDa (Table C-1), and migrated to the same position on the SDS-PAGE gel as the *E. coli*-produced Cry1Ac reference standard (Figure C-1, lane 9). The apparent molecular weight of the full-length *E. coli*-produced Cry1Ac reference protein is 131.7 kDa. The difference in the estimated molecular weights between the MON 87701-produced and *E. coli*-produced Cry1Ac full-length proteins was 1.3% (Table C-1). Because the experimentally determined difference in apparent molecular weight met the pre-set acceptance criteria (≤5% difference), the MON 87701-produced and *E. coli*-produced Cry1Ac proteins are considered equivalent based on their molecular weights.

Table C-1. Molecular Weight Difference between Full-Length MON 87701-Produced and *E. coli*-Produced Cry1Ac Proteins

Molecular Weight of Full-Length MON 87701-Produced Cry1Ac Protein[1]	Molecular Weight of *E. coli*-Produced Cry1Ac Protein[2]	Percent Difference from *E. coli*-Produced Cry1Ac Protein[3]
133.4 kDa	131.7 kDa	1.3 %

[1] Reference Table C-1 for the molecular weight of the full-length MON 87701-produced protein.
[2] Reference the Orion 10000804 COA (Appendix 1) for the molecular weight of the full-length *E. coli*-produced reference protein.
[3] Percent difference was calculated as follows: $\frac{133.4-131.7}{133.4}\times 100\% = 1.3\%$

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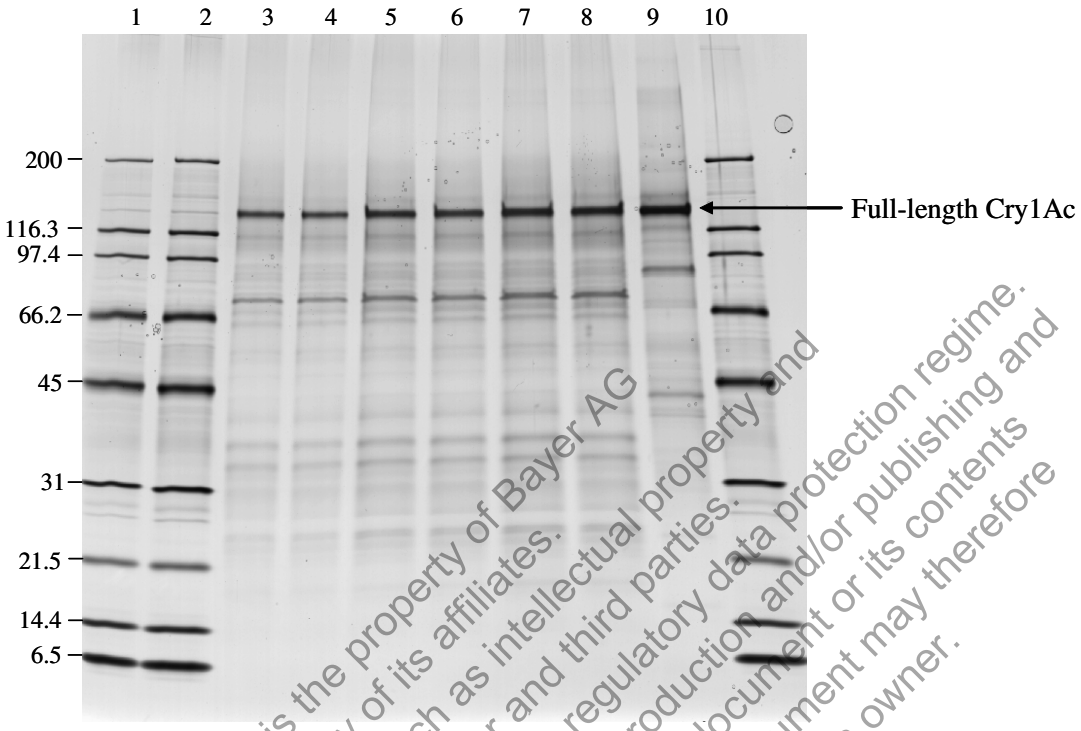

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Figure C-1. Molecular Weight and Purity Analysis of the MON 87701-Produced Cry1Ac Protein

Aliquots of the MON 87701-produced Cry1Ac protein and *E. coli*-produced Cry1Ac reference protein were separated by SDS-PAGE, followed by silver staining. Approximate molecular weights (kDa) are shown on the left and correspond to the marker loaded in lanes 1, 2, and 10.

Lane	Sample	Amount loaded (ng)
1	BioRad Broad Range Marker	360
2	BioRad Broad Range Marker	360
3	MON 87701-produced Cry1Ac protein	95
4	MON 87701-produced Cry1Ac protein	95
5	MON 87701-produced Cry1Ac protein	189
6	MON 87701-produced Cry1Ac protein	189
7	MON 87701-produced Cry1Ac protein	284
8	MON 87701-produced Cry1Ac protein	284
9	*E. coli*-produced Cry1Ac protein	198
10	BioRad Broad Range Marker	360

Results of Cry1Ac Protein Immunoreactivity Equivalence

The MON 87701-produced Cry1Ac protein and corresponding *E. coli*-produced reference protein were loaded on the same gel, which was used for immunoblot analysis. The amount of each sample loaded was based on the concentration of the full-length Cry1Ac protein. The major immunoreactive band observed migrated with an apparent molecular weight of ~133 kDa (Figure C-2), the expected molecular weight of the full-length Cry1Ac protein, and was present in both the MON 87701-produced and *E. coli*-produced Cry1Ac samples. As expected, the immunoreactive signal increased with increased loading levels of both the MON 87701-produced and *E. coli*-produced proteins. Faint immunoreactive bands with molecular weights below ~133 kDa represent degradation products of Cry1Ac. Faint immunoreactive bands with molecular weights around 250 kDa were also observed, and most likely represent aggregation of the Cry1Ac protein. Both protein degradation and protein aggregation are commonly observed during protein purification of Cry proteins. Cry proteins naturally aggregate into crystal structures as has been observed for Cry1A proteins (Guereca and Bravo, 1999), while degradation occurs primarily due to the release of endogenous proteases during the purification procedure (Gao et al., 2006).

Densitometric analysis was conducted to compare the immunoreactivity of full-length MON 87701-produced and *E. coli*-produced Cry1Ac proteins. The relative immunoreactivity of each protein with Cry1Ac-specific antibody was determined by averaging intensity values of six protein bands corresponding to the full-length MON 87701-produced Cry1Ac protein and six bands corresponding to the full-length *E. coli*-produced Cry1Ac protein (Table C-3). The averaged band intensity of the signal from the MON 87701-produced Cry1Ac lanes was 33.3% less than the averaged band intensity of the signal from the *E. coli*-produced Cry1Ac lanes. The observed difference was within the pre-set acceptance criteria for immunoreactivity (±35% difference). Thus, the immunoblot analysis established identity of the MON 87701-produced Cry1Ac protein and demonstrated that the MON 87701-produced and *E. coli*-produced Cry1Ac proteins are equivalent based on their immunoreactivity with Cry1Ac-specific antibody.

Results of MALDI-TOF Trytic Mass Map Analysis

The MON 87701-produced, full-length Cry1Ac protein was assessed by MALDI-TOF mass spectrometry. Prior to analysis, the protein sample was chemically reduced, alkylated, and digested with trypsin. The ability to identify a protein using this method is dependent on matching a sufficient number of observed mass fragments to expected (theoretical) mass fragments. In general, a protein identification made by peptide mapping is considered to be reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen et al., 1997). There were 70 peptides (out of 144 masses) identified that matched the expected masses of the Cry1Ac trypsin-digested peptides (Table C-4). The identified masses were used to assemble a coverage map that indicates those matched peptide sequences within the protein sequence (Figure C-3). The protein was confirmed as Cry1Ac based on the result that a significant portion of the protein, 787 of 1182 amino acids (66.6%), was contained in theoretical mass fragments that matched observed mass fragments.

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Results of N-terminal Sequence Analysis

N-terminal sequencing performed on MON 87701-produced Cry1Ac protein identified seven strong and two tenuous amino acids that matched the predicted N-terminal sequence for Cry1Ac containing four amino acids derived from CTP1 (Figure C-4, panel A). The amino acid cysteine is shown in the predicted sequence at position one based on the coding sequence of the Cry1Ac construct in MON 87701. However, cysteine is unstable during the acid hydrolysis reaction used for N-terminal sequencing, and is usually not explicitly observed (Inglis and Liu, 1970). The clear identification of amino acids in subsequent cycles of the sequencing analysis confirmed that an unidentified amino acid was present at position one. The N-terminal sequencing results for MON 87701-produced Cry1Ac protein were consistent with the sequencing results for the *E. coli*-produced Cry1Ac protein, which was engineered to contain a cysteine as the first amino acid, but which also showed an unidentified amino acid at position one (see Figure C-4).

In addition to analysis of the full-length protein, a second band of approximately 75 kDa, which represented about 10% of total protein based on purity analysis, was also analyzed. Due to the reduced amount of this protein compared to the full-length protein, the signal intensity of the peaks in this analysis was low, and only three strong and three tenuous amino acids were identified. While this number of identified amino acids was insufficient to explicitly align the derived sequence to a known N-terminal sequence, the sequence obtained was consistent with the N-terminal sequence for Cry1Ac (Figure C-4, panel B), suggesting that this protein is a truncated Cry1Ac protein.

Results of Glycosylation Analysis

Many eukaryotic proteins are post-translationally modified with carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex, branched polysaccharide structures or simple monosaccharides. In contrast, strains of *E. coli* used for recombinant protein expression lack the necessary biochemical pathways required for protein glycosylation. To test whether post-translational glycosylation of the MON 87701-produced Cry1Ac protein occurred, it was analyzed for the presence of covalently bound carbohydrate moieties. The *E. coli*-produced Cry1Ac reference protein, horseradish peroxidase (positive control), and transferrin (positive control) were analyzed concurrently with the MON 87701-produced Cry1Ac protein. The results of this analysis are presented in Figure C-5A. The positive controls were detected at the expected molecular weights, in a concentration-dependent manner (Figure C-5A, lanes 2-5). No detectable signal was observed for either the MON 87701-produced or *E. coli*-produced Cry1Ac proteins (Figure C-5A, lanes 6-9) at the expected molecular weight on the blot. Post-analysis staining of this blot with Coomassie stain to detect total protein confirmed that both MON 87701-produced and *E. coli*-produced Cry1Ac proteins were present on the blot at similar protein staining intensities as the positive controls (Figure C-5B, lanes 6-9). Thus, the MON 87701-produced protein has been determined to not be glycosylated and is equivalent to the *E. coli*-produced Cry1Ac reference protein with respect to glycosylation.

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Results of Functional Activity

The functional activity of the MON 87701-produced Cry1Ac protein was determined in an insect bioassay that assesses the impact of the Cry1Ac protein on growth of the test insect, corn earworm. The impact of Cry1Ac on insect growth is expressed as an EC_{50} value, which represents the effective concentration of protein necessary to inhibit insect growth by 50% relative to a control population of insects not exposed to the insecticidal protein. The mean EC_{50} value determined for the MON 87701-produced Cry1Ac protein was 0.0039 µg Cry1Ac/ml diet. This EC_{50} value was very similar to the mean EC_{50} value of 0.0036 µg Cry1Ac/ml diet obtained for the *E. coli*-produced reference protein in the same assay. Because the difference between these values was within the pre-set acceptance criteria (<3 fold difference) for establishing equivalence, MON 87701-produced Cry1Ac is determined to have equivalent functional activity to that of *E. coli*-produced Cry1Ac. These results confirmed that these two proteins are functionally equivalent (Table C-2).

Table C-2. EC_{50} Values and Standard Errors for *E. coli*- and MON 87701-produced Cry1Ac Proteins in a CEW Diet-incorporation Bioassay

		EC_{50} (µg Cry1Ac/ml diet)[1]	
		E. coli-produced	MON 87701-produced
Replicate[2]	1	0.0031 ± 0.00035	0.0050 ± 0.00069
	2	0.0026 ± 0.00022	0.0032 ± 0.00021
	3	0.0050 ± 0.00030	0.0034 ± 0.00035
Overall		0.0036 ± 0.0013	0.0039 ± 0.00098

[1] EC_{50} (mean ± standard error) represents the concentration needed to inhibit the growth of the target insect by 50%.

[2] Each bioassay replicate consisted of a series of six protein levels yielding a dose series with a two-fold separation factor ranging from 0.00065 – 0.020 µg Cry1Ac protein/mL diet and two buffer controls. Insect larvae were placed on these diets using a fine paintbrush, with a target number of 16 insects per treatment. The number of surviving insects and the combined weight of the surviving insects at each dose level was recorded at the end of the 7-day incubation period.

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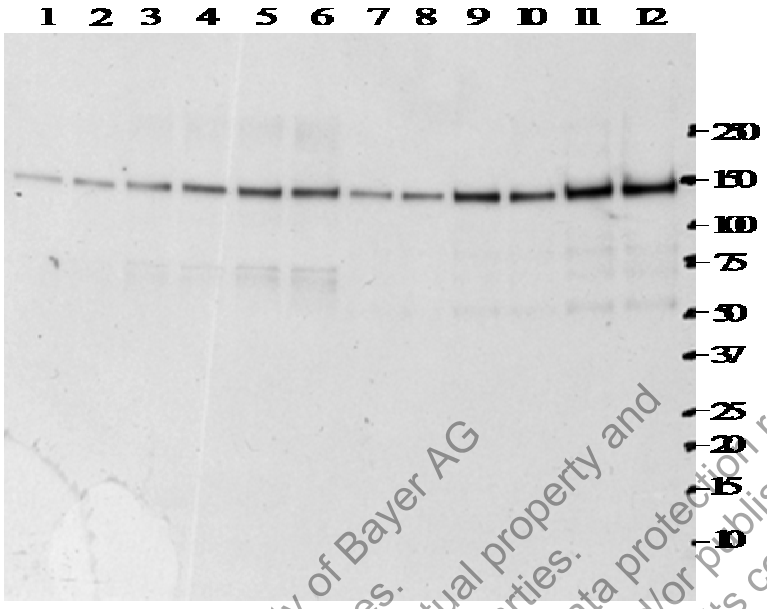

Figure C-2. Western Blot Analysis and Immunoreactivity of MON 87701-Produced and *E. coli*-Produced Cry1Ac Proteins

Aliquots of the plant-produced Cry1Ac protein and *E. coli*-produced Cry1Ac reference protein were separated by SDS-PAGE and electrotransferred to a PVDF membrane. The membrane was incubated with affinity-purified anti-Cry1Ac antibody, and immunoreactive bands were visualized using an ECL system. Approximate molecular weights (kDa) are shown on the right and correspond to the tick marks indicating the position of molecular weight markers loaded beyond lane 12. The 1 min exposure is shown. Amount loaded indicates full-length Cry1Ac amount.

Lane	Sample	Amount Loaded (ng)
1	MON 87701-produced Cry1Ac protein	10
2	MON 87701-produced Cry1Ac protein	10
3	MON 87701-produced Cry1Ac protein	20
4	MON 87701-produced Cry1Ac protein	20
5	MON 87701-produced Cry1Ac protein	30
6	MON 87701-produced Cry1Ac protein	30
7	*E. coli*-produced Cry1Ac protein	10
8	*E. coli*-produced Cry1Ac protein	10
9	*E. coli*-produced Cry1Ac protein	20
10	*E. coli*-produced Cry1Ac protein	20
11	*E. coli*-produced Cry1Ac protein	30
12	*E. coli*-produced Cry1Ac protein	30

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Table C-3. Comparison of Immunoreactive Signal between Full-Length MON 87701-Produced and *E. coli*-Produced Cry1Ac Proteins

Load Amount (ng)	MON 87701-produced Protein Signal Density[1]	*E. coli*-produced Protein Signal Density[1]
10	1.288	1.419
10	1.955	1.798
20	2.908	4.559
20	2.987	3.706
30	4.214	6.547
30	4.140	8.199
Sum	**17.492**	**26.228**
Average Density	**2.915**	**4.371**
Percent difference[2]	**33.3%**	

[1] The density of each band was determined by image analysis of the quantitative western blot shown in Figure C-2. Values shown for signal density are contour quantity, i.e. average OD x contour area in mm^2.

[2] Percent difference is calculated using the equation:

$$\frac{\left|AverageDensityEcoli - AverageDensityPlant\right|}{AverageDensityEcoli} \times 100 = PercentDifference$$

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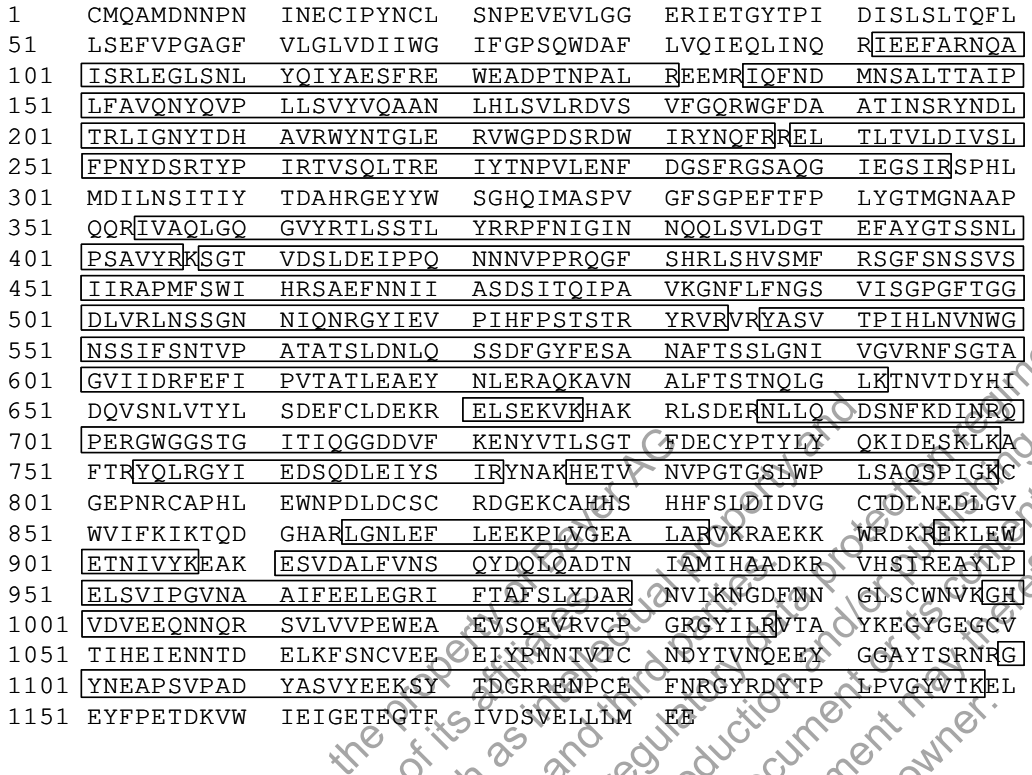

Figure C-3. MALDI-TOF MS Coverage Map of the MON 87701-Produced Cry1Ac Protein

The amino acid sequence of the full-length MON 87701-produced Cry1Ac protein was deduced from the coding region of the full-length *Cry1Ac* gene present in MON 87701 and the observed N-terminal sequence of the protein. Boxed regions correspond to tryptic peptide masses that were identified from the ~131 kDa protein band using MALDI-TOF MS. In total, 70 fragments, covering 66.6% (787 of 1182 total amino acids) of the expected protein sequence, were matched to expected masses.

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Table C-4. Summary of the Tryptic Masses Identified for the Full-Length MON 87701-Produced Cry1Ac Protein Using MALDI-TOF Mass Spectrometry.[1]

1	2	3	4	5	6				
AC-1	AC-2	DHB-1	DHB-2	SA-1	SA-2	Expected	Diff[2]	Fragment	Sequence[3]
579.40		579.38				579.33	0.07 (1)	754-757	YQLR
589.39		589.37				589.28	0.11 (1)	1028-1032	VCPGR
						589.31	0.08 (1)	229-232	DWIR
611.42						611.36	0.06 (1)	941-945	VHSIR
621.46		621.45				621.37	0.09 (1)	1033-1037	GYILR
649.46		649.44				649.37	0.09 (1)	258-262	TYPIR
688.10						688.37	0.27 (1)	98-103	NQAISR
727.46		727.45				727.35	0.11 (1)	233-237	YNQFR
731.46		731.46				731.36	0.10 (1)	428-433	QGFSHR
764.51		764.49				764.39	0.12 (1)	92-97	IEEFAR
		781.51				781.38	0.13 (3)	197-202	YNDLTR
804.41		804.58				804.46	0.05 (1)	263-269	TVSQLTR
816.52		816.52				816.40	0.12 (1)	222-228	VWGPDSR
832.44		832.44	832.62			832.48	0.04 (1)	671-677	ELSEKVK
								743-749	IDESKLK
854.43	854.54	854.55				854.41	0.02 (1)	1119-1125	SYTDGRR
907.60	907.74	907.61				907.46	0.14 (1)	178-185	DVSVFGQR
940.66		940.67				940.51	0.15 (1)	365-372	TLSSTLYR
976.66	976.80	976.67				976.50	0.16 (1)	434-441	LSHVSMFR
1027.69						1027.53	0.16 (1)	696-703	DINRQPER
1038.66	1038.82	1038.68		1038.49	1038.65	1038.50	0.16 (1)	214-221	WYNTGLER
1066.62		1066.66		1066.55		1066.43	0.19 (1)	1126-1133	ENPCEFNR
1074.72		1074.75				1074.55	0.17 (1)	286-296	GSAQGIEGSIR
		1078.73				1078.55	0.18 (3)	687-695	NLLQDSNFK
1144.74	1144.92	1144.78	1145.00	1144.73	1144.84	1144.57	0.17 (1)	454-462	APMFSWIHR

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Table C-4. Summary of the Tryptic Masses Identified for the Full-Length MON 87701-Produced Cry1Ac Protein Using MALDI-TOF Mass Spectrometry (cont.)

1	2	3	4	5	6				
AC-1	AC-2	DHB-1	DHB-2	SA-1	SA-2	Expected	Diff[2]	Fragment	Sequence[3]
1203.87	1204.05	1203.90		1203.83		1203.68	0.19 (1)	354-364	IVAQLGQGVYR
1216.77	1216.96	1216.82				1216.60	0.17 (1)	505-515	LNSSGNNIQNR
1237.78	1237.97	1237.83	1238.08	1237.76		1237.60	0.18 (1)	186-196	WGFDAATINSR
1249.85		1249.88				1249.65	0.20 (1)	595-606	NFSGTAGVIIDR
1253.84	1254.04	1253.87		1253.83		1253.65	0.19 (1)	442-453	SGFSNSSVSIIR
1258.84	1259.04	1258.87				1258.65	0.19 (1)	203-213	LIGNYTDHAVR
1303.85	1304.05	1303.90	1304.23	1303.85	1304.04	1303.67	0.18 (1)	970-980	IFTAFSLYDAR
		1352.96				1352.71	0.25 (3)	1137-1148	DYTPLPVGYVTK
1398.90		1398.95				1398.67	0.23 (1)	120-131	EWEADPTNPALR
1424.88	1425.07	1424.90				1424.65	0.23 (1)	999-1010	GHVDVEEQNNQR
		1552.08		1552.01		1551.81	0.27 (3)	896-907	EKLEWETNIVYK
						1576.81	0.55 (2)	687-699	NLLQDSNFKDINR
	1577.36	1577.19		1577.10		1576.87	0.49 (2)	628-642	AVNALFTSTNQLGLK
1599.06						1598.71	0.35 (1)	1125-1136	RENPCEFNRGYR
1704.09	1704.37	1704.21	1704.57	1704.14		1703.88	0.21 (1)	516-530	GYIEVPIHFPSTSTR
	1795.38	1795.16	1795.59	1795.10		1794.87	0.51 (2)	704-721	GWGGSTGITIQGGDDVFK
1801.13	1801.40	1801.26	1801.64	1801.15		1800.87	0.26 (1)	758-772	GYIEDSQDLEIYSIR
1901.15	1901.47	1901.27	1901.67	1901.21	1901.50	1900.91	0.24 (1)	270-285	EIYTNPVLENFDGSFR
1902.15	1902.49	1902.28		1902.21	1902.47	1901.82	0.33 (1)	1119-1133	SYTDGRRENPCEFNR
						1902.96	0.81 (1)	104-119	LEGLSNLYQIYAESFR
1904.22	1904.49	1904.24	1903.77	1904.24	1904.48	1904.06	0.16 (1)	625-642	AQKAVNALFTSTNQLGLK
1956.29	1956.59	1956.39	1956.82	1956.33	1956.58	1956.01	0.28 (1)	1011-1027	SVLVVPEWEAEVSQEVR
		2088.38				2088.94	0.56 (1)	1100-1118	GYNEAPSVPADYASVYEEK
2098.42	2098.82	2098.55	2098.94	2098.50	2098.81	2098.15	0.27 (1)	865-883	LGNLEFLEEKPLVGEALAR
		2118.44		2118.41		2118.11	0.33 (3)	463-482	SAEFNNIIASDSITQIPAVK
	2142.76	2142.42	2143.20	2142.41	2142.83	2142.08	0.68 (2)	607-624	FEFIPVTATLEAEYNLER
2149.32	2149.71	2149.43				2149.05	0.27 (1)	408-427	SGTVDSLDEIPPQNNNVPPR
2195.47	2195.84	2195.63	2196.12	2195.51	2195.82	2195.16	0.31 (1)	239-257	ELTLTVLDIVSLFPNYDSR

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Table C-4. Summary of the Tryptic Masses Identified for the Full-Length MON 87701-Produced Cry1Ac Protein Using MALDI-TOF Mass Spectrometry (cont.)

1 AC-1	2 AC-2	3 DHB-1	4 DHB-2	5 SA-1	6 SA-2	Expected	Diff[2]	Fragment	Sequence[3]
2211.41	2211.76	2211.49	2212.03	2211.48	2211.80	2211.12	0.29 (1)	483-504	GNFLFNGSVISGPGFTGGDLVR
						2211.13	0.28 (1)	434-453	LSHVSMFRSGFSNSSVSIIR
		2278.60				2278.14	0.46 (3)	203-221	LIGNYTDHAVRWYNTGLER
						2278.21	0.39 (3)	516-534	GYIEVPIHFPSTSTRYRVR
			2376.24	2375.60		2375.24	1.00 (4)	777-799	HETVNVPGTGSLWPLSAQSPIGK
2616.65	2617.12	2616.80	2617.31	2616.79	2617.19	2616.36	0.29 (1)	946-969	EAYLPELSVIPGVNAAIFEELEGR
				3284.93		3284.61 Ma[4]	0.32 (5)	104-131	LEGLSNLYQIYAESFREWEADPTNPALR
				3318.05		3318.71 Ma	0.66 (5)	258-285	TYPIRTVSQLTREIYTNPVLENFDGSFR
				3365.91		3365.71 Ma	0.20 (5)	911-940	ESVDALFVNSQYDQLQADTNIAMIHAADKR
			3374.20			3374.77 Ma	0.57 (4)	595-624	NFSGTAGVIIDRFEFIPVTATLEAEYNLER
	3731.09	3732.34		3731.25	3732.14	3731.12 Ma	0.03 (2)	373-406	RPFNIGINNQQLSVLDGTEFAYGTSSNLPSAVYR
					4371.09	4370.75 Ma	0.34 (6)	704-742	GWGGSTGITIQGGDDVFKENYVTLSGTFDECYPTYLYQK
			4676.21		4676.70	4675.45 Ma	0.76 (4)	136-177	IQFNDMNSALTTAIPLFAVQNYQVPLLSVYVQAANLHLSVLR
	5564.48	5564.43	5563.75			5564.43 Ma	0.05 (2)	136-185	IQFNDMNSALTTAIPLFAVQNYQVPLLSVYVQAANLHLSVLRDVS VFGQR
					6142.52	6141.69 Ma	0.83 (6)	537-594	YASVTPIHLNVNWGNSSIFSNTVPATATSLDNLQSSDFGYFESA NAFTSSLGNIVGVR

[1] Only experimental masses that matched expected masses are listed in the table. All mass values shown were rounded to two decimal places. Columns 1-6 represent experimentally observed masses from Extract 1 or Extract 2 of trypsinized protein mixed with matices α-cyano-4-hydroxy cinnamic acid (AC), dihydroxybenzoic acid (DHB), or 3,5-dimethoxy-4-hydroxycinnamic acid (SA).
[2] Diff represents the difference between the experimental mass and the expected mass; the number in parenthesis indicates the column containing the experimental mass used to calculate the difference.
[3] Sixty-eight unique sequences are shown. Two of the 70 fragments identified were methionine-oxidized versions of two sequences shown.
[4] Ma indicates mass averaged value. Unless Ma is indicated, expected mass is monoisotopic mass.

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A.

Amino acid residue # from the N-terminus	→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Predicted Cry1Ac Sequence[1]	→	C	M	Q	A	M	D	N	N	P	N	I	N	E	C	I
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Observed Sequence	→	X	M	Q	A	M	D	N	(N)	P	(N)	X	X	X	X	X

B.

Amino acid residue # from the N-terminus	→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Predicted Cry1Ac Sequence[1]	→	C	M	Q	A	M	D	N	N	P	N	I	N	E	C	I
		|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Observed Sequence	→	X	M	Q	A	(M)	X	(N)	(N)	X	X	X	X	X	X	X

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Figure C-4. Summary of N-terminal Sequence Analysis

The single letter amino acid codes are: A, Alanine; C, cysteine; D, Aspartic acid; E, Glutamic acid; I, Isoleucine; M, methionine; N, Asparagine, P, Proline; and Q, Glutamine. Amino acids in the experimentally-derived sequence that are in parentheses represent tenuous designations. X indicates an undesignated call in that cycle of the analysis

Panel A: N-terminal sequence determined from full-length (~133 kDa) Cry1Ac band.

Panel B: N-terminal sequence determined from ~ 75 kDa band.

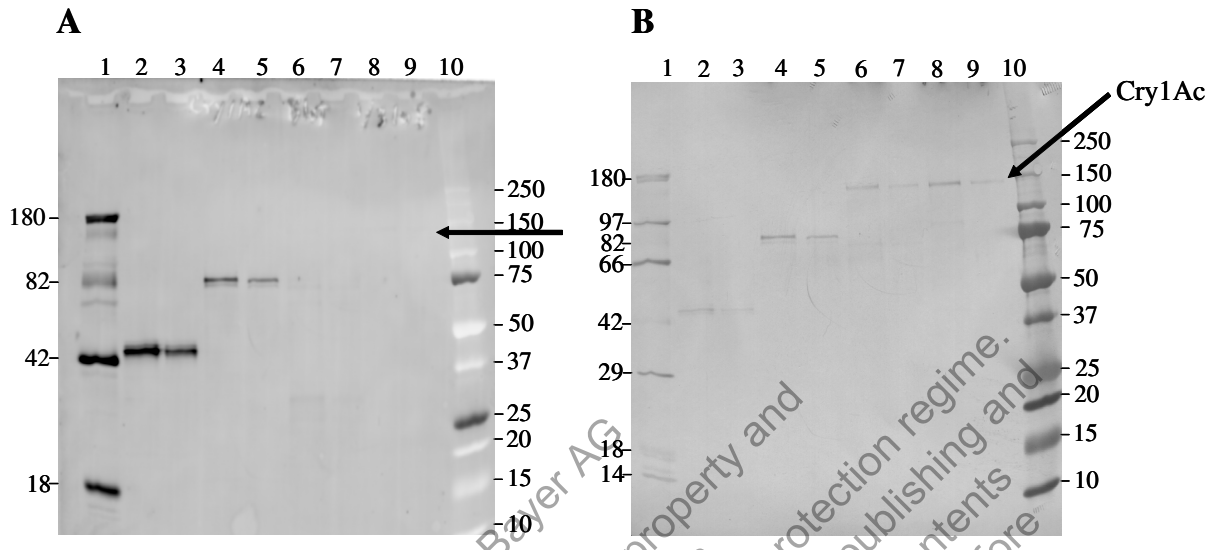

Figure C-5. Glycosylation Analysis of the MON 87701-Produced Cry1Ac Protein

Aliquots of horseradish peroxidase and transferrin (positive controls), MON 87701-produced Cry1Ac protein, and *E. coli*-produced Cry1Ac reference protein (negative control), were separated by SDS-PAGE and transferred to a PVDF membrane. For Cry1Ac samples, amount loaded indicates full-length protein amount. Approximate molecular weights indicated (in kDa) correspond to Candy Cane markers (lane 1) and dual color markers (lane 10).

Panel A: Glycosylation analysis: Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain. The arrow indicates the approximate molecular weight for Cry1Ac.
Panel B: Total protein staining: Following glycosylation analysis, the blot was stained for total protein.

Lane	Sample	Amount Loaded (ng)
1	Candy Cane MW Marker	-
2	Horseradish Peroxidase	100
3	Horseradish Peroxidase	50
4	Transferrin	100
5	Transferrin	50
6	MON 87701-produced Cry1Ac	100
7	MON 87701-produced Cry1Ac	50
8	*E. coli*-produced Cry1Ac	100
9	*E. coli*-produced Cry1Ac	50
10	Precision Plus Dual Color MW marker	-

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Appendix D. Materials and Methods Used for the Analysis of the Levels of Cry1Ac Protein in MON 87701

Materials

Tissue samples analyzed in this study were produced from five field sites in the U.S. during the 2007 season from seed lot GLP-0612-17898-S for MON 87701 and GLP-0612-17895-S for control. The control line was A5547, which is a conventional variety and does not contain the *cry1Ac* coding region. Samples were stored in a -80°C freezer throughout the study. An *E. coli*-produced Cry1Ac protein (Monsanto APS: Orion lot # 10000780) was used as a reference standard for the assay.

Characterization of the Materials

The identities of the test and control substances were confirmed by analysis of the starting seed DNA by an event-specific polymerase chain reaction (PCR) method and the results were archived under the seed lot numbers. The seed samples harvested from the field were also verified by PCR and the resulting Verification of Identity was archived under the starting seed lot numbers, following the Monsanto standard operating procedure.

Field Design and Tissue Collection

Production Plan 07-01-71-01 was initiated during the 2007 planting season to generate test and control substances at various soybean-growing locations in the U.S. The field sites were as follows: Baldwin County, AL (site code AL); Jackson County, AR (site code AR); Clarke County, GA (site code GA); Jackson County, IL (site code IL); and Wayne County, NC (site code NC). These field sites were representative of soybean producing regions suitable for commercial soybean production. At each site, three replicated plots of soybean plants containing MON 87701, as well as the negative control, were planted using a randomized complete block field design. Over-season leaf (OSL 1-4), root, forage, and seed tissues were collected from each replicated plot at all field sites. The over season leaf samples were collected four times at different growth stages: (1) V3 – V4 stage, (2) V6 – V8 stage, (3) V10 – V12 stage, and (4) V14 – V16. Production Plan 07-01-71-02 was initiated during the 2007 planting season to generate test and control substances at Jackson County, IL (site code IL) in the U.S. At this site, plots of plants containing MON 87701, as well as the negative control, were planted using a single plot field design. Pollen/anther tissues were collected from each plot. Throughout both field productions, sample identity was maintained by using unique sample identifiers and proper chain-of-custody documentation. All tissue samples, except harvested seed, were stored in a -80°C freezer and shipped on dry ice to the Monsanto processing facility in Saint Louis, Missouri. Harvested seed samples were stored and shipped at ambient temperature.

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

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plant was labeled as the forage, and the below ground portion was washed and labeled as root tissue. Harvested seed samples were collected at the R8 growth stage.

Tissue Processing and Protein Extraction

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

The Cry1Ac proteins were extracted from soybean tissues using a Harbil mixer (Harbil Industries, Compton, CA) and the appropriate amount of Tris-borate buffer with L-ascorbic acid (TBA) [0.1 M Tris, 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v/v) Tween-20 at pH 7.8, and 0.2% (w/v) L-ascorbic acid]. Insoluble material was removed from the extracts by using a Serum Filter System (Fisher Scientific, Pittsburgh, PA), except pollen, which were centrifuged. The extracts were aliquoted and stored in a -80°C freezer until analyzed.

Anti-Cry1Ac Antibodies

Mouse monoclonal antibody clone M19-N4-A6, also known as M19 (IgG1 isotype, kappa light chain; lot 7495955) specific for the Cry1Ac protein was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography and was used as the capture antibody in the Cry1Ac ELISA. The concentration of the purified IgG was determined to be 6.0 mg/ml by spectrophotometric methods. Production of the M19 monoclonal antibody was performed by Strategic Biosolutions (Newark, DE). The purified antibody was stored in a buffer (pH 7.2) containing 10 mM sodium phosphate, 150 mM sodium chloride, and 15 mM sodium azide.

Goat antibodies (lot G-805044) specific for Cry1Ac were purified using Protein-G Agarose affinity chromatography. The concentration of the purified IgG was determined to be 3.7 mg/ml by spectrophotometric methods. The purified antibody was stored in 1X phosphate-buffered saline (PBS), pH 7.4, and coupled with biotin (Pierce, Rockford, IL) according to the manufacturer's instructions, and assigned lot number G-805045. The detection reagent was NeutrAvidin (Pierce, Rockford, IL) conjugated to horseradish peroxidase (HRP).

Cry1Ac ELISA Method

The Cry1Ac ELISA was performed according to a draft SOP. Mouse anti-Cry1Ac antibody was diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl, pH 9.6) to a final concentration of 2.0 µg/ml, and immobilized onto 96-well microtiter plates followed by incubation in a 4°C refrigerator for ≥8 h. Prior to each step in the assay, plates were washed with 1× phosphate-buffered saline with Tween-20 (PBST). Cry1Ac protein standard or sample extract was added at 100 µl per well and incubated for 1 h at 37°C. The captured Cry1Ac protein was detected by the addition of 100 µl per well of biotinylated goat anti-Cry1Ac antibodies and NeutrAvidin-HRP (Pierce, Rockford, IL). Plates were developed by adding 100 µl per well of HRP substrate, 3,3',5,5'-tetramethyl-benzidine (TMB; Kirkegaard & Perry, Gaithersburg, MD).

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The enzymatic reaction was terminated by the addition of 100 µl per well of 3 M H_3PO_4. Quantification of the Cry1Ac protein was accomplished by interpolation from a Cry1Ac protein standard curve that ranged from 1.0 – 32 ng/ml.

Moisture Analysis

A homogeneous tissue-specific site pool (TSSP) was prepared using the test and control samples of a given tissue type grown at a given site. These pools were prepared for all tissues, except pollen, in this study. All tissues, except pollen, were analyzed for moisture content using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). The mean percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$DWCF = 1 - [Mean\ \%\ TSSP\ Moisture / 100]$$

The DWCF was used to convert protein levels assessed on a µg/g fresh weight (fwt) basis into levels reported on a µg/g dry weight (dwt) basis using the following calculation:

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

The protein levels that were reported to be less than or equal to the limit of detection (LOD) or less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

Data Analyses

All Cry1Ac ELISA plates were analyzed on a SPECTRAmax Plus (Molecular Devices, Sunnyvale, CA) or SPECTRAmax Plus 384 (Molecular Devices, Sunnyvale, CA) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GXP version 5.0.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a "µg/g fwt" basis. For all proteins, this conversion utilized a sample dilution factor and a tissue-to-buffer ratio. The protein values in "µg/g fwt" were also converted to "µg/g dwt" by applying the DWCF, except for pollen which was not analyzed for moisture content due to insufficient sample volume. Microsoft Excel 2002 (Version 10.68241.6839 SP3, Microsoft, Redmond, WA) was used to calculate the Cry1Ac protein levels in soybean tissues.

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Appendix E. Materials and Methods Used for Compositional Analysis of MON 87701 Soybean Harvested Seed and Forage from Five Replicated Field Sites

Materials

MON 87701, a conventional soybean control (A5547), and conventional reference soybean varieties were grown at five U.S. locations in 2007. MON 87701 and the control were grown from seed lots GLP-0612-17898-S and GLP-0612-17895-S, respectively. The control material, A5547, has background genetics representative of MON 87701 but does not contain the *Cry1Ac* gene coding sequence or produce the Cry1Ac protein. In addition, twenty conventional soybean varieties produced alongside of MON 87701 were included for the generation of a 99% tolerance interval. These varieties, locations, and seed lot numbers are listed below:

Material Name	Seed Lot Number	Site Code
A5843	GLP-0702-18243-S	AL
A5959	GLP-0702-18245-S	AL
CMA 5804AOC	GLP-0702-18244-S	AL
H6686	GLP-0702-18247-S	AL
UA 4805	GLP-0702-18123-S	AR
Ozark	GLP-0702-18124-S	AR
Anand	GLP-0702-18122-S	AR
Hornbeck C5894	GLP-0702-18125-S	AR
A5560	GLP-0702-18242-S	GA
CMC 5901COC	GLP-0702-18246-S	GA
LEE 74	GLP-0702-18248-S	GA
A5403	GLP-0702-18241-S	GA
A4922	GLP-0702-18234-S	IL
H4994	GLP-0702-18235-S	IL
H5218	GLP-0702-18236-S	IL
A5427	GLP-0702-18238-S	IL
DP 5989	GLP-0702-18126-S	NC
Hutcheson	GLP-0703-18396-S	NC
USG 5601T	GLP-0703-18402-S	NC
Fowler	GLP-0703-18395-S	NC

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Characterization of the Materials

The identities of the forage and harvested seed samples from MON 87701, control, and reference soybean varieties were verified prior to their use in the study by confirming the chain-of-custody documentation supplied with the forage and harvested seed collected from the field plots. The harvested seed of MON 87701, the conventional soybean control, and reference soybean varieties were also characterized by event-specific polymerase chain reaction (PCR) analysis, for the presence or absence of MON 87701. The results indicate samples from one replicate of MON 87701 at Site AL and one

replicate of A5547 at Site NC contained levels of an unintended trait and, therefore, were deemed unacceptable and were excluded.

Field Production of the Samples

The field design and tissue collection process have been described previously in Appendix C with the addition of reference soybean varieties as described above. A total of twenty different conventional soybean varieties were planted at five field locations with four different varieties grown at each site. Seed were planted in a randomized complete block design with three replicates per block for each of MON 87701, the conventional soybean control and reference soybean varieties. All the samples were grown under normal agronomic field conditions for their respective geographic regions.

Summary of Analytical Methods

Harvested soybean seed and forage samples from MON 87701, the control, and conventional reference soybean varieties were shipped on dry ice to EPL Bio-Analytical Services (EPL-BAS), 9095 W. Harristown Blvd. Niantic, Illinois for compositional analyses. Analyses were performed using methods that are currently used to evaluate the nutritional quality of food and feed.

SOYBEAN FORAGE ANALYTICAL METHOD SUMMARIES:

Ash (SOP-SE-2)
Subsamples of ground forage (3 grams) are ignited in a muffle furnace for three hours at 650°C. The weight of the ash residue remaining after ignition is determined gravimetrically. There is no analytical reference substance for this analysis. Ash results are expressed on a percent fresh weight basis.

Reference:
AOAC International Method 923.03 (2000). In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

Carbohydrates (CHO)
Total carbohydrate content is calculated by difference using the fresh weight-derived data and the formula presented below. There is no analytical reference substance for this analysis.

Carbohydrates (%) = 100 – Moisture (%) -Ash (%) - Fat (%) - Protein (%)

Reference:
United States Department of Agriculture (1973). "Energy Value of Foods", *Agriculture Handbook No. 74,* pp. 2-11.

Crude Fat (SOP-SE-1)
Subsamples of ground forage (2 grams) are dried in an oven for at least 2 hours. The crude fat content is determined gravimetrically after acid hydrolysis and extraction with

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mixed ethers. There is no analytical reference substance for this analysis. Fat results are expressed on a percent fresh weight basis.

Reference:
AOAC International Method 922.06 (2000). In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

Moisture (SOP-SE-25)
Moisture content is determined gravimetrically. Subsamples (2 grams) of ground forage are dried to a constant weight in a forced air oven at 135°C for at least 2 hours. Moisture results are expressed on a percent of fresh weight basis. There is no analytical reference substance for this analysis.

Reference:
AOAC International Method 930.15 (2000). In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

Crude Protein (SOP-SE-20)
Protein content is determined using an automated Kjeldahl technique. A Foss-Tecator 2300 Kjeltec Analyzer Unit is used. Samples are manually digested on a heating block using sulfuric acid and a selenium catalyst then transferred to the analyzer unit where the digests are distilled and titrated. The protein content is calculated by multiplying the amount of nitrogen in the sample by 6.25. Ammonium sulfate is used as an analytical reference substance to verify the accuracy of the distillation step performed by the analyzer unit. The ammonium sulfate reference standard is obtained from Fisher Scientific (Fairlawn, NJ) and has a nitrogen content of 21.0%. The lot number is 043629 Protein results are expressed on a percent fresh weight basis.

Reference:
Foss-Tecator (1999). *Foss-Tecator Kjeltec 2300 Site Preparation, Installation, and Operating Guide*, Foss-Tecator AB, Box 70, S-263 21 Hoganos, Sweden.

Acid Detergent Fiber (SE-3)
Subsamples of ground forage are analyzed to determine the percentage of acid detergent fiber (ADF) by digesting with an acid detergent solution and washing with water. The remaining residue is dried and weighed to determine ADF content. Samples are analyzed with the Ankom Extraction Apparatus. There is no analytical reference substance for this analysis. ADF results are expressed on a percent fresh weight basis.

Reference:
Ankom Technology (1999). ANKOM200 Fiber Analyzer Operator's Manual, Ankom Technology, 140 Turk Hill Park, Fairport, NY 14450.

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Neutral Detergent Fiber (SE-9)
Subsamples of ground forage are analyzed to determine the percentage of neutral detergent fiber (NDF) by digesting with an neutral detergent solution, sodium sulfite and alpha amylase. The remaining residue is dried and weighed to determine NDF content. Samples are analyzed with the Ankom Extraction Apparatus. There is no analytical reference substance for this analysis. NDF results are expressed on a percent fresh weight basis.

Reference:
Ankom Technology (1999). ANKOM^{200} Fiber Analyzer Operator's Manual, Ankom Technology, 140 Turk Hill Park, Fairport, NY 14450.

HARVESTED SOYBEAN SEED ANALYTICAL METHOD SUMMARIES:

Ash (SOP-SE-2)
Subsamples of ground soybean seed are ignited in a muffle furnace for three hours at 650°C. The weight of the ash residue remaining after ignition is determined gravimetrically. There is no analytical reference substance for this analysis. Ash results are expressed on a percent fresh weight basis.

Reference:
AOAC International Method 923.03 (2000). In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

Carbohydrates (CHO)
Total carbohydrate content is calculated by difference using the fresh weight-derived data and the formula presented below. There is no analytical reference substance for this analysis.

Carbohydrates (%) = 100 – Moisture (%) -Ash (%) - Fat (%) - Protein (%)

Reference:
United States Department of Agriculture (1973). "Energy Value of Foods", *Agriculture Handbook No. 74,* pp. 2-11.

Crude Fat (SOP-SE-27)
Subsamples of ground soybean seed are extracted for 16 hours with pentane using soxhlet extraction apparatus. The pentane extract is evaporated to dryness and the crude fat residue is determined gravimetrically. There is no analytical reference standard for this analysis. Fat results are expressed on a percent fresh weight basis.

Reference:
AOAC International Method 960.39 (2000). In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

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Moisture (SOP-SE-4)
Moisture content is determined gravimetrically. Subsamples (2 grams) of ground seed are dried to a constant weight in a vacuum oven at 100°C and 25 inches of mercury pressure for 15 hours. Moisture results are expressed on a percent of fresh weight basis. There is no analytical reference substance for this analysis.

Reference:
AOAC International Method 925.09 (2000). In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

Crude Protein (SOP-SE-20)
Protein content is determined using an automated Kjeldahl technique. A Foss-Tecator 2300 Kjeltec Analyzer Unit is used. Samples are manually digested on a heating block using sulfuric acid and a selenium catalyst then transferred to the analyzer unit where the digests are distilled and titrated. The protein content is calculated by multiplying the amount of nitrogen in the sample by 6.25. Ammonium sulfate is used as an analytical reference substance to verify the accuracy of the distillation step performed by the analyzer unit. The ammonium sulfate reference substance is obtained from Fisher Scientific (Fairlawn, NJ) lot number 043629 and has a nitrogen content of 21.0%. Protein results are expressed on a percent fresh weight basis.

Reference:
Foss-Tecator (1999) *Foss-Tecator Kjeltec 2300 Site Preparation, Installation, and Operating Guide*, Foss-Tecator AB, Box 70, S-263 21 Hoganos, Sweden.

Acid Detergent Fiber (SE-3)
Subsamples of ground soybean seed are analyzed to determine the percentage of acid detergent fiber (ADF) by digesting with an acid detergent solution and washing with water. The remaining residue is dried and weighed to determine ADF content. Samples are analyzed with the Ankom Extraction Apparatus. There is no analytical reference substance for this analysis. ADF results are expressed on a percent fresh weight basis.

Reference:
Ankom Technology (1999). ANKOM200 Fiber Analyzer Operator's Manual, Ankom Technology, 140 Turk Hill Park, Fairport, NY 14450.

Neutral Detergent Fiber (SE-9)
Subsamples of ground soybean seed are analyzed to determine the percentage of neutral detergent fiber (NDF) by digesting with a neutral detergent solution, sodium sulfite and alpha amylase. The remaining residue is dried and weighed to determine NDF content. Samples are analyzed with the Ankom Extraction Apparatus. There is no analytical reference substance for this analysis. ADF results are expressed on a percent fresh weight basis.

Reference:
Ankom Technology (1999). ANKOM200 Fiber Analyzer Operator's Manual, Ankom Technology, 140 Turk Hill Park, Fairport, NY 14450.

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Tryptophan (SE-22)
Subsamples of ground soybean seed are analyzed to determine the amount of tryptophan by hydrolyzing with 4M LiOH and diluting to 50 mL with deionized water. Samples are filtered and analyzed by reverse phase High Performance Liquid Chromatography (HPLC) with ultra-violet (UV) detection. L-Tryptophan is used as the analytical reference substance to verify the accuracy of the method and HPLC. The L-Tryptophan analytical reference substance is purchased from Sigma, has a purity of >99% and lot number 026K0375. The limit of quantitation (LOQ) is 0.125 %. Tryptophan results are expressed on a percent fresh weight basis.

Reference:
Tagers, S.R.; Pesti, G.M. 1990. "Determination of Tryptophan from Feedstuffs Using Reverse Phase High-Performance Liquid Chromatography". Journal of Micronutrient Analysis. 7:27-35.

Amino Acids (SE-58)
Subsamples of ground soybean seed are analyzed to determine the amount of the 15 amino acids by converting the free acids, after acid hydrolysis, to the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatives. Samples are then analyzed by reverse phase Ultra Performance Liquid Chromatography (UPLC) with UV detection. The following amino acids are used as analytical reference substances to verify the accuracy of the method and UPLC:
L-Alanine – lot number 443129/1 and purity 100%. LOQ is 0.375%.
L-Arginine Hydrochloride – lot number 095K0089 and purity >99%. LOQ is 0.310%.
L-Aspartic Acid (Free Acid) – lot number 093K01502 and purity >99%. LOQ is 0.375%.
L-Glutamic Acid (Free Acid) – lot number 085K0713 and purity >99%. LOQ is 0.375%.
Glycine (Free Base) – lot number 034K0166 and purity >99%. LOQ is 0.375%.
L-Histidine Monohydrochloride Monohydrate – lot number 114K0378 and purity >99%. LOQ is 0.278%.
L-Isoleucine – lot number 065K0231 and purity >99%. LOQ is 0.375%.
L-Leucine – lot number 045K0387 and purity >99%. LOQ is 0.375%
L-Lysine Monohydrochloride – lot number 067K0078 and purity 99.4%. LOQ is 0.300%.
L-Phenylalanine – lot number 1166794 and purity 100%. LOQ is 0.375%.
L-Proline- lot number 106K0128 and purity 98.9%. LOQ is 0.370%.
L-Serine – lot number 077K0015 and purity 99.5%. LOQ is 0.375%.
L-Threonine – lot number 095K0374 and purity >99%. LOQ is 0.375%.
L-Tyrosine (Free Base) – lot number 075K0015 and purity 100%. LOQ is 0.375%.
L-Valine – lot number 095K0378 and purity >99%. LOQ is 0.375%.
L-Alpha-Amino-N-Butyric Acid - lot number 126K2666 and purity 100%. No LOQ.
The amino acid analytical reference substances are purchased from Sigma. The amino acids results are expressed on a percent fresh weight basis.

References:
Hong, Ji Liu (1994). "Determination of Amino Acids by Precolumn Derivatization 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and Ultra-Performance Liquid Chromatography with Ultraviolet Detection". Journal of Chromatography A, 670 (1994): 59-66.

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Waters Method, Analysis of Amino Acids in Feeds and Foods Using Modification of the ACCQ•Tag Method ™ for Amino Acid Analysis.

Cystine and Methionine (SE-59)

Subsamples of ground soybean seed are analyzed to determine the amount of cystine and methionine by converting the cystine to cysteic acid and methionine to methionine sulfone after acid oxidation and hydrolysis, to the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatives. Samples are then analyzed by reverse phase UPLC with UV detection. The LOQ is 0.0417 %. The following analytical reference substances are used to verify the method and UPLC:

L-Cysteic Acid - lot number 1215898 and purity 99.3%

L-Methionine Sulfone - lot number 116K1146 and purity 100%

L-Cystine - lot number 037K0148 and purity 100%

L-Methionine - lot number 074K0372 and purity >99%

The analytical reference substances are purchased from Sigma. The cystine and methionine results are expressed on a percent fresh weight basis.

References:

Hong, Ji Liu (1994). "Determination of Amino Acids by Precolumn Derivatization 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and Ultra-Performance Liquid Chromatography with Ultraviolet Detection", Journal of Chromatography A, 670 (1994): 59-66.

Waters Method, Analysis of Amino Acids in Feeds and Foods Using Modification of the ACCQ•Tag Method ™ for Amino Acid Analysis.

Fatty Acid (SE-45)

Subsamples of ground soybean seed are analyzed to determine the fatty acid content by using a soxhlet extraction apparatus. The fatty acids are derivatized into methyl esters with boron trifluoride/methanol. The methyl esters are then assayed by Gas Chromatography (GC) with Flame Ionization Detection (FID). The following analytical reference substances are used to verify the method and the GC:

Fatty Acid Methyl Ester (FAME) Standard (Major Acids) 0.5% C12:0 (LOQ = 0.00505%), 0.5% C14:0 (LOQ = 0.00509%), 10% C16:0 (LOQ = 0.102%), 0.5% C16:1 (LOQ = 0.00512%), 0.5% C17:0 (LOQ = 0.00513%), 0.5% C17:1 (LOQ = 0.00513%), 4% C18:0 (LOQ = 0.0412%), 20% C18:1 (LOQ = 0.206%), 51% C18:2 (LOQ = 0.00926%), 9% C18:3 (LOQ = 0.00165%), 0.5% C20:0 (LOQ = 0.00517%), 0.5% C20:1 (LOQ = 0.00517%), 0.5% C20:2 (LOQ = 0.00517%), 1% C22:0 (LOQ = 0.0104%), and 1% C24:0 (LOQ = 0.0104%), lot number N15-P

FAME Reference Standard (Minor Acids), 10% C8:0 (LOQ = 0.0197%), 10% C10:0 LOQ = 0.0200%), 10% C14:1 (LOQ = 0.0203%), 10% C15:0 (LOQ = 0.0204%), 10% C15:1 (LOQ = 0.0204%), 10% C17:1 (0.00513%), 10% C18:2 (0.00926%), 10% C18:3 (GLA) (LOQ = 0.0206), 10% C20:3 (LOQ = 0.0207%), 10% C20:4 (LOQ = 0.0207%), and 10% C22:1 (LOQ = 0.0207%, lot number N15-P

Tridecanoic Acid (C13:0) - lot number N-13A-JY10-Q and purity >99%. No LOQ.

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Methyl Tridecanoate - lot number N-13M-MA12-R and purity >99%. No LOQ.
The analytical reference substances are purchased from Nu-Chek Prep. The fatty acid results are reported on a percent fresh weight basis.

Reference:
AOAC International Method 939.05 (2000). In Official Methods of Analysis of the AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, Maryland.

Trypsin Inhibitor (SE-12)
Subsamples of ground soybean seed are analyzed to determine trypsin inhibitor content by extracting with sodium hydroxide. Trypsin is added and reacted with the trypsin inhibitor. The amount of trypsin present in the sample is measured using a spectrophotometer, and the amount of inhibitor is calculated based on how much trypsin remains. The trypsin reference substance was purchased from MP Biomedicals. The activity is 245 µ/mg and the lot number is 5432H. There is no LOQ. The trypsin results are reported on a percent fresh weight basis.

Reference:
Anonymous 1997. Trypsin Inhibitor Activity. Official Methods and Recommended Practices of AOCS, Ba 12-75.

Phytic Acid (SE-10)
Subsamples of ground soybean seed are analyzed to determine the amount of phytic acid by extracting the phytic acid with dilute hydrochloric acid and isolating it using an ion-exchange solid phase extraction column. Once isolated and eluted, the phytic acid is analyzed for elemental phosphorus by inductively coupled plasma optical emission spectroscopy (ICP-OES). The phytic acid content is then calculated from the phosphorus concentration. The LOQ is 0.355%. The following analytical reference substances are used to verify the method and the ICP-OES:
Phosphorus Standard - lot number SC7061617 and concentration 10,050 µg/mL
Yttrium Standard – lot number SC7192512 and concentration 1001 µg/mL
Phytic Acid Standard - lot number 035K0590 and purity 97%.
The phosphorus and yttrium were purchased from SCP Science Solution and the phytic acid from Sigma. Phytic acid is reported on a percent fresh weight basis.

Reference:
Anonymous 1988. Phytic Acid in Foods. Official Methods of Analysis of AOAC International, Vol. 2.32.5.18.

Lectins (SE-49)
Subsamples of ground soybean seed are analyzed to determine the amount of lectin by extracting the lectin with potassium phosphate buffer. Lectin was assayed using a hemagglutination test using rabbit red blood cells. The amount of hemagglutination was measured by the amount of turbidity using a spectrophotometer. There are no reference substances and LOQ for the assay. Lectin is reported on a percent fresh weight basis.

Reference:

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Leiner, Irvin, E. 1954. *The Photometric Determination of the Hemagglutination Activity of Soyin and Crude Soybean Extracts.* Scientific Journal Series, Minnesota Agricultural Experiment Station.

Isoflavones (SE-56)

Subsamples of ground soybean seed are analyzed to determine the amount of aglycones by extracting the aglycones with ethanol and hydrochloric acid. The extract is cleaned up using a C18 Sep-PAK and assayed by reverse phase HPLC with UV detection. The LOQ is 10.00 mg/Kg. The following analytical reference substances were used to verify the method and HPLC: Daidzein - lot number DA-120 and purity >99%,
Genistein - lot number CH-147 and purity >99%
Glycitein - lot number 0306103 and purity 97%.
The analytical reference substances daidzein and genistein are purchased from LC Laboratories and glycitein was purchased from Indofine Chemical Company, Inc. Isoflavones are reported on a percent fresh weight basis.

Reference:

Pettersson, H., and Kiessling, K.H., "Liquid Chromatographic Determination of the Plant Estrogens Coumestrol and Isoflavones in Animal Feed." Association of Analytical Chemist Journal, 67 (3):503-506 (1984).

Seo, A., and Morr, C.V., "Improved High Performance Liquid Chromatographic Analysis of Phenolic Acids and Isoflavoids from Soybean Protein Products." J. Agric. Food Chem, 32: 530-533 (1984).

Stachyose/Raffinose (SOP SE-40)

Subsamples of ground soybean seed are analyzed to determine the amount of stachyose and raffinose by extracting with methanol/DI water, partitioning with chloroform and evaporating to dryness. The sample residue is redissolved in DI water and analyzed by reverse phase HPLC with refractive index detection. The following analytical reference substances were used to verify the method and HPLC:
Stachyose Hydrate - lot number 065K3775 and purity 98%. The LOQ is 0.260%
Raffinose Pentahydrate - lot number 035K1371 and purity 99%. The LOQ is 0.200%.
The analytical reference substances were purchased from Sigma. Stachyose and raffinose are reported on a percent fresh weight basis.

Reference:

Anonymous 1985, "Determination of Simple Sugars in Cereal Products – HPLC Method". Approved Methods of the Association of Cereal Chemists, Volume II, 80-04.

Johansen, Helle Nygaard; Glisto, Vibe; Knudsen, Erik Bach. 1996. Influence of Extraction Solvent and Temperature on the Quantitative Determination of Oligosaccharides from Plant Materials by High-Performance Liquid Chromatography. J. Agric. Food Chem., 44, 1470-1474.

Vitamin E (α-tocopherol) (SOP-SE-42)

Subsamples of ground soybean seed are analyzed to determine the amount α-tocopherol by extracting with hexane. The hexane extract is analyzed by HPLC with fluorescence

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detection. The following analytical reference substance was used to verify the method and HPLC: Alpha Tocopherol. The analytical reference substance was purchased from Sigma and has a purity of 97%. The lot number is 066K0667. The LOQ is 2.00 mg/Kg. Alpha tocopherol is reported on a percent fresh weight basis.

Reference:

Anonymous 1984, "High Performance Liquid Chromatography of the Tocols in Corn Grain". JAOCS, Vol. 61 No. 7, July 1984.

Data Processing and Statistical Analysis

After compositional analyses were performed at EPL-BAS, data spreadsheets were forwarded to Monsanto Company. The data were reviewed, formatted, and sent to Certus International, Inc. for statistical analysis.

The following formulas were used for re-expression of composition data for statistical analysis:

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture), Fiber, Phytic Acid, Raffinose, Stachyose, Amino Acids (AA)	% FW	% DW	X/d
Isoflavones	mg/kg FW	mg/kg DW	X/d
Trypsin Inhibitor	TIU/mg FW	TIU/mg DW	X/d
Vitamin E	mg/kg FW	mg/100g DW	X/(10*d)
Fatty Acids (FA)	% FW	% 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 compositional component in this study, at least 50% of the values for an analyte had to be greater than the assay LOQ. Analytes with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following nine analytes with more than 50% of observations below the assay LOQ were excluded from statistical analysis: 8:0 caprylic acid, 12:0 lauric acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 18:3 gamma linolenic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, and 22:1 erucic acid.

Otherwise, individual analyses that were below the LOQ were assigned a value equal to half the LOQ. The following components were assigned values:

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		Obs. Below LOQ				
Component	**Units**	**N**	**(%)**	**Total N**	**LOQ**	**Value Assigned**
Seed Fatty Acid						
17:1 Heptadecenoic Acid	% FW	8	12.7	63	0.0051	0.0026
20:2 Eicosadienoic Acid	% FW	9	14.3	63	0.0052	0.0026

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. The following results had PRESS residual values outside of ± 6 range:

Site	**Rep**	**Description**	**Analyte**	**ID**	**Sent Value**	**Value**	**PRESS Std Residual**
Forage Proximate							
GA	2	CMC 5901COC	Moisture	07017101-00329	38.6	38.6000	-11.4687
Seed Proximate							
NC	2	MON 87701	Total Fat	07017101-00627	33.7	36.3656	9.6571

Both identified values were considered outliers and were removed from further analysis. Because moisture content is required for unit re-expression of forage composition data, all additional forage composition data associated with this sample with an outlier moisture value were removed from the dataset for statistical evaluation.

The outlier test procedure was reapplied to all remaining moisture and total fat data to detect potential outliers that were masked in the first analysis. No further PRESS residuals were outside of ± 6 range.

All soybean compositional analysis components were statistically analyzed using a mixed model analysis of variance. The five replicated sites were analyzed both separately and combined. Individual replicated site analyses used model (1).

(1) $Yij = U + Ti + Bj + eij,$

where Yij = unique individual observation, U = overall mean, Ti = substance effect, Bj = random block effect, and eij = residual error.

Combined site analyses used model (2).

(2) $Yijk = U + Ti + Lj + B(L)jk + LTij + eijk,$

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where Yijk = unique individual observation, U = overall mean, Ti = substance effect, Lj = random location effect, B(L)jk = random block within location effect, LTij = random location by substance interaction effect, and eijk = residual error.

A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured.

For each compositional component, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial conventional substances. Each tolerance interval estimate was based upon one observation per unique reference substance. 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.

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Table E-1. Statistical Summary of Site AL Soybean Forage and Proximate Content for MON 87701 vs the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fiber						
Acid Detergent Fiber (% DW)	34.43 (3.22) [30.04 - 38.83]	39.77 (2.63) [37.44 - 42.06]	-5.34 (4.15) [-12.02 - 1.39]	-18.56, 7.88	0.288	(27.99 - 47.33) [14.93, 56.87]
Neutral Detergent Fiber (% DW)	46.71 (7.87) [42.49 - 49.59]	48.02 (6.52) [42.05 - 59.19]	-1.31 (9.17) [-9.60 - -0.31]	-30.47, 27.86	0.895	(30.96 - 54.55) [21.51, 66.01]
Proximate						
Ash (% DW)	5.52 (0.51) [5.05 - 5.98]	7.27 (0.42) [6.24 - 8.13]	-1.76 (0.66) [-1.47 - -1.19]	-3.85, 0.34	0.076	(4.77 - 8.54) [2.46, 10.14]
Carbohydrates (% DW)	69.48 (1.72) [68.29 - 71.06]	66.97 (1.46) [63.68 - 69.20]	2.51 (1.86) [1.86 - 4.62]	-3.41, 8.43	0.270	(60.61 - 77.26) [56.93, 85.88]
Moisture (% FW)	74.24 (0.91) [72.70 - 75.40]	76.93 (0.78) [75.00 - 78.10]	-2.69 (0.95) [-2.30 - -2.30]	-5.70, 0.32	0.065	(66.50 - 80.20) [57.84, 88.56]
Protein (% DW)	19.86 (1.92) [19.72 - 19.92]	19.84 (1.57) [17.94 - 23.29]	0.026 (2.41) [-3.57 - 1.98]	-7.65, 7.70	0.992	(12.68 - 22.92) [7.05, 27.27]
Total Fat (% DW)	4.90 (0.56) [3.96 - 5.85]	5.98 (0.46) [5.61 - 6.72]	-1.08 (0.72) [-2.76 - 0.25]	-3.37, 1.22	0.232	(3.48 - 7.88) [1.11, 9.11]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2] N=2, sample size =2
[3] N=3, sample size =3
[4]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-2. Statistical Summary of AL Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Alanine (% DW)	1.83 (0.036) [1.83 - 1.84]	1.81 (0.030) [1.81 - 1.82]	0.018 (0.047) [0.018 - 0.020]	-0.13, 0.17	0.731	(1.66 - 1.93) [1.49, 2.02]
Arginine (% DW)	2.97 (0.081) [2.95 - 3.00]	2.79 (0.067) [2.72 - 2.89]	0.18 (0.11) [0.056 - 0.28]	-0.15, 0.52	0.180	(2.54 - 2.99) [2.22, 3.25]
Aspartic Acid (% DW)	5.28 (0.11) [5.23 - 5.26]	5.27 (0.095) [5.15 - 5.34]	0.013 (0.12) [-0.11 - 0.11]	-0.36, 0.39	0.916	(4.74 - 5.50) [4.22, 5.96]
Cystine (% DW)	0.67 (0.044) [0.65 - 0.67]	0.61 (0.037) [0.58 - 0.63]	0.060 (0.049) [0.044 - 0.066]	-0.095, 0.21	0.308	(0.53 - 0.68) [0.45, 0.77]
Glutamic Acid (% DW)	8.24 (0.17) [8.18 - 8.21]	8.18 (0.14) [8.08 - 8.26]	0.057 (0.20) [-0.076 - 0.13]	-0.57, 0.69	0.791	(7.53 - 8.72) [6.60, 9.37]
Glycine (% DW)	1.88 (0.042) [1.86 - 1.89]	1.80 (0.034) [1.76 - 1.85]	0.078 (0.054) [0.0092 - 0.12]	-0.094, 0.25	0.244	(1.67 - 1.99) [1.49, 2.09]
Histidine (% DW)	1.17 (0.030) [1.16 - 1.18]	1.12 (0.024) [1.09 - 1.15]	0.055 (0.038) [0.014 - 0.090]	-0.067, 0.18	0.248	(1.04 - 1.24) [0.94, 1.31]
Isoleucine (% DW)	1.98 (0.046) [1.98 - 1.99]	1.91 (0.037) [1.88 - 1.96]	0.071 (0.059) [0.019 - 0.10]	-0.12, 0.26	0.313	(1.73 - 2.02) [1.54, 2.14]

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Table E-2. Statistical Summary of Site AL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Leucine (% DW)	3.35 (0.074) [3.34 - 3.36]	3.20 (0.060) [3.13 - 3.29]	0.15 (0.095) [0.053 - 0.23]	-0.15, 0.45	0.205	(2.93 - 3.32) [2.64, 3.52]
Lysine (% DW)	2.95 (0.091) [2.92 - 2.99]	2.83 (0.074) [2.67 - 2.91]	0.13 (0.12) [0.022 - 0.077]	-0.25, 0.50	0.357	(2.35 - 3.15) [2.05, 3.47]
Methionine (% DW)	0.58 (0.038) [0.56 - 0.58]	0.53 (0.032) [0.51 - 0.56]	0.042 (0.043) [0.018 - 0.049]	-0.095, 0.18	0.403	(0.49 - 0.62) [0.42, 0.68]
Phenylalanine (% DW)	2.44 (0.089) [2.40 - 2.48]	2.20 (0.073) [2.08 - 2.38]	0.24 (0.12) [0.016 - 0.41]	-0.13, 0.60	0.130	(1.97 - 2.44) [1.66, 2.64]
Proline (% DW)	2.15 (0.044) [2.15 - 2.16]	2.10 (0.036) [2.09 - 2.12]	0.054 (0.057) [0.029 - 0.072]	-0.13, 0.23	0.414	(1.92 - 2.25) [1.73, 2.35]
Serine (% DW)	2.17 (0.045) [2.15 - 2.19]	2.09 (0.037) [2.05 - 2.13]	0.080 (0.058) [0.018 - 0.14]	-0.11, 0.27	0.262	(1.96 - 2.30) [1.75, 2.38]
Threonine (% DW)	1.70 (0.038) [1.69 - 1.72]	1.62 (0.031) [1.58 - 1.68]	0.078 (0.049) [0.010 - 0.13]	-0.079, 0.24	0.210	(1.54 - 1.74) [1.40, 1.83]
Tryptophan (% DW)	0.51 (0.012) [0.50 - 0.52]	0.51 (0.0096) [0.50 - 0.52]	0.00042 (0.014) [-0.0056 - -0.0055]	-0.044, 0.044	0.977	(0.47 - 0.55) [0.43, 0.59]

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Table E-2. Statistical Summary of Site AL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.32 (0.031) [1.28 - 1.33]	1.20 (0.026) [1.17 - 1.22]	0.12 (0.032) [0.11 - 0.12]	0.016, 0.22	0.034	(1.04 - 1.31) [0.85, 1.48]
Valine (% DW)	2.07 (0.043) [2.06 - 2.07]	1.99 (0.035) [1.96 - 2.04]	0.075 (0.056) [0.029 - 0.11]	-0.10, 0.25	0.271	(1.83 - 2.13) [1.64, 2.22]
Fatty Acid (% Total FA)						
10:0 Capric Acid (% Total FA)	0.19 (0.012) [0.18 - 0.19]	0.21 (0.010) [0.18 - 0.23]	-0.025 (0.016) [-0.032 - -0.0018]	-0.076, 0.026	0.215	(0.15 - 0.27) [0.065, 0.34]
14:0 Myristic Acid (% Total FA)	0.10 (0.0013) [0.10 - 0.10]	0.10 (0.0010) [0.10 - 0.11]	-0.00044 (0.0015) [-0.00076 - 0.0021]	-0.0052, 0.0043	0.790	(0.064 - 0.097) [0.052, 0.12]
16:0 Palmitic Acid (% Total FA)	12.07 (0.11) [12.05 - 12.09]	12.04 (0.090) [11.96 - 12.08]	0.032 (0.14) [0.013 - 0.095]	-0.42, 0.48	0.836	(9.80 - 12.38) [8.88, 13.53]
16:1 Palmitoleic Acid (% Total FA)	0.10 (0.0043) [0.091 - 0.11]	0.092 (0.0036) [0.091 - 0.095]	0.0079 (0.0052) [-0.00090 - 0.015]	-0.0087, 0.025	0.225	(0.073 - 0.14) [0.037, 0.15]
17:0 Heptadecanoic Acid (% Total FA)	0.10 (0.00085) [0.10 - 0.10]	0.099 (0.00074) [0.099 - 0.099]	0.0025 (0.00080) [0.0017 - 0.0028]	-0.00008, 0.0050	0.053	(0.076 - 0.10) [0.066, 0.11]
17:1 Heptadecenoic Acid (% Total FA)	0.048 (0.00083) [0.047 - 0.048]	0.047 (0.00068) [0.046 - 0.047]	0.00076 (0.0011) [-0.00020 - 0.0016]	-0.0027, 0.0042	0.529	(0.020 - 0.064) [0.0058, 0.083]

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Table E-2. Statistical Summary of Site AL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fatty Acid (% Total FA)						
18:0 Stearic Acid (% Total FA)	4.42 (0.096) [4.38 - 4.45]	4.51 (0.078) [4.34 - 4.60]	-0.091 (0.12) [-0.14 - 0.037]	-0.48, 0.30	0.512	(3.21 - 5.24) [1.88, 6.25]
18:1 Oleic Acid (% Total FA)	26.17 (1.17) [25.70 - 26.64]	27.43 (0.96) [26.26 - 28.78]	-1.26 (1.52) [-2.14 - -1.55]	-6.08, 3.57	0.468	(16.69 - 35.16) [5.01, 42.01]
18:2 Linoleic Acid (% Total FA)	49.75 (1.07) [49.32 - 50.17]	48.50 (0.88) [47.18 - 49.32]	1.24 (1.38) [1.16 - 2.14]	-3.16, 5.64	0.435	(44.17 - 57.72) [38.57, 66.94]
18:3 Linolenic Acid (% Total FA)	5.60 (0.12) [5.55 - 5.65]	5.47 (0.10) [5.34 - 5.68]	0.13 (0.16) [0.16 - 0.31]	-0.38, 0.64	0.473	(4.27 - 8.81) [2.69, 10.81]
20:0 Arachidic Acid (% Total FA)	0.54 (0.013) [0.54 - 0.55]	0.55 (0.011) [0.53 - 0.57]	-0.0059 (0.017) [-0.016 - 0.0079]	-0.061, 0.049	0.754	(0.36 - 0.55) [0.23, 0.64]
20:1 Eicosenoic Acid (% Total FA)	0.24 (0.015) [0.21 - 0.28]	0.28 (0.012) [0.27 - 0.28]	-0.031 (0.018) [-0.065 - -0.00053]	-0.088, 0.027	0.189	(0.21 - 0.30) [0.16, 0.33]
20:2 Eicosadienoic Acid (% Total FA)	0.033 (0.0060) [0.020 - 0.045]	0.044 (0.0049) [0.040 - 0.047]	-0.011 (0.0077) [-0.020 - -0.0013]	-0.035, 0.014	0.253	(0.016 - 0.054) [0.0029, 0.083]
22:0 Behenic Acid (% Total FA)	0.64 (0.019) [0.62 - 0.65]	0.63 (0.016) [0.61 - 0.65]	0.0064 (0.025) [-0.00071 - 0.018]	-0.074, 0.086	0.816	(0.38 - 0.59) [0.30, 0.67]

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Table E-2. Statistical Summary of Site AL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fiber						
Acid Detergent Fiber (% DW)	14.83 (0.61) [13.66 - 16.00]	14.28 (0.49) [14.00 - 14.72]	0.55 (0.78) [-1.07 - 1.88]	-1.94, 3.04	0.534	(12.79 - 17.98) [11.13, 20.21]
Neutral Detergent Fiber (% DW)	17.37 (1.02) [15.06 - 19.33]	17.49 (0.93) [16.02 - 18.38]	-0.13 (0.84) [-0.97 - 1.25]	-2.80, 2.55	0.890	(13.32 - 23.57) [7.24, 28.70]
Proximate						
Ash (% DW)	5.89 (0.073) [5.87 - 5.90]	5.79 (0.059) [5.69 - 5.88]	0.10 (0.094) [0.025 - 0.18]	-0.20, 0.40	0.358	(4.32 - 5.62) [3.74, 6.45]
Carbohydrates (% DW)	29.12 (0.78) [29.10 - 29.14]	30.31 (0.64) [29.88 - 30.56]	-1.19 (1.01) [-1.39 - -0.74]	-4.41, 2.03	0.324	(31.97 - 38.00) [28.17, 40.99]
Moisture (% FW)	6.94 (1.08) [6.86 - 7.01]	6.85 (0.89) [6.42 - 7.63]	0.082 (1.40) [-0.62 - 0.35]	-4.37, 4.54	0.957	(5.48 - 11.70) [1.45, 12.81]
Protein (% DW)	42.12 (0.53) [42.01 - 42.23]	41.51 (0.43) [41.07 - 41.87]	0.60 (0.68) [0.37 - 0.40]	-1.56, 2.77	0.440	(38.14 - 42.66) [35.30, 45.38]
Total Fat (% DW)	22.92 (0.33) [22.69 - 23.08]	22.40 (0.27) [22.25 - 22.55]	0.52 (0.39) [0.28 - 0.84]	-0.73, 1.77	0.275	(17.90 - 23.56) [14.74, 25.18]
Vitamin						
Vitamin E (mg/100g DW)	7.78 (0.20) [7.58 - 7.98]	6.98 (0.16) [6.86 - 7.21]	0.79 (0.26) [0.70 - 1.12]	-0.022, 1.61	0.053	(1.65 - 8.08) [0, 11.09]

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Table E-2. Statistical Summary of Site AL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Antinutrient						
Lectin (H.U./mg FW)	0.17 (0.20) [0.062 - 0.33]	0.32 (0.17) [0.28 - 0.36]	-0.15 (0.22) [-0.22 - -0.037]	-0.86, 0.55	0.542	(0.090 - 2.47) [0, 3.40]
Phytic Acid (% DW)	2.25 (0.11) [2.22 - 2.29]	2.39 (0.090) [2.23 - 2.66]	-0.14 (0.14) [-0.015 - 0.019]	-0.59, 0.32	0.412	(1.10 - 2.32) [0.54, 3.05]
Raffinose (% DW)	0.51 (0.076) [0.49 - 0.56]	0.49 (0.063) [0.43 - 0.55]	0.021 (0.089) [-0.054 - 0.075]	-0.26, 0.31	0.827	(0.52 - 1.62) [0.038, 2.24]
Stachyose (% DW)	1.84 (0.11) [1.83 - 1.89]	2.37 (0.095) [2.27 - 2.55]	-0.53 (0.13) [-0.72 - -0.38]	-0.93, -0.13	0.024	(1.97 - 5.55) [0.99, 7.93]
Trypsin Inhibitor (TIU/mg DW)	33.46 (2.35) [32.26 - 32.53]	31.07 (2.02) [26.21 - 34.20]	2.39 (2.43) [-0.54 - 6.33]	-5.33, 10.12	0.396	(20.84 - 37.24) [13.58, 46.02]
Isoflavone						
Daidzein (mg/kg DW)	202.56 (28.49) [188.96 - 216.15]	216.48 (23.26) [198.95 - 237.23]	-13.93 (36.78) [-9.99 - 2.88]	-130.99, 103.14	0.730	(213.98 - 1273.94) [0, 1585.14]
Genistein (mg/kg DW)	229.96 (22.24) [214.73 - 245.19]	253.03 (18.16) [244.95 - 259.82]	-23.07 (28.71) [-30.22 - -14.64]	-114.43, 68.29	0.480	(148.06 - 1024.50) [0, 1352.86]
Glycitein (mg/kg DW)	71.34 (5.26) [61.08 - 79.67]	64.86 (4.82) [61.28 - 67.07]	6.47 (4.15) [-0.19 - 12.60]	-6.73, 19.68	0.216	(32.42 - 208.45) [0, 272.12]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2] N=2, sample size=2
[3] N=3, sample size=3
[4]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-3. Statistical Summary of Site AR Soybean Forage Fiber and Proximate Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	37.57 (2.54) [31.80 - 41.20]	36.11 (2.54) [31.86 - 39.42]	1.46 (3.59) [-0.066 - 2.66]	-8.52, 11.43	0.705	(27.99 - 47.33) [14.93, 56.87]
Neutral Detergent Fiber (% DW)	49.83 (2.23) [46.69 - 55.99]	38.62 (2.23) [37.23 - 40.51]	11.21 (3.15) [6.31 - 18.76]	2.45, 19.96	0.023	(30.96 - 54.55) [21.51, 66.01]
Proximate						
Ash (% DW)	5.83 (0.41) [5.29 - 6.52]	6.61 (0.41) [5.58 - 7.23]	-0.78 (0.58) [-1.72 - 0.085]	-2.40, 0.84	0.251	(4.77 - 8.54) [2.46, 10.14]
Carbohydrates (% DW)	70.56 (1.12) [68.75 - 72.89]	70.59 (1.12) [69.06 - 72.99]	-0.030 (1.58) [-0.31 - 0.33]	-4.42, 4.36	0.985	(60.61 - 77.26) [56.93, 85.88]
Moisture (% FW)	72.57 (0.68) [71.60 - 73.30]	72.47 (0.68) [72.20 - 72.60]	0.10 (0.82) [-1.00 - 0.70]	-2.18, 2.38	0.908	(66.50 - 80.20) [57.84, 88.56]
Protein (% DW)	18.53 (0.85) [17.10 - 20.03]	17.37 (0.85) [16.54 - 18.45]	1.16 (1.20) [-0.031 - 1.92]	-2.17, 4.49	0.389	(12.68 - 22.92) [7.05, 27.27]
Total Fat (% DW)	5.20 (0.44) [4.61 - 5.99]	5.33 (0.44) [4.31 - 6.39]	-0.13 (0.48) [-1.41 - 0.70]	-1.47, 1.21	0.798	(3.48 - 7.88) [1.11, 9.11]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3] With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-4. Statistical Summary of Site AR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Alanine (% DW)	1.70 (0.023) [1.66 - 1.77]	1.67 (0.023) [1.65 - 1.69]	0.026 (0.033) [-0.034 - 0.099]	-0.065, 0.12	0.474	(1.66 - 1.93) [1.49, 2.02]
Arginine (% DW)	2.63 (0.064) [2.57 - 2.69]	2.61 (0.064) [2.53 - 2.66]	0.020 (0.091) [-0.061 - 0.092]	-0.23, 0.27	0.838	(2.54 - 2.99) [2.22, 3.25]
Aspartic Acid (% DW)	4.85 (0.091) [4.69 - 5.12]	4.82 (0.091) [4.76 - 4.91]	0.028 (0.13) [-0.16 - 0.31]	-0.33, 0.38	0.837	(4.74 - 5.50) [4.22, 5.96]
Cystine (% DW)	0.62 (0.021) [0.58 - 0.66]	0.61 (0.021) [0.58 - 0.63]	0.0092 (0.018) [-0.011 - 0.043]	-0.042, 0.060	0.642	(0.53 - 0.68) [0.45, 0.77]
Glutamic Acid (% DW)	7.57 (0.12) [7.35 - 7.94]	7.50 (0.12) [7.37 - 7.66]	0.071 (0.18) [-0.23 - 0.46]	-0.42, 0.56	0.705	(7.53 - 8.72) [6.60, 9.37]
Glycine (% DW)	1.73 (0.017) [1.70 - 1.76]	1.69 (0.017) [1.67 - 1.70]	0.040 (0.024) [0.0088 - 0.056]	-0.028, 0.11	0.180	(1.67 - 1.99) [1.49, 2.09]
Histidine (% DW)	1.11 (0.014) [1.09 - 1.12]	1.07 (0.014) [1.05 - 1.09]	0.038 (0.020) [0.027 - 0.054]	-0.018, 0.095	0.132	(1.04 - 1.24) [0.94, 1.31]
Isoleucine (% DW)	1.79 (0.027) [1.74 - 1.86]	1.75 (0.027) [1.72 - 1.79]	0.034 (0.038) [-0.044 - 0.11]	-0.070, 0.14	0.413	(1.73 - 2.02) [1.54, 2.14]

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Table E-4. Statistical Summary of Site AR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Leucine (% DW)	2.99 (0.041) [2.93 - 3.10]	2.93 (0.041) [2.87 - 2.97]	0.066 (0.059) [-0.038 - 0.17]	-0.097, 0.23	0.325	(2.93 - 3.32) [2.64, 3.52]
Lysine (% DW)	2.68 (0.086) [2.58 - 2.83]	2.61 (0.086) [2.45 - 2.74]	0.068 (0.12) [-0.12 - 0.39]	-0.27, 0.40	0.603	(2.35 - 3.15) [2.05, 3.47]
Methionine (% DW)	0.55 (0.018) [0.51 - 0.57]	0.53 (0.018) [0.51 - 0.57]	0.011 (0.022) [-0.013 - 0.049]	-0.051, 0.073	0.648	(0.49 - 0.62) [0.42, 0.68]
Phenylalanine (% DW)	2.06 (0.056) [2.03 - 2.08]	2.02 (0.056) [1.98 - 2.06]	0.042 (0.079) [0.011 - 0.095]	-0.18, 0.26	0.625	(1.97 - 2.44) [1.66, 2.64]
Proline (% DW)	1.99 (0.027) [1.95 - 2.07]	1.96 (0.027) [1.93 - 1.99]	0.034 (0.038) [-0.043 - 0.11]	-0.071, 0.14	0.417	(1.92 - 2.25) [1.73, 2.35]
Serine (% DW)	2.00 (0.021) [1.97 - 2.06]	1.95 (0.021) [1.92 - 1.97]	0.055 (0.026) [0.010 - 0.10]	-0.015, 0.13	0.095	(1.96 - 2.30) [1.75, 2.38]
Threonine (% DW)	1.58 (0.021) [1.55 - 1.62]	1.54 (0.021) [1.51 - 1.55]	0.045 (0.030) [0.0080 - 0.071]	-0.038, 0.13	0.209	(1.54 - 1.74) [1.40, 1.83]
Tryptophan (% DW)	0.51 (0.015) [0.48 - 0.54]	0.49 (0.015) [0.46 - 0.52]	0.021 (0.021) [-0.039 - 0.075]	-0.039, 0.080	0.393	(0.47 - 0.55) [0.43, 0.59]

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Table E-4. Statistical Summary of Site AR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.08 (0.052) [1.04 - 1.13]	1.12 (0.052) [1.03 - 1.17]	-0.037 (0.074) [-0.11 - 0.095]	-0.24, 0.17	0.641	(1.04 - 1.31) [0.85, 1.48]
Valine (% DW)	1.89 (0.025) [1.85 - 1.96]	1.85 (0.025) [1.82 - 1.88]	0.036 (0.035) [-0.033 - 0.098]	-0.062, 0.13	0.365	(1.83 - 2.13) [1.64, 2.22]
Fatty Acid (% Total FA)						
10:0 Capric Acid (% Total FA)	0.18 (0.023) [0.14 - 0.22]	0.23 (0.023) [0.19 - 0.26]	-0.053 (0.024) [-0.11 - -0.012]	-0.12, 0.012	0.085	(0.15 - 0.27) [0.065, 0.34]
14:0 Myristic Acid (% Total FA)	0.083 (0.00059) [0.082 - 0.084]	0.084 (0.00059) [0.083 - 0.085]	-0.00087 (0.00084) [-0.0021 - 0.00099]	-0.0032, 0.0015	0.359	(0.064 - 0.097) [0.052, 0.12]
16:0 Palmitic Acid (% Total FA)	11.65 (0.053) [11.60 - 11.70]	11.65 (0.053) [11.50 - 11.73]	-0.0067 (0.076) [-0.14 - 0.20]	-0.22, 0.20	0.933	(9.80 - 12.38) [8.88, 13.53]
16:1 Palmitoleic Acid (% Total FA)	0.082 (0.0033) [0.073 - 0.088]	0.085 (0.0033) [0.078 - 0.089]	-0.0030 (0.0047) [-0.016 - 0.0071]	-0.016, 0.010	0.558	(0.073 - 0.14) [0.037, 0.15]
17:0 Heptadecanoic Acid (% Total FA)	0.096 (0.00074) [0.095 - 0.097]	0.095 (0.00074) [0.095 - 0.096]	0.00052 (0.0010) [-0.00053 - 0.0017]	-0.0024, 0.0034	0.640	(0.076 - 0.10) [0.066, 0.11]
17:1 Heptadecenoic Acid (% Total FA)	0.037 (0.0042) [0.023 - 0.046]	0.044 (0.0042) [0.043 - 0.045]	-0.0070 (0.0055) [-0.020 - 0.0031]	-0.022, 0.0084	0.275	(0.020 - 0.064) [0.0058, 0.083]

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Table E-4. Statistical Summary of Site AR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fatty Acid (% Total FA)						
18:0 Stearic Acid (% Total FA)	4.01 (0.048) [3.97 - 4.07]	4.11 (0.048) [4.03 - 4.16]	-0.095 (0.041) [-0.16 - -0.058]	-0.21, 0.019	0.082	(3.21 - 5.24) [1.88, 6.25]
18:1 Oleic Acid (% Total FA)	20.21 (0.33) [19.78 - 20.96]	20.62 (0.33) [20.34 - 21.14]	-0.41 (0.43) [-1.36 - 0.59]	-1.59, 0.78	0.395	(16.69 - 35.16) [5.01, 42.01]
18:2 Linoleic Acid (% Total FA)	54.22 (0.30) [53.57 - 54.63]	53.79 (0.30) [53.50 - 54.07]	0.43 (0.29) [-0.23 - 0.98]	-0.37, 1.24	0.207	(44.17 - 57.72) [38.57, 66.94]
18:3 Linolenic Acid (% Total FA)	8.29 (0.062) [8.14 - 8.41]	8.17 (0.062) [8.12 - 8.26]	0.12 (0.087) [-0.12 - 0.27]	-0.12, 0.36	0.234	(4.27 - 8.81) [2.69, 10.81]
20:0 Arachidic Acid (% Total FA)	0.42 (0.0035) [0.41 - 0.42]	0.42 (0.0035) [0.41 - 0.43]	-0.0016 (0.00087) [-0.0026 - -0.00088]	-0.0040, 0.00084	0.145	(0.36 - 0.55) [0.23, 0.64]
20:1 Eicosenoic Acid (% Total FA)	0.22 (0.010) [0.19 - 0.23]	0.21 (0.010) [0.18 - 0.22]	0.0095 (0.014) [-0.026 - 0.046]	-0.030, 0.049	0.543	(0.21 - 0.30) [0.16, 0.33]
20:2 Eicosadienoic Acid (% Total FA)	0.030 (0.0069) [0.021 - 0.046]	0.037 (0.0069) [0.020 - 0.047]	-0.0070 (0.0086) [-0.024 - 0.0015]	-0.031, 0.017	0.461	(0.016 - 0.054) [0.0029, 0.083]
22:0 Behenic Acid (% Total FA)	0.47 (0.0035) [0.46 - 0.48]	0.46 (0.0035) [0.45 - 0.46]	0.014 (0.0046) [0.0047 - 0.024]	0.0013, 0.027	0.037	(0.38 - 0.59) [0.30, 0.67]

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Table E-4. Statistical Summary of Site AR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	16.56 (0.60) [15.87 - 16.91]	15.99 (0.60) [14.46 - 17.77]	0.57 (0.61) [-0.86 - 1.41]	-1.12, 2.25	0.403	(12.79 - 17.98) [11.13, 20.21]
Neutral Detergent Fiber (% DW)	16.40 (1.30) [15.95 - 17.25]	18.91 (1.30) [15.02 - 22.45]	-2.51 (1.82) [-6.43 - 0.93]	-7.57, 2.55	0.240	(13.32 - 23.57) [7.24, 28.70]
Proximate						
Ash (% DW)	4.92 (0.077) [4.77 - 5.11]	4.80 (0.077) [4.72 - 4.89]	0.11 (0.086) [0.047 - 0.21]	-0.13, 0.35	0.260	(4.32 - 5.62) [3.74, 6.45]
Carbohydrates (% DW)	38.33 (0.78) [36.06 - 39.61]	37.81 (0.78) [37.06 - 39.23]	0.53 (1.05) [-1.07 - 2.56]	-2.40, 3.46	0.642	(31.97 - 38.00) [28.17, 40.99]
Moisture (% FW)	7.57 (0.97) [5.93 - 10.70]	6.02 (0.97) [5.44 - 6.63]	1.55 (1.37) [-0.54 - 4.70]	-2.26, 5.36	0.321	(5.48 - 11.70) [1.45, 12.81]
Protein (% DW)	39.01 (0.43) [38.09 - 40.46]	38.64 (0.43) [38.41 - 38.82]	0.37 (0.61) [-0.73 - 2.05]	-1.34, 2.07	0.583	(38.14 - 42.66) [35.30, 45.38]
Total Fat (% DW)	17.79 (0.48) [17.33 - 18.48]	18.73 (0.48) [17.24 - 19.57]	-0.94 (0.59) [-1.82 - 0.090]	-2.58, 0.70	0.185	(17.90 - 23.56) [14.74, 25.18]
Vitamin						
Vitamin E (mg/100g DW)	6.88 (0.12) [6.77 - 7.08]	5.03 (0.12) [4.88 - 5.12]	1.85 (0.17) [1.66 - 2.20]	1.36, 2.33	<0.001	(1.65 - 8.08) [0, 11.09]

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Table E-4. Statistical Summary of Site AR Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Antinutrient						
Lectin (H.U./mg FW)	1.53 (0.66) [0.88 - 2.01]	0.80 (0.66) [0.30 - 1.05]	0.74 (0.93) [0.59 - 0.96]	-1.84, 3.32	0.470	(0.090 - 2.47) [0, 3.40]
Phytic Acid (% DW)	1.70 (0.082) [1.61 - 1.78]	2.01 (0.082) [1.90 - 2.14]	-0.30 (0.11) [-0.53 - -0.17]	-0.61, 0.0017	0.050	(1.10 - 2.32) [0.54, 3.05]
Raffinose (% DW)	1.51 (0.069) [1.44 - 1.54]	1.51 (0.069) [1.40 - 1.61]	-0.0048 (0.098) [-0.073 - 0.040]	-0.28, 0.27	0.963	(0.52 - 1.62) [0.038, 2.24]
Stachyose (% DW)	5.68 (0.17) [5.47 - 5.82]	5.72 (0.17) [5.36 - 5.98]	-0.043 (0.24) [-0.50 - 0.38]	-0.71, 0.62	0.865	(1.97 - 5.55) [0.99, 7.93]
Trypsin Inhibitor (TIU/mg DW)	23.48 (1.42) [23.07 - 23.96]	26.68 (1.42) [23.09 - 30.95]	-3.21 (2.01) [-6.99 - -0.32]	-8.79, 2.38	0.186	(20.84 - 37.24) [13.58, 46.02]
Isoflavone						
Daidzein (mg/kg DW)	767.90 (29.71) [747.32 - 793.95]	658.21 (29.71) [619.71 - 732.57]	109.69 (33.77) [29.86 - 171.61]	15.93, 203.46	0.031	(213.98 - 1273.94) [0, 1585.14]
Genistein (mg/kg DW)	807.35 (25.42) [771.77 - 840.99]	680.07 (25.42) [662.77 - 714.36]	127.28 (25.83) [94.92 - 178.22]	55.57, 198.99	0.007	(148.06 - 1024.50) [0, 1352.86]
Glycitein (mg/kg DW)	182.99 (10.76) [172.51 - 191.49]	163.24 (10.76) [140.43 - 191.71]	19.75 (15.22) [-19.20 - 51.06]	-22.51, 62.02	0.264	(32.42 - 208.45) [0, 272.12]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-5. Statistical Summary of Site GA Soybean Forage Fiber and Proximate Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	31.26 (2.13) [30.21 - 31.87]	32.76 (2.13) [27.42 - 36.30]	-1.50 (2.54) [-4.43 - 2.79]	-8.55, 5.54	0.585	(27.99 - 47.33) [14.93, 56.87]
Neutral Detergent Fiber (% DW)	42.64 (3.78) [37.02 - 52.38]	42.08 (3.78) [34.23 - 46.58]	0.56 (5.34) [-8.40 - 5.81]	-14.26, 15.39	0.920	(30.96 - 54.55) [21.51, 66.01]
Proximate						
Ash (% DW)	5.58 (0.18) [5.50 - 5.69]	5.25 (0.18) [5.10 - 5.38]	0.33 (0.25) [0.17 - 0.42]	-0.37, 1.03	0.262	(4.77 - 8.54) [2.46, 10.14]
Carbohydrates (% DW)	71.49 (0.93) [70.41 - 72.44]	72.52 (0.93) [70.55 - 73.86]	-1.03 (1.32) [-2.23 - -0.14]	-4.68, 2.62	0.478	(60.61 - 77.26) [56.93, 85.88]
Moisture (% FW)	71.13 (0.34) [70.60 - 71.70]	70.13 (0.34) [69.40 - 70.80]	1.00 (0.49) [-0.20 - 1.70]	-0.35, 2.35	0.108	(66.50 - 80.20) [57.84, 88.56]
Protein (% DW)	16.76 (0.64) [15.94 - 17.94]	15.58 (0.64) [14.20 - 16.67]	1.19 (0.91) [0.077 - 2.22]	-1.34, 3.71	0.261	(12.68 - 22.92) [7.05, 27.27]
Total Fat (% DW)	6.22 (0.46) [5.94 - 6.51]	6.61 (0.46) [5.74 - 7.23]	-0.39 (0.65) [-1.00 - 0.20]	-2.19, 1.42	0.584	(3.48 - 7.88) [1.11, 9.11]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-6. Statistical Summary of Site GA Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Alanine (% DW)	1.69 (0.019) [1.68 - 1.70]	1.65 (0.019) [1.63 - 1.67]	0.042 (0.027) [0.0042 - 0.063]	-0.033, 0.12	0.193	(1.66 - 1.93) [1.49, 2.02]
Arginine (% DW)	2.80 (0.036) [2.72 - 2.91]	2.57 (0.036) [2.55 - 2.60]	0.22 (0.050) [0.16 - 0.31]	0.085, 0.36	0.011	(2.54 - 2.99) [2.22, 3.25]
Aspartic Acid (% DW)	4.83 (0.072) [4.80 - 4.87]	4.73 (0.072) [4.59 - 4.90]	0.097 (0.10) [-0.039 - 0.23]	-0.19, 0.38	0.396	(4.74 - 5.50) [4.22, 5.96]
Cystine (% DW)	0.62 (0.013) [0.61 - 0.63]	0.60 (0.013) [0.56 - 0.64]	0.022 (0.018) [-0.018 - 0.052]	-0.027, 0.072	0.279	(0.53 - 0.68) [0.45, 0.77]
Glutamic Acid (% DW)	7.63 (0.098) [7.53 - 7.69]	7.39 (0.098) [7.21 - 7.60]	0.24 (0.14) [0.092 - 0.45]	-0.15, 0.62	0.159	(7.53 - 8.72) [6.60, 9.37]
Glycine (% DW)	1.74 (0.020) [1.73 - 1.78]	1.67 (0.020) [1.64 - 1.72]	0.070 (0.028) [0.059 - 0.089]	-0.0065, 0.15	0.063	(1.67 - 1.99) [1.49, 2.09]
Histidine (% DW)	1.15 (0.011) [1.13 - 1.16]	1.09 (0.011) [1.08 - 1.12]	0.057 (0.015) [0.043 - 0.074]	0.015, 0.098	0.019	(1.04 - 1.24) [0.94, 1.31]
Isoleucine (% DW)	1.81 (0.016) [1.77 - 1.84]	1.74 (0.016) [1.71 - 1.77]	0.074 (0.022) [0.035 - 0.12]	0.012, 0.14	0.029	(1.73 - 2.02) [1.54, 2.14]

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Table E-6. Statistical Summary of Site GA Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Leucine (% DW)	3.04 (0.024) [2.98 - 3.09]	2.91 (0.024) [2.87 - 2.96]	0.13 (0.033) [0.087 - 0.18]	0.043, 0.22	0.014	(2.93 - 3.32) [2.64, 3.52]
Lysine (% DW)	2.75 (0.051) [2.67 - 2.79]	2.60 (0.051) [2.54 - 2.66]	0.15 (0.073) [0.11 - 0.20]	-0.056, 0.35	0.114	(2.35 - 3.15) [2.05, 3.47]
Methionine (% DW)	0.53 (0.018) [0.48 - 0.55]	0.51 (0.018) [0.47 - 0.54]	0.017 (0.026) [-0.059 - 0.080]	-0.055, 0.089	0.548	(0.49 - 0.62) [0.42, 0.68]
Phenylalanine (% DW)	2.24 (0.059) [2.11 - 2.35]	2.06 (0.059) [1.99 - 2.20]	0.18 (0.069) [0.13 - 0.25]	-0.013, 0.37	0.060	(1.97 - 2.44) [1.66, 2.64]
Proline (% DW)	2.00 (0.014) [1.99 - 2.02]	1.94 (0.014) [1.93 - 1.94]	0.069 (0.020) [0.055 - 0.089]	0.014, 0.12	0.025	(1.92 - 2.25) [1.73, 2.35]
Serine (% DW)	2.02 (0.031) [2.00 - 2.04]	1.94 (0.031) [1.91 - 1.99]	0.076 (0.044) [0.046 - 0.11]	-0.045, 0.20	0.157	(1.96 - 2.30) [1.75, 2.38]
Threonine (% DW)	1.60 (0.018) [1.56 - 1.62]	1.53 (0.018) [1.50 - 1.59]	0.061 (0.024) [0.028 - 0.11]	-0.0065, 0.13	0.066	(1.54 - 1.74) [1.40, 1.83]
Tryptophan (% DW)	0.52 (0.0098) [0.50 - 0.54]	0.51 (0.0098) [0.50 - 0.53]	0.0044 (0.014) [-0.026 - 0.026]	-0.034, 0.043	0.764	(0.47 - 0.55) [0.43, 0.59]

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Table E-6. Statistical Summary of Site GA Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.18 (0.044) [1.08 - 1.27]	1.09 (0.044) [1.02 - 1.12]	0.097 (0.062) [-0.043 - 0.25]	-0.076, 0.27	0.195	(1.04 - 1.31) [0.85, 1.48]
Valine (% DW)	1.91 (0.017) [1.88 - 1.94]	1.84 (0.017) [1.80 - 1.87]	0.073 (0.023) [0.034 - 0.12]	0.0078, 0.14	0.035	(1.83 - 2.13) [1.64, 2.22]
Fatty Acid (% Total FA)						
10:0 Capric Acid (% Total FA)	0.20 (0.017) [0.18 - 0.24]	0.18 (0.017) [0.16 - 0.19]	0.022 (0.025) [0.0012 - 0.048]	-0.046, 0.091	0.414	(0.15 - 0.27) [0.065, 0.34]
14:0 Myristic Acid (% Total FA)	0.094 (0.0023) [0.092 - 0.095]	0.097 (0.0023) [0.092 - 0.10]	-0.0031 (0.0033) [-0.0085 - 0.0020]	-0.012, 0.0060	0.395	(0.064 - 0.097) [0.052, 0.12]
16:0 Palmitic Acid (% Total FA)	11.51 (0.12) [11.32 - 11.81]	11.93 (0.12) [11.79 - 12.11]	-0.42 (0.17) [-0.72 - 0.026]	-0.90, 0.062	0.072	(9.80 - 12.38) [8.88, 13.53]
16:1 Palmitoleic Acid (% Total FA)	0.10 (0.0043) [0.097 - 0.11]	0.10 (0.0043) [0.094 - 0.11]	-0.0016 (0.0057) [-0.0042 - 0.0031]	-0.017, 0.014	0.798	(0.073 - 0.14) [0.037, 0.15]
17:0 Heptadecanoic Acid (% Total FA)	0.086 (0.0023) [0.084 - 0.088]	0.087 (0.0023) [0.082 - 0.092]	-0.00085 (0.0026) [-0.0064 - 0.0021]	-0.0081, 0.0065	0.763	(0.076 - 0.10) [0.066, 0.11]
17:1 Heptadecenoic Acid (% Total FA)	0.040 (0.0041) [0.039 - 0.041]	0.032 (0.0041) [0.019 - 0.040]	0.0078 (0.0057) [0.00006 - 0.022]	-0.0081, 0.024	0.244	(0.020 - 0.064) [0.0058, 0.083]

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Table E-6. Statistical Summary of Site GA Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fatty Acid (% Total FA)						
18:0 Stearic Acid (% Total FA)	5.21 (0.12) [5.07 - 5.36]	5.12 (0.12) [4.78 - 5.36]	0.085 (0.14) [-0.042 - 0.29]	-0.31, 0.48	0.581	(3.21 - 5.24) [1.88, 6.25]
18:1 Oleic Acid (% Total FA)	23.10 (0.67) [22.70 - 23.71]	22.28 (0.67) [20.85 - 23.50]	0.82 (0.92) [0.21 - 2.04]	-1.72, 3.37	0.419	(16.69 - 35.16) [5.01, 42.01]
18:2 Linoleic Acid (% Total FA)	50.98 (0.59) [50.39 - 51.53]	51.56 (0.59) [50.31 - 52.88]	-0.57 (0.74) [-1.35 - 0.083]	-2.62, 1.47	0.478	(44.17 - 57.72) [38.57, 66.94]
18:3 Linolenic Acid (% Total FA)	7.23 (0.14) [7.16 - 7.35]	7.24 (0.14) [7.01 - 7.57]	-0.016 (0.20) [-0.40 - 0.20]	-0.56, 0.53	0.937	(4.27 - 8.81) [2.69, 10.81]
20:0 Arachidic Acid (% Total FA)	0.57 (0.012) [0.56 - 0.58]	0.55 (0.012) [0.51 - 0.57]	0.018 (0.014) [-0.0035 - 0.047]	-0.021, 0.057	0.264	(0.36 - 0.55) [0.23, 0.64]
20:1 Eicosenoic Acid (% Total FA)	0.24 (0.0027) [0.23 - 0.24]	0.22 (0.0027) [0.22 - 0.22]	0.012 (0.0038) [0.0040 - 0.016]	0.0013, 0.022	0.035	(0.21 - 0.30) [0.16, 0.33]
20:2 Eicosadienoic Acid (% Total FA)	0.043 (0.0022) [0.039 - 0.049]	0.041 (0.0022) [0.039 - 0.044]	0.0013 (0.0028) [-0.0019 - 0.0049]	-0.0066, 0.0092	0.678	(0.016 - 0.054) [0.0029, 0.083]
22:0 Behenic Acid (% Total FA)	0.60 (0.011) [0.58 - 0.62]	0.55 (0.011) [0.52 - 0.58]	0.046 (0.014) [0.019 - 0.078]	0.0075, 0.084	0.029	(0.38 - 0.59) [0.30, 0.67]

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Table E-6. Statistical Summary of Site GA Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	14.21 (0.35) [13.53 - 14.97]	14.92 (0.35) [14.38 - 15.77]	-0.71 (0.40) [-1.07 - -0.25]	-1.82, 0.40	0.152	(12.79 - 17.98) [11.13, 20.21]
Neutral Detergent Fiber (% DW)	16.23 (0.46) [15.51 - 17.15]	16.14 (0.46) [15.03 - 17.07]	0.095 (0.65) [-1.56 - 2.12]	-1.70, 1.89	0.889	(13.32 - 23.57) [7.24, 28.70]
Proximate						
Ash (% DW)	4.81 (0.079) [4.70 - 4.98]	4.80 (0.079) [4.70 - 4.86]	0.0072 (0.11) [-0.14 - 0.11]	-0.30, 0.32	0.951	(4.32 - 5.62) [3.74, 6.45]
Carbohydrates (% DW)	35.10 (1.46) [34.68 - 35.36]	38.48 (1.46) [35.52 - 43.48]	-3.37 (2.04) [-8.12 - -0.26]	-9.04, 2.29	0.173	(31.97 - 38.00) [28.17, 40.99]
Moisture (% FW)	6.37 (0.40) [5.86 - 7.14]	7.00 (0.40) [6.16 - 8.03]	-0.63 (0.51) [-0.96 - -0.050]	-2.04, 0.77	0.279	(5.48 - 11.70) [1.45, 12.81]
Protein (% DW)	39.33 (1.08) [38.79 - 39.77]	35.93 (1.08) [32.29 - 38.13]	3.39 (1.42) [1.64 - 6.49]	-0.55, 7.34	0.075	(38.14 - 42.66) [35.30, 45.38]
Total Fat (% DW)	20.79 (0.45) [20.29 - 21.20]	20.79 (0.45) [19.39 - 21.68]	-0.0030 (0.63) [-1.39 - 1.80]	-1.76, 1.76	0.996	(17.90 - 23.56) [14.74, 25.18]
Vitamin						
Vitamin E (mg/100g DW)	9.16 (0.22) [8.51 - 9.62]	7.77 (0.22) [7.64 - 7.94]	1.38 (0.32) [0.57 - 1.97]	0.51, 2.26	0.011	(1.65 - 8.08) [0, 11.09]

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Table E-6. Statistical Summary of Site GA Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Antinutrient						
Lectin (H.U./mg FW)	0.90 (0.20) [0.68 - 1.10]	0.84 (0.20) [0.54 - 1.28]	0.055 (0.097) [-0.18 - 0.20]	-0.21, 0.32	0.600	(0.090 - 2.47) [0, 3.40]
Phytic Acid (% DW)	1.50 (0.11) [1.39 - 1.71]	1.52 (0.11) [1.31 - 1.66]	-0.020 (0.16) [-0.26 - 0.11]	-0.47, 0.43	0.907	(1.10 - 2.32) [0.54, 3.05]
Raffinose (% DW)	1.60 (0.098) [1.53 - 1.66]	1.69 (0.098) [1.47 - 1.85]	-0.086 (0.14) [-0.32 - 0.19]	-0.47, 0.30	0.566	(0.52 - 1.62) [0.038, 2.24]
Stachyose (% DW)	4.75 (0.14) [4.40 - 4.96]	4.96 (0.14) [4.74 - 5.19]	-0.21 (0.17) [-0.35 - -0.067]	-0.67, 0.25	0.269	(1.97 - 5.55) [0.99, 7.93]
Trypsin Inhibitor (TIU/mg DW)	23.28 (0.86) [21.65 - 25.24]	29.27 (0.86) [27.29 - 30.69]	-6.00 (1.13) [-7.75 - -4.60]	-9.12, -2.87	0.005	(20.84 - 37.24) [13.58, 46.02]
Isoflavone						
Daidzein (mg/kg DW)	796.62 (53.29) [725.52 - 921.29]	748.07 (53.29) [742.75 - 753.38]	48.55 (75.37) [-27.87 - 178.54]	-160.71, 257.81	0.554	(213.98 - 1273.94) [0, 1585.14]
Genistein (mg/kg DW)	736.27 (36.05) [667.09 - 811.59]	708.78 (36.05) [676.11 - 760.87]	27.49 (50.98) [-9.02 - 50.72]	-114.04, 169.03	0.618	(148.06 - 1024.50) [0, 1352.86]
Glycitein (mg/kg DW)	181.56 (11.88) [171.02 - 195.97]	202.27 (11.88) [179.22 - 227.25]	-20.71 (12.27) [-49.56 - -4.37]	-54.79, 13.37	0.166	(32.42 - 208.45) [0, 272.12]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-7. Statistical Summary of Site IL Soybean Forage Fiber and Proximate Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	36.67 (2.60) [31.94 - 44.08]	36.80 (2.60) [33.62 - 39.28]	-0.13 (3.68) [-5.57 - 10.46]	-10.35, 10.08	0.972	(27.99 - 47.33) [14.93, 56.87]
Neutral Detergent Fiber (% DW)	48.78 (4.07) [46.12 - 53.23]	51.06 (4.07) [43.04 - 64.19]	-2.29 (5.75) [-18.07 - 7.27]	-18.26, 13.69	0.711	(30.96 - 54.55) [21.51, 66.01]
Proximate						
Ash (% DW)	6.56 (0.45) [5.92 - 7.46]	6.48 (0.45) [6.38 - 6.54]	0.079 (0.54) [-0.59 - 0.92]	-1.43, 1.59	0.891	(4.77 - 8.54) [2.46, 10.14]
Carbohydrates (% DW)	74.57 (0.94) [71.98 - 76.73]	74.18 (0.94) [74.04 - 74.26]	0.39 (1.33) [-2.28 - 2.50]	-3.30, 4.08	0.782	(60.61 - 77.26) [56.93, 85.88]
Moisture (% FW)	75.83 (0.32) [75.20 - 76.80]	76.63 (0.32) [76.30 - 77.10]	-0.80 (0.46) [-1.60 - 0.50]	-2.07, 0.47	0.154	(66.50 - 80.20) [57.84, 88.56]
Protein (% DW)	14.53 (0.68) [13.56 - 15.74]	14.87 (0.68) [14.48 - 15.40]	-0.34 (0.97) [-1.18 - 1.26]	-3.03, 2.35	0.742	(12.68 - 22.92) [7.05, 27.27]
Total Fat (% DW)	4.28 (0.39) [3.60 - 4.87]	4.43 (0.39) [4.23 - 4.64]	-0.15 (0.55) [-0.81 - 0.23]	-1.69, 1.38	0.796	(3.48 - 7.88) [1.11, 9.11]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-8. Statistical Summary of Site IL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Alanine (% DW)	1.72 (0.036) [1.66 - 1.80]	1.63 (0.036) [1.59 - 1.71]	0.087 (0.043) [0.079 - 0.095]	-0.033, 0.21	0.115	(1.66 - 1.93) [1.49, 2.02]
Arginine (% DW)	2.61 (0.052) [2.49 - 2.70]	2.44 (0.052) [2.37 - 2.56]	0.17 (0.058) [0.11 - 0.25]	0.0060, 0.33	0.045	(2.54 - 2.99) [2.22, 3.25]
Aspartic Acid (% DW)	4.87 (0.14) [4.68 - 5.20]	4.63 (0.14) [4.46 - 4.96]	0.24 (0.16) [0.23 - 0.25]	-0.21, 0.69	0.214	(4.74 - 5.50) [4.22, 5.96]
Cystine (% DW)	0.63 (0.023) [0.58 - 0.65]	0.66 (0.023) [0.62 - 0.69]	-0.032 (0.033) [-0.11 - 0.032]	-0.12, 0.059	0.378	(0.53 - 0.68) [0.45, 0.77]
Glutamic Acid (% DW)	7.53 (0.20) [7.28 - 8.02]	7.16 (0.20) [6.89 - 7.64]	0.36 (0.24) [0.32 - 0.39]	-0.31, 1.04	0.207	(7.53 - 8.72) [6.60, 9.37]
Glycine (% DW)	1.75 (0.022) [1.71 - 1.80]	1.68 (0.022) [1.64 - 1.72]	0.073 (0.030) [0.042 - 0.10]	-0.011, 0.16	0.074	(1.67 - 1.99) [1.49, 2.09]
Histidine (% DW)	1.11 (0.012) [1.09 - 1.13]	1.05 (0.012) [1.03 - 1.08]	0.052 (0.017) [0.039 - 0.072]	0.0054, 0.098	0.036	(1.04 - 1.24) [0.94, 1.31]
Isoleucine (% DW)	1.78 (0.031) [1.74 - 1.85]	1.69 (0.031) [1.64 - 1.75]	0.099 (0.039) [0.075 - 0.12]	-0.0084, 0.21	0.062	(1.73 - 2.02) [1.54, 2.14]

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Table E-8. Statistical Summary of Site IL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Leucine (% DW)	2.97 (0.053) [2.88 - 3.08]	2.80 (0.053) [2.73 - 2.93]	0.18 (0.067) [0.15 - 0.23]	-0.0079, 0.37	0.056	(2.93 - 3.32) [2.64, 3.52]
Lysine (% DW)	2.78 (0.088) [2.70 - 2.90]	2.57 (0.088) [2.49 - 2.74]	0.21 (0.12) [0.16 - 0.26]	-0.13, 0.55	0.162	(2.35 - 3.15) [2.05, 3.47]
Methionine (% DW)	0.53 (0.028) [0.49 - 0.56]	0.56 (0.028) [0.53 - 0.59]	-0.033 (0.039) [-0.094 - 0.028]	-0.14, 0.076	0.445	(0.49 - 0.62) [0.42, 0.68]
Phenylalanine (% DW)	2.08 (0.048) [1.94 - 2.21]	1.94 (0.048) [1.91 - 1.99]	0.14 (0.066) [0.018 - 0.31]	-0.043, 0.32	0.101	(1.97 - 2.44) [1.66, 2.64]
Proline (% DW)	1.99 (0.040) [1.94 - 2.09]	1.90 (0.040) [1.85 - 1.99]	0.092 (0.049) [0.083 - 0.10]	-0.045, 0.23	0.135	(1.92 - 2.25) [1.73, 2.35]
Serine (% DW)	2.01 (0.034) [1.94 - 2.08]	1.92 (0.034) [1.87 - 1.99]	0.089 (0.049) [0.061 - 0.12]	-0.046, 0.22	0.142	(1.96 - 2.30) [1.75, 2.38]
Threonine (% DW)	1.59 (0.022) [1.56 - 1.63]	1.52 (0.022) [1.49 - 1.58]	0.070 (0.031) [0.052 - 0.10]	-0.015, 0.15	0.083	(1.54 - 1.74) [1.40, 1.83]
Tryptophan (% DW)	0.53 (0.0088) [0.52 - 0.53]	0.51 (0.0088) [0.50 - 0.52]	0.019 (0.011) [0.012 - 0.023]	-0.011, 0.049	0.159	(0.47 - 0.55) [0.43, 0.59]

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Table E-8. Statistical Summary of Site IL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.10 (0.014) [1.07 - 1.13]	1.01 (0.014) [0.98 - 1.04]	0.092 (0.015) [0.067 - 0.12]	0.050, 0.13	0.003	(1.04 - 1.31) [0.85, 1.48]
Valine (% DW)	1.90 (0.033) [1.85 - 1.98]	1.80 (0.033) [1.76 - 1.87]	0.10 (0.040) [0.090 - 0.12]	-0.0073, 0.22	0.060	(1.83 - 2.13) [1.64, 2.22]
Fatty Acid (% Total FA)						
10:0 Capric Acid (% Total FA)	0.18 (0.017) [0.16 - 0.19]	0.20 (0.017) [0.16 - 0.25]	-0.022 (0.024) [-0.092 - 0.028]	-0.089, 0.046	0.424	(0.15 - 0.27) [0.065, 0.34]
14:0 Myristic Acid (% Total FA)	0.089 (0.00087) [0.086 - 0.090]	0.090 (0.00087) [0.089 - 0.091]	-0.00099 (0.0012) [-0.0036 - 0.0017]	-0.0044, 0.0024	0.465	(0.064 - 0.097) [0.052, 0.12]
16:0 Palmitic Acid (% Total FA)	11.53 (0.048) [11.39 - 11.63]	11.71 (0.048) [11.69 - 11.72]	-0.17 (0.050) [-0.30 - -0.075]	-0.31, -0.034	0.025	(9.80 - 12.38) [8.88, 13.53]
16:1 Palmitoleic Acid (% Total FA)	0.093 (0.0021) [0.092 - 0.097]	0.097 (0.0021) [0.097 - 0.097]	-0.0036 (0.0030) [-0.0053 - -0.00043]	-0.012, 0.0048	0.304	(0.073 - 0.14) [0.037, 0.15]
17:0 Heptadecanoic Acid (% Total FA)	0.093 (0.00086) [0.093 - 0.094]	0.094 (0.00086) [0.092 - 0.094]	-0.00023 (0.0011) [-0.00061 - 0.00035]	-0.0032, 0.0027	0.841	(0.076 - 0.10) [0.066, 0.11]
17:1 Heptadecenoic Acid (% Total FA)	0.043 (0.0038) [0.040 - 0.045]	0.042 (0.0038) [0.042 - 0.042]	0.00034 (0.0054) [-0.0019 - 0.0028]	-0.015, 0.015	0.952	(0.020 - 0.064) [0.0058, 0.083]

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Table E-8. Statistical Summary of Site IL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fatty Acid (% Total FA)						
18:0 Stearic Acid (% Total FA)	4.87 (0.047) [4.76 - 4.99]	5.00 (0.047) [4.92 - 5.06]	-0.13 (0.067) [-0.26 - -0.049]	-0.31, 0.059	0.130	(3.21 - 5.24) [1.88, 6.25]
18:1 Oleic Acid (% Total FA)	22.33 (0.23) [21.78 - 22.97]	21.87 (0.23) [21.68 - 22.05]	0.46 (0.21) [0.096 - 0.91]	-0.13, 1.06	0.097	(16.69 - 35.16) [5.01, 42.01]
18:2 Linoleic Acid (% Total FA)	51.78 (0.18) [51.32 - 52.33]	52.08 (0.18) [51.95 - 52.25]	-0.30 (0.19) [-0.63 - 0.078]	-0.84, 0.24	0.201	(44.17 - 57.72) [38.57, 66.94]
18:3 Linolenic Acid (% Total FA)	7.64 (0.087) [7.45 - 7.74]	7.52 (0.087) [7.44 - 7.57]	0.12 (0.092) [0.012 - 0.19]	-0.13, 0.38	0.256	(4.27 - 8.81) [2.69, 10.81]
20:0 Arachidic Acid (% Total FA)	0.51 (0.0055) [0.50 - 0.53]	0.52 (0.0055) [0.51 - 0.52]	-0.0036 (0.0065) [-0.014 - 0.0075]	-0.022, 0.014	0.603	(0.36 - 0.55) [0.23, 0.64]
20:1 Eicosenoic Acid (% Total FA)	0.24 (0.011) [0.23 - 0.25]	0.23 (0.011) [0.22 - 0.23]	0.012 (0.015) [0.0017 - 0.018]	-0.030, 0.053	0.484	(0.21 - 0.30) [0.16, 0.33]
20:2 Eicosadienoic Acid (% Total FA)	0.050 (0.0041) [0.047 - 0.054]	0.045 (0.0041) [0.044 - 0.047]	0.0045 (0.0057) [0.00080 - 0.011]	-0.011, 0.020	0.472	(0.016 - 0.054) [0.0029, 0.083]
22:0 Behenic Acid (% Total FA)	0.54 (0.012) [0.53 - 0.56]	0.52 (0.012) [0.51 - 0.52]	0.026 (0.012) [0.014 - 0.042]	-0.0058, 0.058	0.085	(0.38 - 0.59) [0.30, 0.67]

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Table E-8. Statistical Summary of Site IL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	15.69 (0.78) [14.93 - 16.18]	17.06 (0.78) [15.34 - 19.02]	-1.37 (0.77) [-2.84 - 0.62]	-3.51, 0.77	0.150	(12.79 - 17.98) [11.13, 20.21]
Neutral Detergent Fiber (% DW)	16.74 (0.40) [16.41 - 17.18]	16.21 (0.40) [15.32 - 17.41]	0.53 (0.57) [-1.00 - 1.86]	-1.04, 2.11	0.399	(13.32 - 23.57) [7.24, 28.70]
Proximate						
Ash (% DW)	5.42 (0.083) [5.20 - 5.55]	5.29 (0.083) [5.16 - 5.36]	0.13 (0.042) [0.034 - 0.21]	0.010, 0.25	0.039	(4.32 - 5.62) [3.74, 6.45]
Carbohydrates (% DW)	36.65 (0.71) [35.60 - 37.72]	39.17 (0.71) [37.69 - 39.96]	-2.53 (0.72) [-3.34 - -2.09]	-4.53, -0.52	0.024	(31.97 - 38.00) [28.17, 40.99]
Moisture (% FW)	9.23 (1.03) [6.88 - 10.40]	7.45 (1.03) [6.66 - 8.74]	1.78 (1.12) [0.22 - 3.46]	-1.34, 4.90	0.187	(5.48 - 11.70) [1.45, 12.81]
Protein (% DW)	38.91 (0.63) [37.73 - 40.58]	37.49 (0.63) [36.66 - 38.71]	1.43 (0.78) [1.08 - 1.87]	-0.73, 3.59	0.140	(38.14 - 42.66) [35.30, 45.38]
Total Fat (% DW)	19.05 (0.47) [18.30 - 19.76]	18.05 (0.47) [17.78 - 18.30]	1.00 (0.66) [0.0042 - 1.98]	-0.83, 2.84	0.203	(17.90 - 23.56) [14.74, 25.18]
Vitamin						
Vitamin E (mg/100g DW)	6.72 (0.19) [6.36 - 7.27]	5.31 (0.19) [4.98 - 5.58]	1.41 (0.16) [1.17 - 1.69]	0.98, 1.84	<0.001	(1.65 - 8.08) [0, 11.09]

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Table E-8. Statistical Summary of Site IL Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Antinutrient						
Lectin (H.U./mg FW)	0.98 (0.28) [0.21 - 1.65]	1.04 (0.28) [0.84 - 1.18]	-0.058 (0.39) [-0.88 - 0.47]	-1.15, 1.04	0.889	(0.090 - 2.47) [0, 3.40]
Phytic Acid (% DW)	2.07 (0.085) [1.88 - 2.22]	2.05 (0.085) [1.91 - 2.13]	0.022 (0.12) [-0.25 - 0.31]	-0.31, 0.36	0.865	(1.10 - 2.32) [0.54, 3.05]
Raffinose (% DW)	1.60 (0.065) [1.41 - 1.70]	1.58 (0.065) [1.52 - 1.67]	0.018 (0.092) [-0.16 - 0.18]	-0.24, 0.27	0.852	(0.52 - 1.62) [0.038, 2.24]
Stachyose (% DW)	6.05 (0.29) [5.48 - 6.42]	6.10 (0.29) [5.53 - 6.65]	-0.046 (0.40) [-0.63 - 0.73]	-1.16, 1.07	0.915	(1.97 - 5.55) [0.99, 7.93]
Trypsin Inhibitor (TIU/mg DW)	27.09 (1.77) [22.34 - 31.92]	29.51 (1.77) [28.50 - 30.68]	-2.42 (1.96) [-6.16 - 2.58]	-7.85, 3.02	0.284	(20.84 - 37.24) [13.58, 46.02]
Isoflavone						
Daidzein (mg/kg DW)	890.96 (28.34) [834.82 - 983.26]	803.42 (28.34) [788.95 - 830.65]	87.54 (29.71) [45.87 - 152.61]	5.04, 170.04	0.042	(213.98 - 1273.94) [0, 1585.14]
Genistein (mg/kg DW)	776.22 (31.97) [724.87 - 863.84]	725.36 (31.97) [701.70 - 744.59]	50.87 (42.57) [-19.72 - 162.14]	-67.32, 169.05	0.298	(148.06 - 1024.50) [0, 1352.86]
Glycitein (mg/kg DW)	198.74 (14.52) [164.30 - 228.79]	204.69 (14.52) [177.84 - 219.15]	-5.95 (17.13) [-16.03 - 11.73]	-53.52, 41.62	0.746	(32.42 - 208.45) [0, 272.12]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-9. Statistical Summary of Site NC Soybean Forage Fiber and Proximate Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fiber						
Acid Detergent Fiber (% DW)	44.66 (5.11) [37.12 - 58.25]	36.58 (6.26) [31.54 - 41.61]	8.09 (8.08) [-2.99 - 5.58]	-17.64, 33.82	0.390	(27.99 - 47.33) [14.93, 56.87]
Neutral Detergent Fiber (% DW)	47.51 (2.97) [46.55 - 48.83]	48.85 (3.64) [42.62 - 55.09]	-1.35 (4.70) [-6.26 - 3.93]	-16.29, 13.60	0.792	(30.96 - 54.55) [21.51, 66.01]
Proximate						
Ash (% DW)	5.65 (0.19) [5.42 - 6.03]	5.97 (0.23) [5.93 - 6.01]	-0.32 (0.30) [-0.51 - 0.028]	-1.28, 0.63	0.363	(4.77 - 8.54) [2.46, 10.14]
Carbohydrates (% DW)	71.44 (1.22) [70.23 - 73.06]	70.59 (1.39) [67.72 - 72.82]	0.85 (1.34) [-1.78 - 2.51]	-3.42, 5.13	0.571	(60.61 - 77.26) [56.93, 85.88]
Moisture (% FW)	70.47 (0.30) [70.10 - 71.00]	70.85 (0.37) [70.20 - 71.50]	-0.38 (0.48) [-1.40 - 0.80]	-1.91, 1.14	0.482	(66.50 - 80.20) [57.84, 88.56]
Protein (% DW)	17.17 (0.95) [16.26 - 17.68]	17.62 (1.09) [16.10 - 19.66]	-0.45 (1.06) [-1.98 - 1.46]	-3.83, 2.93	0.700	(12.68 - 22.92) [7.05, 27.27]
Total Fat (% DW)	5.82 (0.43) [5.28 - 6.82]	5.82 (0.49) [5.13 - 6.74]	-0.0020 (0.48) [0.086 - 0.14]	-1.53, 1.52	0.996	(3.48 - 7.88) [1.11, 9.11]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3
[3] N=2, sample size=2
[4]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-10. Statistical Summary of Site NC Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Alanine (% DW)	1.67 (0.016) [1.66 - 1.68]	1.66 (0.018) [1.66 - 1.68]	0.0055 (0.019) [-0.0051 - 0.0048]	-0.056, 0.067	0.795	(1.66 - 1.93) [1.49, 2.02]
Arginine (% DW)	2.42 (0.078) [2.36 - 2.51]	2.50 (0.091) [2.53 - 2.54]	-0.085 (0.094) [-0.16 - -0.014]	-0.38, 0.21	0.432	(2.54 - 2.99) [2.22, 3.25]
Aspartic Acid (% DW)	4.68 (0.073) [4.61 - 4.75]	4.82 (0.085) [4.72 - 4.91]	-0.14 (0.089) [-0.23 - -0.12]	-0.42, 0.14	0.215	(4.74 - 5.50) [4.22, 5.96]
Cystine (% DW)	0.58 (0.016) [0.57 - 0.59]	0.59 (0.019) [0.57 - 0.61]	-0.016 (0.025) [-0.017 - -0.0080]	-0.096, 0.063	0.561	(0.53 - 0.68) [0.45, 0.77]
Glutamic Acid (% DW)	7.30 (0.084) [7.25 - 7.34]	7.47 (0.10) [7.39 - 7.58]	-0.18 (0.12) [-0.29 - -0.14]	-0.56, 0.20	0.228	(7.53 - 8.72) [6.60, 9.37]
Glycine (% DW)	1.67 (0.036) [1.63 - 1.70]	1.66 (0.041) [1.67 - 1.69]	0.0061 (0.037) [-0.0052 - 0.016]	-0.11, 0.12	0.878	(1.67 - 1.99) [1.49, 2.09]
Histidine (% DW)	1.07 (0.028) [1.05 - 1.11]	1.05 (0.032) [1.06 - 1.08]	0.019 (0.027) [-0.00077 - 0.036]	-0.068, 0.11	0.529	(1.04 - 1.24) [0.94, 1.31]
Isoleucine (% DW)	1.71 (0.031) [1.68 - 1.75]	1.70 (0.034) [1.72 - 1.73]	0.0084 (0.027) [-0.016 - 0.016]	-0.077, 0.094	0.775	(1.73 - 2.02) [1.54, 2.14]

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Table E-10. Statistical Summary of Site NC Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Leucine (% DW)	2.85 (0.045) [2.82 - 2.88]	2.87 (0.052) [2.89 - 2.91]	-0.024 (0.053) [-0.044 - -0.024]	-0.19, 0.14	0.684	(2.93 - 3.32) [2.64, 3.52]
Lysine (% DW)	2.55 (0.077) [2.48 - 2.66]	2.42 (0.082) [2.42 - 2.53]	0.14 (0.053) [0.10 - 0.13]	-0.033, 0.31	0.082	(2.35 - 3.15) [2.05, 3.47]
Methionine (% DW)	0.51 (0.014) [0.49 - 0.53]	0.51 (0.017) [0.48 - 0.53]	0.0014 (0.023) [0.0028 - 0.020]	-0.070, 0.073	0.953	(0.49 - 0.62) [0.42, 0.68]
Phenylalanine (% DW)	1.96 (0.096) [1.91 - 2.05]	1.96 (0.11) [1.96 - 2.02]	0.0037 (0.11) [-0.036 - 0.027]	-0.36, 0.37	0.975	(1.97 - 2.44) [1.66, 2.64]
Proline (% DW)	1.89 (0.027) [1.86 - 1.92]	1.92 (0.030) [1.93 - 1.94]	-0.027 (0.028) [-0.058 - -0.0052]	-0.12, 0.063	0.409	(1.92 - 2.25) [1.73, 2.35]
Serine (% DW)	1.94 (0.031) [1.90 - 1.98]	1.92 (0.035) [1.94 - 1.94]	0.027 (0.031) [0.016 - 0.039]	-0.071, 0.12	0.447	(1.96 - 2.30) [1.75, 2.38]
Threonine (% DW)	1.52 (0.029) [1.50 - 1.55]	1.52 (0.032) [1.52 - 1.55]	-0.0018 (0.028) [-0.016 - 0.0045]	-0.090, 0.086	0.951	(1.54 - 1.74) [1.40, 1.83]
Tryptophan (% DW)	0.49 (0.010) [0.47 - 0.51]	0.47 (0.010) [0.47 - 0.49]	0.022 (0.0034) [0.020 - 0.027]	0.012, 0.033	0.006	(0.47 - 0.55) [0.43, 0.59]

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Table E-10. Statistical Summary of Site NC Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.03 (0.041) [0.96 - 1.09]	1.07 (0.050) [1.03 - 1.12]	-0.044 (0.063) [-0.088 - 0.057]	-0.24, 0.16	0.534	(1.04 - 1.31) [0.85, 1.48]
Valine (% DW)	1.82 (0.028) [1.80 - 1.85]	1.82 (0.031) [1.83 - 1.85]	-0.0029 (0.027) [-0.015 - -0.0054]	-0.089, 0.083	0.921	(1.83 - 2.13) [1.64, 2.22]
Fatty Acid (% Total FA)						
10:0 Capric Acid (% Total FA)	0.25 (0.0095) [0.24 - 0.25]	0.22 (0.012) [0.21 - 0.24]	0.022 (0.015) [0.017 - 0.037]	-0.025, 0.070	0.231	(0.15 - 0.27) [0.065, 0.34]
14:0 Myristic Acid (% Total FA)	0.097 (0.0013) [0.094 - 0.10]	0.095 (0.0015) [0.092 - 0.096]	0.0028 (0.0013) [0.0018 - 0.0025]	-0.0013, 0.0070	0.119	(0.064 - 0.097) [0.052, 0.12]
16:0 Palmitic Acid (% Total FA)	12.24 (0.050) [12.19 - 12.30]	12.02 (0.061) [11.91 - 12.13]	0.22 (0.079) [0.057 - 0.40]	-0.036, 0.47	0.072	(9.80 - 12.38) [8.88, 13.53]
16:1 Palmitoleic Acid (% Total FA)	0.086 (0.0016) [0.084 - 0.089]	0.10 (0.0020) [0.098 - 0.10]	-0.014 (0.0026) [-0.018 - -0.0093]	-0.022, -0.0056	0.012	(0.073 - 0.14) [0.037, 0.15]
17:0 Heptadecanoic Acid (% Total FA)	0.093 (0.0014) [0.090 - 0.095]	0.088 (0.0017) [0.088 - 0.090]	0.0044 (0.0019) [0.0033 - 0.0074]	-0.0017, 0.010	0.104	(0.076 - 0.10) [0.066, 0.11]
17:1 Heptadecenoic Acid (% Total FA)	0.038 (0.0032) [0.037 - 0.039]	0.040 (0.0039) [0.040 - 0.040]	-0.0022 (0.0050) [-0.0018 - -0.0011]	-0.018, 0.014	0.690	(0.020 - 0.064) [0.0058, 0.083]

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Table E-10. Statistical Summary of Site NC Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fatty Acid (% Total FA)						
18:0 Stearic Acid (% Total FA)	4.42 (0.065) [4.34 - 4.49]	4.79 (0.080) [4.66 - 4.92]	-0.36 (0.10) [-0.57 - -0.17]	-0.69, -0.035	0.038	(3.21 - 5.24) [1.88, 6.25]
18:1 Oleic Acid (% Total FA)	19.78 (0.35) [19.21 - 20.21]	21.60 (0.43) [20.83 - 22.37]	-1.82 (0.56) [-3.16 - -0.62]	-3.60, -0.036	0.047	(16.69 - 35.16) [5.01, 42.01]
18:2 Linoleic Acid (% Total FA)	54.21 (0.31) [53.89 - 54.61]	52.62 (0.38) [51.93 - 53.30]	1.59 (0.49) [0.58 - 2.68]	0.046, 3.14	0.046	(44.17 - 57.72) [38.57, 66.94]
18:3 Linolenic Acid (% Total FA)	7.45 (0.087) [7.33 - 7.66]	7.11 (0.11) [6.98 - 7.25]	0.34 (0.14) [0.079 - 0.68]	-0.10, 0.78	0.091	(4.27 - 8.81) [2.69, 10.81]
20:0 Arachidic Acid (% Total FA)	0.49 (0.0072) [0.49 - 0.50]	0.51 (0.0088) [0.50 - 0.53]	-0.019 (0.011) [-0.044 - -0.0020]	-0.056, 0.017	0.185	(0.36 - 0.55) [0.23, 0.64]
20:1 Eicosenoic Acid (% Total FA)	0.25 (0.0057) [0.24 - 0.26]	0.23 (0.0064) [0.23 - 0.23]	0.014 (0.0059) [0.0054 - 0.014]	-0.0051, 0.033	0.102	(0.21 - 0.30) [0.16, 0.33]
20:2 Eicosadienoic Acid (% Total FA)	0.043 (0.0012) [0.041 - 0.044]	0.044 (0.0013) [0.042 - 0.045]	-0.0015 (0.0012) [-0.0013 - -0.00084]	-0.0054, 0.0023	0.296	(0.016 - 0.054) [0.0029, 0.083]
22:0 Behenic Acid (% Total FA)	0.55 (0.0059) [0.54 - 0.56]	0.53 (0.0072) [0.52 - 0.54]	0.025 (0.0092) [0.0057 - 0.034]	-0.0038, 0.055	0.069	(0.38 - 0.59) [0.30, 0.67]

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Table E-10. Statistical Summary of Site NC Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Fiber						
Acid Detergent Fiber (% DW)	16.49 (0.69) [16.04 - 17.05]	15.85 (0.85) [15.49 - 16.20]	0.64 (1.09) [-0.16 - 1.56]	-2.84, 4.13	0.597	(12.79 - 17.98) [11.13, 20.21]
Neutral Detergent Fiber (% DW)	20.15 (1.15) [18.97 - 21.80]	17.24 (1.41) [17.16 - 17.32]	2.91 (1.82) [2.53 - 4.47]	-2.87, 8.69	0.207	(13.32 - 23.57) [7.24, 28.70]
Proximate						
Ash (% DW)	4.99 (0.039) [4.92 - 5.04]	5.05 (0.048) [4.98 - 5.12]	-0.063 (0.062) [-0.12 - -0.061]	-0.26, 0.13	0.383	(4.32 - 5.62) [3.74, 6.45]
Carbohydrates (% DW)	31.82 (3.28) [21.58 - 37.50]	36.64 (4.00) [36.15 - 36.58]	-4.82 (4.94) [-15.00 - 0.23]	-20.55, 10.91	0.401	(31.97 - 38.00) [28.17, 40.99]
Moisture (% FW)	7.21 (0.34) [7.08 - 7.33]	6.92 (0.41) [6.77 - 7.06]	0.29 (0.47) [0.27 - 0.31]	-1.22, 1.79	0.585	(5.48 - 11.70) [1.45, 12.81]
Protein (% DW)	37.02 (1.11) [36.49 - 37.46]	35.25 (1.36) [32.37 - 38.18]	1.77 (1.72) [-0.72 - 4.74]	-3.71, 7.25	0.378	(38.14 - 42.66) [35.30, 45.38]
Total Fat (% DW)	21.11 (0.65) [21.02 - 21.20]	20.63 (0.65) [20.59 - 20.66]	0.48 (0.92) [0.61 - 0.61]	-3.47, 4.43	0.651	(17.90 - 23.56) [14.74, 25.18]
Vitamin						
Vitamin E (mg/100g DW)	7.83 (0.29) [7.59 - 8.19]	6.14 (0.34) [5.47 - 6.55]	1.69 (0.35) [1.03 - 2.25]	0.56, 2.82	0.017	(1.65 - 8.08) [0, 11.09]

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Table E-10. Statistical Summary of Site NC Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[3] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[4]]
Antinutrient						
Lectin (H.U./mg FW)	0.96 (0.39) [0.53 - 1.74]	0.54 (0.47) [0.32 - 0.85]	0.42 (0.57) [-0.23 - 1.42]	-1.39, 2.24	0.511	(0.090 - 2.47) [0, 3.40]
Phytic Acid (% DW)	1.76 (0.039) [1.69 - 1.83]	1.85 (0.047) [1.86 - 1.88]	-0.088 (0.051) [-0.11 - -0.026]	-0.25, 0.074	0.181	(1.10 - 2.32) [0.54, 3.05]
Raffinose (% DW)	1.38 (0.067) [1.23 - 1.47]	1.41 (0.082) [1.39 - 1.43]	-0.033 (0.11) [0.0042 - 0.080]	-0.37, 0.31	0.774	(0.52 - 1.62) [0.038, 2.24]
Stachyose (% DW)	4.56 (0.11) [4.32 - 4.72]	5.50 (0.13) [5.36 - 5.73]	-0.94 (0.14) [-1.00 - -0.72]	-1.37, -0.51	0.006	(1.97 - 5.55) [0.99, 7.93]
Trypsin Inhibitor (TIU/mg DW)	24.82 (1.64) [24.57 - 25.04]	25.72 (2.01) [22.49 - 28.96]	-0.90 (2.60) [-4.10 - 2.55]	-9.17, 7.37	0.751	(20.84 - 37.24) [13.58, 46.02]
Isoflavone						
Daidzein (mg/kg DW)	661.66 (45.55) [617.52 - 710.29]	589.26 (55.79) [583.50 - 595.01]	72.41 (72.02) [62.16 - 126.79]	-156.79, 301.60	0.388	(213.98 - 1273.94) [0, 1585.14]
Genistein (mg/kg DW)	715.20 (41.48) [668.18 - 746.88]	598.93 (50.80) [591.01 - 606.84]	116.27 (65.58) [123.71 - 155.87]	-92.43, 324.98	0.174	(148.06 - 1024.50) [0, 1352.86]
Glycitein (mg/kg DW)	187.13 (17.29) [147.65 - 228.15]	145.09 (20.56) [139.44 - 165.70]	42.05 (22.80) [19.91 - 88.71]	-30.52, 114.62	0.162	(32.42 - 208.45) [0, 272.12]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2] N=3, sample size=3, with exception of total fat (N=2)
[3] N=2, sample size=2
[4]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-11. Statistical Summary of Combined Site Soybean Forage Fiber and Proximate Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	37.17 (1.72) [30.04 - 58.25]	36.53 (1.72) [27.42 - 42.06]	0.65 (2.01) [-12.02 - 10.46]	-3.42, 4.71	0.749	(27.99 - 47.33) [14.93, 56.87]
Neutral Detergent Fiber (% DW)	47.16 (2.00) [37.02 - 55.99]	45.57 (2.00) [34.23 - 64.19]	1.59 (2.48) [-18.07 - 18.76]	-3.50, 6.68	0.526	(30.96 - 54.55) [21.51, 66.01]
Proximate						
Ash (% DW)	5.84 (0.30) [5.05 - 7.46]	6.32 (0.30) [5.10 - 8.13]	-0.48 (0.33) [-1.72 - 0.92]	-1.25, 0.29	0.190	(4.77 - 8.54) [2.46, 10.14]
Carbohydrates (% DW)	71.43 (1.12) [68.29 - 76.73]	70.97 (1.12) [63.68 - 74.26]	0.47 (0.61) [-2.28 - 4.62]	-0.79, 1.73	0.452	(60.61 - 77.26) [56.93, 85.88]
Moisture (% FW)	72.86 (1.19) [70.10 - 76.80]	73.41 (1.19) [69.40 - 78.10]	-0.55 (0.49) [-2.30 - 1.70]	-1.67, 0.58	0.296	(66.50 - 80.20) [57.84, 88.56]
Protein (% DW)	17.39 (1.07) [13.56 - 20.03]	17.07 (1.07) [14.20 - 23.29]	0.32 (0.60) [-3.57 - 2.22]	-0.90, 1.54	0.591	(12.68 - 22.92) [7.05, 27.27]
Total Fat (% DW)	5.30 (0.34) [3.60 - 6.82]	5.65 (0.34) [4.23 - 7.23]	-0.35 (0.26) [-2.76 - 0.70]	-0.89, 0.19	0.195	(3.48 - 7.88) [1.11, 9.11]

[1]DW = dry weight; FW = fresh weight; S.E. = standard error; CI = Confidence Interval.
[2] N=14, sample size=14
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-12. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Alanine (% DW)	1.72 (0.029) [1.66 - 1.84]	1.69 (0.029) [1.59 - 1.82]	0.036 (0.016) [-0.034 - 0.099]	0.0044, 0.068	0.027	(1.66 - 1.93) [1.49, 2.02]
Arginine (% DW)	2.68 (0.069) [2.36 - 3.00]	2.58 (0.069) [2.37 - 2.89]	0.096 (0.058) [-0.16 - 0.31]	-0.039, 0.23	0.138	(2.54 - 2.99) [2.22, 3.25]
Aspartic Acid (% DW)	4.90 (0.10) [4.61 - 5.26]	4.85 (0.10) [4.46 - 5.34]	0.053 (0.055) [-0.23 - 0.31]	-0.059, 0.17	0.339	(4.74 - 5.50) [4.22, 5.96]
Cystine (% DW)	0.62 (0.014) [0.57 - 0.67]	0.61 (0.014) [0.56 - 0.69]	0.0051 (0.014) [-0.11 - 0.066]	-0.024, 0.034	0.718	(0.53 - 0.68) [0.45, 0.77]
Glutamic Acid (% DW)	7.65 (0.15) [7.25 - 8.21]	7.53 (0.15) [6.89 - 8.26]	0.12 (0.084) [-0.29 - 0.46]	-0.056, 0.29	0.177	(7.53 - 8.72) [6.60, 9.37]
Glycine (% DW)	1.75 (0.026) [1.63 - 1.89]	1.70 (0.026) [1.64 - 1.85]	0.049 (0.017) [-0.0052 - 0.12]	0.014, 0.083	0.007	(1.67 - 1.99) [1.49, 2.09]
Histidine (% DW)	1.12 (0.015) [1.05 - 1.18]	1.08 (0.015) [1.03 - 1.15]	0.043 (0.011) [-0.00077 - 0.090]	0.021, 0.064	<0.001	(1.04 - 1.24) [0.94, 1.31]
Isoleucine (% DW)	1.81 (0.037) [1.68 - 1.99]	1.76 (0.037) [1.64 - 1.96]	0.052 (0.020) [-0.044 - 0.12]	0.0061, 0.098	0.031	(1.73 - 2.02) [1.54, 2.14]

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Table E-12. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Leucine (% DW)	3.04 (0.066) [2.82 - 3.36]	2.94 (0.066) [2.73 - 3.29]	0.095 (0.040) [-0.044 - 0.23]	0.0018, 0.19	0.046	(2.93 - 3.32) [2.64, 3.52]
Lysine (% DW)	2.74 (0.060) [2.48 - 2.99]	2.62 (0.060) [2.42 - 2.91]	0.12 (0.046) [-0.12 - 0.39]	0.028, 0.21	0.012	(2.35 - 3.15) [2.05, 3.47]
Methionine (% DW)	0.53 (0.012) [0.48 - 0.58]	0.53 (0.012) [0.47 - 0.59]	0.0043 (0.014) [-0.094 - 0.080]	-0.023, 0.032	0.754	(0.49 - 0.62) [0.42, 0.68]
Phenylalanine (% DW)	2.15 (0.056) [1.91 - 2.48]	2.04 (0.056) [1.91 - 2.38]	0.11 (0.052) [-0.036 - 0.41]	-0.013, 0.23	0.073	(1.97 - 2.44) [1.66, 2.64]
Proline (% DW)	2.01 (0.035) [1.86 - 2.16]	1.96 (0.035) [1.85 - 2.12]	0.042 (0.021) [-0.058 - 0.11]	-0.0069, 0.091	0.082	(1.92 - 2.25) [1.73, 2.35]
Serine (% DW)	2.03 (0.032) [1.90 - 2.19]	1.96 (0.032) [1.87 - 2.13]	0.060 (0.019) [0.010 - 0.14]	0.020, 0.10	0.004	(1.96 - 2.30) [1.75, 2.38]
Threonine (% DW)	1.60 (0.020) [1.50 - 1.72]	1.55 (0.020) [1.49 - 1.68]	0.046 (0.016) [-0.016 - 0.13]	0.0078, 0.084	0.024	(1.54 - 1.74) [1.40, 1.83]
Tryptophan (% DW)	0.51 (0.0068) [0.47 - 0.54]	0.50 (0.0068) [0.46 - 0.53]	0.011 (0.0067) [-0.039 - 0.075]	-0.0024, 0.025	0.102	(0.47 - 0.55) [0.43, 0.59]

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Table E-12. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Amino Acid (% DW)						
Tyrosine (% DW)	1.13 (0.034) [0.96 - 1.33]	1.10 (0.034) [0.98 - 1.22]	0.039 (0.029) [-0.11 - 0.25]	-0.028, 0.11	0.213	(1.04 - 1.31) [0.85, 1.48]
Valine (% DW)	1.92 (0.032) [1.80 - 2.07]	1.86 (0.032) [1.76 - 2.04]	0.053 (0.022) [-0.033 - 0.12]	0.0029, 0.10	0.040	(1.83 - 2.13) [1.64, 2.22]
Fatty Acid (% Total FA)						
10:0 Capric Acid (% Total FA)	0.20 (0.014) [0.14 - 0.25]	0.21 (0.014) [0.16 - 0.26]	-0.010 (0.020) [-0.11 - 0.048]	-0.053, 0.032	0.607	(0.15 - 0.27) [0.065, 0.34]
14:0 Myristic Acid (% Total FA)	0.093 (0.0031) [0.082 - 0.10]	0.094 (0.0031) [0.083 - 0.11]	-0.00056 (0.0019) [-0.0085 - 0.0025]	-0.0048, 0.0037	0.769	(0.064 - 0.097) [0.052, 0.12]
16:0 Palmitic Acid (% Total FA)	11.80 (0.12) [11.32 - 12.30]	11.88 (0.12) [11.50 - 12.13]	-0.079 (0.081) [-0.72 - 0.40]	-0.27, 0.11	0.359	(9.80 - 12.38) [8.88, 13.53]
16:1 Palmitoleic Acid (% Total FA)	0.092 (0.0033) [0.073 - 0.11]	0.095 (0.0033) [0.078 - 0.11]	-0.0028 (0.0029) [-0.018 - 0.015]	-0.0097, 0.0041	0.372	(0.073 - 0.14) [0.037, 0.15]
17:0 Heptadecanoic Acid (% Total FA)	0.094 (0.0021) [0.084 - 0.10]	0.093 (0.0021) [0.082 - 0.099]	0.0011 (0.0018) [-0.0064 - 0.0074]	-0.0030, 0.0052	0.553	(0.076 - 0.10) [0.066, 0.11]
17:1 Heptadecenoic Acid (% Total FA)	0.041 (0.0032) [0.023 - 0.048]	0.041 (0.0032) [0.019 - 0.047]	-0.00009 (0.0040) [-0.020 - 0.022]	-0.0092, 0.0090	0.981	(0.020 - 0.064) [0.0058, 0.083]

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Table E-12. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fatty Acid (% Total FA)						
18:0 Stearic Acid (% Total FA)	4.59 (0.22) [3.97 - 5.36]	4.70 (0.22) [4.03 - 5.36]	-0.12 (0.11) [-0.57 - 0.29]	-0.38, 0.14	0.328	(3.21 - 5.24) [1.88, 6.25]
18:1 Oleic Acid (% Total FA)	22.35 (1.28) [19.21 - 26.64]	22.71 (1.28) [20.34 - 28.78]	-0.36 (0.49) [-3.16 - 2.04]	-1.51, 0.79	0.486	(16.69 - 35.16) [5.01, 42.01]
18:2 Linoleic Acid (% Total FA)	52.16 (0.95) [49.32 - 54.63]	51.76 (0.95) [47.18 - 54.07]	0.40 (0.38) [-1.35 - 2.68]	-0.48, 1.29	0.320	(44.17 - 57.72) [38.57, 66.94]
18:3 Linolenic Acid (% Total FA)	7.24 (0.45) [5.55 - 8.41]	7.11 (0.45) [5.34 - 8.26]	0.13 (0.12) [-0.40 - 0.68]	-0.13, 0.40	0.276	(4.27 - 8.81) [2.69, 10.81]
20:0 Arachidic Acid (% Total FA)	0.51 (0.025) [0.41 - 0.58]	0.51 (0.025) [0.41 - 0.57]	-0.0027 (0.013) [-0.044 - 0.047]	-0.032, 0.026	0.836	(0.36 - 0.55) [0.23, 0.64]
20:1 Eicosenoic Acid (% Total FA)	0.24 (0.012) [0.19 - 0.28]	0.23 (0.012) [0.18 - 0.28]	0.0044 (0.010) [-0.065 - 0.046]	-0.020, 0.029	0.683	(0.21 - 0.30) [0.16, 0.33]
20:2 Eicosadienoic Acid (% Total FA)	0.040 (0.0030) [0.020 - 0.054]	0.042 (0.0030) [0.020 - 0.047]	-0.0024 (0.0042) [-0.024 - 0.011]	-0.012, 0.0068	0.585	(0.016 - 0.054) [0.0029, 0.083]
22:0 Behenic Acid (% Total FA)	0.56 (0.028) [0.46 - 0.65]	0.54 (0.028) [0.45 - 0.65]	0.023 (0.0084) [-0.00071 - 0.078]	0.0041, 0.042	0.022	(0.38 - 0.59) [0.30, 0.67]

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Table E-12. Statistical Summary of Combined Site Soybean Seed Amino Acid, Fatty Acid, Fiber, Proximate, Vitamin, Antinutrient and Isoflavone Content for MON 87701 vs. the Conventional Control (A5547) (cont.)

Component (Units)[1]	MON 87701 Mean (S.E.)[2] [Range]	A5547 Mean (S.E.)[2] [Range]	Difference (Test minus Control) Mean (S.E.) [Range]	95% CI (Lower, Upper)	p-Value	Commercial (Range) [99% Tolerance Interval[3]]
Fiber						
Acid Detergent Fiber (% DW)	15.58 (0.49) [13.53 - 17.05]	15.62 (0.49) [14.00 - 19.02]	-0.042 (0.58) [-2.84 - 1.88]	-1.37, 1.28	0.943	(12.79 - 17.98) [11.13, 20.21]
Neutral Detergent Fiber (% DW)	17.33 (0.70) [15.06 - 21.80]	17.28 (0.70) [15.02 - 22.45]	0.057 (0.74) [-6.43 - 4.47]	-1.67, 1.78	0.940	(13.32 - 23.57) [7.24, 28.70]
Proximate						
Ash (% DW)	5.20 (0.18) [4.70 - 5.90]	5.14 (0.18) [4.70 - 5.88]	0.054 (0.043) [-0.14 - 0.21]	-0.046, 0.15	0.246	(4.32 - 5.62) [3.74, 6.45]
Carbohydrates (% DW)	34.22 (1.50) [21.58 - 39.61]	36.44 (1.50) [29.88 - 43.48]	-2.22 (1.02) [-15.00 - 2.56]	-4.31, -0.14	0.037	(31.97 - 38.00) [28.17, 40.99]
Moisture (% FW)	7.52 (0.38) [5.86 - 10.70]	6.84 (0.38) [5.44 - 8.74]	0.68 (0.47) [-0.96 - 4.70]	-0.28, 1.64	0.159	(5.48 - 11.70) [1.45, 12.81]
Protein (% DW)	39.27 (0.86) [36.49 - 42.23]	37.80 (0.86) [32.29 - 41.87]	1.46 (0.54) [-0.73 - 6.49]	0.24, 2.68	0.023	(38.14 - 42.66) [35.30, 45.38]
Total Fat (% DW)	20.29 (0.78) [17.33 - 23.08]	20.12 (0.77) [17.24 - 22.55]	0.17 (0.39) [-1.82 - 1.98]	-0.71, 1.05	0.670	(17.90 - 23.56) [14.74, 25.18]
Vitamin						
Vitamin E (mg/100g DW)	7.69 (0.52) [6.36 - 9.62]	6.24 (0.52) [4.88 - 7.94]	1.45 (0.27) [0.57 - 2.25]	0.81, 2.09	<0.001	(1.65 - 8.08) [0, 11.09]

[1]DW = dry weight; FW = fresh weight; FA = fatty acid; S.E. = standard error; CI = Confidence Interval.
[2] N=14, sample size=14, with the exception of total fat (N=13)
[3]With 95% confidence, interval contains 99% of the values expressed in the population of commercial soybean varieties. Negative limits were set to zero.

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Table E-13. Literature and Historical Ranges for Components in Soybean Forage

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximate (% dw)		
Ash	5.36 – 8.91	6.72 – 10.78
Carbohydrates	62.25 – 72.28	59.8 – 74.7
Moisture (% fw)	68.50 – 78.40	73.5 – 81.6
Protein	16.48 – 24.29	14.38 – 24.71
Total Fat	2.65 – 9.87	1.30 – 5.13
Fiber (% dw)		
Acid Detergent Fiber (ADF)	23.86 – 50.69	not available
Neutral Detergent Fiber (NDF)	19.61 – 43.70	not available

[1]fw=fresh weight; dw=dry weight
[2]Lundry et al. (2008).
[3]ILSI Crop Composition Database,(2006).

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Table E-14. Literature and Historical Ranges for Components in Soybean Seed

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% dw)		
Ash	4.61 – 6.32	3.89 – 6.99
Carbohydrates	32.75 – 40.98	29.6 – 50.2
Moisture (% fw)	6.24 – 11.10	4.7 – 34.4
Protein	34.78 – 43.35	33.19 – 45.48
Total Fat	14.62 – 20.68	8.10 – 23.56
Fiber (% dw)		
Acid Detergent Fiber (ADF)	9.22 – 26.26	7.81 – 18.61
Neutral Detergent Fiber (NDF)	10.79 – 23.90	8.53 – 21.25
Amino Acids (% dw)		
Alanine	1.62 – 1.89	1.51-2.10
Arginine	2.57 – 3.27	2.29-3.40
Aspartic acid	4.16 – 5.02	3.81-5.12
Cystine/Cysteine	0.52 – 0.69	0.37-0.81
Glutamic acid	6.52 – 8.19	5.84-8.20
Glycine	1.59 – 1.90	1.46-2.00
Histidine	0.96 – 1.13	0.88-1.18
Isoleucine	1.59 – 2.00	1.54-2.08
Leucine	2.79 – 3.42	2.59-3.62
Lysine	2.36 – 2.77	2.29-2.84
Methionine	0.45 – 0.63	0.43-0.68
Phenylalanine	1.82 – 2.29	1.63-2.35
Proline	1.83 – 2.23	1.69-2.28
Serine	1.95 – 2.42	1.11-2.48
Threonine	1.44 – 1.73	1.14-1.86
Tryptophan	0.30 – 0.48	0.36-0.50
Tyrosine	1.27 – 1.53	1.02-1.61
Valine	1.68 – 2.09	1.60-2.20
Fatty Acids	**(% dw)**	**(% total)**
8:0 Caprylic	not available	0.148 – 0.148
10:0 Capric	not available	not available
12:0 Lauric	not available	0.082 – 0.132
14:0 Myristic	not available	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

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Table E-14 (continued). Literature and Historical Ranges for Components in Soybean Seed

Tissue/Component[1]	Literature Range[2]	ILSI Range[3]
Fatty Acids	**(% dw)**	**(% total)**
16:0 Palmitic	1.44 – 2.35	9.55 – 15.77
16:1 Palmitoleic	not available	0.086 – 0.194
17:0 Heptadecanoic	not available	0.085 – 0.146
17:1 Heptadecenoic	not available	0.073 – 0.087
18:0 Stearic	0.54 – 1.12	2.70 – 5.88
18:1 Oleic	2.87 – 8.82	14.3 – 32.2
18:2 Linoleic	6.48 – 11.6	42.3 – 58.8
18:3 Gamma Linolenic	not available	not available
18:3 Linolenic	0.72 – 2.16	3.00 – 12.52
20:0 Arachidic	0.04 – 0.7	0.163 – 0.482
20:1 Eicosenoic	0.026 – 0.057	0.140 – 0.350
20:2 Eicosadienoic	not available	0.077 – 0.245
20:3 Eicosatrienoic	not available	not available
20:4 Arachidonic	not available	not available
22:0 Behenic	0.044 – 0.073	0.277 – 0.595
22:1 Erucic	not available	not available
Vitamins (mg/100g dw)		
Vitamin E	1.29 – 4.80	0.19-6.17
Anti-Nutrients		
Lectin (H.U./mg fw)	0.45 – 9.95	0.09 – 8.46
Trypsin Inhibitor (TIU/mg dw)	20.79 – 59.03	19.59 – 118.68
Phytic Acid (% dw)	0.41 – 1.92	0.63 – 1.96
Isoflavones	**(µg/g dw)**	**(mg/kg dw)**
Daidzein	224.03 – 1485.52	60.0 – 2453.5
Genistein	338.24 – 1488.89	144.3 – 2837.2
Glycitein	52.72 – 298.57	15.3 – 310.4

[1]fw=fresh weight; dw=dry weight
[2]Lundry et al. (2008).
[3]ILSI Crop Composition Database,(2006).
Conversions: % dw x 10^4 = µg/g dw; mg/g dw x 10^3 = mg/kg dw; mg/100g dw x 10 = mg/kg dw; g/100g dw x 10 = mg/g dw

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Appendix F. Materials and Methods for Seed Dormancy and Germination Analyses of MON 87701

Materials

MON 87701, a conventional soybean control (A5547), and commercial soybean reference variety starting seed were produced in Washington County, MS; Barnwell County, SC; and Armstrong County, TX in 2007 (Table F-1).

Characterization of the Materials

For the MON 87701, conventional soybean control, and commercial soybean reference variety starting seed, the presence or absence of MON 87701 was verified by event-specific polymerase chain reaction (PCR) analyses. The results of these analyses confirmed the presence of MON 87701 in the test starting seed and the absence of MON 87701 in the control and reference variety seed with a few exceptions. One out of nine control seed samples and seven out of 36 reference seed samples across the three seed production sites contained ≤ 1.84% MON 87701. In addition, one of the control seed samples from the TX1 site contained ≤ 5.65% MON 87701, and one of the reference varieties seed samples from the MS site contained ≤ 3.05% MON 87701. Nevertheless, it was determined the levels of MON 87701 in the conventional soybean control and commercial soybean reference variety seed samples from the isolated plots were sufficiently low and did not negatively affect the quality of the evaluation or interpretation of the results.

Performing Facility and Experimental Methods

Dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was qualified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 2000, 2006, 2007).

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

Germination towels for MON 87701, control, and reference materials were prepared per facility SOPs. Each germination towel represented one replication. The types of data collected depended on the temperature regime. Each rolled germination towel in the AOSA-recommended temperature regime (i.e., 20/30 °C) was assessed periodically during the study for normal germinated, abnormal germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed as defined by AOSA guidelines (AOSA, 2006). Each rolled germination towel in the additional temperature regimes (i.e., 10, 20, 30, 10/20 and 10/30 °C) was assessed periodically during the study for germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed.

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Statistical Analysis

Statistical analyses were performed by the Monsanto Statistics Technology Center. The experimental design for the seed from each site was a randomized complete block with four replications, with the exception that the germination towels were arbitrarily arranged in each bucket and not necessarily randomized. SAS was used to compare MON 87701 to the conventional soybean control within each production site for the following germination characteristics: 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 α=0.05. The test substance was not statistically compared to the reference substances. The minimum and maximum mean values (reference range) were determined from the reference substances at each site.

Individual Site Seed Dormancy and Germination Analysis

After completion of the evaluation, it was determined the seed produced at the MS and SC field sites had high incidences of seed-borne disease (*Phomopsis spp*, *Cercospora spp*) that are documented to adversely affect seed germination (Pathan et al., 1989; Zorrilla et al., 1994). Therefore, it was determined that the data generated on the seed produced at the MS and SC sites were not appropriate for assessing the potential effects of the insect-protected trait on seed dormancy and germination characteristics (data presented in Table F-2). Thus, only the TX data were used to assess whether the introduction of the insect-protected trait altered the dormancy and germination characteristics of MON 87701 compared to the conventional control. Subsequent visual evaluation of seed from the MS and SC sites indicated visual differences in the incidence of seed-borne disease infection between MON 87701 and the parental control (A5547). These observations are further discussed in the environmental interaction section of this Petition (Section VIII.D.2.2).

No statistical differences were detected between MON 87701 and the control for percent viable firm-swollen seed in any temperature regime for seed produced at the MS or SC sites. At these sites, a total of 17 statistically significant differences were detected out of 48 comparisons between MON 87701 and the control (Table F-2). In the 20/30 ºC AOSA temperature regime, MON 87701 had lower normal germination than the control for seed produced at the MS (62.7 vs. 77.8%) and SC sites (35.8 vs. 47.8%). Percent abnormal germinated seed was also higher for MON 87701 than the control at the MS site (17.7 vs. 11.0%) in the 20/30 ºC AOSA temperature regime. Percent germinated seed was lower for MON 87701 than the control in the 10 ºC (86.0 vs. 94.5%), 20 ºC (74.7 vs. 85.8%), 30 ºC (70.8 vs. 84.0%), 10/20 ºC (79.8 vs. 90.8%), and 10/30 ºC (83.3 vs. 90.3%) temperature regimes for seed produced at the MS site and in the 10/20 ºC (69.5 vs. 81.8%) temperature regime for seed produced at the SC site (Table F-2). Percent dead seed was concomitantly higher for MON 87701 than the control in the 20/30 ºC AOSA temperature regime (19.7 vs. 11.3%) and in the 10 ºC (13.3 vs. 5.3%), 20 ºC (25.0 vs. 14.0%), 30 ºC (29.3 vs. 16.0%), and 10/20 ºC (20.3 vs. 9.3%),10/30 ºC (16.3 vs. 9.8%) temperature regimes for seed produced at the MS site and in the 10/20 ºC (28.5 vs.16.8%) temperature regime for seed produced at the SC site (Table F-2). Percent viable hard seed was higher for MON 87701 than the control (0.5 vs. 0.0%, or two hard

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seed vs. zero hard seed) in the 10/30 ºC temperature regime for seed produced at the MS site (Table F-2).

While there is no plausible hypothesis that the insect-protected trait would be associated with increased disease susceptibility, follow up evaluations of the starting seed from the MS and SC sites were conducted. This included a visual evaluation and a subsequent disease screening for the identified pathogen(s) to determine potential differences in the prevalence of seed-borne disease infection between MON 87701 and the conventional soybean control (A5547). The diseases were identified as *Phomopsis* complex and *Cercospora kikuchii*. This preliminary qualitative evaluation suggested that infection from the two seed-borne diseases may have been higher in seed of MON 87701 than the conventional soybean control.

A subsequent comparative disease screening was conducted on seed from multiple production environments, genetic backgrounds, and maturity groups. Each seed source consisted of MON 87701 and an appropriate control (Table F-3). Seed from each source was tested in a laboratory using a standard assay accepted by the seed industry (USDA NSHS) for incidence of *Phompsis* complex or *Cercospora kikuchii*. While seven statistical differences were detected out of 31 comparsions ($p \leq 0.05$), three differences indicated increased susceptibility and four indicated decreased susceptibility to these pathogens. Overall these results support a conclusion of no increase or decrease in the percentage of seed infected between MON 87701 and its control by *Phompsis* complex or *Cercospora kikuchii* (Tables F-4 and F-5). These results and their potential impact on altered disease susceptibility of MON 87701 are further discussed in the environmental interaction section of this Petition (Section VIII.D.2.2).

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Table F-1. Starting Seed of MON 87701, Control and Commercial Soybean Reference Varieties Used in Dormancy Assessment

Production Site	Substance Name	Substance Type	Phenotype[1]	Sample ID Number
MS	MON 87701	Test	Insect-protected	07199-001
MS	A5547	Control	Conventional	07199-004
MS	Asgrow A5427	Reference	Conventional	07199-008
MS	Asgrow A5403	Reference	Conventional	07199-009
MS	Asgrow A5560	Reference	Conventional	07199-010
MS	Asgrow A5843	Reference	Conventional	07199-011
SC	MON 87701	Test	Insect-protected	07199-002
SC	A5547	Control	Conventional	07199-005
SC	Delta & Pine 5634 RR	Reference	Glyphosate-tolerant[2]	07199-012
SC	Hornbeck C5894	Reference	Conventional	07199-013
SC	CMA5804A0C	Reference	Conventional	07199-014
SC	Asgrow A5959	Reference	Conventional	07199-015
TX	MON 87701	Test	Insect-protected	07199-003
TX	A5547	Control	Conventional	07199-006
TX	Delta & Pine 5414RR	Reference	Glyphosate-tolerant[2]	07199-007
TX	Delta & Pine 5989	Reference	Conventional	07199-016
TX	Crows C5215R	Reference	Glyphosate-tolerant[2]	07199-017
TX	Crows C5515R	Reference	Glyphosate-tolerant[2]	07199-018

[1] MON 87701 expresses the insect-protected trait; the control and reference soybean varieties do not express the insect-protected trait.
[2] Commercially-available glyphosate-tolerant (Roundup Ready 40-3-2) soybean variety.

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

Temp. Regime	Category	MS[1] Mean %[1]			SC[1] Mean %[1]			TX[1] Mean %[1]		
		MON 87701 (SE)	Control (SE)	Reference Range[2]	MON 87701 (SE)	Control (SE)	Reference Range[2]	MON 87701 (SE)	Control (SE)	Reference Range[2]
10°C	Germinated	86.0* (4.3)	94.5 (1.8)	72.0 – 96.0	71.0 (3.5)	80.0 (5.4)	57.3 – 89.7	96.7 (0.3) §	93.8 (1.4)	86.0 – 98.8
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 – 3.3	2.3 (0.3)	2.3 (1.3)	4.0 –15.5	0.3 (0.3) §	0.8 (0.3)	0.3 – 4.3
	Dead	13.3* (3.7)	5.3 (1.6)	4.0 – 24.5	25.5 (2.9)	16.5 (4.1)	4.7 – 23.3	2.7 (0.3) §	5.5 (1.3)	0.8 – 9.0
	Viable Firm Swollen	0.5 (0.5)	0.3 (0.3)	0.0 – 1.0	1.3 (0.5)	1.3 (0.5)	1.3 – 4.0	0.3 (0.3) §	0.0 (0.0)	0.0 – 0.8
20°C	Germinated	74.7* (2.7) §	85.8 (1.3)	62.8 – 92.5	64.0 (1.0)	69.0 (3.2) §	69.5 – 89.3	95.0 (0.7)	94.5 (1.2)	89.3 – 98.3
	Viable Hard	0.3 (0.3) §	0.3 (0.3)	0.0 – 1.0	0.8 (0.5)	1.3 (0.9) §	2.0 – 6.3	0.0* (0.0)	0.5 (0.3)	0.0 – 4.3
	Dead	25.0* (2.6) §	14.0 (1.4)	7.5 – 36.5	35.3 (1.3)	29.7 (3.8) §	8.3 – 23.8	5.0 (0.7)	5.0 (0.9)	1.8 – 6.3
	Viable Firm Swollen	0.0† (0.0) §	0.0 (0.0)	0.0 – 0.0	0.0 (0.0)	0.0 (0.0) §	0.3 – 0.5	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
30°C	Germinated	70.8* (1.4)	84.0 (1.5)	53.8 – 88.3	58.8 (1.4)	64.8 (4.2)	67.3 – 87.3	97.5 (0.3)	97.3 (0.9)	90.8 – 99.3
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.5 (0.5)	0.5 (0.3)	0.3 – 6.3	0.3 (0.3)	0.0 (0.0)	0.0 – 0.3
	Dead	29.3* (1.4)	16.0 (1.5)	11.8 – 45.8	40.8 (1.0)	34.8 (4.3)	12.3 – 26.5	2.3 (0.3)	2.8 (0.9)	0.8 – 6.3
	Viable Firm Swollen	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3

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

Temp. Regime	Category	MS[1] Mean %[1]			SC[1] Mean %[1]			TX[1] Mean %[1]		
		MON 87701 (SE)	Control (SE)	Reference Range[2]	MON 87701 (SE)	Control (SE)	Reference Range[2]	MON 87701 (SE)	Control (SE)	Reference Range[2]
10/20°C	Germinated	79.8* (0.8)	90.8 (0.8)	63.7 – 96.0	69.5* (1.6)	81.8 (1.7)	69.8 – 91.0	94.8 (1.4)	93.8 (1.6)	86.3 – 99.5
	Viable Hard	0.0 (0.0)	0.0 (0.0)	0.3 – 2.7	1.5 (0.6)	1.5 (0.6)	2.3 – 13.0	0.5 (0.3)	0.3 (0.3)	0.0 – 6.5
	Dead	20.3* (0.8)	9.3 (0.8)	3.8 – 33.3	28.5* (2.2)	16.8 (1.6)	6.0 – 16.5	4.8 (1.7)	6.0 (1.5)	0.5 – 7.3
	Viable Firm Swollen	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.5 (0.3)	0.0 (0.0)	0.0 – 1.8	0.0† (0.0)	0.0 (0.0)	0.0 – 0.0
10/30°C	Germinated	83.3* (2.4)	90.3 (1.4)	63.0 – 95.5	72.3 (2.3)	74.8 (1.4)	71.5 – 92.0	94.8 (1.1)	98.8 (0.6)	92.3 – 98.3
	Viable Hard	0.5* (0.3)	0.0 (0.0)	0.0 – 2.3	0.8 (0.5)	1.0 (0.6)	1.3 – 8.3	0.8 (0.5)	0.5 (0.3)	0.0 – 3.5
	Dead	16.3* (2.3)	9.8 (1.4)	4.5 – 34.3	27.0 (2.5)	24.3 (1.9)	5.5 – 20.3	4.5 (0.9)	0.8 (0.8)	1.8 – 4.0
	Viable Firm Swollen	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3	0.0 (0.0)	0.0 (0.0)	0.0 – 0.5	0.0 (0.0)	0.0 (0.0)	0.0 – 0.3
20/30°C (AOSA)	Normal	62.7* (2.8)	77.8 (2.6)	39.3 – 85.0	35.8* (2.2)	47.8 (1.8)	44.8 – 68.0	72.3 (3.9) §	67.3 (4.4)	52.3 – 84.5
	Abnormal	17.7* (0.9)	11.0 (0.7)	8.3 – 19.5	24.8 (1.4)	20.3 (0.9)	16.5 – 22.3	25.7 (4.4) §	30.0 (3.7)	14.0 – 39.0
	Viable Hard	0.0 (0.0) §	0.0 (0.0)	0.0 – 2.0	1.8 (0.9)	2.0 (0.4)	2.0 – 6.5	0.7 (0.3) §	0.8 (0.5)	0.0 – 4.3
	Dead	19.7* (2.9)	11.3 (2.7)	6.8 – 39.0	37.3 (3.1)	29.8 (1.8)	13.5 – 26.0	1.3 (0.7) §	1.8 (0.5)	1.3 – 4.3
	Viable Firm Swollen	0.0† (0.0)	0.0 (0.0)	0.0 – 0.0	0.5 (0.5)	0.3 (0.3)	0.0 – 0.5	0.0 (0.0) §	0.3 (0.3)	0.0 – 0.3

Note: The experimental design was a randomized complete block within each site. SE = Standard Error. Means based on four replicates (N=4) of 100 seed except where denoted by §, in which means are based on three replicates (N=3) of 100 seeds.

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

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

[1] In some instances, the total percentage of both MON 87701 and the control did not equal exactly 100% due to numerical rounding of the means.

[2] Reference range is the minimum and maximum mean value observed among the four reference soybean varieties.

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

Production Site[1]	Material Type[2]	Production Year	Material Name[3]	Phenotype	Monsanto Source Lot
MS	Control	2007	A5547	Conventional	11217152
MS	Test	2007	MON 87701	Insect-Protected	11217153
MS	Reference	2007	A5427	Conventional	11217154
MS	Reference	2007	A5403	Conventional	11217155
MS	Reference	2007	A5560	Conventional	11217156
MS	Reference	2007	A5843	Conventional	11217157
SC	Control	2007	A5547	Conventional	11219208
SC	Test	2007	MON 87701	Insect-Protected	11219209
SC	Reference	2007	HBK C5894	Conventional	11219210
SC	Reference	2007	CMA5804A0C	Conventional	11219211
SC	Reference	2007	A5959	Conventional	11219212
SC	Reference	2007	DP 5634 RR	Glyphosate-tolerant[4]	11219213
TX	Control	2007	A5547	Conventional	11219214
TX	Test	2007	MON 87701	Insect-Protected	11219215
TX	Reference	2007	DP 5989	Conventional	11219216
TX	Reference	2007	DP 5414 RR	Glyphosate-tolerant[4]	11219217
TX	Reference	2007	C5215R	Glyphosate-tolerant[4]	11219218
TX	Reference	2007	Crows C5515R	Glyphosate-tolerant[4]	11219219
AL	Reference	2007	H6686	Conventional	11219225
AL	Control	2007	A5547	Conventional	11219554
AL	Reference	2007	CMA5804A0C	Conventional	11219224
AL	Reference	2007	A5959	Conventional	11219223
AL	Reference	2007	A5843	Conventional	11219222
AL	Test	2007	MON 87701	Insect-Protected	11219221
AR	Test	2007	MON 87701	Insect-Protected	11219227
AR	Reference	2007	Anand	Conventional	11219230
AR	Reference	2007	UA4805	Conventional	11219228
AR	Reference	2007	Hornbeck C5894	Conventional	11219231
AR	Control	2007	A5547	Conventional	11219226
AR	Reference	2007	Ozark	Conventional	11219229
GA	Reference	2007	A5560	Conventional	11219234
GA	Reference	2007	CMC5901C0C	Conventional	11219235
GA	Reference	2007	Lee74	Conventional	11219236
GA	Test	2007	MON 87701	Insect-Protected	11219233
GA	Reference	2007	A5403	Conventional	11219237
GA	Control	2007	A5547	Conventional	11219232
IL	Test	2007	MON 87701	Insect-Protected	11219406
IL	Control	2007	A5547	Conventional	11219407
IL	Reference	2007	A4922	Conventional	11219408
IL	Reference	2007	A5427	Conventional	11219416
IL	Reference	2007	H4994	Conventional	11219412
IL	Reference	2007	H5218	Conventional	11219415
NC	Test	2007	MON 87701	Insect-Protected	11219418
NC	Control	2007	A5547	Conventional	11219417
NC	Reference	2007	DP 5989	Conventional	11219419
NC	Reference	2007	USG 5601T	Conventional	11219421
NC	Reference	2007	Fowler	Conventional	11219427
NC	Reference	2007	Hutcheson	Conventional	11219420

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

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

[1] MS = Washington County, Mississippi; SC = Barnwell County South Carolina; TX = Armstrong County, Texas; AL = Baldwin County, Alabama; AR = Jackson County, Arkansas; GA = Clarke County, Georgia; IL = Jackson County, Illinois; NC = Wayne County, North Carolina; ARNE = Jackson County, Arkansas; GACH = Tift County, Georgia; SCEK = Barnwell County, South Carolina; MSLE = Washington County, Mississippi; PR = Isabella, Puerto Rico.

[2] MON 87701 expresses the insect-protected trait. The control, reference varieties, and negative (-) isoline do not express the insect-protected trait.

[3] MON 87701 and conventional soybean control, with the exception of those materials followed by an asterisk (*) all have the A5547 genetic background. The genetic background of MON 87701 and conventional soybean control denoted by an asterisk are provided parenthetically.

[4] Commercially-available glyphosate-tolerant (Roundup Ready 40-3-2) soybean variety.

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

Sites[1]	Genetic Background	MON 87701	MON 87701(-)	Control	Reference Range[2]	
		Mean (SE)	Mean (SE)	Mean (SE)	Minimum	Maximum
MS	A5547	39.5 (1.0)	–	31.3 (3.8)	23.3	57.0
SC	A5547	14.8 (3.1)	–	17.5 (1.0)	3.5	10.0
TX	A5547	0.0† (0.0)	–	0.0 (0.0)	0.0	0.0
AL	A5547	89.3 (3.9)	–	86.0 (1.1)	41.8	87.0
AR	A5547	2.5 (0.3)	–	3.5 (1.8)	0.0	4.0
GA	A5547	1.5 (0.6)	–	1.8 (0.5)	0.3	6.0
IL	A5547	0.0 (0.0)	–	0.0 (0.0)	0.0	2.0
NC	A5547	7.8* (2.5)	–	0.8 (0.5)	4.8	10.8
ARNE	A5547	0.5 (0.3)	–	0.0 (0.0)	0.0	0.5
GACH	A5547	16.5 (1.6)	–	17.5 (0.6)	11.8	30.3
SCEK	A5547	7.3 (1.7)	–	7.8 (0.9)	10.8	20.8
MSLE (2002)	A5547	0.8 (0.3)	2.0 (0.9)	–	–	–
PR (2002)	A5547	0.0† (0.0)	0.0 (0.0)	–	–	–
PR (2005)	A5547	0.3* (0.3)	–	1.0 (0.0)	–	–
PR (2005)	A5602	0.3 (0.3)	–	0.3 (0.3)	–	–
PR (2007)	M-Soy 8329	1.8 (0.9)	2.3 (0.3)	0.3 (0.3)	–	–

Note: The experimental design was a completely randomized design (CRD) within each set of sites separated by a bold line. SE = Standard Error. Means based on four replications (N=4) of 100 seed from each lot evaluated. MON 87701 (-) indicates negative isoline.

* Indicates a significant difference was detected between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

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

[1] MS = Washington County, Mississippi; SC = Barnwell County, South Carolina; TX = Armstrong County, Texas = CRD1. AL = Baldwin County, Alabama; AR = Jackson County, Arkansas; GA = Clarke County, Georgia; IL = Jackson County, Illinois; NC = Wayne County, North Carolina = CRD2; ARNE = Jackson County, Arkansas; GACH = Tift County, Georgia; SCEK = Barnwell County, South Carolina = CRD3. MSLE = Washington County, Mississippi; PR = Isabella, Puerto Rico = CRD4

[2] Reference range = Minimum and maximum mean values from among the 12 (CRD1), 8 (CRD2) and 20 (CRD3) commercially-available reference soybean varieties.

Dash (−) indicates seed was not available for evaluation.

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

Sites[1]	Genetic Background	MON 87701 Mean (SE)	MON 87701(-) Mean (SE)	Control Mean (SE)	Reference Range[2] Minimum	Reference Range[2] Maximum
MS	A5547	5.5 (2.4)	–	1.8 (1.0)	1.0	6.5
SC	A5547	4.0 (1.5)	–	4.0 (0.7)	1.5	2.3
TX	A5547	0.8 (0.5)	–	0.5 (0.5)	0.0	0.0
AL	A5547	0.0 (0.0)	–	0.0 (0.0)	0.0	1.8
AR	A5547	10.8 (1.7)	–	6.8 (1.3)	0.0	0.8
GA	A5547	7.5 (1.8)	–	9.0 (1.9)	0.0	5.3
IL	A5547	17.5 (2.5)	–	12.3 (1.7)	1.8	14.0
NC	A5547	12.0* (1.5)	–	21.3 (4.8)	2.0	13.3
ARNE	A5547	0.8 (0.3)	–	1.0 (0.4)	0.0	1.3
GACH	A5547	14.5* (0.9)	–	8.8 (1.0)	2.3	5.5
SCEK	A5547	23.5* (0.5)	–	15.8 (2.5)	2.8	17.3
MSLE (2002)	A5547	24.5 (4.6)	33.8 (4.1)	–	–	–
PR (2002)	A5547	0.0† (0.0)	0.0 (0.0)	–	–	–
PR (2005)	A5547	4.5* (1.7)	–	14.0 (2.7)	–	–
PR (2005)	A5602	2.0* (0.9)	–	13.8 (1.8)	–	–
PR (2007)	M-Soy 8329	38.8 (1.9)	33.5 (4.0)	36.0 (2.1)	–	–

Note: The experimental design was a completely randomized design (CRD) within each set of sites separated by a bold line. SE = Standard Error. Means based on four replications (N=4) of 100 seed from each lot evaluated. MON 87701(-) indicates negative isoline.

* Indicates a significant difference was detected between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

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

[1] MS = Washington County, Mississippi; SC = Barnwell County, South Carolina; TX = Armstrong County, Texas = CRD1. AL = Baldwin County, Alabama; AR = Jackson County, Arkansas; GA = Clarke County, Georgia; IL = Jackson County, Illinois; NC = Wayne County, North Carolina = CRD2; ARNE = Jackson County, Arkansas; GACH = Tift County, Georgia; SCEK = Barnwell County, South Carolina = CRD3. MSLE = Washington County, Mississippi; PR = Isabella, Puerto Rico = CRD4

[2] Reference range = Minimum and maximum mean values from among the 12 (CRD1), 8 (CRD2) and 20 (CRD3) commercially-available reference soybean varieties.

Dash (−) indicates seed was not available for evaluation.

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

Materials

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

Field Sites and Plot Design

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

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

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

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Table G-1. Starting Seed for Phenotypic Assessments

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

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

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

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

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Table G-2. Field and Planting Information

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

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

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

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

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

Planting and Field Operations

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

Phenotypic Observations

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

Ecological Observations

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

Abiotic Stress Response, Disease Damage, and Arthropod Damage

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

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

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

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

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

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

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

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

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

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

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

Arthropod Abundance

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

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

Arthropods were collected using a beat sheet sampling method (Kogan and Pitre, 1980). The beat sheet was an approximately 36 × 42 inch white, vinyl sheet spread between the plants of two adjacent rows. Plants were shaken vigorously along the length of each side of the beat sheet to dislodge arthropods from the plants. A total of four sub-samples were

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

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

Ecological Interactions Evaluation Criteria

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

Data Assessment

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

Statistical Analysis

Analysis of variance was conducted according to a randomized complete block design using SAS (Version 9.1.3). The level of significance was α=0.05. MON 87701 was compared to the control substance within each site (individual-site analysis) and pooled across all sites (combined-site analysis) for early stand count, seedling vigor, days to 50% flowering, plant height, lodging, shattering, final stand count, seed moisture, 100 seed weight, seed test weight, and yield. Yield data from the SC site were calculated using standard soybean moisture of 13% as opposed to using measured seed moisture from the SC site (as was done for the other sites) due to measurements being taken by a moisture

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meter with poor resolution. Therefore, yield from the SC site was statistically analyzed within the site, but was not included in the combined-site analysis. Growth stage, flower color, plant pubescence, abiotic stress response, disease damage, and arthropod damage data were not statistically analyzed. Arthropod pest abundance and beneficial arthropod abundance data were statistically analyzed only within individual collection intervals and sites due to the variation in temporal activity and geographical distribution of the taxa.

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

Individual Field Site Plant Growth and Development Results and Discussion

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

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References:

Kogan, M. and H. N. Pitre. 1980. General Sampling Methods for Above-Ground. In Populations of Soybean Arthropods. New York, Springer-Verlag. Pp 30-60.

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

	Phenotypic Characteristic (units)									
	Early stand count (#/plot)		Seedling vigor (1-9 scale)		Days to 50% flowering		Flower color[2]		Plant pubescence[2]	
Site[1]	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701	Control	MON 87701	Control
AL	256.3 (7.22)	272.3 (4.98)	3.0 (0.00)	3.0 (0.00)	199.0† (0.00)	199.0 (0.00)	White	White	Hairy	Hairy
AR1	182.7 (6.84)	179.7 (21.36)	4.0 (0.00)	4.7 (0.33)	198.0 (0.58)	198.7 (0.33)	White	White	Hairy	Hairy
AR2	211.3 (30.69)	248.0 (11.55)	4.3 (1.20)	5.0 (0.58)	195.0 (1.15)	195.0 (1.15)	White	White	Hairy	Hairy
AR3	166.0 (12.86)	190.0 (10.41)	4.3 (0.33)	4.0 (0.00)	207.3* (0.33)	208.7 (0.33)	White	White	Hairy	Hairy
GA1	229.0 (33.13)	217.0 (16.17)	4.3 (0.33)	4.0 (0.00)	210.0* (0.00)	209.3 (0.33)	White	White	Hairy	Hairy
GA2	167.3 (11.70)	203.7 (6.49)	3.7 (0.33)	3.3 (0.33)	209.7 (0.33)	207.7 (0.33)	White	White	Hairy	Hairy
IL	235.7 (8.29)	241.0 (5.20)	3.7 (0.33)	3.7 (0.33)	217.3* (0.33)	218.7 (0.33)	White	White	Hairy	Hairy
IN	243.7 (18.77)	220.7 (31.93)	5.0* (0.00)	5.7 (0.33)	213.7*(0.33)	217.3 (0.33)	White	White	Hairy	Hairy
KS	272.7 (10.17)	269.0 (7.23)	5.0† (0.00)	5.0 (0.00)	227.0 (0.00)	227.3 (0.33)	White	White	Hairy	Hairy
LA1	325.7* (14.84)	274.3 (4.67)	4.0 (1.00)	3.0 (1.00)	217.0† (0.00)	217.0 (0.00)	White	White	—	—
LA2	248.0 (11.36)	245.3 (10.17)	4.7 (0.33)	4.3 (0.67)	189.7 (0.33)	190.0 (0.00)	White	White	Hairy	Hairy
MS	206.3 (35.53)	185.7 (25.10)	3.3 (1.86)	5.0 (1.00)	185.3 (2.33)	187.7 (2.33)	White	White	Hairy	Hairy
NC	224.3 (9.06)	230.7 (8.51)	2.0 (0.00)	2.0 (0.00)	211.0† (0.00)	211.0 (0.00)	White	White	Hairy	Hairy
SC	276.0 (5.00)§	286.7 (2.85)	2.5 (0.50) §	3.0 (0.58)	192.0† (0.00)§	192.0 (0.00)	White	White	Hairy	Hairy
TX1	—	—	1.3 (0.33)	1.0 (0.00)	208.0 (0.00)	208.0 (0.00)	White	White	Hairy	Hairy
TX2	—	—	3.0 (0.00)	3.3 (0.33)	219.0 (0.00)	219.0 (0.00)	White	White	Hairy	Hairy

Note: The experimental design was a randomized complete block. SE = Standard Error. Mean based on three replicates (N=3) except where denoted by §, in which means are based on two replicates (N=2).

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

† No statistical comparisons were made due to lack of variability in the data.

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

[2] Flower color and plant pubescence data were categorical and were not statistically analyzed.

Dash (—) indicates data not available or excluded from the data analysis.

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

	Phenotypic Characteristic (units)							
	Plant height (in)		Lodging (0-9 scale)		Pod Shattering (0-9 scale)		Final stand count (#/plot)	
Site[1]	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)
AL	31.6 (1.03)	31.3 (0.41)	2.3 (0.33)	2.3 (0.33)	0.0† (0.00)	0.0 (0.00)	231.3* (10.68)	264.0 (9.29)
AR1	26.5 (1.31)	25.4 (1.00)	3.0 (0.00)	2.7 (0.67)	0.0† (0.00)	0.0 (0.00)	171.3 (8.01)	172.7 (13.86)
AR2	37.2 (1.10)	35.8 (1.47)	0.3 (0.33)	1.3 (0.33)	0.0† (0.00)	0.0 (0.00)	203.7 (17.74)	231.3 (9.28)
AR3	35.9 (0.68)	32.8 (1.27)	5.7 (0.33)	5.0 (1.00)	0.0† (0.00)	0.0 (0.00)	157.0 (7.23)	183.7 (12.00)
GA1	28.2 (1.67)	25.5 (1.12)	1.7 (0.67)	1.3 (0.33)	—	—	163.3 (9.39)	172.0 (14.64)
GA2	28.3 (1.19)	30.0 (1.55)	0.0† (0.00)	0.0 (0.00)	0.0 (0.00)	0.3 (0.33)	153.3 (10.99)	172.7 (2.40)
IL	33.0 (0.95)	32.3 (0.84)	0.0* (0.00)	1.0 (00.0)	0.0† (0.00)	0.0 (0.00)	241.7 (10.90)	239.7 (13.64)
IN	36.7 (0.84)	35.8 (1.11)	2.7 (0.33)	3.0 (0.58)	1.0 (0.58)	1.0 (0.00)	197.0 (10.15)	185.7 (22.53)
KS	27.3 (0.82)	26.4 (0.87)	1.7 (0.33)	1.7 (0.33)	2.0 (0.00)	1.7 (0.33)	252.0 (10.02)	238.0 (13.23)
LA1	29.9 (0.96)	30.1 (0.64)	0.0† (0.00)	0.0 (0.00)	0.0† (0.00)	0.0 (0.00)	322.3* (16.19)	273.0 (4.00)
LA2	31.1 (0.27)	29.7 (0.37)	3.0 (0.58)	2.7 (0.33)	0.0 (0.00)	0.3 (0.33)	213.0 (10.41)	209.7 (6.64)
MS	33.4 (2.27)	31.1 (0.90)	4.3 (0.67)	4.0 (0.58)	0.0† (0.00)	0.0 (0.00)	135.3 (22.17)	127.7 (11.46)
NC	38.0* (0.92)	34.6 (0.64)	6.7* (0.33)	3.3 (0.33)	1.7* (0.33)	0.7 (0.33)	214.3 (1.45)	232.3 (5.49)
SC	32.7 (0.05)§	28.8 (1.76)	1.0 (1.00)§	0.3 (0.33)	3.0† (0.00)§	1.0 (0.00)	244.0 (5.00)§	261.3 (8.69)
TX1	33.7 (0.42)	33.9 (0.48)	0.0† (0.00)	0.0 (0.00)	1.7 (0.33)	0.7 (0.33)	—	—
TX2	26.9 (2.45)	28.1 (0.29)	0.0† (0.00)	0.0 (0.00)	—	—	—	—

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3) except where denoted by §, in which means are based on two replicates (N=2).

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

† No statistical comparisons were made due to lack of variability in the data.

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

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

	Phenotypic Characteristic (units)							
	100 seed weight (g)		Seed moisture (%)		Test weight (lb/bu)		Yield (bu/ac)	
Site[1]	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)	MON 87701 Mean (SE)	Control Mean (SE)
AL	—	—	—	—	—	—	—	—
AR1	18.5 (0.09)	18.3 (0.38)	12.7 (0.19)	12.9 (0.69)	53.3 (0.35)	53.5 (0.09)	74.6 (3.06)	74.7 (2.43)
AR2	15.8 (0.02)	15.8 (0.06)	10.4 (0.06)	10.5 (0.06)	51.7 (0.17)	51.7 (0.09)	74.2 (0.84)	75.1 (3.48)
AR3	20.7 (0.22)	20.2 (0.46)	11.6* (0.07)	10.6 (0.07)	54.2 (0.03)	53.9 (0.15)	73.0 (2.08)	71.3 (4.13)
GA1	—	—	—	—	—	—	—	—
GA2	17.2 (0.29)	17.2 (0.12)	13.8 (0.55)	13.5 (0.12)	53.6 (2.25)	55.7 (0.56)	13.4* (3.06)	29.4 (1.92)
IL	15.7 (0.32)	15.3 (0.70)	12.0 (0.15)	12.5 (0.20)	59.7 (0.33)	59.0 (0.00)	43.3 (2.87)	40.9 (1.64)
IN	16.5 (0.21)	15.9 (0.43)	12.5 (0.42)	12.0 (1.22)	56.9 (1.77)	58.4 (1.33)	30.4* (1.22)	34.4 (2.45)
KS	13.6 (0.15)	13.2 (0.39)	8.6 (0.06)	8.6 (0.12)	55.2 (2.48)	54.0 (1.16)	48.6 (4.94)	48.6 (2.82)
LA1	16.7 (0.70)	16.4 (0.66)	17.5* (0.07)	16.5 (0.20)	49.7 (0.67)	49.3 (0.88)	36.4 (2.27)	31.8 (1.72)
LA2	19.8 (0.89)	19.6 (0.23)	16.3* (0.75)	15.0 (0.19)	49.7 (0.33)	51.7 (0.33)	36.1* (0.60)	46.5 (1.94)
MS	18.2 (0.44)	17.5 (0.29)	14.3* (0.57)	13.2 (0.09)	53.1 (1.09)	52.3 (1.72)	45.9 (2.05)	54.8 (3.87)
NC	13.6 (0.58)	14.2 (0.64)	13.2 (0.19)	13.5 (0.32)	53.3 (0.55)	54.3 (0.03)	60.1 (4.95)	62.4 (5.30)
SC	17.9 (1.00)[§]	17.0 (0.90)	—	—	66.8 (1.50)[§]	69.6 (0.67)	29.0 (1.69)[§]	26.1 (0.99)
TX1	14.9 (0.67)	14.0 (0.30)	13.6 (0.26)	14.1 (0.12)	60.2 (0.93)	59.3 (0.50)	62.9* (1.04)	58.8 (2.07)
TX2	—	—	—	—	—	—	—	—

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3) of except where denoted by [§], in which means are based on two replicates (N=2).

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

[†] No statistical comparisons were made due to lack of variability in the data.

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

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

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

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

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

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

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

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

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

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

[1] Site codes are as follows: LA2 = Rapides Parish, LA; MS = Washington County, MS; NC = Wayne County, NC; SC = Barnwell County, SC.
[2] Obs. = Observation number; dates in month/day/year format
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Table G-4 (continued). Growth Stage Monitoring of MON 87701, the Conventional Soybean Control, and the Reference Soybean Varieties

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

[1] Site codes are as follows: ; TX1 = Armstrong County, TX; TX2 = Hockley County, TX.
[2] Obs. = Observation number; dates in month/day/year format.
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Table G-5. Abiotic Stressor Evaluation Using Observational Severity Scale for MON 87701 and the Conventional Soybean Control

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

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

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

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

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

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

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

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

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

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*Indicates a difference observed between MON 87701 and the control for green cloverworms at AR1 site (none vs. moderate; Observation 4) and the LA1 site (slight vs. moderate; Observation 4); soybean loopers at the LA1 site (slight vs. moderate; Observation 4); velvetbean caterpillars at the LA1 site (slight vs. moderate; Observation 4); bean leaf beetles at the AR1 site (none vs. moderate; Observation 4) and the LA2 site (none vs. slight; Observation 3). Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with three replications. Observational data were collected at four crop development stages with the exception of the GA1 site where data were collected at only the first three observations, Observation 1 = V2-V4, Observation 2 = R1-R2, Observation 3 = R3-R5, and Observation 4 = R6-R8.

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

[2] Includes soybean aphids.

[3] Includes green stink bugs.

[4] Includes fall armyworms and beet armyworms.

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

Arthropod	Site[1]	Abundance of Pest Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
		MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]
Bean leaf beetle	GA1	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	—	—	—
	LA1	2.0 (1.53)	1.3 (0.67)	0.0 – 1.3	0.3 (0.33)	0.3 (0.33)	0.3 – 3.3	2.0 (0.00)[§]	3.0 (1.15)	2.0 – 7.5
	SC	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)	0.0 (0.00)	0.0 – 0.3
	TX1	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	—	—	—
Corn earworm	SC	—	—	—	0.0* (0.00)	4.3 (1.45)	0.0 – 5.3	0.7* (0.67)	4.0 (1.15)	0.7 – 2.3
Green cloverworm	GA1	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	—	—	—
	LA1	0.3 (0.33)	2.0 (1.00)	0.0 – 1.3	0.0 (0.00)	1.0 (1.00)	1.3 – 2.7	0.0[†] (0.00)[§]	0.0 (0.00)	0.0 – 0.0
	SC	1.3 (1.33)	5.0 (2.08)	3.7 – 10.0	0.0 (0.00)	4.7 (2.03)	4.7 – 5.3	52.7 (26.98)	58.3 (14.84)	63.0 – 83.3
	TX1	0.0 (0.00)	0.0 (0.00)	0.0 – 1.0	0.3* (0.33)	16.7 (3.18)	25.3 – 45.7	—	—	—
Potato leafhopper	SC	0.0 (0.00)	0.0 (0.00)	0.0 – 1.7	—	—	—	—	—	—
Soybean looper	GA1	—	—	—	0.0 (0.00)	2.0 (2.00)	2.0 – 16.0	—	—	—
	LA1	—	—	—	0.3 (0.33)	1.0 (0.58)	0.0 – 1.3	0.0 (0.00)[§]	1.0 (1.00)	0.0 – 0.5
	SC	—	—	—	0.0 (0.00)	2.3 (1.86)	1.3 – 3.7	0.0* (0.00)	8.7 (1.20)	7.3 – 14.7

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3) of except where denoted by [§], in which means are based on two replicates (N=2). Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

[†] No statistical comparisons were made due to lack of variability in the data.

[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.

[2] Reference range is the minimum and maximum mean value observed among the four reference soybean varieties.

Dash (—) indicates arthropod not evaluated.

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

Arthropod	Site[1]	Abundance of Pest Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
		MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]
Stink bug	GA1	0.0 (0.00)	0.0 (0.00)	0.0 – 0.3	11.3 (4.67)	18.0 (4.93)	6.0 – 29.7	—	—	—
	LA1	0.3 (0.33)	0.3 (0.33)	0.0 – 3.3	2.0 (1.53)	6.3 (2.33)	2.3 – 7.7	3.5 (2.50) [§]	3.7 (1.20)	1.5 – 5.7
	SC	4.3 (2.19)	5.3 (3.84)	0.7 – 10.3	12.3 (2.96)	14.3 (1.86)	6.7 – 16.3	38.0* (38.00)	85.0 (8.54)	26.0 – 59.7
	TX1	0.0 (0.00)	0.0 (0.00)	0.0 – 0.7	1.3 (0.67)	0.0 (0.00)	1.3 – 1.3	—	—	—
Thrips	SC	231.3 (15.32)	116.0 (101.37)	114.7 – 220.0	—	—	—	—	—	—
Velvetbean caterpillar	GA1	—	—	—	0.0 (0.00)	0.3 (0.33)	1.0 – 1.7	—	—	—
Webworm	GA1	—	—	—	0.0* (0.00)	6.7 (2.33)	4.0 – 16.0	—	—	—
	SC	—	—	—	0.3* (0.33)	8.7 (1.67)	9.3 – 30.7	0.0* (0.00)	4.7 (0.67)	1.7 – 4.7

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3) of except where denoted by [§], in which means are based on two replicates (N=2). Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

[†] No statistical comparisons were made due to lack of variability in the data.

[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.

[2] Reference range is the minimum and maximum mean value observed among the four reference soybean varieties.

Dash (—) indicates arthropod not evaluated.

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

		Abundance of Beneficial Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	Site[1]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]
Spiders	GA1	0.7 (0.33)	1.0 (0.58)	0.0 – 1.0	4.7 (0.33)	5.0 (2.08)	2.7 – 6.0	—	—	—
	LA1	4.3 (2.33)	4.7 (2.03)	3.0 – 4.0	1.3 (0.88)	0.3 (0.33)	0.3 – 3.3	0.0 (0.00) §	0.3 (0.33)	0.0 – 1.0
	SC	6.3 (3.18)	6.7 (0.33)	4.3 – 5.3	2.3 (1.20)	2.7 (0.88)	3.3 – 9.7	1.0 (0.58)	1.0 (0.58)	0.0 – 3.3
	TX1	5.0 (1.00)	4.3 (2.19)	0.0 – 2.7	1.7 (0.67)	0.0 (0.00)	1.3 – 4.7	—	—	—
Big-eyed bug	GA1	0.3 (0.33)	1.3 (1.33)	0.3 – 2.0	30.7 (6.57)	53.0 (13.05)	11.7 – 70.7	—	—	—
	SC	10.7 (1.45)	7.3 (2.33)	3.3 – 10.7	3.7 (1.76)	4.3 (1.45)	3.0 – 7.7	6.0 (1.00)	8.7 (6.67)	6.0 – 8.7
	TX1	—	—	—	2.3 (1.20)	1.3 (0.67)	0.3 – 1.0	—	—	—
Carabids	LA1	1.0 (0.58)	0.0 (0.00)	0.0 – 1.0	0.7 (0.67)	0.0 (0.00)	0.0 – 2.0	—	—	—
Lacewing	GA1	0.3 (0.33)	0.3 (0.33)	0.0 – 1.0	—	—	—	—	—	—
Ladybird beetle	GA1	0.0 (0.00)	0.3 (0.33)	0.0 – 3.0	2.0 (1.53)	2.0 (1.15)	1.0 – 7.3	—	—	—
	LA1	—	—	—	—	—	—	0.0 (0.00) §	0.7 (0.67)	0.5 – 1.3
	SC	1.7 (0.88)	0.3 (0.33)	0.0 – 1.3	0.0* (0.00)	1.7 (0.67)	0.3 – 2.3	—	—	—
	TX1	0.3 (0.33)	0.0 (0.00)	0.0 – 0.7	0.7 (0.33)	0.3 (0.33)	0.3 – 2.3	—	—	—

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3) of except where denoted by §, in which means are based on two replicates (N=2). Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5, and Collection 3 = R6-R8.

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

† No statistical comparisons were made due to lack of variability in the data.

[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.

[2] Reference range is the minimum and maximum mean value observed among the four reference soybean varieties.

Dash (—) indicates arthropod not evaluated.

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

		Abundance of Beneficial Arthropods[2]								
		Collection 1			Collection 2			Collection 3		
Arthropod	Sites[1]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]	MON 87701 Mean (SE)	Control Mean (SE)	Reference Range[2]
Micro-parasitic parasitoid	LA1	2.7 (2.19)	0.7 (0.67)	0.0 – 0.3	—	—	—	0.0[§]	0.0	0.0 – 9.5
	SC	1.7 (0.67)	1.7 (1.20)	0.0 – 1.7	—	—	—	—	—	—
Damsel bug	GA1	0.3 (0.33)	0.7 (0.33)	0.0 – 0.3	34.7 (2.33)	58.3 (8.88)	22.0 – 58.7	—	—	—
	LA1	0.3 (0.33)	0.7 (0.67)	0.0 – 1.0	0.7 (0.33)	0.3 (0.33)	0.3 – 1.0	0.0[†] (0.00) [§]	0.0 (0.00)	0.0 – 0.0
	SC	0.3 (0.33)	0.0 (0.00)	0.0 – 0.7	0.7 (0.67)	0.0 (0.00)	0.0 – 0.7	0.0 (0.00)	0.0 (0..00)	0.3 – 2.0
	TX1	1.3 (0.33)	2.3 (1.45)	1.0 – 3.7	1.3 (0.88)	1.3 (1.33)	1.0 – 2.0	—	—	—
Orius	GA1	0.0 (0.00)	0.0 (0.00)	0.0 – 0.3	0.3 (0.33)	0.0 (0.00)	0.0 – 1.0	—	—	—
	LA1	0.3 (0.33)	0.7 (0.67)	0.0 – 1.3	0.0 (0.00)	0.0 (0.00)	0.0 – 0.3	0.0†(0.00) §	0.0 (0.00)	0.0 – 0.0
	SC	9.3 (1.33)	8.0 (2.08)	7.3 – 10.7	0.3 (0.33)	0.3 (0.33)	0.0 – 1.7	0.0* (0.00)	1.3 (0.67)	0.0 – 1.3
	TX1	0.0[†] (0.00)	0.0 (0.00)	0.0 – 0.0	0.0 (0.00)	0.0 (0.00)	0.0 – 0.3	—	—	—

Note: The experimental design was a randomized complete block. SE = Standard Error. Means based on three replicates (N=3) of except where denoted by [§], in which means are based on two replicates (N=2). Data were from arthropod collections performed at four crop developmental stages: Collection 1 = R1-R2, Collection 2 = R3-R5 and Collection 3 = R6-R8.

* Indicates a statistically significant difference between MON 87701 and the conventional soybean control A5547 ($p \leq 0.05$).

[†] No statistical comparisons were made due to lack of variability in the data.

[1] Site codes are as follows: GA1 = Tift County, GA; LA1 = St. Landry Parish, LA; SC = Barnwell County, SC; TX1 = Armstrong County, TX.

[2] Reference range is the minimum and maximum mean value observed among the four reference soybean varieties.

Dash (—) indicates arthropod not evaluated.

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

Plant Production

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

Flower Collection

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

Pollen Sample Preparation

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

Data Collection

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

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

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

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

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

Statistical Analysis

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

Reference:

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

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Appendix I. Materials and Methods for Symbiont Evaluation

Materials

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

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

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

Greenhouse Phase and Experimental Design

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

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

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

Plant Harvesting/Data Collection

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

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

Statistical Analysis

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

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

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Appendix J. Summary of Non-target Organism Evaluations

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[redacted] *2009. Evaluation of Potential Dietary Effects of Cry1Ac on the Ladybird Beetle, Coleomegilla maculata (Coleoptera: Coccinellidae). Monsanto Study Number REG-08-337.*

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

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

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

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

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

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

[redacted] *and* [redacted]. *2009. Evaluation of Potential Dietary Effects of the Cry1Ac Protein on the Parasitic Wasp, Pediobius foveolatus (Hymenoptera: Eulophidae). Monsanto Study Number REG-08-467.*

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

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

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

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

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

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

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

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Appendix K. Petitioner's Environmental Assessment

A. Background

This section provides a brief summary of three key areas to be covered in an environmental assessment prepared by APHIS for MON 87701 under the National Environmental Policy Act (NEPA) – Alternatives, the Affected Environment, and Potential Environmental Consequences. The significance of the potential environmental impact takes into consideration both the context and the intensity of the proposed action. This assessment provides data and analysis that appropriately addresses and evaluates relevant factors indicative of the intensity of the proposed action as described in implementing NEPA regulations (40 CFR § 1508.27).

MON 87701 has been the subject of numerous field trials conducted in the U.S under APHIS notifications and permits since 2001. Information has been developed from these field trials, other tests, and the literature to specifically assess whether the insect-protected trait (production of the Cry1Ac protein) or the plant transformation process altered MON 87701 in any way that would impart plant pest characteristics or cause significant environmental impacts, including cumulative impacts.

Purpose and Need

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act, as amended (7 U.S.C. § 7701–7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered (GE) organisms and products. An 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. A GE organism is considered a regulated article if APHIS has reason to believe it could pose a plant pest risk. 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 as a potential plant pest. (7 CFR § 340.6 "Petition for Determination of Nonregulated Status"). The petitioner is required to provide information (§ 340.6(c)(4)) related to plant pest risk that the USDA may use to determine whether the regulated article is unlikely to present a plant pest risk. If, based on this information, the USDA determines that the article is unlikely to pose a plant pest risk, the article may be granted deregulated status.

Monsanto Company (Monsanto) has submitted this Petition to APHIS for the determination of non-regulated status for MON 87701 soybean plants genetically engineered to express the Cry1Ac protein which is not typically found in soybean. Researchers have found that when soybean plants produce the Cry1Ac protein, they are protected from feeding damage from certain lepidopteran insects. Monsanto has requested that APHIS make a determination that these soybean plants will no longer be considered regulated articles under 7 CFR Part 340.

Soybean is attacked by numerous insects throughout the growing season, but only a few pose a significant economic threat, and not to all production regions (Higley and Boethel, 1994). The occurrence of soybean insects follows a north-south gradient (Higley and

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Boethel, 1994). Generally, soybean insect pest problems are less severe in the midwest states than in other soybean producing areas (Higley and Boethel, 1994). Insect pressure is generally greatest in the Southeast region. Four lepidopteran insects are considered major insect pests of soybean in the Southeast. Velvetbean caterpillar and soybean looper infestations are greatest in the southeastern states because of their close proximity to the tropics where these insect pests overwinter and because the warm climate facilitates multiple generations per year (Heatherly and Hodges, 1999).

Chemical insecticides are used for controlling lepidopteran infestations in soybean, but are not always effective. Narrow application windows, the emergence of insecticide resistance, and public pressure for reduced pesticide use limit the desirability of this approach to pest management (Thomas and Boethel, 1994). Monsanto has developed soybean plants (MON 87701) through the use of biotechnology that produce the Cry1Ac protein for control of lepidopteran pests. Production of the Cry1Ac protein in soybean leaf and other tissues is highly effective for control of certain lepidopteran pests that feed on soybean. MON 87701 would improve upon current agricultural practices by eliminating or reducing insecticide use for targeted lepidopteran pests, reduce the risks posed to non-target species, and improve the efficiency of soybean production systems by increasing or maintaining yield potential while reducing insecticide costs.

B. Affected Environment

The proposed deregulation would be relevant to the production of an intensively cultivated row crop – soybean. Soybean is grown as a commercial crop in over 35 countries. In the United States, it is generally grown on greater than 70 million acres in at least 31 states with over a million acres grown in each of the following states: IA, IL, MN, IN, MO, NE, OH, SD, AR, ND, KS, MI, MS, WI, NC, KY, TN (USDA-NASS, 2006a-b). While soybean is one of the largest row crops grown in the U.S., only approximately 16% of soybean acres receive an insecticide application on an annual basis (see Table IX-4). Thus, it is not expected that MON 87701 has a commercial fit on the majority of U.S. soybean acres. MON 87701 produces the Cry1Ac protein providing protection against targeted lepidopteran insect pests of soybean. Lepidopteran insect pressure is greatest in the U.S. Southeast region, particularly in the southern states bordering the Gulf of Mexico and the Atlantic Ocean.

As discussed throughout the Petition, initial planting of MON 87701 in the U.S. will be solely for soybean breeding and seed multiplication purposes and will likely be limited to the states of GA, NC, SC, IL, IN, IA, and MO. This breeding and seed increase activity will support the South American commercial soybean production market. EPA approval is only being sought at this time for a seed increase registration to support breeding and seed multiplication activities in the U.S.

If, at some future date, MON 87701 is approved by EPA for commercial planting and the crop is commercialized in the U.S., it is not certain how many acres of MON 87701 would be grown; however, growers in the states of AL, AR, GA, LA, MS, NC, SC, and TN would be the most likely to use MON 87701, due to infestations of lepidopteran insect pests requiring control measures. Nevertheless, because pest pressure in any given geographic area may change over time, all areas of U.S. soybean cultivation are included here as the affected environment. At some time in the future, Monsanto may seek EPA approval to allow the commercial planting of MON 87701.

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Broad commercial use is expected in South America (e.g., Brazil) where lepidopteran insects have a larger economic impact on commercial production. This environmental assessment does not address potential environmental impacts on countries outside the U.S. in which MON 87701 may eventually be grown. The agency action triggering this environmental assessment, APHIS's determination of nonregulated status, only determines the plant pest risk posed by MON 87701 when grown within the U.S. and, subject to other necessary clearances by other U.S. federal regulatory agencies, allows the crop to be grown in the U.S. Each country in which MON 87701 will be planted has an independent regulatory system in place to address that country's own environmental protection concerns.

This deregulation is being sought in an environment that has rapidly adopted biotechnology-derived soybean varieties (James, 2007). Thirteen different biotechnology-derived soybean crop products have been deregulated by USDA since 1994 (www.aphis.usda.gov). Biotechnology-derived herbicide-tolerant soybean varieties were grown on approximately 69 million of the 75 million acres of soybean grown in the U.S. in 2008 (USDA-ERS, 2008). Thus, soybean seed breeders, seed manufactures, and soybean producers are accustomed to the presence of biotechnology-derived soybean and are capable of breeding, manufacturing seed, and producing harvested seed to meet the needs of various markets. MON 87701 is the first *Bacillus thuringiensis* (Bt)-based insect-protected soybean crop product for which deregulated status is being requested. However, Bt-producing corn and cotton varieties have been deregulated and on the market for several years (de Maagd et al., 1999; Mendelsohn et al., 2003).

The affected environment is described in detail in Sections II through X of this Petition. The environment includes commercial soybean, *Glycine max*, its uses as human food, animal feed and industrial products; lepidopteran insects that feed on soybean plants; lands where soybean is grown; and adjacent non-agricultural land. Related agricultural practices such as tillage, crop rotation, pesticide use, weed management, irrigation practices, and non-agricultural lands are considered part of the affected environment. Specialty soybean production, including organic soybean production is also considered part of the affected environment. Seed production and related human activities associated with marketing of harvested soybean seed are also included in the affected environment.

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C. Alternatives

The action 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 from Monsanto's Petition:

- **No action**
  - MON 87701 would remain a regulated article
- **Approval in part**
  - MON 87701 would be granted deregulated status with some restrictions (e.g., geographic)
- **Approval in whole**
  - MON 87701 would be granted full deregulated status

Rejection of the "no action" and "approval in part" options and adoption of approval in whole is dependent upon a finding of no plant pest risk for MON 87701. MON 87701 has been thoroughly characterized and extensive information presented in Sections I through X of this Petition demonstrates that MON 87701 does not present a plant pest risk. On the basis of this analysis, Monsanto is requesting as the "preferred" alternative, an "approval in whole" or full deregulated status for MON 87701. Information and arguments presented in this section will further demonstrate that MON 87701 does not present a significant environmental impact; thus, the requirements of NEPA can be satisfied by an Environmental Assessment and Finding of No Significant Impact (FONSI).

Table K-1 below summarizes the results for each of the issues raised in the Environmental Consequences Section (see Section C. below) for the *"no action"* or *approval in whole* alternative. *Approval in part* is not discussed further because there is no increased plant pest risk associated with MON 87701; specifically, there is no geographic variation in plant pest risk for MON 87701 and no basis for approval in part.

Table K-1. Comparison of Alternatives

Attribute/Measure	Alternative A No Action	Alternative B Deregulation in Whole
Meets Purpose and Need Objectives	No	Yes
Unlikely to Pose Plant Pest Risk	Satisfied through use of regulated field trials	Satisfied through use of regulated field trials and safety assessment
Commercial Use	Unchanged	Unchanged; separate approval required from EPA for commercial planting, not being sought at this time
Management Practices		
Certified Seed Production	Adverse risk for release of unapproved event and continued regulation	Unchanged; seed production will be in accordance with AOSCA standards; deregulation would allow MON 87701 to be grown without APHIS permit/notification
Soybean Production	Unchanged	Potential increased efficiency of soybean production
Pesticide Use	Unchanged	Potential for reduction in use of broad spectrum insecticides; unchanged if available only to breeders and seed producers who will continue to use broad spectrum insecticides
		Unchanged for all other pesticide applications
Resistance Management	Unchanged	Unchanged; multiple alternative hosts available when only approved for seed multiplication and broad spectrum insecticides are applied
		IRM plan would be required by U.S. EPA for commercial production approval to address potential for resistance development

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Table K-1. (continued). Comparison of Alternatives

Attribute/Measure	Alternative A No Action	Alternative B Deregulation in Whole
Human and Animal Health		
Risk to Human and Animal Health	Unchanged	Unchanged; lack of effect of MON 87701 on public health or safety and no change in composition of harvested seed or forage
Worker Safety	Unchanged	Unchanged for breeding, seed multiplication and certified seed production; pesticide use practices will not change Possible benefit under commercial production through decreased exposure to insecticides
Environment		
Risk to Plants	Unchanged	Unchanged due to no increase in plant pest potential, including weediness, and low potential for gene introgression
Risk to Animals	Unchanged	Unchanged; supported by low oral toxicity, protein safety, and compositional equivalence
Biodiversity	Unchanged	Unchanged due to plant pest characteristics; possible increase in biodiversity due to reduced use of broad spectrum insecticides (if introduced for commercial production)
Land Use	Unchanged	Unchanged, no change in rotational crops or increase in acreage; MON 87701 would be grown on land previously used for agricultural production
Cumulative Impacts		
Land use, Insect Resistance Management, Human and Animal Health, Plant Health, and Specialty Soybean Production	Unchanged	Unchanged • No change in cultivation practices • Low likelihood for development of resistance due to use of IRM strategies • No acute toxicity associated with Cry1Ac • Lack of interactions with previously deregulated biotechnology-derived soybean traits • Wide use of other biotechnology-derived soybean traits since 1996 with continued production of specialty soybean
Management Practices	Unchanged	Unchanged for breeding and seed multiplication Potential decreased application of insecticides to control lepidopteran insects under commercial production
Biodiversity, Risk to Threatened or Endangered Species and Non-target Organisms	Unchanged	Cry1Ac protein is highly specific and does not accumulate in soil Possible increase in biodiversity (including non-target organisms) due to reduction in broad spectrum insecticide use if introduced for commercial production
Economic and Environmental Interests	Unchanged	Increased efficiency in soybean production may lead to improved farm income if introduced for commercial production

[1]In all cases the *"no action"* alternative considers the impact due to continued confined release trials under USDA notification with MON 87701.

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D. Potential Environmental Consequences

In considering potential environmental impacts, factors related to the intensity of the proposed action on the effected environment are addressed throughout this section. Analysis of these factors considered the "no action" and the "preferred" alternative (deregulation in whole). The differences between the two alternatives address the question of whether deregulation of MON 87701 results in a significant impact to the quality of the human environment. In most cases, there are no differences between the two alternatives. Where differences were noted, these differences are described and their significance evaluated. Factors evaluated as part of the assessment of significance include: potential impacts to land use patterns, farming practices, specialty and organic soybean production and to non-agricultural lands, impacts to the marketability of soybean seed for planting and harvested seed for commodity markets, impacts to public health, impacts to non-target organisms, and threatened or endangered species and biodiversity. Finally, cumulative impacts are considered in light of this action combined with past and future actions.

Methodology and Assumptions

The "preferred" alternative would allow planting of MON 87701 throughout the U.S. A list of the states where soybean is produced is presented in Table IX-2.

As discussed throughout the Petition, initial introduction of MON 87701 will be for soybean breeding and seed multiplication purposes on limited acreage, most likely in the states of GA, NC, SC, IL, IN, IA, and MO, to support the South American commercial soybean production market. EPA approval is only being sought at this time for a seed increase registration for breeding and seed multiplication in the U.S.

If a future decision is made to commercialize MON 87701 in the U.S., it will be used where lepidopteran pest pressure will make its use economically viable. Information presented in this Petition indicates that insect populations and damage to soybean is highly variable throughout the U.S. MON 87701 has a commercial fit in southern soybean production areas in the states of AL, AR, GA, LA, MS, NC, SC, and TN. While approximately 16% of the soybean acres received an insecticide application in 2006, future insect pest pressure is not predictable. Thus, at some future date, MON 87701 may be grown widely in soybean production regions depending upon insect pest pressure and product benefits. Therefore, the states mentioned in Table IX-2 are included in the analysis.

Broad commercial use is expected in South America (e.g., Brazil) where lepidopteran insects have a larger economic impact on commercial production. This environmental assessment does not address potential environmental impacts MON 87701 may pose in South America. The agency action triggering this environmental assessment, APHIS's determination of nonregulated status, only determines the plant pest risk posed by MON 87701 when grown within the U.S. and, subject to other necessary clearances by other U.S. federal regulatory agencies, allows the crop to be grown in the U.S. Each country in which MON 87701 will be planted has an independent regulatory system in place to address the country's own environmental protection concerns.

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D.1. Impacts on Land Use

Monsanto considered potential impacts associated with the cultivation of MON 87701 on land use. Soybean fields are typically highly managed agricultural areas that can be expected to be dedicated to crop production for many years and cultivation of MON 87701 is not expected to differ from typical soybean cultivation. The insect protection trait would provide a benefit to growers by simplifying agricultural practices associated with soybean production. MON 87701 will likely be used in common rotations on land previously used for agricultural purposes. Based on these considerations, there is no difference between the "no action" and "preferred" alternative because there is no apparent potential for significant impact on land use if APHIS grants non-regulated status to MON 87701.

D.2. Potential for Non-crop and Non-agricultural Impacts

G. max has never been found in the wild in the U.S. (Hymowitz and Singh, 1987; CFIA, 1996; OECD, 2000). Soybean does not grow and persist in unmanaged habitats and would not be expected to invade and/or persist in the natural environment including, streams, lakes, oceans or other aquatic environments. With the exception of production of the Cry1Ac protein, MON 87701 is similar to other commercial soybean varieties currently grown in the U.S. and would be expected to similarly have no significant impact to non-agricultural lands and aquatic systems. Under the "preferred" alternative, MON 87701 could be produced broadly on land where soybean may be grown. Insecticide applications on commercial fields containing MON 87701 may be eliminated or reduced, thereby decreasing the chance for pesticide drift to non-agricultural lands adjacent to soybean fields. Under the "no action" alternative, MON 87701 may still be grown under USDA notification and some of these same benefits would occur in confined release field trials although at a much reduced scale.

D.3. Potential Impacts to Agricultural Practices

MON 87701 has been shown to be no different from conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics (see Sections VII, VIII, IX, and X of this Petition), and has the same levels of resistance to most insects and diseases as current commercial soybean. With the exception of insect control practices, no changes to agronomic practices typically applied in management of conventional soybean are required for MON 87701. Specifically no increases in pesticides and fertilizers are required as well as no changes in cultivation, planting, or harvesting practices. Thus, there is no change in the agricultural practices listed above should APHIS grant either alternative. Potential impacts to agricultural practices that could occur are largely associated with a possible decrease in pesticide applications. As with any insecticide, the use of MON 87701 has the potential to cause development of resistance to Cry1Ac. An analysis of expected impact to agricultural practices for commodity seed and certified seed production as well as potential impacts to pesticide application practices and potential for development of resistance are discussed below.

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Potential impact to soybean commodity seed production

A summary of agronomic practices and expected impacts is presented in Section IX of this Petition. The extent to which MON 87701 would be grown in the U.S. if it were to be approved by EPA and released for commercial planting is unknown. However, even if grown throughout the U.S., no significant impact would be expected from the introduction of MON 87701 on current cultivation and management practices or in crop rotation practices for soybean. MON 87701 has been shown to be no different from conventional soybean in its agronomic, phenotypic, ecological, and compositional characteristics (refer to Sections VII, VIII, IX and X), and has the same levels of resistance to insects and diseases as current commercial soybean, except for the introduced trait of enhanced protection from feeding damage caused by targeted lepidopteran pests. Like the other Bt-based crops, such as Bt cotton and Bt corn that have been cultivated and consumed in the U.S. since 1996, the "preferred" alternative would allow for unconfined release of MON 87701. In this case, insect-protected soybean MON 87701 may improve the current agricultural practices by eliminating or reducing insecticide use for targeted lepidopteran pests, reduce the risks for non-target species, and improve the efficiency in soybean production systems by increasing or maintaining yield potential while reducing insecticide and labor costs. According to Brookes and Barfoot (2008), the introduction and use of Bt-producing corn in the U.S. has resulted in increased annual yields of 5% resulting in increased farm income. Similarly, Bt-cotton growers have experienced yield increases ranging from 9-11%, thereby increasing farm profitability.

Under the "no action" alternative, MON 87701 would still be allowed to be grown under USDA notification, however, the potential benefits discussed above would not be possible. Moreover, MON 87701 would remain a regulated article for purposes of 7 CFR Part 340. Although it is unlikely given limited acreage and product stewardship practices, this regulatory status could adversely affect the continued flow of soybean exports from the U.S. and cause unnecessary commercial disruption of the soybean market.

Potential impact to certified seed production

Certified seed production is a carefully managed process (see Section IX.B.2). MON 87701 is not expected to impact certified seed production practices or production of other certified conventional, specialty or organic soybean seed for the following reasons. MON 87701 would be produced using practices already in place for production of certified seed. The implementation of management practices to avoid pollen from a biotechnology-derived crop in organic, specialty or conventional soybean seed or commodity seed production operations is directly impacted by the nature of soybean pollination. Soybean is a highly self-pollinated species and exhibits very low levels of outcrossing. When plants are grown in very close proximity to each other (15 cm), average cross-pollination rates were 1.8% (Ray et al., 2003). At greater distances, cross-pollinations rates were 0.41 and 0.03% at 0.9 and 5.4 m, respectively. Hence, certified soybean seed producers can and have effectively implemented practices (i.e., isolation distances during the growing season, equipment cleaning during harvest and post-harvest separation of harvested seed) that allow them to maintain commercially acceptable levels of varietal purity. Under the "no action" alternative, MON 87701 would remain a

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regulated article. Although it is unlikely given limited acreage, and production under confined release conditions, this regulatory status could adversely affect the continued flow of soybean exports from the U.S. and cause unnecessary commercial disruption of the soybean market. This would also result in a wasteful use of APHIS resources through continued administrative costs associated with permits and notifications and potential field inspections which is not necessary given that there is no increased plant pest potential for MON 87701. Under the "preferred" alternative, seed production could occur with production systems already developed by seed producers for certified seed varieties. MON 87701 has been thoroughly characterized and (with the exception of insect tolerance) is not phenotypically different from conventional soybean and similarly is not likely to impact seed production practices.

Potential impact of MON 87701 to insect control practices

MON 87701 would control the two most damaging defoliating insects in the south, velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*). According to USDA-NASS statistics, about 16% of the U.S. soybean acreage in 2006 received an insecticide treatment (Table IX-4). Insecticide applications vary considerably across the U.S with approximately 15% of the Midwest acres treated compared to 75% of the acres in some of the southern states (see Section IX.E). It was estimated that 40-50% of the soybean acreage in the southeastern states such as Georgia and Louisiana were treated with insecticides to control lepidopteran pests, with velvetbean caterpillar and soybean looper being the main target pests (Gianessi et al., 2002). Three insecticides (chlorpyrifos, esfenvalerate, lambda-cyhalothrin) account for almost all the soybean treated acreage. Each of these insecticides controls a similar broad spectrum of insect pests, including lepidopteran and non-lepidopteran pests. Approximately 1.8 million pounds of these three insecticides were applied to soybean acreage in 2006 (see Table IX-4). Thus, under the "no action" alternative, growers would continue to apply insecticides to control lepidopteran insects. Under the "preferred" alternative, a reduction in insecticide use to a level below the 1.8 million pounds currently used could occur.

As discussed above, if MON 87701 is deregulated by APHIS and, at some point, approved for commercial planting by EPA, some reduction in pesticides applied for control of lepidopteran insects may occur for commodity soybean production. In contrast, soybean certified seed production requires greater inputs and the economic threshold for insect control is lower compared to commodity soybean production. Because of this, breeders conducting seed multiplication activities continue to use chemical pesticides even when growing plants producing PIPs. Accordingly, despite the lepidopteran protection provided by the Cry1Ac protein, it is unlikely that certified seed producers will change this practice due to the need to preserve yield and seed quality. Thus, no changes in pesticide use for certified seed production are predicted for either alternative. Because no commercial planting of MON 87701 is currently anticipated, no immediate changes in pesticide use are anticipated. However, subject to EPA commercial planting approval, some reduction in insecticide use may occur at a later date.

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Resistance management

EPA requires as a condition of registration that Bt-producing plants (PIPs) sold for commercial planting implement Insect Resistance Management (IRM) programs to prevent or delay the onset of resistance in the target insect species. These programs have been highly effective for delaying the development of resistance to Cry proteins produced by biotechnology-derived crops (EPA, 2000; Tabashnik et al., 2003). IRM programs traditionally rely on the use of a non-Bt producing crop refuge planted in close proximity to the Bt-producing crop, thereby preserving Bt-susceptible insects in the population and reducing the likelihood for selection of resistant alleles.

Initially, Monsanto is seeking from EPA a registration that would allow only MON 87701 seed breeding and seed multiplication. Given the potential acreage and use of MON 87701 in this limited case, no IRM plan or refuge is warranted. According to EPA's guidance, implementation of an IRM plan is not required if the seed multiplication covers less than 20,000 acres per county and up to a total of 250,000 acres per PIP active ingredient per registrant per year (see Section IX.I). Less than 1% of the soybean certified seed producing acres (approximately 15,000 acres) are targeted to be devoted to production of MON 87701 seed, representing less than 0.02% of total U.S. soybean acreage. At this acreage and crop density, the risk for developing resistance to Cry1Ac due to deregulation of MON 87701 is extremely remote, because a natural refuge exists in the vast amount of non-Bt producing soybean already growing, thereby supporting the conclusion that no IRM plan is warranted. The risk for development of resistance is further minimized since certified seed growers will use broad spectrum insecticides to control insect infestations in order to preserve yield, ensure seed quality, and to maximize profit. Thus, under the "preferred" alternative, no impact to the rate or incidence of the development of insect resistance to Cry1Ac is predicted due to deregulation in whole of MON 87701 for certified seed production. Under the "no action" alternative, MON 87701 could still be grown on limited acres under USDA notification and present minimal risk to the development of resistance to Cry1Ac.

Should Monsanto decide at some future date to offer MON 87701 to U.S. growers for larger scale commercial production, Monsanto would be required to submit and implement an IRM plan to EPA as part of a commercial use registration application. If approved, the registration would require the implementation of an IRM plan to mitigate the development of resistance to Cry1Ac. Thus, there is no difference between the "no action" and "preferred" alternatives based on the effectiveness of IRM plans implemented for previously approved Bt-producing crops.

Summary of impacts to agricultural practices

The potential changes to agricultural practices (decreased pesticide applications, increased yield, and enhanced farm profitability) that could result from commercialization of MON 7701 in the U.S. do not constitute significant environmental impacts. They are considered potential benefits that may improve the efficiency of farming when added to other improvements gained through agricultural practices, advances in germplasm, weed and insect control practices. APHIS has already approved several Bt-producing crops and other biotechnology-derived soybean crop products.

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MON 87701 may add to the benefits of these commercial products continuing the trend towards increasing yield and farm profitability.

D.4. Potential Impact of MON 87701 to Organic or Specialty Soybean Production

Organic farming operations as described by the National Organic Program, which is administered by USDA's Agricultural Marketing Service, requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition of the use of excluded methods. Excluded methods include a variety of methods used to genetically engineered 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 87701 is an excluded method under the National Organic Program [7 C.F.R. § 205.2].

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 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. 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 of a production or handling operation is considered a process claim, not a product claim.

Production systems designed prior to the introduction of MON 87701 or even prior to the introduction of biotechnology-derived soybean have allowed for production of soybean to meet customer demands. In addition to the market segments to produce organic or conventional soybean, distinct identity-preserved specialty soybean with such traits as clear hilum or high protein have also been grown and successfully marketed for specific food uses in domestic and export markets for many years (Zhanglin et al., 2004). The choice to grow biotechnology-derived, organic or conventional soybean depends on market dynamics. The dynamics of the marketplace, choice between various varieties of soybean, and the existing production practices will not be impacted by the introduction of MON 87701.

Organic soybean producers utilize production practices designed to specifically avoid the presence of both soybean products using conventional herbicide or other pesticide treatments, as well as biotechnology-derived crops. These well established practices to avoid "excluded methods" will continue with the introduction of MON 87701 varieties. They include isolation zones, use of buffer rows surrounding the organic crop, adjusted planting dates and varietal selection (www.attra.ncat.org). The implementation of

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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. As noted previously in this Petition, soybean is a highly self-pollinated species and exhibits very low levels of outcrossing. When plants are grown in very close proximity to each other (15 cm), average cross-pollination rates were 1.8% (Ray et al., 2003). At greater distances, cross-pollinations rates were 0.41 and 0.03% at 0.9 and 5.4 m, respectively. Hence, organic or conventional soybean producers can and have effectively implemented practices (i.e., isolation during the growing season, equipment cleaning during harvest, and post-harvest separation of harvested seed) that allow them to avoid the presence of biotechnology-derived soybean and maintain organic or conventional production status.

In 2006, Roundup Ready soybean was planted on an estimated 67 million U.S. acres, representing 89% of the U.S. soybean crop (USDA-NASS, 2007a; James, 2007). Despite the high adoption rates of Roundup Ready soybean by growers, organic and conventional soybean production remains an option for farmers who choose to produce these varieties of soybean. The decision to grow organic, conventional, or biotechnology-derived soybean varieties is typically an economic one based on market dynamics. Organic soybean producers and those growing conventional soybean for sensitive non-biotechnology markets typically enjoy a market premium offsetting the additional production and record-keeping costs. While the widespread adoption of Roundup Ready soybean has reduced the number of conventional soybean varieties that are available, conventional and organically produced soybean seed is currently available from numerous seed suppliers (Table K-2). Thus, growers have a choice in the soybean variety they plant, and this is not expected to change with the introduction of MON 87701.

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Table K-2. Organic and Conventional Soybean Seed Sources

Organic Soybean Seed Sources*:	Conventional Soybean Seed Sources
Albert Lea Seed House	Garst Seed
Blue River Hybrids	Monsanto (Asgrow/DEKALB)
Golden Grains	Monsanto (Delta and Pine Land)
Great Harvest Organics	Monsanto (Schillinger Seed)
Greis Seed Farm	Pioneer
Lancaster Ag Products	Soy Genetics
Lawler Farm Center	Stine Seed
Prairie Gold Seeds	Syngenta - multiple brands
Superior Organic Grains, Ltd	Terral Seed
Walter Seed and Honey Co	Various State Crop Improvement Organizations

* From: www.organicgrains.ncsu.edu

Based on the above information, there is no difference between the "no action" and "preferred" alternatives. There is widespread use of biotechnology-derived soybean in the marketplace, and systems have been developed to produce soybean to meet customer needs. These systems will not change under either alternative.

D.5. Potential Impacts on Raw or Processed Agricultural Commodities

Extensive data have been presented with this Petition relating to plant growth parameters, disease susceptibility, insect susceptibility, and forage and harvested seed composition of MON 87701 compared to conventional soybean varieties. Analysis of these data indicate no differences between MON 87701 and the conventional soybean varieties that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity. Compositional analysis of MON 87701 demonstrated that MON 87701 and conventional soybean are compositionally equivalent. Consequently, no significant effect on raw or processed commodities is expected if APHIS were to grant nonregulated status to MON 87701.

As previously mentioned (Section II.E and Table X-4), given the reproductive biology of soybean there is a very low likelihood for economic impact to conventional raw and processed soybean products should commingling or inadvertent outcrossing occur as a result of mechanical or physical interaction between MON 87701 fields and conventional or organic production fields in close proximity.

The low level presence of MON 87701 will not impact the quality of raw or processed soybean. Most organic production is done on a contract basis, and buyers of organic commodity seed recognize that for crop species where there are biotechnology-derived crop varieties on the market, a guarantee that a commodity crop is 100% "free" of biotechnology-derived material is not feasible based on testing and sampling methodology (Born, 2005). Thus, in some instances buyer allowances between 0.1 to 5% biotechnology-derived commodity seed in organic grains are often specified (www.attra.ncat.org). This also is consistent with the USDA National Organic Program allowing for detectable residues of excluded methods (including biotechnology-derived crop products) as long as the producer has taken steps to avoid those methods (www.ams.usda.gov/nop/Q&A.html).

Similarly, international regulatory organizations have recognized that testing and sampling methodologies limit the ability to confirm that conventional commodity seed is 100% free of biotechnology-derived material. Thus, they have set allowable tolerances for this material in conventional products to support food labelling and traceability laws. These tolerances allow from 0.9% (European Union) up to 5% (Japan) of the food to be biotechnology derived in products considered "conventional." Levels above the threshold will trigger special labelling. Thus, *de minimis* levels of approved biotechnology-derived soybean would be allowable in certified organic or conventional soybean. Based on this analysis, no impact on raw or processed agricultural commodities would be anticipated under either alternative.

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D.6. Potential Impacts on Commercial Use

Soybean is a globally traded commodity and the U.S. is the single largest exporter. The commercial use of MON 87701 in the U.S. would not be feasible without approvals from key trading partners. A description of the key export markets in which Monsanto intends to obtain safety authorizations for importation of soybean and soybean products is presented in Section I.C. of this Petition. Meanwhile, given the relatively small market opportunity of MON 87701 in U.S., the initial commercialization of MON 87701 is targeted in South America. The initial planting of MON 87701 in the U.S. will be limited to breeding and seed multiplication actitivies to support the commercial launch in South America.

The decision to deregulate MON 87701 would allow for breeding of this product into conventional and biotechnology-derived soybean varieties and would make MON 87701 available to breeders, certified seed producers, and potentially growers. Like other biotechnology-derived traits, it is expected that breeders and certified seed producers would use MON 87701 to supply seed for planned commercial markets in South America (e.g., Brazil). U.S. growers may eventually use MON 87701 if Monsanto obtains appropriate registrations from the U.S. EPA.

The marketability of organic, specialty, conventional, and biotechnology-derived soybean will not change with the introduction of MON 87701. The majority of soybean grown in the U.S. is currently produced using a biotechnology-derived trait. Monsanto has presented submission plans in the Petition highlighting the need for approvals of MON 87701 in key soybean exporting countries prior to full-scale commercial launch. Monsanto would not commercially release MON 87701 until all key soybean import markets with functioning regulatory systems have also granted approval of MON 87701 as described in detail in Monsanto's stewardship program in Section IX.J.

Data on MON 87701 presented in this Petition and data on all its progeny have shown no significant adverse effects to non-target organisms, no increase in fitness or weediness characteristics, and no effect on the health of other plants. Based on all these considerations, there is no apparent potential for impacts on commercial use if APHIS grants the "preferred" option.

Under the "no action" alternative MON 87701 might not be available to breeders or growers. Although breeding and seed increase could take place in the U.S. under APHIS notification, the additional costs associated with the regulatory process could significantly reduce the product's economic viability. The "no action" alternative would not affect the systems already used to separate biotechnology-derived soybean from specialty soybean.

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D.7. Health and Safety

Prior to the introduction of a biotechnology-derived crop product to the marketplace, Monsanto conducts tests to assure that all products are safe for their intended use and appropriately labelled. Under the Federal Food, Drug, and Cosmetic Act (FFDCA) [21 U.S.C. 301 et seq.], pesticide residues in or on raw agricultural commodities or processed

foods are considered to be safe only after a tolerance or exemption from tolerance has been established. Residue tolerances and exemptions for pesticides are established by EPA under the FFDCA. Currently, tolerance exemptions have been granted for residues of the *Bacillus thuringiensis* Cry1Ac protein and the genetic material necessary for its production for all crops when applied/used as a plant-incorporated protectant (PIP) (EPA, 1997). The FDA enforces the tolerances set by the EPA. As previously mentioned, Monsanto will also prepare a submission to the U.S. EPA requesting a Section 3 seed increase registration to allow for breeding and seed multiplication plantings. Monsanto will also consult with the FDA on the food and feed safety assessment for the whole food produced by MON 87701.

Human Health. Potential impacts to human health could occur if there were harmful properties associated with the Cry1Ac protein or introduced compositional changes to the harvested seed used for food or feed purposes. Other potential impacts that could occur in MON 87701 are increased susceptibility to insects or disease requiring additional applications of pesticides, thereby increasing worker exposure to these pesticides. Movement of the inserted genetic material in MON 87701 to other sexually compatible species is not possible in the U.S. (discussed in Section D.8), therefore, exposure to MON 87701 can only come from soybean.

MON 87701 was developed through *Agrobacterium*-mediated transformation of soybean meristem tissue using the binary transformation plasmid PV-GMIR9 (Section IV; Figure IV-1, and Table IV-1). MON 87701 contains one copy of the insert at a single integration locus. No additional genetic elements from the transformation vector were detected in the genome of MON 87701, including backbone sequence from plasmid PV-GMIR9. Additionally the data confirm the organization and sequence of the insert, demonstrate the stability of the insert over several generations, and demonstrate that the genomic DNA sequences flanking the 5' and 3' ends of the insert are native to the soybean genome. On the basis of these data, it is concluded that only the expected Cry1Ac protein is produced from the inserted DNA.

For MON 87701, the available data demonstrate that harvested seed is as safe as conventional soybean for food and feed uses; and is safe and wholesome for consumption. The only compositional change is associated with the presence of minor amounts of the Cry1Ac protein. To assess the impact of the Cry1Ac protein on food and feed safety, bioinformatic analyses were used to establish the lack of both structurally and immunologically-relevant similarities between allergens or toxins, based on the amino acid sequence of the Cry1Ac protein in MON 87701. Furthermore, digestive fate experiments conducted with the Cry1Ac protein demonstrated that the full-length protein is rapidly digested in simulated gastric fluid (SGF), a characteristic shared among many proteins with a history of safe consumption. A small transiently stable Cry1Ac protein fragment is very quickly (within 30 sec) degraded during short exposure to simulated intestinal fluid (SIF). Rapid digestion of the full-length Cry1Ac protein in SGF and SIF, together with complete degradation of the small transiently stable fragment in SIF, indicates that it is highly unlikely that the Cry1Ac protein and its fragment will reach absorptive cells of the intestinal mucosa. This, combined with the history of safe exposure to the donor organism and the Cry1Ac protein and the lack of homology of the amino acid sequence of this protein to known allergens and toxins, supports a conclusion that Cry1Ac has low

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allergenic and toxic potential. Finally, mouse acute oral toxicity evaluations also have demonstrated that the Cry1Ac protein is not acutely toxic and does not cause any adverse effect, even at the highest dose levels tested, which were 1290 mg/kg body weight for females and 1460 mg/kg body weight for males. There was no mortality and no reports of any adverse clinical effects. At necropsy, the macroscopic appearance of the protein-dosed mice was within normal limits and similar to the controls. No toxicity was observed in any of the groups. These assessments lead to the conclusion that there is no meaningful risk to animal or human health from dietary exposure to Cry1Ac from MON 87701.

Extensive analysis of the composition of MON 87701 demonstrated that no biologically-relevant changes were detectable, outside of the intended presence of the Cry1Ac protein in soybean tissues. A detailed compositional assessment of soybean harvested seed and forage is presented in Section VII of this Petition. The levels of key nutrients, anti-nutrients, and other components in MON 87701 were examined and compared to that of the conventional soybean control, A5547, a conventional soybean variety with background genetics representative of MON 87701. Additionally, tolerance intervals representing 99% of the values of each analyte for a commercial conventional soybean population were established. Results demonstrate that the levels of key nutrients, anti-nutrients, and other components of MON 87701 are compositionally and biologically equivalent to conventional soybean.

A summary of the impacts to the health and phenotype of MON 87701 due to the trait and transformation process is presented in Section VIII.E. No biologically-meaningful differences were observed between MON 87701 and the conventional soybean control (A5547). These assessments included 14 plant growth and development characteristics; five seed dormancy/germination parameters under six different temperature regimes; two pollen characteristics; and more than 500 observations for abiotic stressor, disease susceptibility, arthropod damage, arthropod abundance, and plant-symbiont interaction. Data on environmental interactions also indicate that MON 87701 does not confer any increased susceptibility or tolerance to specific disease, insect, or abiotic stressors compared to conventional soybean. Compositional assessments (discussed above) conducted on harvested seed and forage also support a conclusion of no impact to human or animal health when MON 87701 is compared to conventional soybean.

Worker Safety. As discussed in this Petition, MON 87701 has the potential to reduce worker exposure to pesticides applied to commercial planting of soybean. Fewer insecticide applications result in less worker exposure, thereby benefiting the safety of workers. Certified seed production utilizes similar agronomic practices as commodity seed production (see Section IX). However, due to the economic value associated with certified seed production, these seed producers will often control insects to preserve yield and quality. Thus, no change in worker exposure to conventional pesticides is expected due to deregulation of MON 87701 for breeding and seed multiplication purposes.

Under the "preferred" alternative, additional exposure to the Cry1Ac protein to humans and animals is expected. Considering soybean is not sexually compatible with any other plant in the U.S., the source of exposure is only through soybean plants and harvested seed. Regardless, the Cry1Ac protein is not a harmful substance nor does it impact human and animal safety and health. Furthermore, soybean produced by MON 87701 is found to be compositionally equivalent to conventional soybean and is wholesome for

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consumption. Thus, the "preferred" alternative poses no significant impact to human health and animal safety. A potential benefit could occur to commercial soybean field worker safety through reduced exposure to insecticides applied to control lepidopteran insects. Under the "no action" alternative, this benefit could not occur.

D.8. Plant and Animal Communities Including Threatened or Endangered Species

Gene Movement. In assessing the risk of gene introgression from MON 87701 into its sexually compatible relatives, Monsanto considered two primary issues: 1) the potential for gene flow and introgression, and 2) the potential impact of introgression. The genus *Glycine* has approximately nine species, with *G. max* being placed in the subgenus *Soja* along with one other species, *G. soja*. *G. max* is sexually compatible with only *G. soja* and no other *Glycine* species. *G. max* is the only *Glycine* species located in the United States. Therefore, the probability of gene flow and introgression of MON 87701 into other species in the U.S. is essentially zero (Stewart et al., 2003); thus, the potential impact of introgression is nonexistent if APHIS were to grant the Petition for non-regulated status in whole. For these reasons, there is no impact to animal and plant communities or threatened and endangered species due to gene movement for either alternative.

Non-Target and Beneficial Organisms. Monsanto evaluated the potential for deleterious effects or significant impacts on non-target and beneficial organisms. Cry1Ac protein originates from *Bacillus thuringiensis* (Bt), a ubiquitous gram-positive soil bacterium that accumulates crystal proteins during sporulation. These crystal (Cry) proteins bind to the specific receptors on the midgut epithelium of the target lepidopteran insects and form cation-selective pores, which lead to the inhibition of the digestive process and result in the insecticidal activity (Hofmann et al., 1988; Slaney et al., 1992; VanRie et al., 1990). One valuable feature of this activity is that it is targeted to specific categories of insects, and does not impact broader insect populations or other organisms. This target specificity is determined by discrete structural features of the Cry protein or proteins that accumulate in different Bt subspecies and due to the specific high-affinity receptors present on specific insect species' gut epithelium.

Studies have previously been conducted to evaluate the spectrum of insecticidal activity of Cry1Ac protein produced from *Bacillus thuringiensis* var. *kurstaki* HD-73 against a variety of agronomically-important insects and one non-insect arthropod taxon (MacIntosh et al., 1990). Species tested included seven species of Lepidoptera: beet armyworm (*Spodoptera exigua*), black cutworm (*Agrotis ipsilon*), cabbage looper (*Trichoplusia ni*), corn earworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*), tobacco budworm (*Heliothis virescens*), and tobacco hornworm (*Manduca sexta*); five species of Coleoptera: alfalfa weevil (*Hypera postica*), cotton boll weevil (*Anthonomis grandis*), horseradish flea beetle (*Phyllotreta armoraciae*), southern corn rootworm (*Diabrotica undecimpunctata howardi*), and Japanese beetle (*Popillia japonica*); one species of Diptera: yellow fever mosquito (*Aedes aegypti*); one species of Blattodea: German cockroach (*Blatella germanica*); one species of Hemiptera: green peach aphid (*Myzus persicae*); one species of Isoptera: termite (*Reticulitermes flavipes*); and one species of mite: two-spotted spider mite (*Tetranychus urticae*). The results showed that the Cry1Ac protein had activity against all seven of the representative

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lepidopteran insects. However, there was no indication of activity of the Cry1Ac protein against any of the ten non-lepidopteran species.

Additional studies were conducted to evaluate the spectrum of activity for the Cry1Ac protein against major lepidopteran insect pests of soybean and a variety of other lepidopteran insects of importance to soybean. This information is presented in Section X.A.3. The results from these assays confirmed that the Cry1Ac protein produced by MON 87701 is effective against lepidopteran insects.

In addition to biological activity screens, Monsanto analyzed data to determine if there were changes to phenotype, germination, vegetative growth, reproductive parameters and response to biotic stressors (insect and disease stress) associated with MON 87701 in comparison to the various control lines (conventional soybean). These experiments are designed to document how MON 87701 performs in the field environment compared to conventional soybean varieties (e.g., do the plants/seeds look, germinate, grow, flower, respond to insect and disease pressures similar to a conventional soybean variety). Data presented in Section VIII indicate that the ecological interactions between MON 87701 and the conventional soybean control were similar. Monsanto also noted no differences in field interactions with beetles, aphids, whiteflies, or other organisms that were different from control plants. Thus, it is highly unlikely that MON 87701 would have a negative impact to non-target and beneficial organisms. In general, non-target invertebrates are more abundant in Bt cotton and Bt corn fields compared to insecticide treated controls (Marvier et al., 2007; Wolfenbarger et al., 2008). As with other Bt-producing crops, the reduction in broad spectrum insecticide applications would likely have a positive benefit to non-target and beneficial organisms. Therefore, under the "preferred" alternative this benefit could be realized for larger scale production. Under the "no action" alternative, insecticide applications would continue on their current course depending on insect pressure and economic thresholds.

Impacts to soil microorganisms. Monsanto has conducted tests to examine the soil degradation of Cry1Ac and the impact of MON 87701 on symbiotic soil microorganisms. The Cry1Ac protein does not persist in soils when evaluated under laboratory or field conditions (see Section X). Thus, no accumulation of the Cry1Ac protein is expected in soils where MON 87701 will be grown. Monsanto also presented data in the Petition (Section VIII) demonstrating the lack of impact to symbiotic microbes associated with soybean plants. The *B. japonicum*-soybean symbiosis of MON 87701 was not altered as a result of the introduction of the *cry1Ac* gene and the Cry1Ac protein production compared to a conventional soybean control. On the basis of these observations and in conjunction with related phenotypic measurements for MON 87701, and lack of Cry1Ac activity against non-lepidopteran arthropods, no impact on soil microorganisms and other soil arthropods is expected for either alternative.

Potential impacts to threatened or endangered animals and plants. Monsanto considered the potential impact on federally listed Threatened or Endangered Species (TES) and species proposed for listing, as provided under Section 7 of the Endangered Species Act. In this analysis, Monsanto considered the biology of MON 87701, as well as typical agricultural practices associated with cultivation of soybean. As previously noted, consumption of Cry1Ac protein has shown no toxicity in laboratory testing with mice. MON 87701 does not express any additional proteins, natural toxicants,

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allelopathic chemicals, pheromones, hormones, etc. that are known to directly or indirectly affect a listed TES or species proposed for listing. MON 87701 is not sexually compatible with a federally listed TES or a species proposed for listing. The only TES animal listed that occupies habitat that is likely to include soybean fields and that might feed on soybean is the federally Endangered Delmarva Peninsula Fox Squirrel, found in areas of the mid-Atlantic Eastern seaboard (*Sciurus niger cinereus*) [http://ecos.fws.gov/tess_public/SpeciesReport.do]. 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. Given all these factors and the lack of noted adverse effects on mice and other non-target organisms, it is concluded that MON 87701 will not have an effect on the Delmarva Peninsula Fox Squirrel.

No impact to any threatened or endangered plant species is expected. *G. max* has no sexually-compatible relatives in the U.S. and does not survive outside of cultivation. Thus, there is no opportunity for MON 87701 to interbreed with any plant species or displace natural vegetation in the U.S.

Potential impacts to threatened or endangered arthropods. As noted previously, MON 87701 differs from conventional soybean only in the expression of the Cry1Ac protein and the presence of the *cry1Ac* gene that are responsible for controlling targeted lepidopteran insects. Some lepidopteran insects are listed as threatened or endangered on the U.S. Fish and Wildlife data base (http://ecos.fws.gov/tess_public/StateListing.do?state=all). According to the database only one lepidopteran, the Saint Francis' satyr butterfly (*Neonympha mitchellii francisci*), is listed as endangered in any of the states where MON 87701 is expected to be grown for breeding and seed multiplication purposes. The Saint Francis' satyr is endangered in North Carolina. Threatened and endangered lepidopterans in the U.S. have very restrictive habitat ranges, and their larvae typically feed on specific host plants, none of which include soybean (http://ecos.fws.gov/tess_public/StateListing.do?state=all). Thus, it is highly unlikely that MON 87701 would have an impact on the Saint Francis' satyr butterfly because larval host plants are believed to be graminoids such as grasses, sedges, and rushes. Based on this information, the "preferred" and "no action" alternatives are not different.

D.9. Potential Impacts on Biodiversity

Analysis of available information indicates that MON 87701 exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other sexually compatible relatives (of which there are none in the United States), and it is likely to have no effect on non-target organisms common to agricultural ecosystems or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. According to Wolfenberger et al. (2008) and Marvier et al. (2007), Bt-producing crops generally increase the abundance of non-target organisms compared to insecticide treated crops. Thus, deregulation in whole of MON 87701 may have similar effects when used on agricultural lands. Based on this analysis, it is concluded that if APHIS adopts the "preferred" alternative, and MON 87701 is offered to commercial growers, biodiversity may increase. This would not occur under the "no action" alternative or for use of MON 87701 for breeding and seed multiplication purposes.

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E. Potential for Cumulative Impacts

According to the Council on Environmental Quality (CEQ) regulations (40 CFR § 1508.27), as part of the assessment of significance, one must consider potential impacts of this action in light of previous related and reasonably foreseeable future related actions. Therefore, cumulative impacts of a proposed action must be evaluated in order to determine whether a proposal significantly affects the quality of the human environment. Factors addressed in the previous sections largely focused on potential significant effects directly associated with a decision to deregulate MON 87701. Many of these same factors must be considered in order to assess possible cumulative effects. Factors addressed under cumulative impacts take into account previous and future actions which of themselves may not constitute a significant impact, but in combination with this action and possible future actions may result in a significant effect to the quality of the human environment. Direct effects that may be cumulative as well as past and possible future actions are considered in this section. Past actions include deregulation of other biotechnology-derived crop products including those that produce Bt proteins and soybean crop products that MON 87701 may be bred with.

E.1. Potential for Cumulative Impacts to Public and Animal Health

Stacking through conventional breeding: As previously mentioned, several biotechnology-derived soybean crop products have been deregulated or are under consideration for deregulation, and a list of the event codes approved by USDA is presented in the chart below. MON 87701 may be bred with these deregulated biotechnology-derived soybean crop products as well as with conventional soybean, creating new improved varieties. For biotechnology-derived products, APHIS has determined that these individual soybean products do not display increased plant pest characteristics and any progeny derived from crosses of these soybean products with other conventional or biotechnology-derived soybean are unlikely to exhibit new plant pest properties. All biotechnology-derived soybean products on the market today have satisfactorily completed the FDA consultation process expressly established to review the safety of whole foods derived from biotechnology-derived crops for human and animal consumption (see Table K-3). Thus, combining the unrelated single events through conventional breeding should not pose any new characteristics which would change the safety assessment conclusions. An assessment of the stability of the genetic insert in MON 87701 was conducted, and data have been presented in the Petition demonstrating that MON 87701 is stable in progeny. Having established that the genetic material is stable and that MON 87701 is inherited in a Mendelian fashion, and based on experience with MON 87701 in Monsanto's plant breeding program, it can be concluded that the phenotype of MON 87701 is likewise stable. Given that there have been no plant pest or plant health characteristics associated with MON 87701, or with any of the previously deregulated events listed below, no significant impacts are expected through the use of MON 87701 in breeding programs and in combination with any of the previously deregulated soybean crop products.

Furthermore, the use of conventional breeding to produce combined trait or combined event products would identify off-types and non-performing germplasm during

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development of new inbreds and new varieties and be removed from further development. Breeders use standard testing and assessment procedures to further examine and confirm the equivalence of the combined trait products to the single event products in terms of phenotypes, agronomic characteristics, and the efficacy of the traits.

It may be noted that there are no pesticidal protein-producing soybean products on this list; thus, there is no possibility for synergistic effects to insects due to stacking with another deregulated pesticidal trait. No impacts to public health (e.g., food or feed safety) are expected due to the breeding of MON 87701 with these soybean varieties because the proteins have an established history of safe use and none of the pathways are expected to interact resulting in the production of novel untested compounds.

Table K-3. Deregulated Biotechnology-derived Soybean Products

Phenotype	ID Code(s)	Institution	Date Deregulated
High Oleic Acid	DP-3Ø5423-1	Pioneer	Submitted
Glyphosate Tolerant	MON 89788	Monsanto	February, 2007
Phosphinothricin Tolerant	GU262	AgrEvo	October, 1998
Phosphinothricin Tolerant	A5547-127	AgrEvo	May, 1998
Altered Oil Profile	G94-1, G94-19, G-168	DuPont	May, 1997
Phosphinothricin Tolerant	W62, W98, A2704-12, A2704-21, A5547-35	AgrEvo	August, 1996
Glyphosate tolerant	40-3-2	Monsanto	May, 1994

A potential cumulative impact to human and animal health to consider is the impact from all sources of Cry1 proteins. Sprays of sporulated *B. thuringiensis* have a long history of safe use for pest control in agriculture, especially in organic farming (Cannon, 1993; EPA, 1988; WHO, 1999). Microbial pesticides containing *B. thurigiensis* Cry1A proteins have been used for more than 45 years and endured extensive toxicity testing showing no adverse effects to human health (Baum et al., 1999; Betz et al., 2000; EPA, 2000; EPA, 2001; McClintock et al., 1995; Mendelsohn et al., 2003). During the last decade a variety of biotechnology-enhanced crops containing Cry1 proteins from *B. thuringiensis* have been commercialized, thus, rendering these plants resistant to several insect pests (De Maagd et al., 1999; Mendelsohn et al., 2003). For example, corn that produces the Cry1Ab (YieldGard, Bt11) and Cry1F (Herculex I) proteins, as well as cotton producing the Cry1Ac and Cry2Ab2 (Bollgard II) proteins are currently registered and sold on the market (EPA, 2008; Mendelsohn et al., 2003). Compositional equivalence of these products to conventional varieties has been demonstrated (Berberich et al., 1996). Detailed human and animal safety assessments and almost a decade of safe human and animal consumption of these crops confirm their safety (Betz et al., 2000; Mendelsohn et al., 2003). Based on these data, the deregulation of MON 87701 in combination with other Cry1 protein products would not present any cumulative impact to human or animal health.

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Under the "preferred" alternative, MON 87701 would be available for stacking with other deregulated biotechnology traits through conventional breeding, and an additional Cry1Ac producing crop could increase the exposure to Cry1Ac in food, feed and to the environment by adding to the Cry1Ac produced from previously approved crops and used in microbial sprays. Having established that the phenotypes are stable and that there are no expected interactions of the biochemical pathways, and considering the safety of the Cry1Ac protein as well as its established history of safe use, there is no cumulative impact due to the "preferred" alternative. Under the "no action" alternative, stacking of biotechnology-derived traits through conventional breeding would occur and exposure to the Cry1 protein would continue from all sources, including exposure to the Cry1Ac protein through use of Bt cotton and Bt corn and microbial sprays.

E.2 Potential for Cumulative Impacts on Biodiversity, Preservation of Soybean Germplasm Purity, and Specialty Soybean Production

The "preferred" alternative would allow broad scale production of MON 87701 in an environment where other Cry1 producing crops as well as Bt sprays containing Cry1 are used. As discussed previously in Section D.9, use of Bt crops in comparison to insecticide treated crops have in general shown increases in the abundance of non-target organisms. Based on these analyses, there is negligible potential for any adverse cumulative impacts on biodiversity from the commercialization of MON 87701 combined with other Cry protein crops and sprays that are already on the market or can be reasonably foreseen to enter the market in the future. Broad scale use of MON 87701 may result in a net positive benefit to biodiversity compared to use of insecticide sprays.

Data have been presented in this Petition demonstrating that the insert in MON 87701 is inherited in a predictable Mendelian fashion. Thus, MON 87701 may be easily incorporated or removed from germplasm using well established breeding techniques. Soybean breeders have developed numerous biotechnology improved varieties over the past 13-years with traits that have been previously approved by APHIS using well established breeding systems based on knowledge of gene inheritance. MON 87701 in conjunction with other biotechnology-derived traits does not impact the breeding strategies soybean breeders use to select and produce improved varieties.

Biotechnology-derived soybean varieties have been on the market since 1996. While there has been a reduction in the number of conventional soybean varieties available, this is due to the demand for biotechnology-derived varieties not to the failure of maintaining varietal purity. In fact, organic, conventional, and specialty soybean varieties are available even though the vast majority of soybean grown contains a biotechnology-derived trait (see Section D.4.). Thus, introduction of another biotechnology-derived soybean, in an environment where greater than 90% of the soybean grown already contain a biotechnology trait, is unlikely to impact the production of specialty soybean varieties. For these reasons, long-term use of MON 87701 ("preferred" alternative) is not expected to impact soybean germplasm and specialty soybean production. Under the "no action" alternative, these breeding systems will still be in place and used to preserve the integrity of soybean germplasm.

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E.3 Potential for Cumulative Impacts on Land Use and Agronomic Practices

E.3.1 Cumulative Impacts to Land Use

The cumulative land area in the U.S. planted to principal crops, which include corn, sorghum, oats, barley, winter wheat, rye, durum, spring wheat, rice, soybean, peanuts, sunflower, cotton, dry edible beans, potatoes, canola, proso millet, and sugar beets, has remained relatively constant over the past 25 years. From 1983 to 1995, the average yearly acreage of principal crops was 328 million. This average is statistically unchanged since the introduction of biotechnology-derived crops in 1996 (http://usda.mannlib.cornell.edu/usda/current/htrcp). Therefore, there is no indication that the introduction and widespread adoption of biotechnology-derived crops has resulted in a significant change to the total U.S. cropland acres.

Following APHIS deregulation and commercial introduction, the acres of insect-protected crop products (i.e., corn, cotton) planted for each crop has steadily increased (James, 2007). However, rapid adoption of insect-tolerant crops has not correlated with an increase in the total acres for that particular crop. Specific crop acres do vary from year-to-year, but these fluctuations occur routinely and are due to a myriad of factors based largely around grower economic returns for each crop, crop rotation practices, and government programs. MON 87701 will likely be stacked with MON 89788 (Roundup Ready 2 Yield soybean) and is expected to be grown on land that is currently devoted to the production of commercial biotechnology-derived soybean varieties. Thus, a decision to deregulate MON 87701 in whole or the "no action" alternative would not be expected to impact the cumulative acres devoted to soybean production in the U.S., or the cumulative acres devoted to any other crop for which an insect-protected plant has been deregulated.

E.3.2 Insect Resistance Management

Bt proteins are very important biological pesticides that are used by growers planting biotechnology-derived Bt-containing crop products and organic growers. Thus, a potential cumulative impact from widespread adoption of Bt-based crops combined with the use of Bt sprays is the development of resistance to the Cry1Ac protein through commercial use and subsequent exposure to lepidopteran insect pests.

The Cry1Ac protein expressed in MON 87701 shares >99% amino acid identity with Cry1Ac from Bt and 100% amino acid sequence identity with the Cry1Ac protein present in Bollgard and Bollgard II cotton, with the exception of the four additional amino acids at the N-terminus of the MON 87701-produced Cry1Ac protein that are derived from a chloroplast targeting sequence. Bollgard cotton expressing the Cry1Ac protein was deregulated by USDA in 1995 and the second generation cotton (Bollgard II) producing Cry1Ac and Cry2Ab was deregulated in 2002. Additionally, other crops expressing Bt-proteins have also been deregulated.

The EPA regulates all plant incorporated protectants (PIPs), which includes all Bt-containing crops. As a condition of registration for any Bt-containing crop, all applicants must develop, implement and oversee an IRM plan that growers must use to minimize the development of resistance. The IRM plans that are currently in place for Bt-containing crops were a condition of registration required by EPA. No field-based resistance has

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been observed to any Bt-containing crop in the continental U.S. despite more than a decade of intensive use of various Bt cotton and Bt corn products (see Section IX.I).

As discussed previously (Section I.C), Monsanto intends to apply for an EPA registration that would only allow planting of MON 87701 for breeding and seed multiplication purposes. Given the limited acreage for MON 87701 needed for such breeding and seed multiplication use, under EPA policy, no IRM plan or refuge is warranted because selection pressure from the use of MON 87701 will be negligible under these circumstances (see Section IX.I). Furthermore, adequate refuge is available from non Bt-producing soybean. Moreover, during seed multiplication, breeders will not depend on the Cry1Ac produced by MON 87701 as the primary insecticide. Instead, breeders will employ broad spectrum insecticides, thereby further limiting selection pressure from the Cry1Ac protein.

Should commercial intentions change and Monsanto decide to offer MON 87701 to U.S. growers, Monsanto would be required by EPA to submit an IRM plan consistent with the intended use of MON 87701 and taking into account other Bt-producing crops, particularly those using the Cry1Ac protein. Thus, either in the context of breeding and seed multiplication or full-scale commercialization, EPA has in place systems and requirements to address the potential for the development of resistance that accompanies the use of any Bt-containing crop.

E.4 Potential for Cumulative Impacts on Non-agricultural Environments and Threatened and Endangered Species

Basic to all evaluations of biotechnology-derived soybean's potential for cumulative impact to the environment or threatened and endangered species is the fact that soybean cannot persist as a weed. It is an annual, largely self-pollinated crop that lacks sexually compatible wild relatives in the U.S. (including threatened or endangered plant species). Soybean exhibits extremely limited seed dormancy, has no weedy characteristics, and volunteer plants are easily controlled. It is not capable of establishing persistent populations in unmanaged environments. As demonstrated previously, the presence of the trait and transformation process in no way alters the weediness potential or gene flow potential of MON 87701.

The safety of the Cry1 family of proteins has been established through their use in Bt-producing corn and cotton crops and through their use in microbial formulations. Bollgard cotton producing the Cry1Ac protein was deregulated by APHIS in 1995 (http://www.aphis.usda.gov/brs/not_reg.html) and has been on the market since 1996 and cultivated on millions of acres globally. No credible negative cumulative impacts of any significance have been associated with Bollgard cotton or other Cry1 producing crops. Similarly, long term use of MON 87701 would not be expected to cause any significant cumulative impacts to non-agricultural land or threatened and endangered species. According to Brookes and Barfoot (2008), cumulatve insecticide use on Bt cotton and Bt corn, has decreased. Reduction in the use of pesticides reduces the chance for spray drift to non-agricultural lands and may reduce runoff to streams, rivers and lakes. Hence, the "preferred" alternative may provide greater benefits to non-agricultrural lands and endangered species than the "no action" alternative.

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E.5. Potential for Cumulative Impacts on Economic and Environmental Interests

Biotechnology-derived crop products have contributed to increased yields, enhanced simplicity and flexibility of insect pest control and weed management, reduced chemical insecticide and herbicide use, and increased no-till acreage that resulted in less soil erosion and less runoff of pesticides and water (Koziel et al., 1993; Martin and Hyde, 2001; Carpenter and Gianessi, 2001; Gianessi et al., 2002; Shelton et al., 2002; Fawcett and Towery, 2002; Hyde et al., 2003; Carpenter et al., 2004; Sankula and Blumenthal, 2004; Sankula et al., 2005; Sankula, 2006).

In a recent study, economists Brookes and Barfoot (2008) quantified the cumulative economic and environmental impacts of biotechnology-derived crops grown during the past eleven years (1996-2006). The authors report that biotechnology-derived crops have resulted in substantial global economic and environmental benefits. Over the past 11 years biotechnology-derived crop adoption has positively impacted the environment by reducing greenhouse gas emissions from agriculture and reducing pesticide spraying. This technology has also contributed to higher yields for many growers contributing to increased grower incomes. A new study issued by the National Center for Food Agricultural Policy reported an increased net return to U.S. growers of [redacted] (http://www.ncfap.org) for 2006 alone. The estimated farm income benefit to growers worldwide for all biotechnology-derived crops is $[redacted] (Brookes and Barfoot, 2005). Additionally, there is speculation that, without wide adoption of this technology, world prices for corn and soybean could be even higher than the current prices. In 2004, the United Nations Food and Agriculture Organization noted that agricultural biotechnology is a complementary tool to traditional farming methods that can help poor farmers and consumers and improve food security (UN-FAO, 2004).

These benefits are from biotechnology-derived crops that were designed to be tolerant of or resistant to biotic environmental stressors such as weeds (via novel herbicide tolerances) and insects. MON 87701 is the first Bt-producing soybean plant controlling selected lepidopteran insects. It is not known how many U.S. acres MON 87701 would be grown on if commercialized. However, pending EPA registration of MON 87701 for commercial use in the U.S., MON 87701 may provide benefits to U.S. soybean producers similar to other Bt-producing crops commercialized on broad acres across the U.S. On a global basis, MON 87701 will help reduce carbon emissions by reducing pesticide sprays and contribute to improved yields. Given the above, under the "preferred" alternative, insect-protected soybean MON 87701 has the potential to add to the positive cumulative impact of biotechnology products on the U.S. economy and the environment. These benefits would not occur under the "no action" alternative.

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F. Highly Uncertain, Unique or Unknown Risks

MON 87701 has been thoroughly characterized and data submitted in the Petition demonstrate that it poses no increased plant pest risk compared to conventional soybean. USDA-APHIS has previously deregulated 13 biotechnology-derived soybean crop products. While MON 87701 represents the first Bt-producing soybean crop product reviewed by APHIS, Bt-producing corn and cotton plants have been commercialized and

grown on millions of acres globally. Introduction of these crops have provided control of target insects while showing no unintended effects on non-target or beneficial insects and have provided predictable benefits to growers. In this respect, a decision to deregulate a new biotechnology-derived soybean crop product is not precedent setting nor are the effects to the quality of the human environment highly uncertain or unpredictable.

G. Summary

MON 87701 has been thoroughly characterized and the extensive body of information presented in Sections I through X of this Petition demonstrates that MON 87701 does not present a plant pest risk, has no significant impact on threatened or endangered species or biodiversity, and will not impact the commercial interests of soybean producers or those involved in the marketing and sale of soybean and soybean products. The introduction and adoption of Bt-producing crops have increased yields and benefited farm income in the U.S. However, the amount of land devoted to farming (specifically to corn or cotton) has not changed with the introduction of Bt-producing crops. Similarly, no change in the use of agricultural land or amount of land devoted to farming would be expected to occur with the commercial introduction of MON 87701. With the exception of an anticipated reduction of insecticide use pending EPA approval and commercial planting of MON 87701 in the U.S., agricultural practices are not likely to change. The potential reduction in pesticide use is a positive benefit from an environmental and worker safety perspective. Soybean products produced by MON 87701 have been demonstrated to be safe and wholesome for food and feed purposes. The Cry1Ac protein produced by MON 87701 has been characterized and demonstrated to be safe for consumption. The Cry1 family of proteins are used extensively in Bt-producing corn and cotton plants and in microbial sprays, and have an established history of safe use for food, feed, and the environment.

Factors related to "significance" as described by CEQ regulations have been adequately addressed in this analysis. It can be concluded from the analysis of these factors that deregulation of MON 87701 does not represent a significant impact to the quality of the human environment either directly or in combination with past and reasonably foreseeable future actions. For these reasons, the requested action of deregulation in whole does not present a significant environmental impact and should lead to a Finding of No Significant Impact.

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USDA/APHIS Petition #09-082-01p
December 9, 2009

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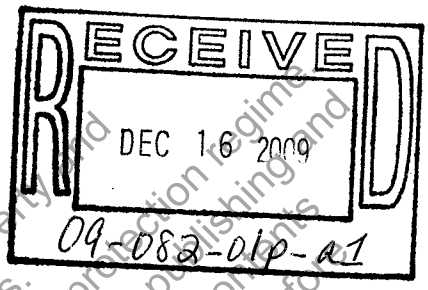

[redacted]
Director, Environmental Risk Analysis Programs
USDA-APHIS-BRS
4700 River Road
Riverdale, MD 20737

RE: Summary of additional information, clarifications and corrections to the Petition for Determination of Non-regulated Status MON 87701; Petition #09-082-01p

Dear [redacted]

This letter summarizes the additional information, clarifications and corrections to the Petition for Determination of Non-regulated Status of MON 87701; Petition #09-082-01p as outlined in the November 19, 2009 letter from USDA/APHIS to Monsanto, and as discussed during the conference call between USDA/APHIS and Monsanto on November 12, 2009.

After reviewing the petition, USDA/APHIS requested additional information and clarification before declaring this petition technically complete. Monsanto's responses to the questions posed are in the attached Addendum and appropriate revisions have been made in the revised petition in the sections indicated. All table, figure and section numbers refer to both the original and revised versions of the petition, except where noted.

The enclosed response addendum contains an appendix containing confidential business information (CBI); therefore a CBI-deleted version has been supplied. The enclosed CBI copy and CBI-deleted versions of the response are being submitted to provide the correct format, consistent with APHIS' website directions (http://www.aphis.usda.gov/brs/pdf/Doc_Prep_Guidance.pdf), for a petition containing confidential information.

In addition to the clarifications and corrections requested by USDA/APHIS, minor changes were made to the document to improve consistency, clarity and accuracy of the document. The list of these clarifications and corrections can be found in the table attached to this letter. These changes include renaming Section X "Plant Pest Assessment" as "Environmental Consequences and Impact on Agronomic Practices", moving Section XI "Summary of

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Environmental Assessment" to Appendix K and renaming it "Petitioner's Environmental Assessment", and renaming Section XII to Section XI. None of the minor corrections or edits listed in the attached table change the conclusion of the assessment that MON 87701 is unlikely to pose an increased plant pest potential or to have an adverse environmental consequence compared to conventional soybean.

As requested by USDA/APHIS, two printed copies and an electronic version of the revised petition are enclosed. Should you have any questions concerning this letter, or wish to set up a meeting for further discussion, please contact [redacted] Sr. Director U.S. Regulatory Affairs & Policy, Washington DC, at [redacted] or me at [redacted].

Sincerely,

[redacted]

Regulatory Affairs, Oilseeds

cc: [redacted] Monsanto
Regulatory files/09-SY-194U

See attachment

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Attachment

Clarifications and Corrections to Petition #09-082-01p for the Determination of Non-regulated Status for MON 87701

Page, Section (changes)	Existing Text or Information	Revised Text or Information
Page 52, Section V Changed the sentence in the legend of Figure V-1	Identified on the map are genetic elements within the insert, as well as restriction sites with positions relative to the size of the linear map for enzymes used in the Southern analyses.	Identified on the map are genetic elements within the insert, including restriction sites with positions relative to the size of the genomic flanking sequences and the insert sequence for enzymes used in the Southern analyses.
Page 53, Section V Added ~1.9Kb in Table V-1 when digested with *Xho I/Nde I* (probes 9, 11)	~5.7 Kb ~2.7 Kb	~5.7 Kb ~2.7 Kb ~1.9Kb
Page 56, Section V.A.2 Changed the sentence in the 2nd paragraph	The ~1.9 kb band observed in Figure V-3 (lanes 4 and 11) represents **the 3' border fragment containing the 3' end of the inserted DNA along with the adjacent genomic DNA flanking the 3' end of the insert.**	The ~1.9 kb band observed in Figure V-3 (lanes 4 and 11) represents an internal fragment contained in the inserted T-DNA.
Page 59, Section V.A.3 Changed the sentence in the legend of Figure V-3	The blot was hybridized with two **overlapping** ^{32}P-labeled probes that spanned portions of the T-DNA I sequence (Figure IV-1, Probes 8 and 10).	The blot was hybridized with two ^{32}P-labeled probes that spanned portions of the T-DNA I sequence (Figure IV-1, Probes 8 and 10).
Page 60, Section V.A.3 Changed the sentence in the legend of Figure V-4	The blot was hybridized with two **overlapping** ^{32}P-labeled probes that spanned portions of the T-DNA I sequence (Figure IV-1, Probes 9 and 11).	The blot was hybridized with two ^{32}P-labeled probes that spanned portions of the T-DNA I sequence (Figure IV-1, Probes 9 and 11).
Page 61, Section V.B Changed the	The presence or absence of plasmid backbone sequences in the soybean genome was evaluated by	The presence or absence of plasmid backbone sequences in the soybean genome was evaluated by digesting the

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sentence in the first paragraph.	digesting the test and control genomic DNA samples with *Nco* I / *Vsp* I or *Xho* / *Nde* I and hybridizing with **overlapping** backbone probes spanning the entire backbone sequence of PV-GMIR9 (Figure IV-1, Probes 1, 2, 3, and 4).	test and control genomic DNA samples with *Nco* I / *Vsp* I or *Xho* / *Nde* I and hybridizing with backbone probes spanning the entire backbone sequence of PV-GMIR9 (Figure IV-1, Probes 1, 2, 3, and 4).
Page 66, Section V.D. Changed the 4th sentence.	The DNA sequence of the MON 87701 insert is 6426 base pairs long, beginning at base 3908 of PV-GMIR9 located in the right border region and ending at base **10334** in the left border region of PV-GMIR9.	The DNA sequence of the MON 87701 insert is 6426 base pairs long, beginning at base 3908 of PV-GMIR9 located in the right border region and ending at base 10333 in the left border of PV-GMIR9.
Page 67, Section V.E. Changed the sentence in 1st paragraph	In order to demonstrate the stability of MON 87701, Southern blot analyses were performed using DNA obtained from multiple generations of MON 87701.	In order to demonstrate the stability of **T-DNA** in MON 87701, Southern blot analyses were performed using DNA obtained from multiple generations of MON 87701.
Page 141 Section X. in 3rd paragraph.	APHIS has recently proposed to amend **40** CFR Part 340 to include its noxious weed authority.	APHIS has recently proposed to amend **7** CFR Part 340 to include its noxious weed authority.
Page 185 (originally Page 212, Appendix B Changed the lane numbering in the legend of Figure B-1.	PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 87701 were performed on MON 87701 genomic DNA extracted from leaf tissue (Lanes 3, **6, 11, 15, 21, 25, and 30**).	PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 87701 were performed on MON 87701 genomic DNA extracted from leaf tissue (Lanes 3, 7, 10, 14, 18, 23, 27, 31 and 35).
Page 211 (originally Page 238) A sentence was added under Characterization of the Materials		…for the presence or absence of MON 87701. "**The results indicate samples from one replicate of MON 87701 at Site AL and one replicate of A5547 at Site NC contained levels of an unintended trait and, therefore, were deemed unacceptable and were excluded**".

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Response to Review for Completeness and Acceptability of Monsanto Petition Number 09-082-01p for a Determination of Non-regulated Status for MON 87701 soybean

This response document provides clarification and corrections to questions on the Petition for Determination of Non-regulated Status of MON 87701, Petition #09-082-01p, as outlined in the November 19, 2009 letter from USDA-APHIS to Monsanto. This response contains confidential business information (CBI) that Monsanto is requesting be held confidential. The CBI information is provided in Appendix 1 to this response, with the appendix provided as *CBI copy* and *CBI-deleted* versions.

USDA Question #1 (General Issue): Standard deviation (or standard error) values are presented for some analyses in the petition, but not others. Those statistics are useful for reviewers to make inferences about data presented in tables or to cross check petitioner's interpretation of data. Please provide SD (or SE) values for all mean values in tables. Also, provide sample sizes for all measures of central tendency presented in the petition.

Monsanto Response: Standard error (SE) values have been added to Tables VIII-3, VIII-5, VIII-6, VIII-7, F-2, F-4, F-5, G-3, G-8 and G-9.

SE values have not been added to Table VII-1 because this table is a summarization from Tables E-1 through E-12 (see Appendix E in Petition #09-082-01p) to show the significant differences among analytes between MON 87701 and the conventional soybean control A5547. Tables E-1 through E-12, with the associated footnotes, provide the standard error and sample size. The detailed analytical methodology and statistical analysis for all measures of central tendency can be found in Appendix E.

USDA Question #2 (General Issue): Please submit all final field test reports for those notifications that are cited in the petition. While we recognize that some of are not yet due, we cannot complete our review until all are received.

Monsanto Response: All field test reports for notifications cited in the petition (Table A-1) have been submitted to USDA-APHIS, and the last field report corresponding to Notification 08-261-101n was delivered to USDA on Nov 13, 2009.

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

Monsanto Response: The T-DNA insert and flanking DNA sequence in MON 87701 (Figure 1, see Response Appendix 1) was compared to the transformation vector PV-GMIR9 sequence and to the conventional soybean sequence (Figures 3, 4 and 5, see Response

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Appendix 1) using the MegaBLAST[1] program. The comparison between MON 87701 and PV-GMIR9 sequences identified the insert and flanking sequences in MON 87701. The comparison between MON 87701 flanking and conventional soybean sequences allowed for the determination of any rearrangement (deletion or insertion) that may have occurred during the transformation process.

a) **Alignment of MON 87701 sequence with plasmid PV-GMIR9 sequence** (Figure 3, Response Appendix 1**).** Alignment of MON 87701 sequence with PV-GMIR9 sequence identified a 6426 bp sequence that is identical between MON 87701 and PV-GMIR9. This 6426 bp sequence is the T-DNA insert in MON 87701, represented as base pairs 2001-8426 in the MON 87701 DNA sequence (Figure 1) and base pairs 3908-10333 in the PV-GMIR9 sequence. Base pairs 1-2000 and 8427-10535 in MON 87701 represent the flanking soybean genomic DNA sequences (Figure 1).

b) **Alignments of the conventional soybean sequence with MON 87701 flank sequences** (Figures 4 and 5, Response Appendix 1**).** Alignment of the conventional soybean sequence with MON 87701 5′ flank sequence (Figure 4) shows that base pairs 1-1440 of conventional soybean DNA (Figure 2) are 100% identical to the MON 87701 5′ flank sequence (Figure 1, base pairs 547-1986). In addition, Figure 5 shows that base pairs 1473-2968 of the conventional soybean DNA sequence are 100% identical to the MON 87701 3′ flank sequence (Figure 1, base pairs 8427-9922). The alignments of conventional soybean DNA with the MON 87701 flanking DNA sequences do not show any unexpected rearrangement of the native soybean genomic DNA flanking the T-DNA (1440 bp of conventional genomic DNA on the 5′ flank of the T-DNA and 1496 bp of conventional soybean genomic DNA on the 3′ flank of the T-DNA).

Based on the alignments, the following rearrangements have been identified. A 14 bp sequence (1987-2000 bp) in MON 87701 was identified as an insertion, because it is not present in the conventional soybean DNA (bold letters in Figure 1 and Figure 4). Conversely, conventional soybean genomic sequence base pairs 1441-1472 are not present in the MON 87701 flanking sequences and are identified as a 32 bp deletion (bold letters in Figure 2 and Figure 4). As noted in the petition, such minor rearrangements are commonly observed during *Agrobacterium*-mediated transformation.

USDA Question #4 (Specific Issue): (pg 76-77, Figure VI-1) please highlight differences in sequence as described in the caption.

Monsanto Response: The differences of amino acid sequence from comparisons between Cry1Ac from *Bacillus thuringiensis* and two plant-produced Cry1Ac proteins expressed in Bollgard® cotton and MON 87701 are highlighted on pages 76-77 in the revised petition #09-082-01p.

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[1] MegaBLAST is a proprietary Monsanto DNA sequence alignment tool.
® Bollgard is a registered trademark of Monsanto Technology LLC.

USDA Question #5 (Specific Issue): (pg 212) There are errors in the lane numbering in the caption.

Monsanto Response: The lane numbing errors have been corrected in the legend of Figure B-1 (see Appendix B of revised petition #09-082-01p).

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Response Appendix 1:

DNA Sequences Alignment Between MON 87701 and the Conventional Soybean Control (A5547) at Insertion Site

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Figure 1. DNA Sequence of the Insert and Adjacent Genomic DNA in MON 87701

The following DNA sequence represents the consensus sequence of the insert in MON 87701. Base pairs 1-2000 (underlined) represent soybean genomic DNA flanking the 5′ end of the insert. Base pairs 2001-8426 represent the inserted DNA corresponding to base pairs 3908-10333 from PV-GMIR9. A small insertion of 14 bp is bolded (base pairs 1987-2000). Base pairs 8427-10535 (double underlined) represent soybean genomic DNA flanking the 3′ end of the insert.

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Figure 2. DNA Sequence of the PCR Product from Conventional Soybean.

Base pairs 1-1440 (underlined) represent genomic soybean DNA sequence that is identical to the 5' genomic DNA sequence flanking the MON 87701 insert (547-1986 bp, Figure 1). Base pairs 1441-1472 represent a 32 bp deletion located at the 5' end to the MON 87701 insertion site of the T-DNA (Bolded). Base pairs 1473-2968 (double underlined) represent genomic soybean DNA sequence that is identical to the 3' genomic DNA sequence flanking the MON 87701 insert (8427-9922 bp, Figure 1).

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Figure 3. MegaBLAST Alignment of PV-GMIR9 DNA Sequence with MON 87701 Sequence

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Figure 4. MegaBLAST Alignment of MON 87701 5′ Flank Sequence with Conventional Soybean DNA Sequence

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Figure 5. MegaBLAST Alignment of MON 87701 3′ Flank Sequence with Conventional Soybean DNA Sequence

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Petition for Determination of Non-Regulated Status for Insect-Protected Soybean MON 87701

CLAIM OF DATA CONFIDENTIALITY

Monsanto is claiming the DNA sequence of plasmid PV-GMIR9, the T-DNA insert of MON 87701, and the 3' and 5' flanking sequences of the insert in MON 87701 as Confidential Business Information (CBI). This information is located in Monsanto's response document (dated December 9, 2009) as Appendix 1, Figures 1 through 5. The following justification is made for this CBI claim.

i. Legal Background

The Freedom of Information Act (FOIA), 5 U.S.C. § 552, specifically exempts from release "trade secrets and commercial or financial information obtained from a person as privileged or confidential" ("Exemption 4") 5 U.S.C. § 552(b)(4). Exemption 4 applies where the disclosure of information would be likely to cause substantial harm to the competitive position of the owner, National Parks & Conservation Association v. Morton, 498 F.2d 765, 770 (D.C. Cir. 1974); Gulf & Western Industries, Inc. v. U.S., 615 F.2d 527, 530 (D.C. Cir. 1979), or where, in the case of information voluntarily submitted to the government, the information is not customarily made available to the public by the provider. Critical Mass Energy Project v. NRC, 975 F.2d 871 (D.C. Cir. 1992) ("financial or commercial information provided to the Government on a voluntary basis is 'confidential' for the purpose of exemption 4 if it is of a kind that would customarily not be released to the public by the person from whom is was obtained.").

A party seeking to demonstrate "substantial competitive harm" need not show actual competitive harm, but must only demonstrate the presence of competition and the likelihood of substantial competitive injury. Id. at 530; National Parks & Conservation Association v. Kleppe, 547 F.2d 673, 679 (D.C. Cir. 1976); Miami Herald Pub. Co. v. U.S. Small Business Administration, 670 F.2d 610, 614 (5th Cir. Unit B 1982).

For the purposes of FOIA, courts have defined the term "trade secret" to mean a "secret, commercially valuable plan, formula, process, or device that is used for the making, preparing, compounding, or processing of trade commodities and that can be said to be the end product of either innovation or substantial effort." Public Citizen Health Research Group v. FDA, 704 F.2d 1280, 1288 (D.C. Cir. 1983); Anderson v. Dept. of Health & Human Services, 907 F.2d 936, 943-44 (l0th Cir. 1990).

Where, as in the case of Monsanto's product subject to an FOIA request, the development time and costs of the products have been substantial and the information can only be obtained by competitors at considerable cost, disclosure is prohibited. Greenberg v. Food and Drug Administration, 803 F.2d at 1213, 1216-1218 (D.C. Cir. 1986); Worthington Compressors, Inc. v. Costle, 622 F.2d 45, 51-52 (D.C. Cir. 1981).

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Information on commercial development falls squarely within this definition, and is the type of information accorded trade secret protection by the courts under Exemption 4 of the FOIA request. The courts have been very clear in finding commercial development information covered by Exemption 4 where the release of such information could allow competitors to procure a clear understanding of a company's business practices and allow a competitor to cause harm to a company's competitive standing. See, e.g., Braintree Electric Light Dept. v. Dept. of Energy, 494 F. Supp. 287, 289-291 (D. D.C. 1980).

The U.S. Department of Agriculture's APHIS has defined "Confidential Business Information" for the purposes of biotechnology submissions within the boundaries of these statutory and court interpretations of Exemption 4. "Policy Statement on the Protection of Privileged or Confidential Business Information," (the CBI Policy Statement), 50 Fed. Reg. 38561 (Sept. 23, 1985). The CBI Policy Statement defines CBI to consist of "Trade Secrets" and "Commercial or Financial Information." "Trade Secrets" are, in turn, defined as: "information relating to the production process. This includes production data, formulas, and processes, and quality control tests and data, as well as research methodology and data generated in the development of the production process. Such information must be (1) commercially valuable, (2) used in one's business and (3) maintained in secrecy."

The CBI Policy Statement states that "Commercial or Financial Information will also be deemed confidential if review establishes that substantial competitive harm would result from disclosure." Information such as the sequence of the inserted DNA or flanking regions appears to fall squarely within this description. *See e.g.,* USDA-APHIS BIOTECHNOLOGY USER'S GUIDE, GENERAL DOCUMENT PREPARATION GUIDELINES FOR SUBMISSION TO BRS, 9 (February 5, 2008).

ii. **Justification**

The DNA sequence of plasmid PV-GMIR9, the T-DNA insert of MON 87701, and the 3' and 5' flanking sequences of the insert in MON 87701 fall within the well-established boundaries of CBI as recognized by the federal courts and by APHIS. This information is either protected because it was voluntarily submitted by Monsanto and Monsanto has not released this information to the public,[1] and/or this information is protected because it constitutes Monsanto's trade secrets or commercial or financial information, as APHIS and the courts have defined those terms.[2] As discussed more fully below, this information comprises the results of extensive research and intellectual property required both for the commercial viability and regulatory authorization of this product. This information would be worth millions of dollars to one of Monsanto's competitors in this field, and should be accorded the protections due such confidential and valuable information.

Monsanto is at the leading edge in the development of biotechnology products in a rapidly growing and highly competitive industry. Monsanto faces a number of strong,

[1] See, e.g., Critical Mass Energy, 975 F.2d at 878.

[2] See, e.g., National Parks, 498 F.2d at 770.

multinational competitors in this field, including BASF, Bayer, Dow AgroSciences, DuPont and Syngenta. Monsanto's competitors, both domestic and international, have the expertise not only to replicate Monsanto's products, but also to use Monsanto's technology to develop other, competing products, thereby saving millions of dollars and years of development efforts.

Monsanto has been working on the development of biotechnology-derived crops since the early 1980s, and has become a leader in the field through the expenditure of several billion dollars in research and testing costs. Monsanto can document the development and testing costs by means of monthly summaries of the worker hours devoted to these projects, budgetary documents, field test agreements and project documents.

Presently, Monsanto's competitors cannot duplicate Monsanto's commercially valuable products from information in the public domain without going through the same painstaking trial and error development and testing that Monsanto has undertaken. Although certain information regarding Monsanto products has been made available, *e.g.,* in the context of patent applications, this information is voluminous and general in nature, and does not identify information Monsanto has found most effective for a particular product. A competitor cannot determine from the patent applications which particular combination of genes and transgenic products will prove to be commercially valuable.

The release of the DNA sequence of plasmid PV-GMIR9, the T-DNA insert of MON 87701, and the 3' and 5' flanking sequences of the insert in MON 87701 could provide competitors with commercially valuable knowledge regarding the characteristics of particular products Monsanto is planning to commercialize and the likely time frame for commercialization. This information would be extremely helpful to these companies in developing their own marketing strategies and development plans in a highly competitive market.

The commercial value of the information regarding the sequence of the inserted DNA has been recognized by Congress in its enactment of FIFRA and the FFDCA. Section 3 of FIFRA sets up an elaborate system of protections for these types of data, protecting them from any use by other manufacturers for a period of ten years, and requiring compensation for the use of these data by competitors after that initial ten year period. In 1996, Congress amended the FFDCA to provide both disclosure protections and compensation equivalent to that provided by FIFRA for health and safety data submitted to support pesticide residue tolerance and tolerance exemption applications. FFDCA § 408(i). APHIS should recognize the Congressional action to protect the commercial nature of these types of documents. APHIS's failure to do so could result in the loss of millions of dollars to Monsanto in data use and compensation rights.

In addition to the compensation provisions for these types of data set forth by FIFRA and FFDCA, each statute contains independent provisions for the protection from disclosure of this information. FIFRA § 10(g); FFDCA § 408(i). FOIA prohibits the disclosure

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of information specifically protected by statutes such as these. 5 U.S.C. § 552(b)(3). This prohibition provides additional justification for the protection of these data.

In summary, the DNA sequence of plasmid PV-GMIR9, the T-DNA insert of MON 87701, and the 3' and 5' flanking sequences of the insert in MON 87701 provided in Monsanto's response document (dated December 9, 2009) as Appendix 1, Figures 1 through 5, are required in order for Monsanto to obtain non-regulated status of MON 87701 and, thereby, commercial approval for this product. This information could save such competitors millions of dollars in research. Monsanto has demonstrated, and Congress has recognized, the commercial value and confidential nature of these data. The DNA sequence of plasmid PV-GMIR9, the T-DNA insert of MON 87701, and the 3' and 5' flanking sequences of the insert in MON 87701 are an integral part of Monsanto's business and should be protected as such.

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